Appendix

List of papers

Paper I

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Frozen Antarctic path for dispersal initiated parallel host-parasite evolution on different continents

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ABSTRACT

After the break-up of Gondwana dispersal of organisms between America, Australia and Africa became more complicated. One of the possible remaining paths led through Antarctica, that was not yet glaciated and it remained habitable for many organisms. This favourable climate made Antarctica an important migration corridor for organisms with good dispersal ability, such as Aculeata (Hymenoptera), till the Oligocene cooling. Here we tested how cooling of Antarctica impacted global dispersal of Aculeata parasites (Strepsiptera: Xenidae). Our data set comprising six nuclear genes from a broad sample of Xenidae. Bayesian dating was used to estimate divergence times in phylogenetic reconstruction. Biogeography was investigated using event-based analytical methods: likelihood-based dispersal–extinction–cladogenesis and Bayesian models. The Bayesian model was used for reconstruction of ancestral host groups. Biogeographical methods indicate that multiple lineages were exchanged between the New World and the Old World + Australia until the Antarctica became completely frozen over. During the late Paleogene and Neogene periods, several lineages spread from the Afrotropics to other Old World regions and Australia. The original hosts of Xenidae were most likely social wasps. Within one lineage of solitary wasp parasites, parallel switch to digger wasps (Sphecidae) occurred independently in the New World and Old World regions. The biogeography and macroevolutionary history of Xenidae can be explained by the combination of dispersal, lineage extinction and climatic changes during the Cenozoic era. A habitable Antarctica and the presence of now-submerged islands and plateaus that acted as a connection between the New World and Old World + Australia provided the possibility for biotic exchanges of parasites along with their hymenopteran hosts. Although Xenidae are generally host specialists, there were significant host switches to unrelated but ecologically similar hosts during their evolution. There is little or no evidence for cophylogeny between strepsipteran parasites and hymenopteran lineages.

1. Introduction

Vicariance and long distance dispersion (LDD) are two of the most important mechanisms in biogeography and are often considered as competing hypotheses. Vicariance and LDD events have been of major interest for studies of global terrestrial historical biogeography and continental drift (Sanmartín and Ronquist, 2004). The break-up of the Gondwana supercontinent began during the Mesozoic era and lasted until the beginning of the Oligocene epoch, about 30 million years ago (Ma). This resulted in the separation of its components into Africa, Antarctica, Australia, India, Madagascar, New Zealand, and South America. Proper understanding of the sequence and timing of geological events associated with the break-up of the southern continents (Jokat et al., 2003; Scotese et al., 1988) is crucial for interpreting biogeographical histories of biological taxa. The many disjunctions of distribution areas are usually explained by the breakup of Gondwana, causing successive divisions of an ancestral biota. However, recent biogeographic studies, based on molecular estimates and more accurate paleogeographic reconstructions, indicate that dispersal may have been more important than traditionally assumed (Sanmartín and Ronquist, 2004). Although the break-up of Gondwana started in the Cretaceous period, the connection between Australia and South America via Antarctica allowed biotic interchanges until the late Eocene epoch (McLoughlin, 2001). These interchanges were possible due to the habitable Antarctic environment under Eocene climate conditions (Pross et al., 2012). Antarctica was covered with a subtropical rain forest with southern beeches (*Nothofagus*) as dominant plants (Iglesias et al., 2011). A similar type of forest can be found at present in New Zealand,

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Tasmania or in the South American region of Patagonia (Swenson et al., 2001). At the Eocene-Oligocene boundary there was a significant cooling of Antarctica which resulted in the formation of an ice sheet accompanied by glacial cycles between 34 and 31 Ma (Galeotti et al., 2016). Despite this cooling, multiple refuges with mosaics of *Nothophagus* and conifer-dominated woodlands and tundra continued to occur in Antarctica during the Oligocene epoch (approximately 34–23 Ma) (Anderson et al., 2011). Based on pollen records, Warny et al. (2009) suggest that woodlands existed on Antarctica at least until 15 Ma, when there was a short period of rapid warming between 15 and 16 Ma. Furthermore, a recent geochemical study supports the presence of woodlands in the Neogene period (Rees-Owen et al., 2018).

According to current studies, as the connection between South America, Australia and Africa, Antarctica and adjacent island archipelagos in the Indian Ocean played a crucial role, in the evolution of highly mobile Aculeate Hymenoptera. Almeida et al. (2012) found multiple lineage exchanges between South America and Australia in bees of the Colletidae family during the Paleogene period, which is consistent with the hypothesis of Antarctica as a migration bridge (McLoughlin, 2001). Studies of allodapine bees (Allodapini) indicate that there was a single colonization event from Africa to Australia during the Eocene (Chenoweth and Schwarz, 2011; Schwarz et al., 2006). The authors of the studies suggest that this bee group, which diversified in Africa, expanded via the stepping stones of the now-submerged Kerguelen and Crozet Plateaus and via Antarctica because the node ages are too recent for the Gondwanan vicariance hypothesis, but too early for dispersal through the Sunda archipelago. Kayaalp et al. (2017) use the example of colletid bees to demonstrate the path from Australia to Africa. These bees diverged from their Australian ancestors during the Eocene and expanded into Africa at a similar time and in possibly the same way as Allodapini, but in the opposite direction.

Host specific parasites are often good models to track the biogeography of their hosts (Štefka et al., 2011). We assume that southern hemisphere Hymenoptera carried their parasites during dispersal events and also acquired parasites in their new environment. Parasites and their hosts are in a continuous 'arms race'; therefore, transitions of parasites to new hosts are limited by host specificity (Dawkins and Krebs, 1979). When the number of suitable hosts increases, host specificity decreases (Poulin, 2007). Within these narrower and wider host-parasite associations, species are able to influence the selective pressures that they enact on each other in the coevolutionary relationship (Agosta, 2006). Here we focused on the Xenidae family, one of the most evolutionarily derived and cosmopolitan groups of Strepsiptera, which parasitize different lineages of wasps, including: pollen wasps (Vespidae: Masarinae), sphecid and digger wasps (Sphecidae, Crabronidae), solitary wasps (Vespidae: Eumeninae, Zethinae), and social wasps (Vespidae: Polistinae, Vespinae) (Kinzelbach, 1971). Systematic division of Xenidae into various genera is based on known host associations and consists of: *Paragioxenos*, *Paraxenos*, *Pseudoxenos*, and *Xenos* (Kinzelbach, 1971). Xenidae and their sister group Stylopidae form a large monophyletic clade, which is considered to be one of the largest adaptive radiations in Strepsiptera (McMahon et al., 2011; Pohl and Beutel, 2005). The monophyly of this group is supported by both morphological and molecular studies (McMahon et al., 2011; Pohl and Beutel, 2005). The origin and diversification of these Hymenopteran parasites has been dated to the Paleogene (McMahon et al., 2011). Females of most Strepsiptera are permanently endoparasitic, neotenic, legless and immobile, and disperse only with their hosts. Adult males are always free-living and actively fly using their hindwings. Males of Strepsiptera are also well-known for their short lifespan of only several hours, during which they must find a female (Straka et al., 2011) that is releasing a powerful sex pheromone (Lagoutte et al., 2013; Zhai et al., 2016).

Here, we aimed to study the global biogeography and host specialization of parasites of the highly mobile Hymenoptera to test the possibility of transcontinental dispersals during the Eocene epoch when Antarctica was habitable. The stability of host associations and systematics of Xenidae were also tested. We combined phylogenetic inference, divergence dating, ancestral states analysis, and biogeographic inference to generate a deeper understanding of the evolutionary history of this remarkable group of insect parasites.

2. Material and methods

2.1. Material and data sets

Our data set consisted of 84 specimens from the Xenidae family and five outgroup specimens from the Stylopidae family (Strepsiptera). We selected all available material from supposed species rank of Xenidae, identified either by morphology, distinct host genus, or by barcode sequence in some specimens. Similar specimens in presented criteria, but from distant locations, such as different continents were considered as different species for the purpose of our research. Voucher names, hosts, and collection localities are listed in Supplementary data 1, Table S1. Individuals were extracted from metasoma of their host specimens (Hymenoptera: Aculeata). A large majority of sequences were newly obtained for this study. Several sequences of COI were acquired from Jůzová et al. (2015) (GenBank accession numbers: KF803415, KF803417, KF803418, KF803419) and one COI sequence was acquired from McMahon et al. (2011) (NCBI code: JN082809). Sequences of all genes of voucher *Stylops* were obtained from transcriptome shotgun assembly (Misof et al., 2014) (GenBank accession number: GAZM00000000.2).

Vouchers of newly obtained specimens came from personal collections of Jakub Straka (Charles University, Prague), Daniel Benda (Charles University, Prague), Yuta Nakase (Shinshu University, Matsumoto), and from museum collections of CNC (Ottawa, Canada), KUNHM (Lawrence, USA), AMNH (New York, USA), and BMNH (London, UK).

2.2. Preparation of DNA sequences

The entire body of male and female individuals of Strepsiptera, removed from their hosts, was lysed in Proteinase K (Qiagen). DNA was isolated using a DNA isolation kit (Qiagen) according to the manufacturer's protocol. Partial sequences of the following mitochondrial and five nuclear protein coding genes were amplified by polymerase chain reaction (PCR): cytochrome *c* oxidase subunit I (COI), Cinnamoyl alcohol dehydrogenase (CAD), RNA helicase DDX23 (DDX23), AP-2 complex subunit alpha (AP2A), AFG3-like protein 2 (AFG3L2), and BTB/POZ domain-containing protein 6 (BTBD6). Primers were developed using the Primer3 program (Rozen and Skaletsky, 2000) implemented in GENEIOUS 9.1 (Kearse et al., 2012). Subsequently, primers were manually modified based on insect sequences available from NCBI and compared with Hymenoptera sequences to prevent amplification of host DNA. The resulting primer parameters were checked with OligoAnalyzer 3.1 (Owczarzy et al., 2008). Primers for the COI gene were modified according to Jůzová et al. (2015) and for nuclear genes, primers were newly developed. See Table S2 in Supplementary data 2 for a list of the primers and PCR conditions.

Obtained sequences were edited and aligned in GENEIOUS 9.1 (Kearse et al., 2012). Each sequence was checked for possible contamination by host DNA and to determine whether the sequences matched the Strepsiptera order using BLAST (http://blast.ncbi.nlm.nih.gov). Sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov; National Center for Biotechnology Information, NCBI). The concatenated alignment was created in GENEIOUS 9.1 (Kearse et al., 2012), including partial sequences of all six genes for a total of 3371 nucleotide sites (606 base pairs (bp) for COI, 432 bp for CAD, 635 bp for DDX23, 454 bp for AP2A, 576 bp for AFG3L2 and 668 bp for BTBD6). All of the alignments are available from www.aculeataresearch.com.

2.3. Phylogenetic analysis

Maximum likelihood (ML) and Bayesian inference (BI) analyses were carried out on the combined data set of all genes. PartitionFinder v. 2.1 (Lanfear et al., 2017) was used to determine the best partitioning scheme for codon positions of each gene and the corresponding best evolutionary models for the data set (Supplementary data 2, Table S3). Bayesian information criterion (BIC) was used for BI analysis and Akaike information criterion corrected for sample size (AICc) was used for ML analysis. The 'greedy' algorithm was set for both analyses.

Bayesian analysis was conducted using MrBayes (Huelsenbeck and Ronquist, 2001). Four simultaneous Markov chains were run for 50 million generations. Convergence of chains was inspected by checking the posterior distributions of log likelihoods using the TRACER v. 1.6 program (Rambaut and Drummond, 2009). All of the parameters from Markov chain Monte Carlo (MCMC) analyses were summarized using the sump command in MrBayes and the first 25% were discarded as burn-in. Bayesian analysis was carried out using MrBayes v. 3.2.2 on XSEDE utility on the CIPRES Web Portal at http://www.phylo.org/portal2/ (Miller et al., 2010).

ML analyses were calculated using the GARLI 2.0 program (Zwickl, 2006). Ten independent search replicates were performed for each analysis. One thousand bootstrap replicates were performed for calculating branch support values. A consensus tree with bootstrap values was constructed from the bootstrap replicates in GENEIOUS 9.1 (Kearse et al., 2012).

2.4. Divergence time estimation

To estimate the divergence times of Xenidae lineages, we employed BEAST 1.8.3 (Drummond et al., 2012). Taxon sampling was left unchanged for all analyses because it was optimised *a priori* (taxon balanced for data mapping). The best-fitting nucleotide substitution model was determined by PartitionFinder 2.1 (Lanfear et al., 2017). Lognormal distribution in uncorrelated relaxed clock (Drummond et al., 2006) was implemented under Yule process prior (Gernhard, 2008). Because there are no known fossils of the Xenidae and Stylopidae families, appropriate divergence times that resulted from analysis of complete Strepsiptera order (McMahon et al., 2011) were used for calibration of our analysis. Divergence time of Xenidae and Stylopidae was set to 62 Ma (StDev 9.0) and the inner dating point of the Xenidae family was set to 46 Ma (StDev 8.5). The inner dating point was accurately set according to results from Bayesian analysis and it consists of parasites from Sphecidae and Vespidae hosts except Epiponini and *Vespula*. Both dating points, outgroup and ingroup, were constrained in prior settings and it is consistent with the high posterior probability support of exact nodes revealed by independent unconstrained analysis in MrBayes. Twelve independent runs were conducted for 100 or 200 million generations, sampling every 3000 generations. The initial 10% of trees were discarded as burn-in. Convergence of runs was checked using TRACER 1.6 (Rambaut and Drummond, 2009). However, analyses resulted in two competing likelihood islands and we had to select only runs within the higher likelihood island. Finally, eight runs with more than a billion states were combined and ESS values for prior and posterior higher than 120 were regarded as sufficient support (ESS values for other features was always higher than 200). A maximum clade credibility tree was generated by TreeAnnotator 1.8.3 (Drummond et al., 2012) and visualized with Figtree 1.4.0 (Rambaut, 2016).

2.5. Reconstruction of ancestral states

2.5.1. Biogeographic inference

For the purpose of inferring the distributional history of Xenidae in post-gondwanan distribution of continents, we adopted a coarse classification of six classic biogeographic areas: the Neotropical, Nearctic, Afrotropical, Palearctic, Indomalayan, and Australasian regions (Cox, 2001). The Wallace line (Wallace, 1876) was set as the boundary between the Indomalayan and Australasian regions. We merged the Nearctic region with the Neotropical as the "New World" for the purposes of our coarse analysis due to a scarce sampling of North America. Moreover, every clade with a North American representative also occurred in the Neotropical region.

To estimate ancestral biogeographic areas, we used maximum likelihood and Bayesian approaches implemented in RASP 3.1 (Yu et al., 2015). For the analysis, 2000 trees were selected from the output phylogenetic trees obtained from BEAST (every 520,230th output tree) using LogCombiner v 1.8.3 (Rambaut and Drummond, 2010). As a maximum likelihood approach, we used the dispersal-extinction-cladogenesis continuous-time model for geographical range evolution (DEC) (Ree et al., 2005; Ree and Smith, 2008) in the package LAGRANGE 2 (Likelihood Analysis of Geographic RANGe Evolution) implemented in RASP. Maximum likelihood (ML) parameters are estimated for the rates of migratory events between areas (range expansions) and local extinctions within areas (range contraction). The DEC model resembles a character-state reconstruction approach (Ree and Smith, 2008) but differs markedly from this class of methods in its treatment of cladogenesis events. Unlike character states, which are assumed to be inherited identically by daughter lineages, geographical ranges can potentially be inherited in a non-identical manner, as a consequence of spatial subdivision of the ancestral range (Ree and Sanmartín, 2009). In the analysis, we used the default settings and the maximum number of states was set to 2. Movement between areas at any time was unconstrained.

For Bayesian biogeographic reconstruction, we used the Bayesian Binary MCMC approach (BBM) (Yu et al., 2015) models dispersal among areas as a stochastic process represented by a Markov chain, involving the transition between two or more discrete states with different rates or probabilities. We used the most general and complex model implemented in RASP (F81 + Γ) with equal among-site rate variation and the maximum number of states set to 2. MCMC chains were run for 1 million generations (10 chains, temperature of 0.1) with a sampling frequency of 100 and a subsequent exclusion of 1000 generations as burn-in.

2.5.2. Ancestral hosts

Because the Strepsipterans of the Xenidae family are easily found together with their hosts, we have information about host identity for all but one sample. We divided the host groups into four categories as a character state. Wasps of the Vespidae family were separated into two categories - solitary wasps and social wasps. Subfamilies Eumeninae and Zethinae were included in the category 'solitary wasps'. The category 'social wasps' is represented by subfamilies Polistinae and Vespinae, which form a monophyletic group (Bank et al., 2017). The third category comprised digger wasps of Crabronidae, a paraphyletic family, while the fourth category represented the Sphecidae family (Peters et al., 2017).

To assess ancestral host conditions throughout the tree, we used the BBM approach, incorporated in RASP v. 3.1 (Yu et al., 2015). For the RASP analysis, we used the same subsample of 2000 output phylogenetic trees obtained from BEAST for the inference of biogeography. BBM is a statistical method mainly used for geographical distributions but can also be used for traits such as host exploitation (Winter et al., 2017). We used the most general and complex model implemented in RASP (F81 + Γ) with an equal among-site rate variation. The maximum number of states was set to 1. MCMC chains were run for 1 million generations (10 chains, temperature of 0.1) with a sampling frequency of 100 and a subsequent exclusion of 1000 generations as burn-in.

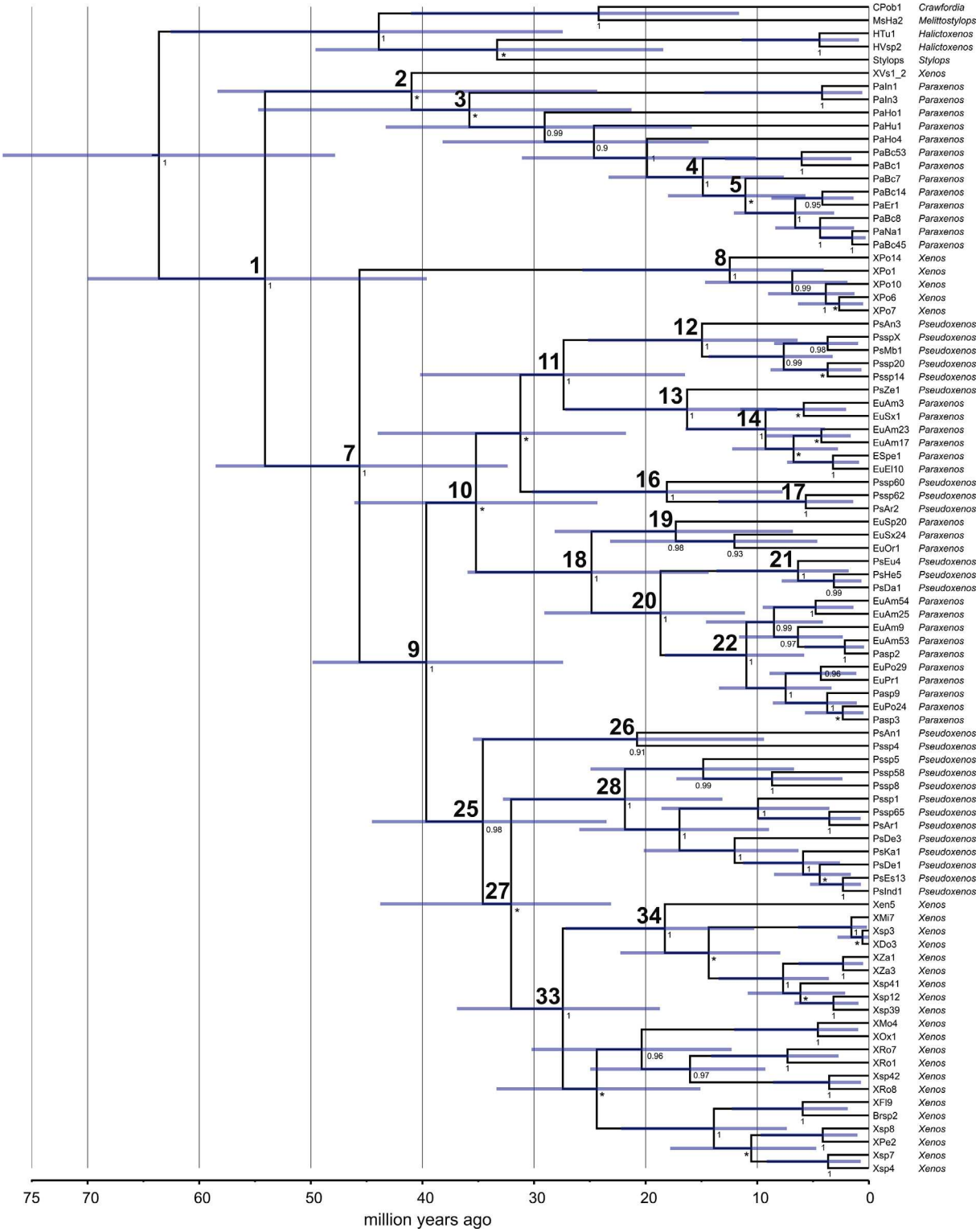

Fig. 1. Bayesian maximum credibility chronogram of Xenidae, from a BEAST analysis, with outgroups. Blue bars depict the 95% HPD (highest probability density). Numbers above the nodes mark important phylogenetic events and numbers under the nodes indicate posterior probability. Posterior probabilities lower than 0.9 are indicated by stars at the nodes. On the right are the vouchers of the samples and names of Xenidae genera. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Phylogeny of Xenidae and break-up of traditional genera

Based on analysis of the six-gene data set, we confirmed the Xenidae family as a monophyletic group (the posterior probability (PP) was equal to 1 in MrBayes; Fig. 1, node 1; Fig. S1). The phylogenetic analyses make traditional genera para or polyphyletic. Genus *Xenos* is divided into three groups. The first branch is represented by a single sample, XVs1_2 (*Xenos vespularum*), and it is unclear whether this

sample represents the basal branch for the family (MrBayes, PP = 1; Fig. S1) or whether it is a sister taxon to the *Paraxenos* clade (Fig. 1, node 2). The positions of the next two independent lineages of *Xenos* are well supported (Fig. 1, nodes 8, 33; Fig. S1). Node 7 (Fig. 1) illustrates evidence that the *Xenos* lineage of node 8 is a sister group to all remaining Xenidae. The third *Xenos* group represents the crown lineage of this family (Fig. 1, node 33). Genus *Paraxenos* represents a polyphyletic taxon. Species assigned to this genus are scattered across the family, with clades indicated by nodes 3, 14, 19 and 22 (Fig. 1). Most *Paraxenos* lineages are related to *Pseudoxenos* lineages with high support (Fig. 1, nodes 11, 18; Fig. S1). We recovered the *Pseudoxenos* genus as a paraphyletic group with one *Xenos* and two *Paraxenos* groups nested within it. Support for the whole clade is strong (PP = 1) (Fig. 1, node 9; Fig. S1), but the position of node 33 as internal to the clade is still speculative due to week or moderate support at nodes 25 (PP = 0.98) and 27 (PP < 0.9) (Fig. 1).

3.2. Divergence dating and biogeographic history

Xenidae is a post-gondwanan group with a median age of 54 Ma (40–70 Ma is 95% highest posterior density – HPD). According to the DEC and BBM analyses, the biogeographic origin of the family is uncertain due to a very early split into Palearctic and New Word clades (Figs. 2 and 3; nodes 2, 7). Probabilities for a New World-Palearctic origin are almost identical in both DEC and BBM analyses (Figs. 2 and 3; node 1). The age of the basal clade, including *Xenos vespularum* and the first *Paraxenos* group, is estimated to 41 Ma (24–58 Ma) (Fig. 1; node 2). The origin of this basal lineage is convincingly estimated as Palearctic with very high probability (Figs. 2 and 3; nodes 2, 3). The subsequent clade of Xenidae from Epiponini paper wasps (Figs. 2 and 3; node 7) is very well supported as New World. It separated from other Xenidae at 46 Ma (32–59 Ma) and consisted of South American representatives that diversified 12 Ma (4–26 Ma) (Figs. 2 and 3; node 8). It is striking that this early-separated lineage has a relatively young crown group compared to other clades (Fig. 1, node 8). The *Pseudoxenos-Xenos* group, sister clade to Epiponini parasites (Fig. 1, node 9; Fig. S1), represents a diversified group with an age of 40 Ma (27–50 Ma) and a well-supported New World origin according to BBM analysis (Fig. 3, node 9). The DEC analysis suggest variable state in node 9, but highest probability of presence of Xenidae in the New World. High ancestral state probabilities in nodes 10 and 25 (Figs. 2, 3) show that early evolution of this xenid lineage continued in the New World during the Eocene.

We have evidence that there were two dispersals of xenid parasites from the New World to Australasian and Afrotropical regions during the Late Eocene/Early Oligocene in a strongly supported clade delimited by node 9 (Fig. 1). The DEC model suggests early dispersal from New World to Australia with high probability in node 10 (Fig. 2). It is likely that these dispersions occurred via Antarctica. The duration of a habitable Antarctica is shown as a light green background in Figs. 2 and 3. The DEC model suggests that lineage of the node 11 remained in the New World and Australasian ancestors extinct. The dispersion event from Australasian to Afrotropical region occurred in sister lineage (Fig. 2; nodes 15,18). Results from BBM analysis also suggest that the direction of dispersal was from the New World to Australasian and Afrotropical regions; however, it is unclear whether the path to Africa led through Australia (Fig. 3; nodes 10, 15, 18).

Subsequent late Eocene dispersion event from the New World to Africa also occurred in parallel during Xenidae evolution, presented at nodes 25 and 27. The result of this colonization was two lineages with primary Afrotropical distribution represented by nodes 28 and 34 (Figs. 2, 3). Both DEC and BBM analyses suggest a New World origin for node 25 and according to DEC, the lineage in nodes 27 and 33 have the highest probability of New World-Afrotropical distribution (Fig. 2). The following dispersals events lasting from Oligocene to Middle Miocene led mainly from Afrotropical to Palearctic and Indomalayan regions (Fig. 2; nodes 20, 28, 35, 38). On the contrary, there is evidence of dispersal from Palearctic to Afrotropics within the *Paraxenos* goup (Figs. 2, 3; node 4). A slightly younger lineage, presented at node 20 (Figs. 2, 3), indicates an interconnection between the Afrotropical and Palearctic regions which lasted until recent times and whose Australasian ancestors probably extinct. However, according to DEC model, the connection of Afrotropical, Oriental and may be also Palearctic regions are dated back to 30 Ma (nodes 27, 33; Fig. 2).

In comparison to the Eocene and Oligocene, the second half of the Miocene epoch, approximately between 15 and 7 Ma, is characterized by little or questionable evidence for migration between continents. Evidence for dispersion from the Australasian to Indomalayan regions is presented in node 17 (Figs. 2 and 3). The *Paraxenos* lineage of node 22 shows connectivity between Afrotropical and Palearctic regions, which is clearly supported by the DEC and BBM analyses (Figs. 2 and 3). Although the BBM model suggests a reverse dispersion event from the Indomalayan region to Africa, the DEC model show lasting presence of Afrotropical-Indomalayan ancestral distribution (Figs. 2, 3; nodes 29, 30). Nodes 6 (11 Ma, 6–18 Ma) and 32 (6 Ma, 3–11 Ma) (Figs. 2 and 3) show the parallel dispersion of originally Afrotropical and Palearctic lineages to Indomalayan, and Australasian regions in *Paraxenos* and *Pseudoxenos* lineages. The grey windows in Figs. 2 and 3 represent precipitous expansions; however, the direction of dispersal is uncertain.

3.3. Host switches

BBM analysis suggests that the social wasps are ancestral hosts of Xenidae with high probability (Fig. 4, node 1). A host switch to Crabronidae digger wasps occurred only once during the evolution of Xenidae and they have remained faithful to this host group for tens of millions of years (Fig. 4; node 3).

Clade 7 comprises, with very high probability, parasites of social wasps. The basal lineage (Fig. 4, node 8) is specialized to Neotropical tribe Epiponini. A switch to solitary wasp hosts likely occurred only once in the evolution of Xenidae, with the ancestors being social wasps (Fig. 4; node 9). The origin of the clade parasitizing solitary wasps is dated to 40 Ma (27–50 Ma) (Fig. 1, node 9). Solitary wasps are also the probable ancestral host group for several ancestral linages deeper in time (Fig. 4, nodes 10, 25, 27), perhaps acting as a kind of ‘core’ host group, from which several subsequent host switches occurred. Most host solitary wasps belong to the Eumeninae subfamily. Within Eumeninae, parasitism of the Eumenini tribe (Fig. 4, node 31) occurred only once, about 12 Ma (6–20 Ma). Only one sample (PsZe1) was recorded as a parasite of the Zethinae subfamily, which is more related to social wasps than to other solitary wasps.

According to the analysis, two host transitions to social wasps occurred. The first switch was associated with the origin of Xenidae, while the second happened later, around 24 Ma (19–37 Ma) (Fig. 4, node 33). The latter lineage includes hosts of the Vespini, Polistini and Ropalidini tribes and the switch was from solitary to social wasps, as suggested by strongly supported node 27 (Fig. 4). The most remarkable switches occurred in the lineage of node 10, which has solitary wasps as the ancestral hosts. There were two or three independent switches from solitary wasps to digger wasps of the Sphecidae family, which happened in parallel in the New World and Old World + Australia regions, as shown in the blue and green windows in Fig. 4. The New World host switch is illustrated at nodes 13 and 14, while the Old World + Australia host switch is illustrated at nodes 15, 18 and 20 (Fig. 4). In the latter case, two alternative interpretations are possible. The parasites may have switched to sphecid wasps once and subsequently returned to solitary wasps, or two independent switches to sphecid wasps may have occurred.

4. Discussion

Strepsiptera are an ancient group of insect parasites with late

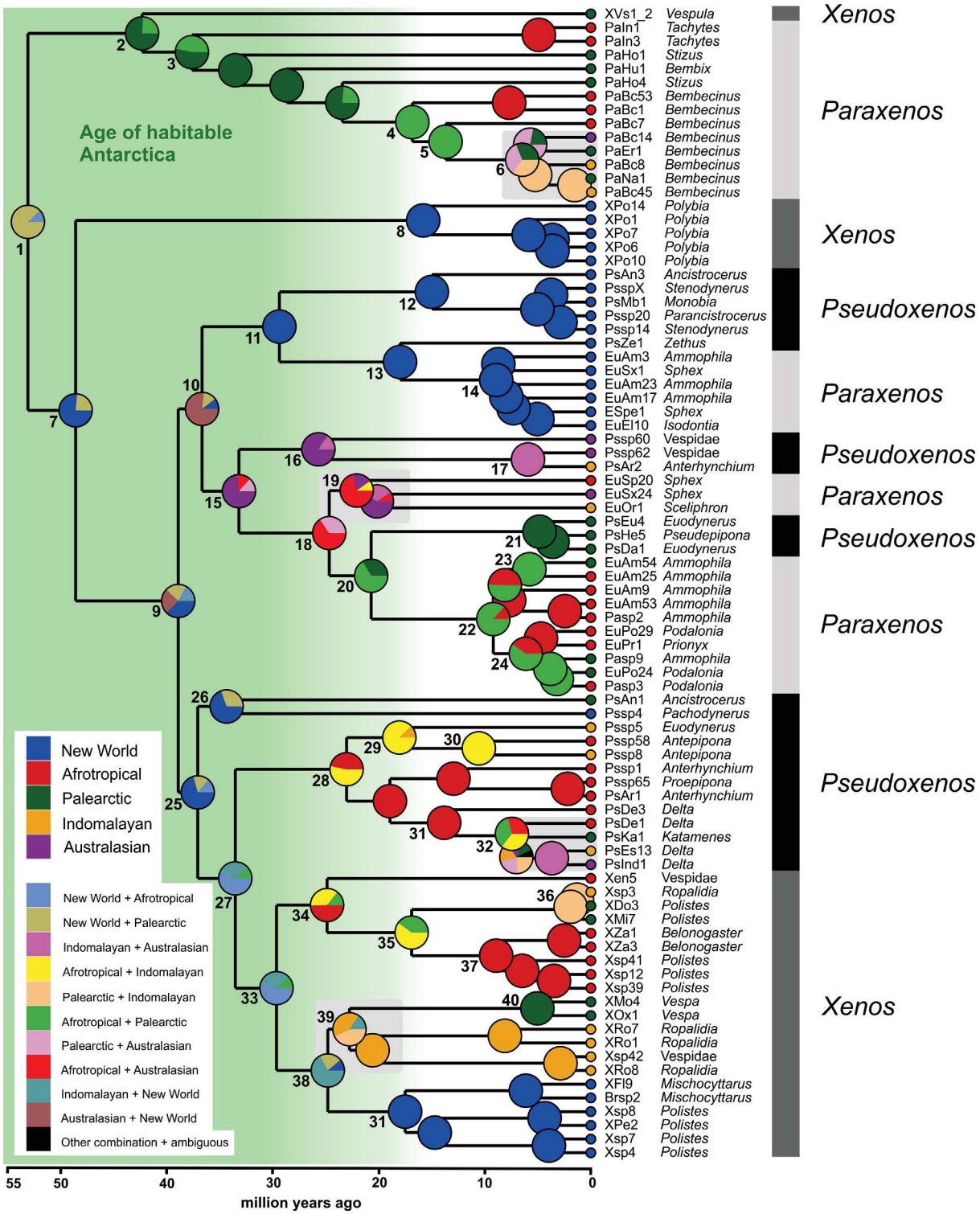

Fig. 2. Results of the RASP analysis (Lagrange, the DEC model) of the biogeographic history of Xenidae. Pie charts indicate the probability of the character states. The green window represents the duration of a habitable Antarctica during the Eocene (darker green) and after cooling of the climate during the Oligocene and Miocene (lighter green) (Galeotti et al., 2016; Warny et al., 2009). Grey windows represents the important dispersal events. On the right are the vouchers, names of host genera, and names of Xenidae genera. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Paleozoic origin in early Carbon period (Toussaint et al., 2017). The Xenidae family is a highly derived modern group arising during the Paleogene after the breakup of Gondwana (McMahon et al., 2011). According to our results, the phylogeny of Xenidae is fundamentally different than formerly conceived. A previous phylogenetic study based solely on morphological data (Pohl and Beutel, 2005) suggests that the *Paraxenos* genus is a basal group with *Pseudoxenos* and *Xenos* together as a crown group. Nevertheless, the relationships within this family are more complex. Present tree topology shows that all three traditional genera are polyphyletic or paraphyletic. In this context, the results also change the historical concept of host specificity that was used, along with morphological characters, to make determinations on a generic

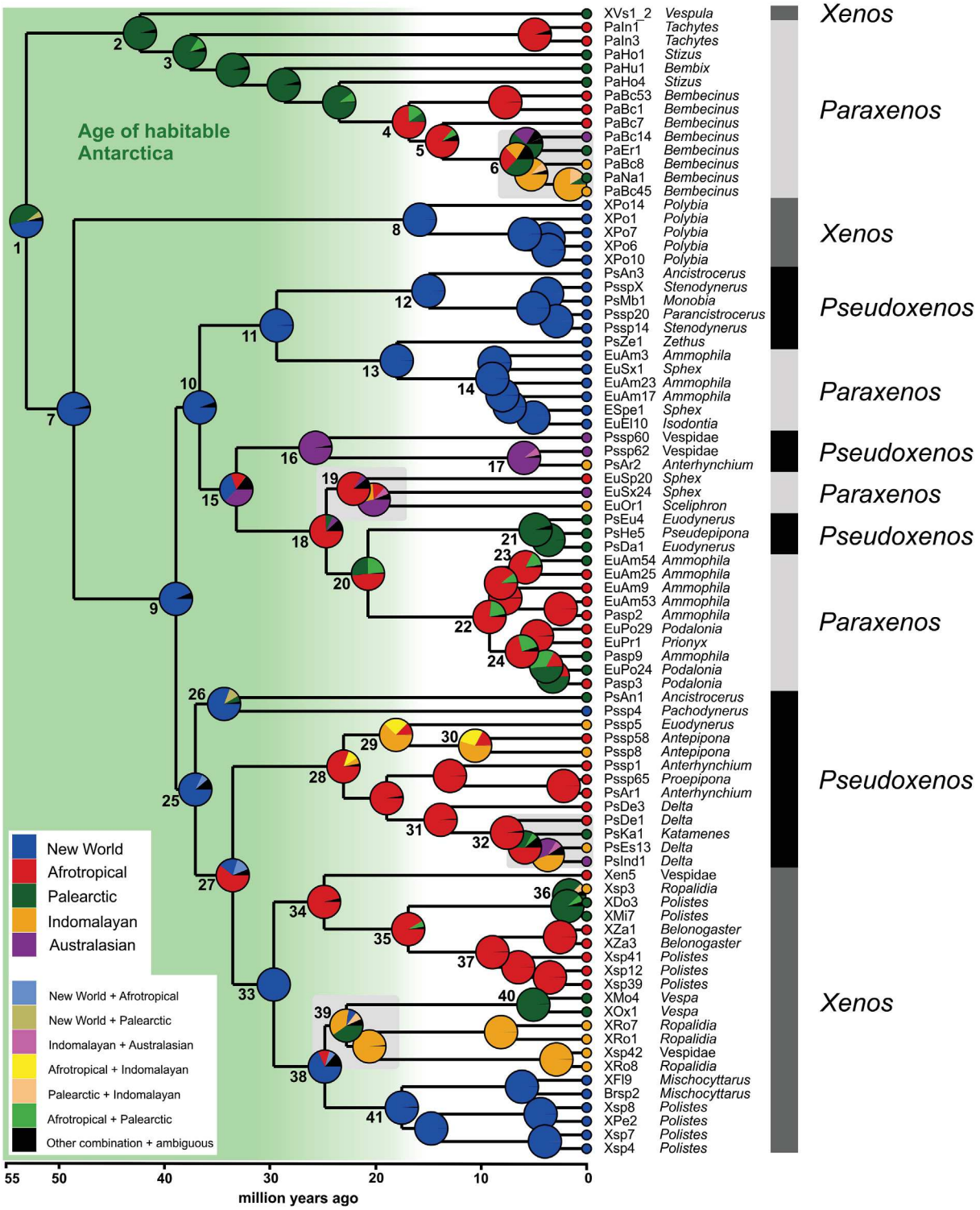

Fig. 3. Results of the RASP analysis (Bayesian Binary MCMC – BBM) of the biogeographic history of Xenidae. Pie charts indicate the probability of the character states. The green window represents the duration of a habitable Antarctica during the Eocene (darker green) and after cooling of the climate during the Oligocene and Miocene (lighter green) (Galeotti et al., 2016; Warny et al., 2009). Grey windows represent the important dispersal events. On the right are the vouchers, names of host genera, and names of Xenidae genera. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

level (Kinzelbach, 1978). We conclude that results of this study provide fundamental information for a necessary revision of generic classification across the Xenidae family.

4.1. Historical biogeography

Xenidae are a diverse family that arose in the post-gondwanan time when the continents were already distantly separated from each other. Xenidae females remain permanently in the host throughout their life (Kathirithamby, 1989; Pohl and Beutel, 2008); therefore, their dispersal

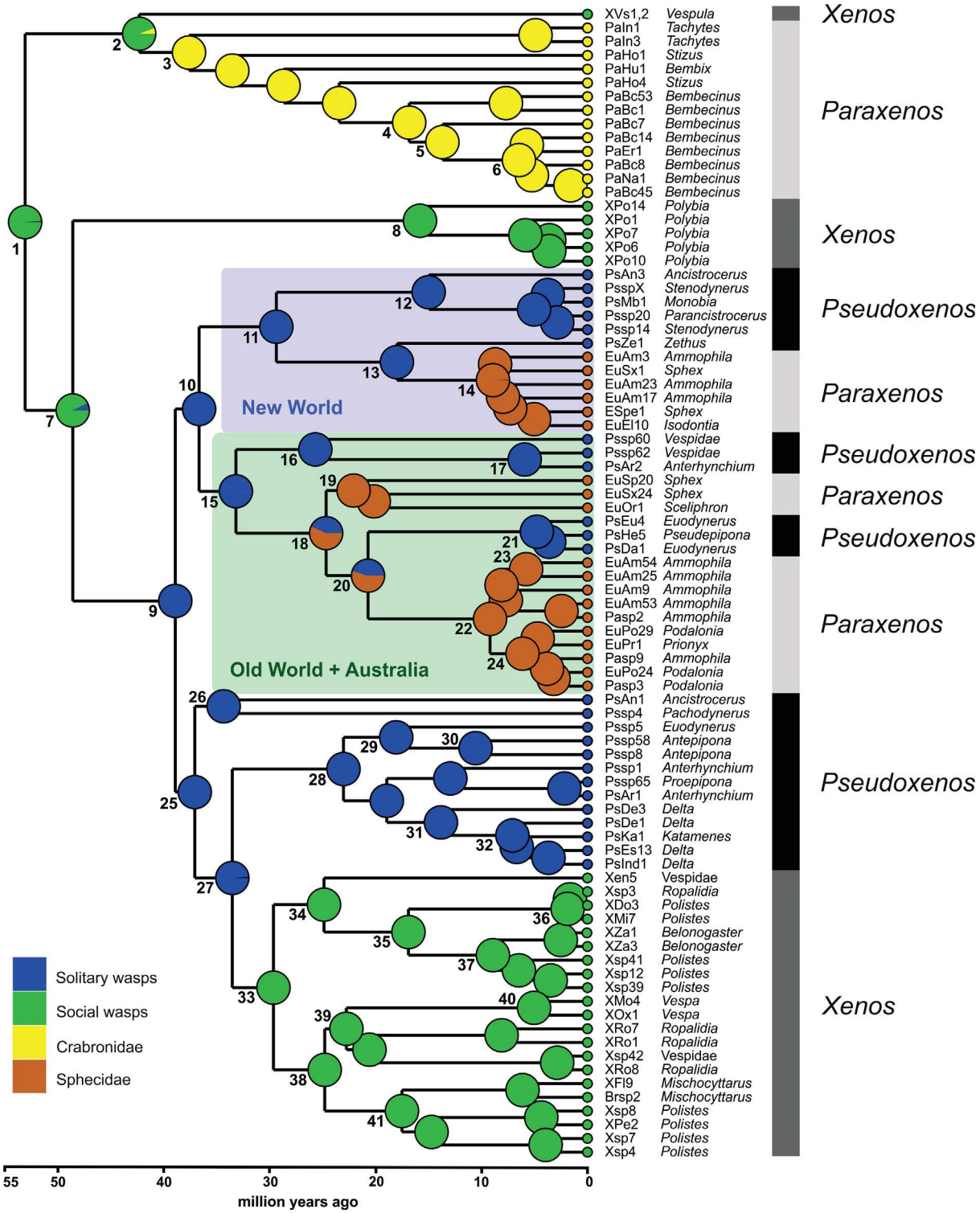

Fig. 4. Results of the RASP analysis (Bayesian Binary MCMC – BBM) of the ancestral hosts of Xenidae. Pie charts indicate the probability of the character states. The light green window represents the New World lineage and the light blue window represents the Australian and Old World lineage. On the right are the vouchers, names of host genera, and names of Xenidae genera. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

is fully dependent on hymenopteran hosts. This situation provides a good opportunity to test hypotheses, especially those regarding long distance dispersion or dispersal via stepping stones. Although the ultimate geographical origin of the family remains uncertain from both of our biogeographic analyses, there is very good evidence for a divergence into two lineages – one with Palearctic origin and another with New World origin.

Migration events deeper in the evolution of Xenidae are quite complex. To better illustrate and interpret results of the ancestral reconstruction analyses by disperasal-extinction-cladogenesis model, we visualized a summary of the dispersion events on maps in Fig. 5A–D. We divided the evolution of Xenidae into four migration periods, which

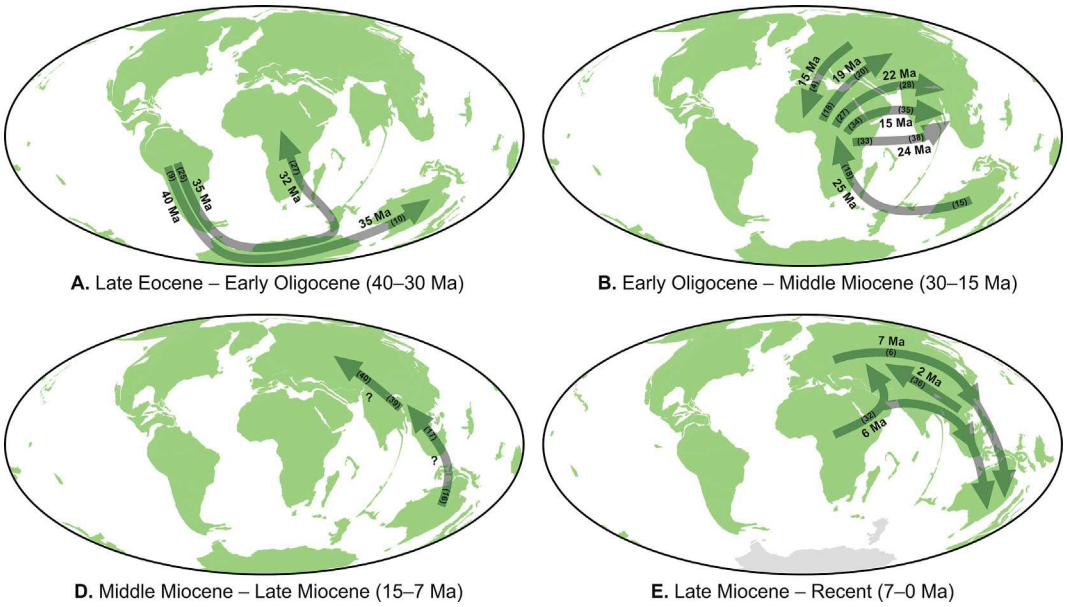

Fig. 5. A–D. Schematic maps of dispersals during the evolution of Xenidae. Arrows indicate the direction of spreading. Numbers in brackets inside the arrows indicate the number of nodes from Fig. 2. Numbers outside the arrows indicate the estimated timing of dispersal (millions of years ago (Ma)) from the BEAST analysis (Fig. 1, median value was used). Maps were developed and modified from the ODSN service (http://www.odsn.de/odsn/services/paleomap/paleomap.html), accessed in May 2018.

are related to paleoclimatic and geological events. The first period (40–30 Ma; Fig. 5A) involves transcontinental dispersions between the New World and Old World + Australia in the southern hemisphere, with no intercontinental dispersals occurring in the northern hemisphere. We have evidence for dispersal of Xenidae in lineage 10 (Figs. 2 and 3) from the New World to Old World region, which have followed a path through Australia with very high probability.

This trans-Australian path is supported by the both DEC and BBM analyses. In lineage 25 (Fig. 2; nodes 25, 27, 33), the DEC analysis suggest dispersal from New World to Afrotropical and following connection and share of fauna between New World and Afrotropics. The timing of these events, estimated with molecular dating, is remarkably congruent with the timing of geological connections between Antarctica, Australia, and South America, which were closely associated until the beginning of the Oligocene (Lawver et al., 1992). These dispersion events also correspond with the presence of the Kerguelen and Crozet Plateaus, which are hypothesized to be functional stepping stones connecting Antarctica, Africa, and India (Lawver and Gahagan, 2003; McLoughlin, 2001). Results of the biogeographic analyses indicate that Antarctica was an important crossroad that allowed transcontinental biota dispersals due to favourable climate conditions (Iglesias et al., 2011). Habitable conditions during the late Cretaceous and Paleogene periods are also documented by a number of fossils, mainly ratite birds, marsupials, and plants (Dettmann, 1990; Tambussi et al., 1994; Woodburne and Case, 1996). Modern biogeographic studies strongly suggest that Antarctica and the adjacent now-submerged plateaus were an important corridor for aculeate Hymenoptera (Almeida et al., 2012; Kayaalp et al., 2017; Schwarz et al., 2006) to disperse between South America and Australia and between Australia and Africa in both directions. This conclusion is supported by climatic and geological data providing evidence that Aculeata were capable migrants with the ability to disperse over water, as was already assumed by Michener (1979). In accordance with these works, our results support the importance of Antarctica as a migration bridge in the early evolution of Xenidae. In accordance with cooling of Antarctica at Eocene-Oligocene boundary, we do not have evidence of any dispersal from New World to Old World or Australia in the time after 30 Ma.

The next migration period, 30–15 Ma (Early Oligocene – Middle Miocene), is illustrated in schematic map B (Fig. 5). During this time period, it is more complicated to explain the dispersion events by the 'southern way'. Opening of the Drake and Tasmanian Passages was the most likely cause for the rapid planetary cooling that occurred due to the subsequent development of a south circumpolar current and glaciation of Antarctica (McLoughlin, 2001). Despite this cooling, recent studies indicate the presence of refuges with mosaic woodlands and tundra in Antarctica until the Neogene period (Anderson et al., 2011; Rees-Owen et al., 2018; Warny et al., 2009). Migration through Antarctica was definitely limited but not completely prevented during that time, as some *Pseudoxenos* hosts are cold-adapted species, such as the Andean genus *Hypodynerus* (Teson and Remes-Lenicov, 1979). With both the ability for long distance dispersion and the potential of cold-adapted hosts, we cannot unequivocally rule out the 'southern way' hypothesis. Dispersion from Australasian to Afrotropical region between nodes 15 and 18 (Fig. 2) can best be explained by the southern way. The alternative hypothesis could be by the northern way through Indomalayan followed by extinction in this region but it is not parsimonious explanation. The problematic expansion shown on the grey widow at nodes 38–39 (Figs. 2, 3) cannot be well explained based on current data. This lineage includes *Xenos* individuals that parasitize *Ropalidia* paper wasps. However, *Xenos* from *Ropalidia* is also widely distributed in Africa (Kinzelbach, 1975) and Madagascar (Straka and Benda unpubl.), but we have DNA from Indomalayan specimens only. The Early Oligocene – Middle Miocene period is also conspicuous of several dispersals from Afrotropical to Palearctic and Indomalayan regions (Fig. 5B). These events support the importance of Afrotropical region as a rich source of diversity for recent distribution of Xenidae. In contrast, *Paraxenos* lineage (node 3; Fig. 2) had the ancestral distribution in Palearctic and dispersed to Afrotropical region in the same period.

The Middle to Late Miocene migration period of 15–7 Ma is shown in Fig. 5C. This time period can be characterized as a 'period of calm', during which most divergences lacked a distinctive dispersal event, except for the two dispersions from Indomalayan to Palearctic and from Australian to Indomalayan regions. Unfortunately, the timing of these dispersions is unclear because of long branches (Fig. 2; 16–17, 39–40). The situation of dispersal stagnation can be explained by the occurrence of multiple changes. Firstly, the climate conditions in Antarctica and the adjacent islands have become too harsh and overall uninhabitable

because of an event called 'Middle Miocene disruption' (Lewis et al., 2008). This event is characterized by rapid cooling of the Earth about 14 Ma and is considered to be a significant period of extinction (Raup and Sepkoski, 1984). Moreover, the Kerguelen and Crozet plateaus near Antarctica were, by this time, largely submerged, and the continents likely became too distant due to their continuous movement away from each other to provide a migration corridor for the aculeate hosts (Scotese and Golonka, 1997).

The most recent migration period from 7 to 0 Ma is depicted by the map in Fig. 5D. The two expansive dispersions during this period are highlighted by the grey windows of nodes 32 and 6 (Figs. 2 and 3). The first migration event led from the Afrotropics through Palearctic and Indomalayan regions to Australia and the parallel one from Afrotropical-Palearctic in the same direction. This supports the hypothesis above that Afrotropics was a main source of xenid diversity for the Old World and Australia from the Miocene period until the present. The increasing incidence of dispersion events in the last 7 Ma could be caused by the emerging climate change at the end of the Miocene. Jansen et al. (1990) describe a series of glacial episodes in the area surrounding the Norwegian-Greenland Sea from the late Miocene (5.45 Ma) through the Pliocene period that had a smaller magnitude than those of the period postdating the major onset of large scale northern hemisphere glacial cyclicity at 2.57 Ma and after 1.2 Ma. These climate fluctuations could have uncovered ecological niches for Xenidae hosts, resulting in expansions, during which they brought along their parasites.

The situation during the time of the Eocene/Oligocene boundary as well as the phase before the 'Middle Miocene disruption' are characterized by a subsequent cooling of Antarctica (Galeotti et al., 2016). Both events likely had a very similar effect on Strepsipteran host biota as laid out above for Xenidae hosts during the more recent glacial cyclicity of the northern hemisphere. Such conditions could be a trigger for expansions and transcontinental dispersions through the interchange of fauna.

4.2. Ancestral hosts and parallel evolution

According to the analysis, the ancestral host group of Xenidae were social wasps (Fig. 4, node 1). The host switch from social to solitary wasps was secondary and probably only occurred once (Fig. 4, node 9). This contradicts the traditional hypothesis that parasites are tied to their hosts and follow host evolution (Brooks, 1979; Eichler, 1948). Results of the Hymenoptera phylogeny, published by Peters et al. (2017), estimated social wasp (Vespinae) origin to be about 50 Ma. On the other hand, the timing of Xenidae origin may be linked to the origin of social wasp primary hosts. As recent phylogenetic studies show (Branstetter et al., 2017; Peters et al., 2017), other major Xenidae host groups, including the solitary wasps, Crabronidae and Sphecidae, are significantly older than Xenidae. In this context, the remarkable host switches supported by the Xenidae phylogenetic tree (Fig. 3) also suggest that Xenidae evolution was not bound by the evolution of their hosts.

The relative generalism of Xenidae and their ability to colonize new host lineages is best illustrated by the parallel host switches from solitary to sphecid wasps (Sphecidae) (Fig. 4, blue and green window). These switches occurred in the New World and Old World + Australia areas independently. Beginning of this remarkable parallel host-parasite evolution was preceded by the Eocene/Oligocene expansion from the New World to Old World + Australia region via the Antarctic path. Colonization of the same host lineage on different continents demonstrates their ability to utilize opportunity for potential hosts in various environments.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2019.02.023.

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Paper II

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Unexpected cryptic species diversity of parasites of the family Xenidae (Strepsiptera) with a constant diversification rate over time

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Abstract. Parasitism is one of the most successful and ancient strategies. Due to the specialized lifestyle of parasites, they are usually affected by reductions and changes in their body plan in comparison with nonparasitic sister groups. Extreme environmental conditions may impose restraints on behavioural or physiological adaptations to a specific host and limit morphological changes associated with speciation. Such morphological homogeneity has led to the diversity of parasites being underestimated in morphological studies. By contrast, the species concept has dramatically changed in many parasitic groups during recent decades of study using DNA sequence data. Here we tested the phenomenon of cryptic species diversity in the twisted-wing parasite family Xenidae (Strepsiptera) using nuclear and mitochondrial DNA sequence data for a broad sample of Xenidae. We used three quantitative methods of species delimitation from the molecular phylogenetic data – one distance-based (ABGD) and two tree-based (GMYC, bPTP). We found 77–96 putative species in our data and suggested the number of Xenidae species to be more diverse than expected. We identified 67 hosts to species level and almost half of them were not previously known as hosts of Xenidae. The mean number of host species per putative species varied between 1.39 and 1.55. The constant rate in net diversification can be explained by the flexibility of this parasitic group, represented by their ability to colonize new host lineages combined with passive long-range dispersal by hosts.

Introduction

Parasitism is probably the most common life history that has repeatedly evolved across the tree of life in all major groups of organisms (Windsor, 1998; Poulin, 2007). Parasites are organisms that stay in a close physical contact with their hosts for a significant part of their lives and together are in a continuous 'arms race'. Whereas a parasite does not kill its host, a parasitoid will usually kill the host as part of its development from immature (Eggleton & Gaston, 1990); moreover, parasites feed on the host during immature and adult stages, while parasitoids only feed on the host as an immature. Through natural selection hosts develop various preventative mechanisms to combat the parasite, similarly parasites respond in various ways to overcome these defensive mechanisms (Dawkins & Krebs, 1979). During these 'arms races' parasites can influence the condition, growth, reproduction and ultimately the fitness of their hosts; thus, they are important agents of natural selection (Poulin, 2007; Schmid-Hempel, 2009).

The diversity of parasites and parasitoids is frequently discussed in the context of hidden diversity and cryptic species (Smith *et al.*, 2008; de León & Poulin, 2018; Santos *et al.*, 2018). Cryptic species are species that are genetically distinct but morphologically indistinguishable, although slight differences are often detected. Extreme environmental conditions might impose stabilizing selection on morphology, reducing or eliminating morphological changes that may accompany speciation (Bickford *et al.*, 2007). Schönrogge *et al.* (2002) have claimed that species experiencing strong selection on behavioural or

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physiological characters for adaptation to a specific host might not be expected to show morphological changes among species. Despite the cryptic species problem, correct identification of species is essential in estimating parasite host specificity. If species are incorrectly or poorly defined, then host specificity may be over- or underestimated (Poulin, 2007). With the advent of rapid DNA sequencing methods during recent decades, molecular taxonomy has been proposed for quick species diversity assessment. It has helped to resolve the species diversity of various little-known taxa, and the enormous diversity in morphologically homogeneous groups has been successfully uncovered due to the use of the species delimitation method (Tautz *et al.*, 2003; Blaxter, 2004). For insect parasites and parasitoids especially, the species concept has been dramatically changed by molecular taxonomy (Smith *et al.*, 2008; Hayward *et al.*, 2011; Veijalainen *et al.*, 2012).

Some of the least known and inconspicuous insect parasites are members of the order Strepsiptera. Strepsipterans are obligate entomophagous parasites of seven insect orders and are characterized by extreme sexual dimorphism. They undergo a dramatic hypermetamorphosis of body structures during development. Females of most Strepsiptera are permanently 'quasi'-endoparasitic, neotenic and immobile. They disperse only with their hosts and viviparously produce the first-instar larvae, which have three pairs of walking legs, live freely and invade the host body (Pohl & Beutel, 2008; Kathirithamby, 2009). Females also release a powerful sex pheromone (Lagoutte *et al.*, 2013; Zhai *et al.*, 2016) to attract males for mating. Males have a short lifespan of only several hours, during which they must find a female and mate (Straka *et al.*, 2011).

Although the phylogenetic relationships within Strepsiptera are mostly resolved (except the family Bohartillidae) (McMahon *et al.*, 2011), only a few studies have dealt with species diversity and host specificity in Strepsiptera with molecular phylogenetic methods. The first species delimitation analysis was published by Halbert *et al.* (2001), who identified several species of the family Myrmecolacidae. Subsequent molecular phylogenetic works studied the host specificity of myrmecolacid species and confirmed that the extremely dimorphic sexes utilize hosts from separate orders (Kathirithamby & Johnston, 2003; Kathirithamby *et al.*, 2010). Hayward *et al.* (2011) performed a phylogeography analysis of the species *Caenocholax fenyesi* Pierce (Myrmecolacidae) in Central America and discovered at least 10 clades that could be considered as separate species. These lineages show some degree of biogeographical separation and host specificity. Host specificity sometimes differs between males and females, with changes in host preference accompanying diversification. Fossil evidence of the *C. fenyesi* complex suggests a very low molecular clock rate and an ancient origin of cryptic lineages that supports the theory of slow changes in anagenesis (Kathirithamby & Henderickx, 2008; Hayward *et al.*, 2011). Matsumoto *et al.* (2011) focussed on the phylogeography of the species *Elenchus japonicus* (Esaki & Hashimoto) (Elenchidae) in Southeast Asia. The results of this study revealed three species-like lineages with varying host specificity. Jůzová *et al.* (2015) performed a molecular phylogeny of the species-rich genus *Stylops* Kirby (Stylopidae), which parasitizes *Andrena* Fabricius bees. This study revealed that *Stylops* species are mostly specialized on particular host subgenera, as predicted in previous morphology-based studies by Bohart (1941) and Luna de Carvalho (1974). The authors also reject the more conservative hypothesis about host specificity based on morphological features postulated in previous studies, which either assigned each *Stylops* species to one host species of *Andrena* (Kifune & Hirashima, 1985; Kifune, 1991) or considered *Stylops* species to be generalists with a large number of host species (Kinzelbach, 1978).

In our study, we focus on the family Xenidae, one of the most derived groups of Strepsiptera based on recent phylogenetic studies (Pohl & Beutel, 2005; McMahon *et al.*, 2011). Previously, the family was divided into three subfamilies, Xeninae (including *Paragioxenos* Ogloblin, *Xenos* Rossi, *Pseudoxenos* Saunders), Paraxeninae (*Paraxenos* Saunders) and Stylopinae (including seven genera now placed in Stylopidae) based on morphological characters (Kinzelbach, 1971a). Later, Xeninae, Paraxeninae and Stylopinae were all placed in Stylopidae (Kinzelbach, 1978). Pohl (2002) provided evidence for the paraphyly of Stylopidae and re-established the family Xenidae (including Xeninae and Paraxeninae). Pohl & Beutel (2005) confirmed the monophyly of Xenidae + Stylopidae, which was subsequently supported using molecular data by McMahon *et al.* (2011). The first occurrence of the cryptic species phenomenon in Xenidae was published by Nakase & Kato (2013), who focussed on the giant *Xenos* strepsipteran parasites in large hornets (*Vespa* L.). They found two distinct species of *Xenos* with different host specificity and inconspicuous distinguishing characteristics. Benda *et al.* (2019) performed a comprehensive molecular phylogeny of Xenidae and examined historical biogeography and host switches at various taxonomic levels. They proposed that this family may represent a model for host-parasite interactions as hosts are known for all described xenid species (see. Cook, 2019). Based on three species delimitation methods, we explored the species diversity of Xenidae, propose estimates for host specificity and investigate diversification rates throughout the family.

Material and methods

Taxon sampling

Our data set consisted of 209 specimens from three genera of Xenidae (*Paraxenos*, *Pseudoxenos*, *Xenos*) and five outgroup specimens from the sister family Stylopidae. The outgroup sampling consisted of five individuals from four genera: *Crawfordia* Pierce, *Halictoxenos* Pierce, *Melittostylops* Kinzelbach and *Stylops*. We selected all available material of Xenidae, identified by morphology, affiliation to host taxon or both. Assignment of specimens to each species was carried out according to biogeographical and host data found in the literature. Host specimens were identified to the lowest taxonomic unit possible. We used the updated classification for the host families established based on recent phylogenomic works on

Aculeate Hymenoptera (Bank *et al.*, 2017; Sann *et al.*, 2018). Voucher names, hosts and collection localities are listed in Table S1. Individuals were extracted from the metasoma of their host specimens (Hymenoptera: Aculeata). A large majority of sequences were newly obtained for this study. All NCBI codes are listed in Table S1. The sequences of 89 specimens were taken from a previous study (Benda *et al.*, 2019). Four sequences of COI were acquired from Jůzová *et al.* (2015) (NCBI codes: KF803415, KF803417, KF803418, KF803419), five were acquired from McMahon *et al.* (2011) (NCBI codes: JN082805, JN082806, JN082809, JN082810, JN082811), and six from Nakase & Kato (2013) (NCBI codes: AB759562, AB759563, AB759570, AB759572, AB759577, AB759584). Sequences of all genes of voucher *Stylops* were obtained from a transcriptome assembly (Misof *et al.*, 2014) (GenBank accession number: GAZM00000000.2). Vouchers of newly obtained specimens were obtained from the personal collections of Jakub Straka (Charles University, Prague), Daniel Benda (Charles University, Prague) and from the museum collections of CNC (Ottawa, Canada), KUNHM (Lawrence, USA), AMNH (New York, USA) and BMNH (London, UK).

Preparation of DNA sequences

The entire body of male and female individuals of Strepsiptera, removed from their hosts, was lysed in Proteinase K (Qiagen). DNA was isolated using a DNA isolation kit (Qiagen) according to the manufacturer's protocol. Partial sequences of one mitochondrial and five nuclear protein-coding genes were amplified by polymerase chain reaction (PCR): cytochrome *c* oxidase subunit I (COI), cinnamoyl alcohol dehydrogenase (CAD), RNA helicase DDX23 (DDX23), AP-2 complex subunit alpha (AP2A), AFG3-like protein 2 (AFG3L2), and BTB/POZ domain-containing protein 6 (BTBD6). Primers for all genes were taken from Benda *et al.* (2019). See Table S2 for a list of the primers and PCR conditions.

Obtained sequences were edited and aligned in GENEIOUS 9.1 (Kearse *et al.*, 2012). Each sequence was checked for a possible contamination by host DNA and the sequences were analysed by BLAST (http://blast.ncbi.nlm.nih.gov) in order to determine whether the sequences matched Strepsiptera. Sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov; National Center for Biotechnology Information, NCBI). The concatenated alignment was created in GENEIOUS 9.1 (Kearse *et al.*, 2012), including partial sequences of all six genes for a total of 3371 nucleotide sites (606 base pairs [bp] for COI, 432 bp for CAD, 635 bp for DDX23, 454 bp for AP2A, 576 bp for AFG3L2, and 668 bp for BTBD6). All alignments are available on www.aculeataresearch.com.

Phylogenetic analysis

Maximum likelihood (ML) and Bayesian inference (BI) analyses were carried out on the combined data set of all genes. PartitionFinder 2.1 (Lanfear *et al.*, 2017) was used to determine the best partitioning scheme for codon positions of each gene and the best corresponding evolutionary models for the data set (Table S2). Bayesian information criterion (BIC) was used for BI analysis and Akaike information criterion corrected for the sample size (AICc) was used for ML analysis. The 'greedy' algorithm was set for both analyses. Bayesian analyses were conducted using MrBayes (Huelsenbeck & Ronquist, 2001). Four simultaneous Markov chains were run for 50 million generations. The convergence of chains was inspected by checking the posterior distributions of log-likelihoods using TRACER 1.6 (Rambaut & Drummond, 2009). All of the parameters from Markov chain Monte Carlo (MCMC) runs were summarized using the sump command in MrBayes and the first 25% were discarded as burn-in. ML analyses were calculated using the GARLI 2.0 program (Zwickl, 2006). Ten independent search replicates were performed for each analysis. One thousand bootstrap replicates were performed for the calculation of branch support values. A consensus tree with bootstrap values was constructed from the bootstrap replicates in GENEIOUS 9.1 (Kearse *et al.*, 2012).

Divergence time estimation

Estimates of divergence time in Xenidae were performed using BEAST 1.8.3 (Drummond *et al.*, 2012). The best partitioning scheme was determined by PartitionFinder v. 2.1 (Lanfear *et al.*, 2017) under the BIC criterion. A lognormal distribution in uncorrelated relaxed clock (Drummond *et al.*, 2006) was implemented under Yule process prior (Gernhard, 2008). Tree calibration was done based on the results of study of Strepsiptera phylogeny by McMahon *et al.* (2011). Divergence of Xenidae from Stylopidae was set to 62 million years ago (Ma) (StDev 9.0) and the inner dating point of Xenidae was set to 46 Ma (StDev 8.5). Nine independent runs were conducted for 40 or 80 million generations, sampling every 3000 generations. The first 20% of trees were discarded as burn-in. TRACER 1.6 was used for the convergence statistics of runs (Rambaut & Drummond, 2009). However, analyses resulted in two competing likelihood islands and we had to select only runs within the higher likelihood island. Only three runs with more than 162 million states were combined. All ESS values higher than 150 were regarded as having a sufficient support, and all except three values were higher than 200. A maximum clade credibility tree was generated by TreeAnnotator 1.8.3 (Drummond *et al.*, 2012) and visualized with Figtree 1.4.0 (Rambaut, 2016).

Species delimitation

We analysed sequence data using three quantitative methods of species delimitation. One distance-based – automatic barcode gap discovery (ABGD) (Puillandre *et al.*, 2012a), and two tree-based – generalized mixed Yule coalescent analysis (GMYC) (Pons *et al.*, 2006; Fujisawa & Barraclough, 2013) and Poisson tree processes model (PTP) (Zhang *et al.*, 2013). GMYC and PTP were originally designed for the analysis of single-locus

data, but have also been applied to concatenated multilocus data (Luo *et al.*, 2018). Each method uses its own terminology to refer to the delimited taxa-like 'groups' (ABGD), 'entities' (GMYC) or 'phylogenetic species' (PTP), thus acknowledging that they may not represent biologically meaningful species. In barcoding, 'molecular operational taxonomic units' (MOTUs) is also a widely used term (Floyd *et al.*, 2002), which taxonomically specifies a specimen based on provided DNA sequence. For clarity and consistency with other studies (Hayward *et al.*, 2011; Schwarzfeld & Sperling, 2015), we are using the term 'putative species' instead of any of these other terms.

The ABGD method was used to sort the available COI sequences into genetic clusters that could be considered putative species. This program was originally developed for the COI barcoding sequence. The ABDG algorithm automatically finds the inflection point in the frequency distribution of ranked pairwise genetic distances between aligned homologous sequences and does so recursively to obtain the best partition of the data set into candidate species. The calculation of confidence limit for intraspecific divergence is model-based (Puillandre *et al.*, 2012a). We first calculated the uncorrected p-distance matrices for the COI dataset in Geneious (Kearse *et al.*, 2012). Calculation of the number of clusters was done on the ABGD web server (Puillandre *et al.*, 2012a) available at https://bioinfo.mnhn.fr/abi/public/abgd/. The analysis was performed using default values, employing the Kimura (K80) model.

The GMYC method (Pons *et al.*, 2006; Fujisawa & Barraclough, 2013) is a tree-based method that uses a maximum likelihood approach for delimiting an independently evolving species. The GMYC method requires an ultrametric tree without identical sequences to avoid zero-length terminal branches that hamper the likelihood estimation. A likelihood-ratio test is used to determine if the model with a shift in the branching processes provides a better fit to the data than the null model lacking a shift in branching processes. The ultrametric tree was obtained in a previous analysis using BEAST 1.8.3. (Drummond *et al.*, 2012). The GMYC was run as implemented in the GMYC web server (http://species.h-its.org/gmyc/). The analysis was launched in two modes with single- (sGMYC) and multiple-thresholds (mGMYC).

The PTP method is a tree-based approach that delimits species using phylogenetic trees (Zhang *et al.*, 2013). PTP uses only nucleotide substitution information and implements a model using the gene tree branch lengths generated by two independent Poisson process classes (within- and among-species substitution events). The fundamental assumption of this method is that the number of substitutions is significantly higher between species than within species (Zhang *et al.*, 2013). The bPTP is a version of the original maximum likelihood PTP that provides posterior delimitation probabilities (PP value) to delimited species on the input phylogenetic tree. The maximum likelihood PTP result is part of the results from bPTP analysis. A higher PP value on a node indicates all descendants from this node are more likely to be from one phylogenetic species (Zhang *et al.*, 2013). The analysis was conducted on the web server for bPTP (http://species.h-its.org/ptp/), using the MrBayes phylogenetic tree (with outgroup taxa removed) obtained in a previous analysis. We ran 500 000 MCMC generations with a thinning value of 100 and 10% burn-in.

Mean divergence age of two sister species and number of hosts

The age of the most recent common ancestor (MRCA) for all sister species pairs was obtained from the dated tree (Fig. 1). The mean age of all sister species pair divergence was calculated for each species delimitation method (ABGD, sGMYC, mGMYC, bPTP). Differences in age between species delimitation methods were tested by ANOVA on logarithmic data. A TukeyHSD posthoc test was used to determine the significance of the difference between each pair of methods. The program R (R Core Team, 2014) was used for the analysis. The mean number of host species per strepsipteran species in Xenidae was also calculated. The mean was acquired for each species delimitation method. The difference between single host and multiple host species was tested between methods. Differences between methods were tested using the binomial generalized linear model in the R program (R Core Team, 2014). List of analysed data using R software are available in Table S3.

Exploring diversification rates using a lineage through time (LTT) plot

The diversification of Xenidae was investigated by generating a semi-logarithmic lineage-through-time (LTT) plot with 95% confidence intervals using TRACER 1.6 (Rambaut & Drummond, 2009) and modified output dated trees from Bayesian phylogenetic analysis conducted in BEAST 1.8.3 (Drummond *et al.*, 2012). The post-burn-in output was resampled to 2000 selected trees using LogCombiner 1.8.3 that is part of the BEAST 1.8.3 package. Generation of an LTT plot is frequently used to graphically explore the diversification rates (Ricklefs, 2007). The output is a graphical representation of the cumulative number of reconstructed lineages over time based on a chronogram.

Results

Phylogenetic relationships

Based on our concatenated alignment of 3371 bp and 209 specimens from one mitochondrial (COI) and five nuclear markers (CAD, DDX23, AP2A, AFG3L2, BTBD6), we found support for monophyly of Xenidae (the posterior probability [PP] was equal to 1 in BEAST and MrBayes analyses; Fig. 1, node 1; Fig. S1). The topology displayed paraphyly of *Pseudoxenos* and polyphyly of the genera *Xenos* and *Paraxenos*. *Xenos* was divided into three lineages, including the lineage of *Xenos vespularum* Kifune & Maeta (XVs1_2), a neotropical lineage (node 8) parasitizing Epiponini hosts (e.g., *Polybia* Lepeletier de Saint Fargeau spp.) (Fig. 1; Fig. S1, PP = 1), and a highly derived crown group (Fig. 1, node 27; Fig. S1, PP = 1). *Paraxenos* was

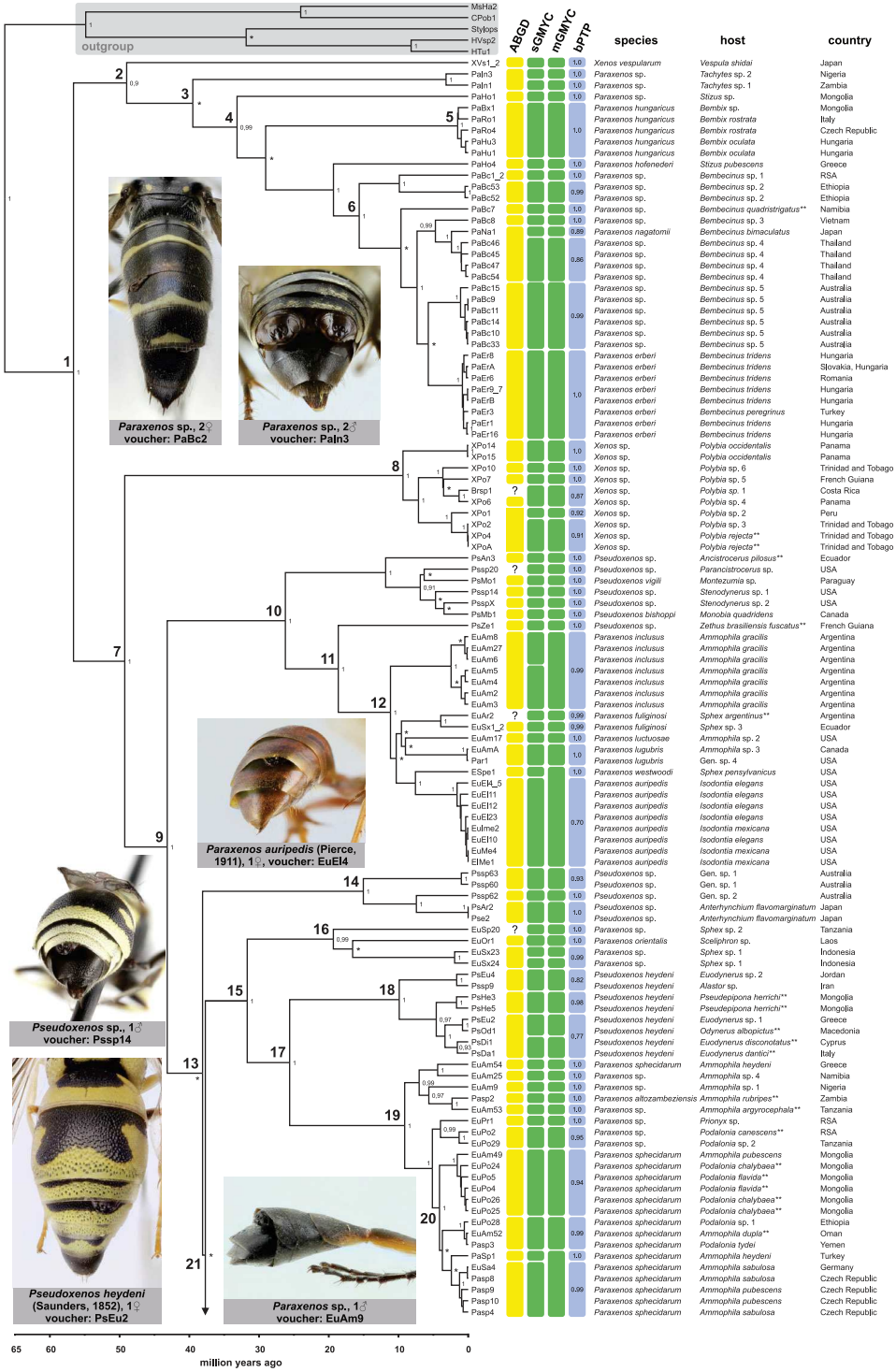

Fig. 1. Bayesian maximum credibility chronogram of Xenidae from the BEAST analysis. Numbers on nodes indicate posterior probability. Posterior probabilities lower than 0.9 are indicated by stars (*). Terminals are listed as voucher codes. Some vouchers with identical sequences were interchanged using the following abbreviations: PaErA (PaEr2, PaCr2), PaErB (PaEr14, PaEr15), XPoA (XPo12, Xpo13), EuAmA (EuAm23, EuAm24), XenA (Xen1, Xen6, Xen7), XenB (Xen8, Xen9, Xen10), XspA (Xsp39, Xsp40), XVeA (XVe1, XGa1), XDoA (XDo5, XDo6, XDo7, XDo8, XDo42). Putative species clusters obtained using each delimitation method are shown as coloured columns to the right of terminal labels. Column ABGD: question marks (?) indicate the absence of COI sequence. Column bPTP: inside numbers indicate bPTP posterior probability values, grey clusters represent an incongruent topology between MrBayes tree and BEAST tree. The text columns on the right indicate putative species of Xenidae, their hosts, and the country of collection. New host records are indicated by a double asterisk (**) [Colour figure can be viewed at wileyonlinelibrary.com].

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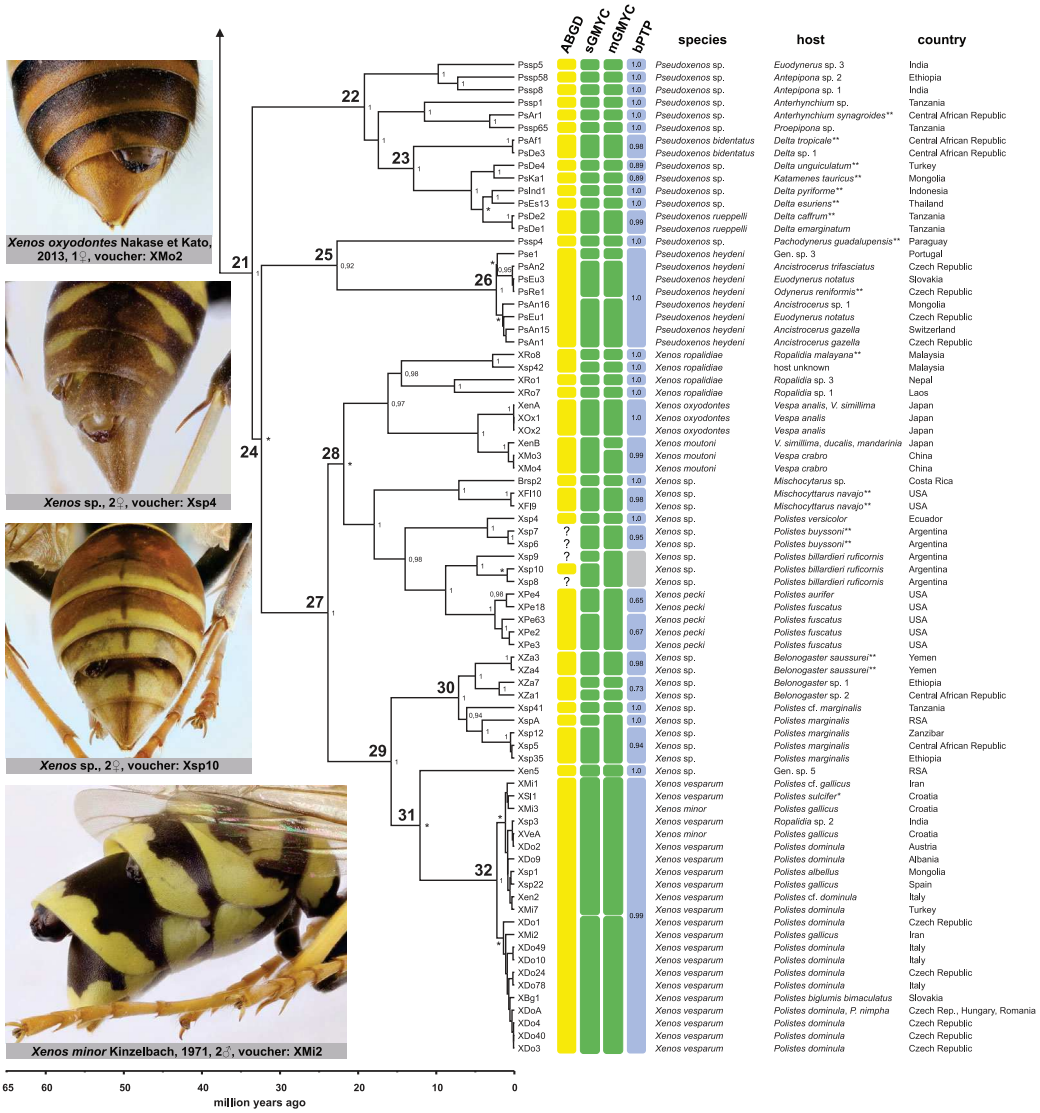

Fig. 1. Continued. [Colour figure can be viewed at wileyonlinelibrary.com].

recovered in four separate lineages. The first lineage being parasites on Crabronidae and Bembicidae (Fig. 1, node 3 while the other three lineages parasitize Sphecidae (Fig. 1, nodes 12, 16, 19, PP = 0.99–1).

Species delimitation

In total, the ABGD method identified 77 putative species; however, we had eight specimens with missing COI sequences that were needed for a complete resolution of the ABGD delimitation. The bPTP analysis delimited 86 putative species. When comparing the tree-based methods, bPTP estimated the fewest putative species. However, an entirely clear interpretation was not possible for Xsp8, Xsp9 and Xsp10 because of the incongruent topology between the MrBayes and BEAST results. Results from the mGMYC analysis delimitated 91 putative species, while the sGMYC method resulted in 96 putative species.

The phylogenetic position of *X. vespularum* was not congruent between MrBayes and BEAST analyses, having lower support in the latter (Fig. 1, node 2, PP = 0.9, Fig. S1). The position of this species was placed as sister to all remaining Xenidae (Fig. 1, node 2) or sister to the lineage with Crabronidae-Bembicidae hosts (Fig. S1). However, *X. vespularum* is well delineated as a putative species by all species delimitation methods (Fig. 1).

The species of *Paraxenos* in node 4 (Bembicidae hosts) represent a monophyletic group but the sister position of this clade to those *Paraxenos* species parasitic on *Tachytes* Panzer is not well supported (Fig. 1, node 3, 4; Fig. S1). The specimens that parasitize *Bembix* Fabricius and *Bembecinus* Costa form two monophyletic well-supported lineages (Fig. 1, node 5, 6; Fig. S1). There is strong congruence in the delimitation of

putative species within the *Paraxenos* lineage at node 3 (number of putative species: ABDG = 11, sGMYC = 13, mGMYC = 13, bPTP = 13); we assigned four putative species to the described species in this lineage.

We obtained relatively strong support for the monophyly of the *Xenos* lineage that parasitize members of Epiponini (*Polybia*) in South America (Fig. 1, node 8; Fig. S1). Six species were congruently delimited by three of the methods (sGMYC, mGMYC, bPTP) but it was not possible to assign any putative species to the described species. The well-supported New World lineage of node 10 (*Pseudoxenos* + *Paraxenos*) parasitize various different hosts, such as Vespidae (Odynerini and Zethini) and Sphecidae. The ABGD and bPTP delimitation methods resulted in 14 putative species within this group, but two COI sequences were missing for the ABGD analysis. The GMYC methods resulted in a difference of two putative species (number of putative species: sGMYC = 15, mGMYC = 13). The eight described species were assigned to putative species.

Node 15 (Fig. 1) represents a well-supported lineage with the Old World and Australian species (Fig. S1, MrBayes, PP = 1). This clade comprises *Paraxenos* rendered paraphyletic by a *Pseudoxenos* species group (node 18). There was congruence in the ABGD and bPTP delimitation methods circumscribing species; however, one COI sequence was missing in the ABGD analyses (17 putative species were delimited by bPTP). The output of these methods differed slightly from the results of the GMYC methods (number of putative species: sGMYC = 19, mGMYC = 18). The results of all species delimitation methods supported splitting *Ps. heydeni* (Saunders) and *Pa. sphecidarum* (Dufour) into several different putative species (Fig. 1, nodes 18, 20). In both cases, these lineages were split into biogeographically disparate species.

The monophyly of species of *Pseudoxenos* in lineage 22 was strongly supported, as well as the branching of its internal clades (Fig. 1, Fig. S1). This lineage utilizes a diverse range of hosts from in Odynerini and Eumenini (Vespidae: Eumeninae). Species of Xenidae that parasitize Eumenini formed a monophyletic group (Fig. 1, node 23). All species delimitation methods were congruent in the number and delineation of putative species. The PP values in the bPTP analysis were very high for all 12 putative species. *Pseudoxenos heydeni* was recovered as a monophyletic lineage (node 26) and delimited as a single putative species by both ABGD and bPTP, while both GMYC approaches splitted it into three putative species.

The largest clade of the three lineages of *Xenos* was recovered with relatively high support (Fig. 1; node 27), which represents a highly derived clade in Xenidae. This lineage utilizes a diverse range of vepid hosts from Vespini, Mischocyttarini, Ropalidiini and Polistini. The putative species number was slightly different among all delimitation methods (ABDG = 17, sGMYC = 22, mGMYC = 20, bPTP = 19), although four COI sequences were missing for the ABGD analysis that could affect the result for that analysis. All methods were relatively congruent regarding the split of *X. ropalidiae* (Kinzelbach) into 3–4 putative species, all of which parasitize species of *Ropalidia* Guérin-Méneville. *Xenos pecki* Kirby was delineated as a single species by ABGD and mGMYC and splitted into two species using the bPTP method (albeit with very low PP values). Both GMYC and bPTP methods showed that *X. vesparum* Rossi is a well-supported species, but the GMYC methods splitted it into two putative species. *Xenos minor* Kinzelbach was recovered within a larger clade of *X. vesparum* rendering the latter paraphyletic and had no support as a putative species from species delimitation methods.

Table 1. TukeyHSD test of the difference in the mean divergence age of two sister species between each species delimitation method

Methods	diff	lwr	upr	*P*-value adj
mGYMC - ABDG	−0.6620	−1.3167	−0.0073	**0.0464**
sGYMC - ABDG	−0.6774	−1.3190	−0.0357	**0.0343**
bPTP - ABDG	−0.2445	−0.8904	0.4013	0.7574
bPTP - mGYMC	0.4175	−0.1916	1.0267	0.2852
sGYMC - mGYMC	−0.0153	−0.6202	0.5895	0.9999
sGYMC - bPTP	−0.4329	−1.0280	0.1623	0.2356

Divergence age of two sister species and diversification rates over time

We investigated the age of the most recent common ancestor (MRCA) of all putative sister species pairs and made a comparison of all species delimitation methods (Fig. 2). The mean age of divergence determined by each of the methods is as follows: ABGD 6.54 Ma (SD = 4.6752, N = 25), sGMYC 4.09 Ma (SD = 2.2691, N = 34), mGMYC 4.28 Ma (SD = 2.4764, N = 31) and bPTP 5.71 Ma (SD = 4.4015, N = 33). We found a significant difference in the mean divergence age of two sister species determined by different methods (ANOVA on logarithmic data, F = 3.6226, P = 0.0152). The post-hoc TukeyHSD test showed a significant difference between the ABGD and mGMYC methods (P = 0.0464) and also between the ABDG and sGYMC methods (P = 0.0343). No significant differences were detected between the other methods (Table 1). The LTT plot reconstructed from the MCC chronogram (Fig. 1) of Xenidae revealed a constant rate in net diversification throughout the past 45 million years (Fig. 3). A slight increase in diversification rate could be detected for the most recent 2 million years.

Diversity of hosts

We managed to obtain host specimens from all known host families, including Bembicidae, Crabronidae, Sphecidae and Vespidae. This included 35 host genera, all of which were previously known as hosts of Xenidae except the genus *Zethus* Fabricius. Furthermore, our results showed evidence of 122 sorted host species, from which 67 were identified to species level (name assigned). Twenty-nine of the named host species were not found in the literature as hosts of Xenidae previously.

Most species of Xenidae were found in a single host species (Fig. 4). This pattern is evident in all species delimitation

methods applied to hosts (ABGD = 70.27%, sGMYC = 77.08%, mGMYC = 75.82%, bPTP = 77.01%). There was no significant difference in the proportion of species with single- and multiple-host species among the delimitation methods (binomial GLM, deviance = 1.29, df = 3, P = 0.7327). The mean number of hosts in Xenidae was 1.55 (ABGD), 1.39 (sGMYC), 1.42 (mGMYC) and 1.44 (bPTP). The putative species assigned to *X. vesparum* (node 32) had the largest number of hosts (mostly *Polistes*), with the ABGD and bPTP methods confirming nine species. The greatest number of host genera was found in the putative species of node 26 (Fig. 1, ABGD, bPTP), which had six host species belonging to four genera.

Discussion

Strepsiptera are an archaic order that originated during the early Carboniferous period, with Xenidae arising much later during the Paleogene (McMahon *et al.*, 2011; Toussaint *et al.*, 2017). The evolution and diversification of Xenidae was accompanied by numerous transcontinental dispersals and disparate host switches (Benda *et al.*, 2019). These findings are supported by this study using the same markers but with denser taxon sampling. This analysis also supports suggested taxonomic changes of traditional genera (Benda *et al.*, 2019). We confirm the paraphyly of the genus *Pseudoxenos* and polyphyly of the genera *Xenos* and *Paraxenos*. There are similar cases in other Strepsiptera families; McMahon *et al.* (2011) reported the polyphyly of the genus *Halictophagus* Perkins (Halictophagidae) and paraphyly of the family Myrmecolacidae. Our results are in accordance with Hayward *et al.* (2011), who postulated that morphological similarity among specimens is not a reliable indicator of relationship in parasites. Moreover, the clear lack of monophyly in all three traditional genera compels us to re-evaluate the status of each through more detailed morphological and taxonomic study of the family. Based on our results, in accordance with Benda *et al.* (2019), we propose that the existence of at least 11 lineages could each be considered as separate genera, even if no morphological apomorphies have been identified as yet.

Diversification rate of xenid parasites

The large number of parasitic species has led to a speculation about whether rates of speciation and extinction are dependent on parasite diversity; if these factors are mutually exclusive, species diversity should fluctuate instead of remaining relatively constant over time (McLeish *et al.*, 2010). The key situation occurs after the parasitic lineage evolves from nonparasitic ancestors. Although such specialized changes may facilitate the exploitation of new ecological niches, such intimate specialization subsequently binds the evolutionary fate of the parasite to their host (Krüger *et al.*, 2009). A majority of phylogenetic studies investigating diversification rates have shown high rates of lineage accumulation early in a group's evolution (McLeish *et al.*, 2010). A possible scenario for subsequent evolution is a remarkable radiation, such as occurred in the megadiverse parasitoid Hymenoptera and the parasitic Platyhelminthes (Littlewood *et al.*, 1999; Whitfield, 2003). In contrast, Litman *et al.* (2013) explored the evolution of brood parasitism in long-tongued bees and found decreased rates of diversification in eight out of ten brood parasitic clades. Vanhove *et al.* (2015) observed a decrease in diversification rate over time in *Gyrodactylus* Nordmann parasitising tropheine cichlids in Lake Tanganyika. This phenomenon might be explained due to an increased risk of extinction associated with a parasitic life-history strategy, which may in fact be an evolutionary 'dead end' (Wiegmann *et al.*, 1993). In the case of Xenidae parasites, we found the diversification of Xenidae lineages to be nearly constant through time (Fig. 3). This constant diversification could be explained by increased flexibility of this group, represented by the relatively low host fidelity of Xenidae and their ability to colonize new host lineages, combined with their frequent passive (host mediated) long-range dispersal (Benda *et al.*, 2019). Opportunities for host switching may be more frequent in hosts that feed on various flowers, where incidental encounters of non-hosts are frequent. Stylopidae parasitize bees and larvae are deposited on flowers where they wait for phoretic transport by host females to their nests (Linsley & MacSwain, 1957; Batra, 1965). Although this behaviour was not observed in Xenidae, we expect a similar larval transport system occurring on flowers and as such, exposure to a diverse spectrum of potential hosts visiting these flowers presents an ideal opportunity for host switches. This phenomenon of low-host fidelity in Xenidae may provide a mechanism to escape from possible evolutionary dead ends and could explain the large diversity of the family, one of the most species-rich in Strepsiptera (Pohl & Beutel, 2008).

Species delimitation methods

We investigated species diversity with phylogenetic data for the whole Xenidae family using three species delimitation methods. Although a complete resolution of the dataset by ABGD was impossible because of several missing COI sequences, the results from this method tend to underestimate the number of putative species compared to the other methods used. Consistent with other empirical studies (e.g., Pentinsaari *et al.*, 2017; Renner *et al.*, 2017), the distance-based ABGD method is more conservative, and joins lineages and identifies fewer putative species than alternative methods. A clear example is our analysis of the divergence age calculated from all putative sister species pairs (Fig. 2, Table 1). Our comparison of the methods suggests a similarity between bPTP and mGMYC. For our data, the sGMYC method separated lineages into the greatest number of putative species. There was a relatively smaller difference between the single and multiple threshold methods for the GMYC analysis. While the multiple threshold method of GMYC was developed to take into account the different branching patterns and rates of evolution across a phylogenetic tree (Monaghan *et al.*, 2009; Papadopoulou *et al.*, 2009), evidence suggests that it is consistently less accurate than the single threshold method (Esselstyn *et al.*, 2012;

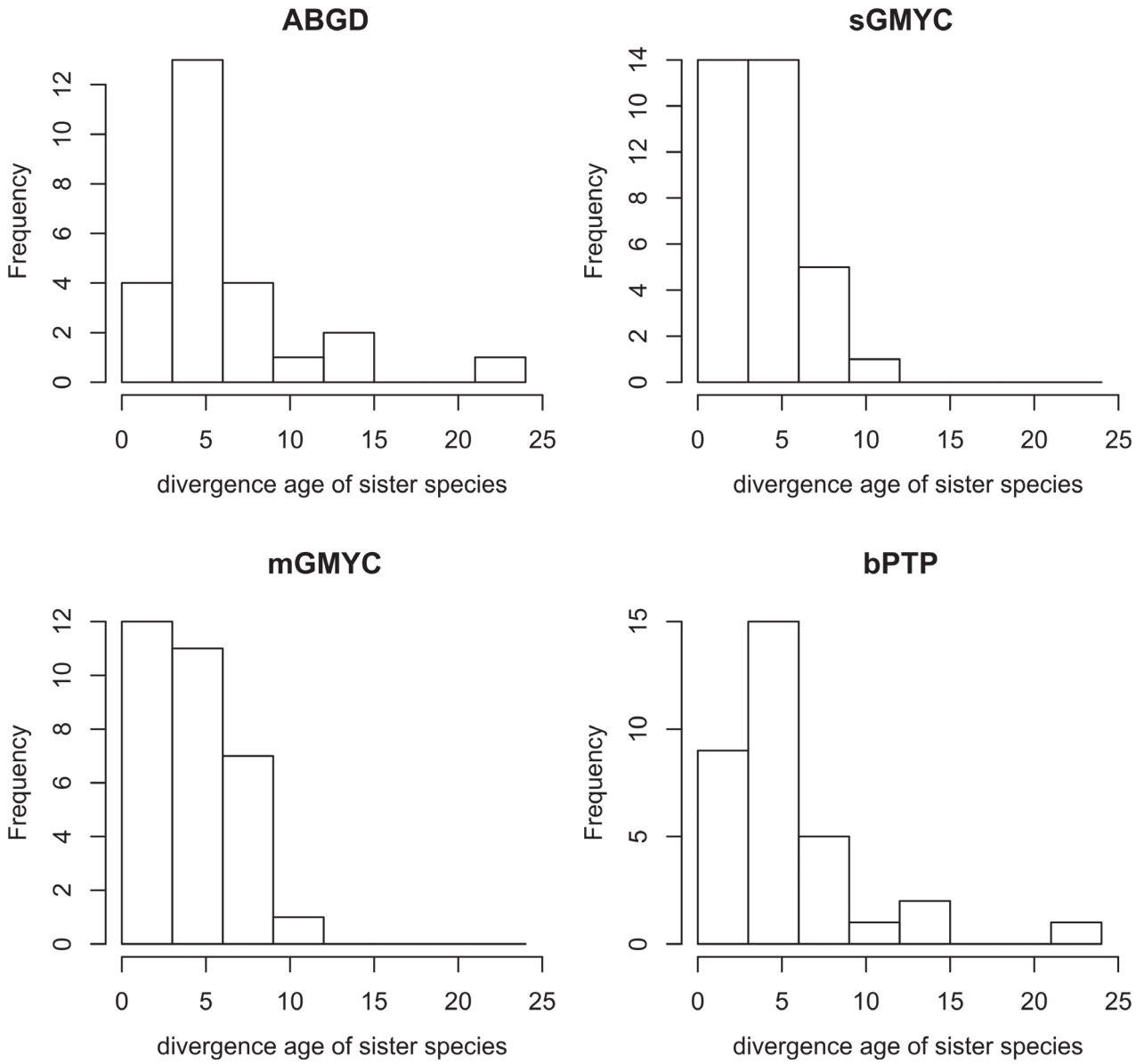

Fig. 2. Comparison of four species delimitation methods with regard to the divergence age of sister species.

Fujisawa & Barraclough, 2013; Schwarzfeld & Sperling, 2015). Schwarzfeld & Sperling (2015) also observed that, in all cases, the sGMYC method recovered a higher number of species than mGMYC, but the multiple-threshold method still overestimates the number of species. Our results are consistent with this suggestion that the GMYC method shows a tendency for oversplitting (Pentinsaari *et al.*, 2017).

Results produced by the bPTP method show very low PP values in some delimited putative species. These low values (0.65–0.77) are associated with the putative species that are delimited inconsistent with results from other methods (ABGD, GMYC), and reveal some problems with the delimitation of these lineages into putative species. These problems can be overcome by more sophisticated tools of population genetic methods. The PTP method requires a phylogenetic tree for analysis, but with branch lengths proportional to the amount of genetic change rather than to the time as used by the GMYC method. The PTP method tends to obtain more precise results than GMYC when interspecific distances are small (Zhang *et al.*, 2013); nevertheless, these two methods often produce similar estimates of delimitation among putative species (Arrigoni *et al.*, 2016; Luo *et al.*, 2018).

Luo *et al.* (2018) showed that tree-based methods like PTP and GMYC are negatively influenced by gene flow and are sensitive to the ratio of population size to divergence time, reflecting the impact of incomplete lineage sorting on species

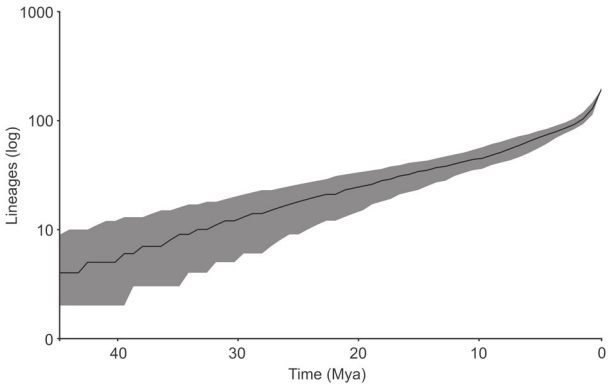

Fig. 3. Semi-logarithmic lineages-through-time plot reconstructed from the MCC chronogram for the representatives of the entire Xenidae. Bold line represents median values of lineage diversification and grey area corresponding to 95% highest posterior density (HPD) intervals.

delimitation. Unexpectedly, they found only a modest effect of increasing the number of loci and the sample size per species on delimitation accuracy. In the context of integrative taxonomy, various molecular species delimitation methods provide only 'primary species hypotheses', which need to be assessed using multiple lines of evidence (Puillandre *et al.*, 2012b). For example, groups of sequences that are consistently delimited by a variety of quantitative methods are good candidate species to be examined in more detail using comparative morphology or ecology that either supports or refutes the putative species (Kekkonen & Hebert, 2014). On the other hand, species that are inconsistently delimited are good candidates for more fine-scaled analyses, such as quantitative morphometrics or population genetics (Schwarzfeld & Sperling, 2015).

Species delimitation

Before a comprehensive revision of taxa is possible, rapid insight into putative species diversity is required, especially in species-rich groups of morphologically hard-to-distinguish parasites. Hayward *et al.* (2011) emphasized that high morphological similarity among specimens of poorly studied parasitic species cannot be taken as indicative of a close genetic relationship. They suggested that if cryptic species commonly exist in Strepsiptera, current order-wide estimates of diversity may be underestimated by more than an order of magnitude. Our data also assume a considerable underestimation of Strepsiptera biodiversity.

Our results support *Ps. heydeni* as two widely separate lineages. Kinzelbach (1978) validated *Ps. heydeni* as a species with Palearctic distribution parasitizing diverse host genera of tribe Odynerini (Vespidae). Interestingly, one *Ps. heydeni* lineage (node 26) represents one putative species with a large geographic area (Portugal to Mongolia) and a wide range of Odynerini hosts. The second *Ps. heydeni* lineage (node 18) contains a mixture of closely related species. This complex of species exhibits different distributions, which points to a possible vicariant evolution. We could divide this species complex into Middle East, Mediterranean and East Asian taxa. Although the putative species have distinct areas, they have very similar hosts from tribe Odynerini (e.g. *Euodynerus* Dalla Torre). Cook (2019) restored several synonyms for *Ps. heydeni*, although we retain this name until the species can be examined further in a taxonomic context.

Another split of a previously described taxon was found in *Pa. sphecidarum* that Kinzelbach (1978) identified in all Palearctic region as a parasite of the genera *Ammophila* Kirby, *Podalonia* Fernald and *Sphex* Linnaeus. In spite of this, the *Pa. sphecidarum* complex consists of four putative species with different distributions in Mongolia, Middle East + Ethiopia, Turkey and Central Europe. *Xenos ropalidiae*, also consists of three to four putative species distributed in Malaysia, Nepal and Laos. Although Kinzelbach (1975) recorded this species in Afrotropical and Indomalayan biogeographic regions, we support the contention that it represents a complex of putative species. We have only recorded genus *Ropalidia* as a host, and Kinzelbach (1975) suggests is the exclusive host lineage for this species complex. However, we found that *Ropalidia* can also be a host of *X. vesparum*.

The preponderance of non-monophyletic genera is not unique to Xenidae. Hayward *et al.* (2011) reported similar occurrences of non-monophyly in Myrmecolacidae and also considered *Caenocholax fenyesi* Pierce as a complex of at least 10 cryptic species in Central America. Although multiple *C. fenyesi* putative species occur sympatrically, they proposed that observed levels of phylogenetic separation imply that co-occurrence is a consequence of secondary contact rather than recent sympatric speciation. Furthermore, they discussed that *C. fenyesi* specimens revealed slight variation in key characters among several proposed species. Nakase & Kato (2013) distinguished two cryptic species of *Xenos* that are parasitic on large *Vespa* hornets using barcode sequences and proposed subtle morphological characters to discriminate them. These findings are consistent with the statement of Bickford *et al.* (2007) that cryptic species seem to be morphologically indistinguishable, but differences are often detected once researchers are prompted to look. Moreover, Hayward *et al.* (2011) reported the age of the *C. fenyesi* complex as approximately 25–38 Ma, which suggests a relatively long time of evolutionary stasis with little change in external morphology evident in the fossil record (Kathirithamby & Henderickx, 2008).

Our results also suggest a likely synonymy between two European species in Xenidae. *Xenos minor* Kinzelbach has a limited distribution in Croatia (Kinzelbach, 1971b) but is probably a synonym of the more widely distributed *X. vesparum*.

Host diversity and specificity

An important feature of parasite evolution is host specificity. It can better explain the breadth of a parasite's ecological niche than other parameters. It can tell us how likely a parasite may switch hosts or the likelihood of changes in distribution (Poulin & Mouillot, 2003). It is necessary to approach the question

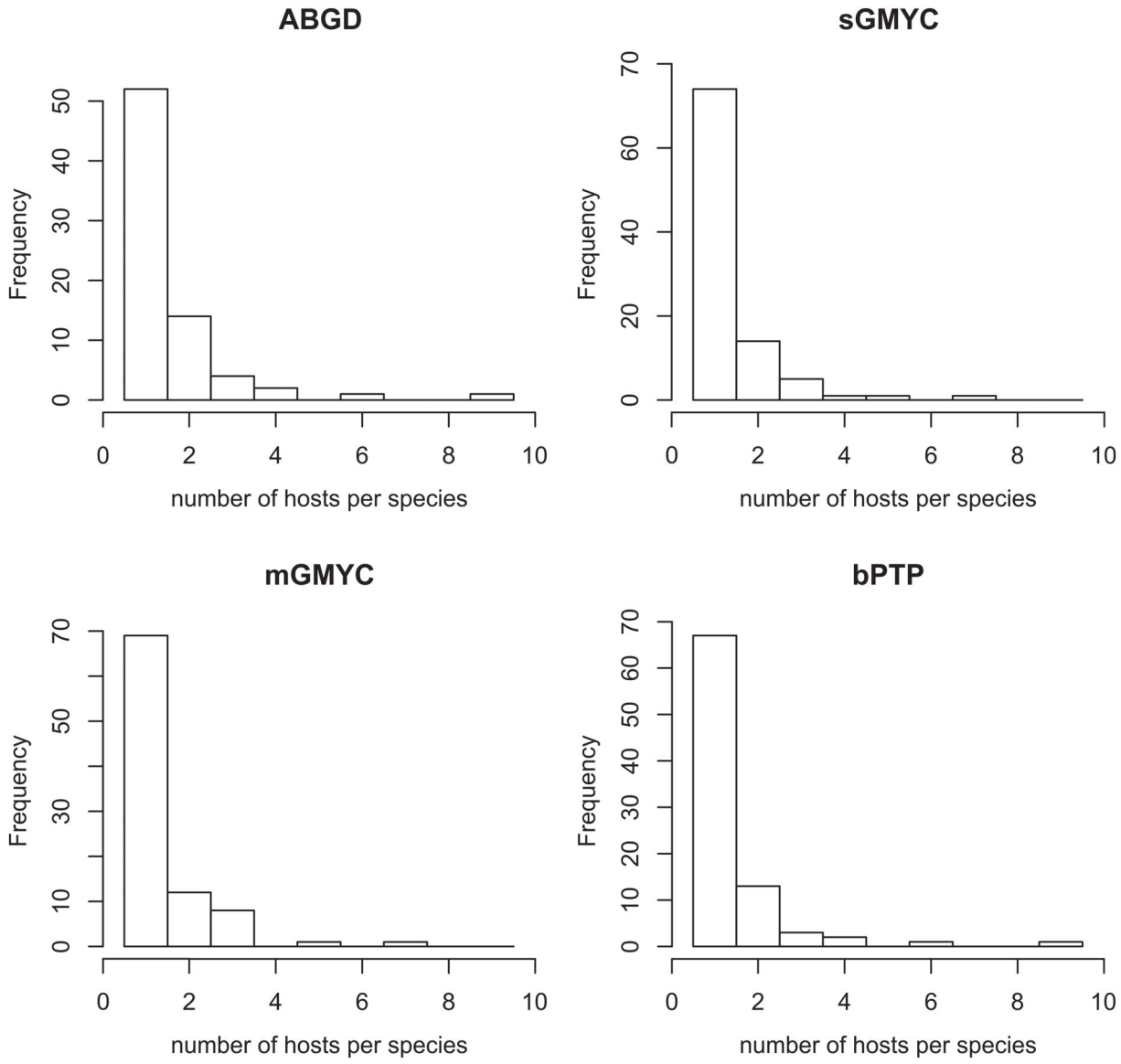

Fig. 4. Comparison of four species delimitation methods with regard to the number of hosts per putative species of Xenidae.

of host specificity very carefully regarding the species concept and delimitation. Incorrect species synonymy or the presence of undetected cryptic species may undermine estimates of the host specificity (Poulin, 2007). In spite of the small number of described species, the spectrum of hosts in Strepsiptera is relatively wide and comprises seven insect orders (Kathirithamby, 2008; Pohl & Beutel, 2008). However, there can be significant differences in host specificity at the species level. Before the era of molecular phylogenetic studies, the prevailing agreement was that there is high host specificity in the more derived families parasitizing Hymenoptera hosts (Stylopidae, Xenidae, Myrmecolacidae), while some other families (e.g. Halictophagidae) exhibit lower host specificity (Bohart, 1941; Riek, 1970; Kathirithamby, 1989). Matsumoto *et al.* (2011) suggested that the species from the genus *Elenchus* Curtis (Elenchidae) can parasitize more than one host genus of the hemipteran family Delphacidae. In contrast, Jůzová *et al.* (2015) revealed that species of *Stylops* are mostly restricted to host subgenera within *Andrena* (Andrenidae). Nakase & Kato (2013) reported different host specificity in sister species of *Xenos* parasitizing *Vespa*. Although *X. moutoni* Buysson was found to be a parasite of several *Vespa* species, *X. oxyodontes* Nakase & Kato predominantly parasitises *Vespa analis* Fabricius.

Our results suggest relatively high host specificity in Xenidae. Depending on the species delimitation method, there are 70–77% of putative species with only one host. We found no significant variance among all delimitation methods in the proportion of species with single and multiple host species. The mean number of host species per putative species varied between 1.39 and 1.55. Although there could be a biasign effect

from singletons, the effect of a dominant host could also play a role. The effect of the dominant host was suggested by Nakase & Kato (2013) in some species like *Pa. erberi* Saunders, *Pa. inclusus* (Oliveira & Kogan), and *X. vesparum*. In the case of *Pa. erberi*, the main host could be *Bembecinus tridens* (Fabricius) because only one other species, *B. peregrinus* (F. Smith), was recorded in our dataset. *Bembecinus tridens* is the most numerous host recorded in the literature (Kinzelbach, 1978). High specificity is also reflected in other putative *Paraxenos* species from *Bembecinus* hosts on different continents.

Our results revealed nine host species in *X. vesparum*, which was the highest number of the putative species in our dataset. However, more than half of the host records were of *Polistes dominula* (Christ), which is also recorded as the main host of *X. vesparum* (e.g., Batelka & Straka, 2005). An interesting finding is the stylopization of *P. sulcifer* Zimmermann from the obligate social parasitic subgenus *Sulcopolistes* (Blüthgen). This parasitic subgenus is not recorded as a host for Xenidae, but Smit & Smit (2014) recorded stylopization of two species from the subgenus *Sulcopolistes* (*P. atrimandibularis* Zimmermann, *P. semenowi* Morawitz). Stylopization of social parasitic Hymenoptera is very rare and occurs only in the subgenus *Sulcopolistes* and the facultative social parasitic hornet *Vespa dybowskii* André, the latter being parasitised by *Xenos moutoni* in Japan (Nakase & Kato, 2013). All three *Sulcopolistes* species are social parasites of *P. dominula*, which is the main host of *X. vesparum*. Stylopized specimens of *P. dominula* form an aggregation outside nests and often return to the nest where the parasitic larvae of *X. vesparum* are released (Hughes *et al.*, 2003, 2004). Therefore, parasitic *Sulcopolistes* could be easily infected by their hosts in the nest. We also have evidence for the extraordinary host switch of *X. vesparum* from Polistini to Ropalidiini (*Ropalidia*). This unusual switch between tribes (Polistini - Ropalidiini) is previously not recorded as *X. vesparum* is previously known to exclusively parasitise species of *Polistes*.

Rarely, some species are relative generalists and parasitise several genera within a tribe. For example, putative species from the *Pa. sphecidarum* clade seem to be specific to *Ammophila* and *Podalonia* (Ammophilini). Furthermore, at least one lineage of *Ps. heydeni* (node 26) parasitise four genera of Odynerini with no obvious specific preference. These patterns of specificity are relatively consistent with the host summary of *Pa. specidarurum* and *Ps. heydeni* by Kinzelbach (1978), who identified a wide range of host species, but exclusively from related genera.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Bayesian maximum credibility tree (MrBayes) of Xenidae.

Table S1. List of all specimens analysed in the present study, with GenBank accession numbers for sequenced genes.

Table S2. List of the primers and PCR conditions.

Table S3. List of analysed data in R and additional outputs.

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Data availability statement

DNA sequences are stored in the Genbank database with accession numbers listed in Table S1. All other data are presented within this manuscript or in the online Supporting Information.

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Paper III

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A generic classification of Xenidae (Strepsiptera) based on the morphology of the female cephalothorax and male cephalotheca with a preliminary checklist of species

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Abstract

The generic taxonomy and host specialization of Xenidae have been understood differently by previous authors. Although the recent generic classification has implied a specialization on the level of host families or subfamilies, the hypothesis that each xenid genus is specialized to a single host genus was also previously postulated. A critical evaluation of the classification of the genera of Xenidae is provided here based on morphology in accordance with results of recent molecular phylogenetic studies. External features of the female cephalothoraces and male cephalothecae were documented in detail with different techniques. Diagnoses and descriptions are presented for all 13 delimited genera. The earliest diverging genera are usually well characterized by unique features, whereas deeply nested genera are usually characterized by combinations of characters. Three new genera are described: *Sphecixenos* **gen. nov.**, *Tuberoxenos* **gen. nov.**, and *Deltoxenos* **gen. nov.** Five previously described genera are removed from synonymy: *Tachytixenos* Pierce, 1911, **stat. res.**; *Brasixenos* Kogan & Oliveira, 1966, **stat. res.**; *Leionotoxenos* Pierce, 1909, **stat. res.**; *Eupathocera* Pierce, 1908, **stat. res.**; and *Macroxenos* Schultze, 1925, **stat. res.** One former subgenus is elevated to generic rank: *Nipponoxenos* Kifune & Maeta, 1975, **stat. res.** *Monobiaphila* Pierce, 1909, **syn. nov.** and *Montezumiaphila* Brèthes, 1923, **syn. nov.** are recognized as junior synonyms of

Leionotoxenos Pierce, 1909, **stat. res.** *Ophthalmochlus* Pierce, 1908, **syn. nov.**, *Homilops* Pierce, 1908, **syn. nov.**, *Sceliphronechthrus* Pierce, 1909, **syn. nov.**, and *Ophthalmochlus* (*Isodontiphila*) Pierce, 1919, **syn. nov.** are recognized as junior synonyms of *Eupathocera* Pierce, 1908, **stat. res.** A preliminary checklist of 119 described species of Xenidae with information on their hosts and distribution is provided. The following 14 species are recognized as valid and restituted from synonymy: *Tachytixenos indicus* Pierce, 1911, **stat. res.**; *Brasixenos acinctus* Kogan & Oliveira, 1966, **stat. res.**; *Brasixenos araujoi* (Oliveira & Kogan, 1962), **stat. res.**; *Brasixenos bahiensis* Kogan & Oliveira, 1966, **stat. res.**; *Brasixenos brasiliensis* Kogan & Oliveira, 1966, **stat. res.**; *Brasixenos fluminensis* Kogan & Oliveria, 1966, **stat. res.**; *Brasixenos myrapetrus* Trois, 1988, **stat. res.**; *Brasixenos zikani* Kogan & Oliveira, 1966, **stat. res.**; *Leionotoxenos hookeri* Pierce, 1909, **stat. res.**; *Leionotoxenos jonesi* Pierce, 1909, **stat. res.**; *Leionotoxenos louisianae* Pierce, 1909, **stat. res.**; *Eupathocera luctuosae* Pierce, 1911, **stat. res.**; *Eupathocera lugubris* Pierce, 1909, **stat. res.**; *Macroxenos piercei* Schultze, 1925, **stat. res.** New generic combinations are proposed for 51 species: *Leionotoxenos arvensidis* (Pierce, 1911), **comb. nov.**; *Leionotoxenos bishoppi* (Pierce, 1909), **comb. nov.**; *Leionotoxenos foraminati* (Pierce, 1911), **comb. nov.**; *Leionotoxenos fundati* (Pierce, 1911), **comb. nov.**; *Leionotoxenos huastecae* (Székessy, 1965), **comb. nov.**; *Leionotoxenos itatiaiae* (Trois, 1984), **comb. nov.**; *Leionotoxenos neomexicanus* (Pierce, 1919), **comb. nov.**; *Leionotoxenos prolificum* (Teson & Remes Lenicov, 1979), **comb. nov.**; *Leionotoxenos robertsoni* (Pierce, 1911), **comb. nov.**; *Leionotoxenos tigridis* (Pierce, 1911), **comb. nov.**; *Leionotoxenos vigili* (Brèthes, 1923), **comb. nov.**; *Eupathocera argentina* (Brèthes, 1923), **comb. nov.**; *Eupathocera auripedis* (Pierce, 1911), **comb. nov.**; *Eupathocera bucki* (Trois, 1984), **comb. nov.**; *Eupathocera duryi* (Pierce, 1909), **comb. nov.**; *Eupathocera erynnidis* (Pierce, 1911), **comb. nov.**; *Eupathocera fasciati* (Pierce, 1909), **comb. nov.**; *Eupathocera fuliginosi* (Brèthes, 1923), **comb. nov.**; *Eupathocera inclusa* (Oliveira & Kogan, 1963), **comb. nov.**; *Eupathocera insularis* (Kifune, 1983), **comb. nov.**; *Eupathocera mendozae* (Brèthes, 1923), **comb. nov.**; *Eupathocera piercei* (Brèthes, 1923), **comb. nov.**; *Eupathocera striati* (Brèthes, 1923), **comb. nov.**; *Eupathocera taschenbergi* (Brèthes, 1923), **comb. nov.**; *Eupathocera westwoodii* (Templeton, 1841), **comb. nov.**; *Macroxenos papuanus* (Székessy, 1956), **comb. nov.**; *Sphecixenos abbotti* (Pierce, 1909), **comb. nov.**; *Sphecixenos astrolabensis* (Székessy, 1956), **comb. nov.**; *Sphecixenos dorae* (Luna de Carvalho, 1956), **comb. nov.**; *Sphecixenos erimae* (Székessy, 1956), **comb. nov.**; *Sphecixenos esakii* (Hirashima & Kifune, 1962), **comb. nov.**; *Sphecixenos gigas* (Pasteels, 1950), **comb. nov.**; *Sphecixenos kurosawai* (Kifune, 1984), **comb. nov.**; *Sphecixenos laetum* (Ogloblin, 1926), **comb. nov.**; *Sphecixenos orientalis* (Kifune, 1985), **comb. nov.**; *Sphecixenos reticulatus* (Luna de Carvalho, 1972), **comb. nov.**; *Sphecixenos simplex* (Székessy, 1956), **comb. nov.**; *Sphecixenos vanderiisti* (Pasteels, 1952), **comb. nov.**; *Tuberoxenos altozambeziensis* (Luna de Carvalho, 1959), **comb. nov.**; *Tuberoxenos sinuatus* (Pasteels, 1956), **comb. nov.**; *Tuberoxenos sphecidarum* (Siebold, 1839), **comb. nov.**; *Tuberoxenos teres* (Pasteels, 1950), **comb. nov.**; *Tuberoxenos tibetanus* (Yang, 1981), **comb. nov.**; *Deltoxenos bequaerti* (Luna de Carvalho, 1956), **comb. nov.**; *Deltoxenos bidentatus* (Pasteels, 1950), **comb. nov.**; *Deltoxenos hirokoae* (Kifune & Yamane, 1992), **comb. nov.**; *Deltoxenos iwatai* (Esaki, 1931), **comb. nov.**; *Deltoxenos lusitanicus* (Luna de Carvalho, 1960), **comb. nov.**; *Deltoxenos minor* (Kifune & Maeta, 1978), **comb. nov.**; *Deltoxenos rueppelli* (Kinzelbach, 1971a), **comb. nov.**; *Xenos ropalidiae* (Kinzelbach, 1975), **comb. nov.** *Xenos minor* Kinzelbach, 1971a, **syn. nov.** is recognized as a junior synonym of *X. vesparum* Rossi, 1793. *Ophthalmochlus duryi* Pierce, 1908, **nomen nudum** and *Eupathocera lugubris* Pierce, 1908, **nomen nudum** are recognized as nomina nuda and therefore unavailable in zoological nomenclature. The species diversity of Xenidae probably remains poorly known: the expected number of species is at least twice as high as the number presently described.

Keywords

Cephalotheca, cephalothorax, generic revision, morphology, Strepsiptera, taxonomy, wasp parasite, wasps, Xenidae

Table of contents

Introduction

Strepsiptera are a highly derived group of insect endoparasites and one of the smallest orders of holometabolous insects, comprising approximately 600 described species

(Pohl and Beutel 2008; Cook 2019). Phylogenetic analyses of molecular data suggest an origin of Strepsiptera in the early Carboniferous (Toussaint et al. 2017; McKenna et al. 2019), even though the oldest fossils are known from Cretaceous Burmese amber (Pohl et al. 2020). The phylogenetic position of Strepsiptera was one of the most intractable enigmas in insect systematics ('the Strepsiptera problem', Kristensen 1981). Finally, a sister-group relationship with Coleoptera was convincingly confirmed by transcriptomic and genomic analyses (Boussau et al. 2014; Misof et al. 2014), and has been also supported by morphological data (Beutel et al. 2019).

Strepsipterans are obligate entomophagous parasites of species of seven insect orders (Zygentoma, Blattodea, Mantodea, Orthoptera, Hemiptera, Hymenoptera, and Diptera). Their morphology is strongly modified in all life stages and both sexes, which is clearly correlated with their highly specialized life cycle and endoparasitic habits. Strepsiptera undergo a dramatic hypermetamorphosis of body structures during development. Adult males and females are characterized by extreme sexual dimorphism (Pohl and Beutel 2008; Kathirithamby 2009). Conspicuous features of males are mesothoracic halteres, fan-shaped hind wings, specialized compound eyes (Buschbeck et al. 2003) with cornea lenses separated by chitinous bridges densely covered with microtrichia, and antler-shaped flabellate antennae (Ulrich 1930; Pohl and Beutel 2005). Adult males always leave the host and have an excellent flying capacity. In their very short life span of only few hours they must find a female and mate (Pix et al. 1993; Beani et al. 2005; Straka et al. 2011). Adult females are wingless, neotenic, and either free living (Mengenillidae and probably Bahiaxenidae) or permanently endoparasitic (remaining Strepsiptera: Stylopidia) (Pohl et al. 2018). They release a potent sex pheromone (Lagoutte et al. 2013; Zhai et al. 2016) to attract males for mating. Females produce numerous first-instar larvae viviparously. The miniaturized primary larvae, with an average length of ca. 230 μm (Pohl 2002), have three pairs of walking legs, an abdominal jumping device (with the exception of Stylopidae), and are very agile. They are well equipped with light sense organs and penetrate the body wall of the host using their mandibles (Pohl 2002; Pohl and Beutel 2008).

Xenidae and its sister taxon Stylopidae are groups with the highest degree of specialization in Strepsiptera. They belong to Stylopidia, a clade containing more than 97% of species of the order (Pohl and Beutel 2008). In contrast to Mengenillidae, which are restricted to Zygentoma as hosts, species of Stylopidia parasitize only pterygote insects. The dramatic change in life history linked with endoparasitic females caused far-reaching transformations of morphological characters (Pohl and Beutel 2008). Adult females of Stylopidia form a functional unit with the exuvia in contrast to the free-living wingless females of the family Mengenillidae (and probably Bahiaxenidae) (Kinzelbach 1971b, Pohl and Beutel 2005). The permanently endoparasitic females of Stylopidia are legless and extremely simplified morphologically. The anterior body regions form a compact sclerotized cephalothorax as a secondary tagma extruded from the host abdomen. The sack-shaped unsclerotized and unpigmented posterior body remains inside the host (Kinzelbach 1971b; Kathirithamby 1989).

The female cephalothorax in Xenidae and Stylopidae and all other groups of Stylopidia is in fact a product of fusion comprising the head, the thorax, and the anterior part of abdominal segment I (Löwe et al. 2016; Richter et al. 2017). This fusion of primary tagmata and segments increases the mechanical stability of the body part extruded from the host (Pohl and Beutel 2008). Likewise the flattening of the cephalothorax is interpreted as an adaptation to mechanical strain caused by the cuticle of the host's abdominal segments (Kinzelbach 1971b). The distinct constriction in the middle region of abdominal segment I in Xenidae and Stylopidae marks the penetration point of the host's body wall where the parasite is in direct contact with host intersegmantal membrane. It probably prevents the extruded anterior body part from slipping back into the body lumen of the host (Lauterbach 1954; Löwe et al. 2016; Richter et al. 2017).

The female cephalothoracic capsule includes the exuviae of the secondary and tertiary larval stages, forming a functional unit (puparium) with the female integument below these layers (Richter et al. 2017). The cephalothoracic part of the exuvia of secondary larvae is several times thicker than that of the tertiary stage. It is sclerotized and forms the main protective layer of the exposed part of the body (Richter et al. 2017). Many structures of cephalic and thoracic origin are distinctly or completely reduced, including the compound eyes, antennae, mouthparts, and legs, obviously correlated with endoparasitism (Kinzelbach 1971b; Pohl and Beutel 2005; Löwe et al. 2016; Richter et al. 2017). The spiracles on abdominal segment I are the only functional pair preserved in the females of Stylopidia. The absence of spiracles on segments II–VIII is very likely correlated with permanent endoparasitism (Pohl and Beutel 2005). Linked with the reduction of the primary female genital apparatus (e.g., ovaries and oviducts), novel structures involved in reproduction have evolved, such as a birth opening on the ventral side of the cephalothorax between the cephalic and prosternal regions. The birth opening is connected with birth organs by the brood canal. There, the copulation takes place and numerous first instar larvae are released (Kinzelbach 1971b, Kathirithamby 1989; Peinert et al. 2016).

The male puparium is similar to that of the female in some aspects, also involving the exuvia of the secondary larva, and also possessing a strongly sclerotized exposed anterior part and a large, distinctly less pigmented posterior region (Kinzelbach 1971b, Pohl and Beutel 2008). Ecdysial sutures are absent in the male puparium of Strepsiptera including the exuvia of the secondary larva. The anterior part of the puparium, the cephalotheca, is opened when the adult male leaves the host abdomen after finishing the development (Pohl and Beutel 2005). It is homologous to the head capsule of the secondary larva in the female cephalothorax. The cephalotheca is separated from the posterior part of the puparium by a circular furrow, a zone of weakness of the cuticle of the puparium. Kathirithamby (1990) described this structure as a preformed ecdysial line of weakness. To emerge, males of some genera (*Xenos*, *Stylops*) use their mandibles to open the cephalotheca, first piercing through it, and then cutting along a furrow in a scissor-like fashion (Grabert 1953; Kinzelbach 1971b, Hrabar et al. 2014). Once the cephalotheca is cut free, the male pushes it open with his head (Hrabar et al. 2014).

Xenidae originated relatively late, approximately 50–60 million years ago (McMahon et al. 2011). They are parasites of wasps from four families, viz. Hymenoptera: Aculeata: Crabronidae, Bembicidae, Sphecidae, and Vespidae (Benda et al. 2021). Xenidae are mainly distinguished from the closely related Stylopidae by the exclusive use of wasps as hosts (in contrast to bee hosts in Stylopidae) and unique characters of first instar larvae. The latter are adaptations to the smooth body surface of the hosts and enhance the attachment capacity. This includes enlarged and rounded adhesive tarsal pads and filamentous cuticular outgrowths of the labium which strongly increase the wettability (Pohl and Beutel 2004, 2008).

This group appeared in the literature as a subfamily "Xenides" inside the family Stylopidae in Saunders (1872) who made the first attempt to divide strepsipterans into taxonomic groupings and separating "Xenides" from "Pseudoxenides" (Cook 2019). Pierce (1908) was the first to use the name Xenidae as a family designation within the Strepsiptera. The taxonomic rank was changed by Kinzelbach (1971b) who treated Xeninae, Paraxeninae and Stylopinae as subfamilies of Stylopidae in a broader sense (Cook 2019). Pohl (2002) re-established Xenidae based on a cladistic analysis of morphological characters of the first instar larvae. He placed Xenidae as sister group of Stylopidae + Myrmecolacidae, rendering the Stylopidae in their former concept paraphyletic. Pohl and Beutel (2005), analyzing morphological characters of males, females and first instars, established Xenidae and Stylopidae as sister taxa, which was later supported by the molecular phylogeny of McMahon et al. (2011).

The first generic classification of Xenidae was provided by Pierce (1908, 1909, 1911) who described several genera based on a concept that each genus of Xenidae is specialized on one host genus of wasps. This concept was later rejected by Bohart (1941). A more recent classification of Xenidae has proposed four genera, each specialized on one or several families or subfamilies of hosts (Kinzelbach 1971b, Cook 2019). *Paragioxenos* Ogloblin is an enigmatic genus specialized on pollen wasps (Masarinae) with an endemic distribution in Australia. *Paraxenos* Saunders is distributed worldwide and specialized on wasps of the families Crabronidae, Sphecidae and Bembicidae. *Pseudoxenos* Saunders is also cosmopolitan and specialized on solitary potter wasps (Eumeninae). *Xenos* Rossi, which occurs on all continents except for Australia and Antarctica, parasitises social wasps of the subfamilies Polistinae and Vespinae. In clear contrast to this taxonomic concept, Benda et al. (2019) found little or no evidence for cophylogenetic links between strepsipteran parasites and hymenopteran host lineages, and refuted the monophyly of three of the traditional genera. These results were confirmed by a recent analysis with a denser taxon sampling, and it was suggested to re-evaluate the status of each genus in a more detailed taxonomic revision of the family, also based on morphology (Benda et al. 2021). Consequently, the main aim of the present study is a critical evaluation of the relationships and classification of the genera of Xenidae. Using various microscopic methods, we explore the morphology of the female cephalothorax and

male cephalotheca. We compare our findings with results of previous molecular phylogenetic studies. Additionally, we provide a preliminary checklist of all described species of Xenidae. We also summarize host and distributional data for each described species. We understand this study as a first step towards a modern taxonomy of Xenidae. This should be crucial for a better understanding and easier investigation of these remarkable parasites in the future.

Materials and methods

Material

A total of 234 females and male puparia of Xenidae were obtained from hosts of the families Vespidae, Crabronidae, Bembicidae, and Sphecidae. Voucher names, hosts, and collection localities are listed in Suppl. material 1: Table S1. Material from the following public and private collections were examined:

AMNH American Museum of Natural History, New York, USA;
CNC Canadian National Collection of Insects, Arachnids, and Nematodes, Ottawa, Ontario, Canada;
CUNHM Chulalongkorn University Natural History Museum, Bangkok, Thailand;
DBPC Daniel Benda personal collection, Prague, Czech Republic;
JSPC Jakub Straka personal collection, Prague, Czech Republic;
KUNHM Natural History Museum, Division of Entomology, University of Kansas, Lawrence, Kansas, USA;
NMPC National Museum, Prague, Czech Republic;
OLML Oberösterreichisches Landesmuseum, Linz, Austria;
YNPC Yuta Nakase personal collection, Matsumoto, Japan.

Fixation and preparation

All host individuals were first relaxed in water vapor and then immediately dissected. The endoparasitic females and males were removed from the host body. Females and male puparia used for morphological study were cleared using a mixture of lysis buffer ATL and proteinase K (Qiagen) heated to 56 °C. The lysis procedure took several hours or overnight. Cleared specimens were cleaned in distilled water several times and then stored in vials with 96% ethanol. Complete female cephalothoraces and male puparia were air-dried using a micro-pad inserted into the cephalothorax to prevent the cuticle from collapsing during the process. The female body was usually extracted from the cephalothorax before drying. After this step and the removal of the micro-pad, the dried specimens were glued onto card mounting points, which were pinned.

Measurements

The width and length of the female cephalothorax, the female head capsule and the male cephalotheca were measured using a Leica S9D Stereomicroscope with a calibrated ocular micrometer. The cephalothorax length was measured from the apex of the clypeal lobe to the constriction of abdominal segment I; the cephalothorax width is the maximum distance between its lateral margins.

Photomicrography

The general habitus of stylopized host specimens and the host abdomen with protruding strepsipterans were documented. Multifocus images were taken using Canon EOS 550D or 70D cameras equipped with EF 50 mm and MP-E 65 mm macro lenses. Lateral lights and a diffuser were used.

For the documentation of the original coloration of the female larval cephalothorax and the male cephalotheca, air-dried specimens glued to the card mounting points were used. They were photographed with a Canon EOS 7D digital SLR equipped with a Canon MP-E 65 mm macro lens (Canon, Krefeld, Germany) fitted with a StackShot macro rail (Cognisys, Traverse City, MI, USA). Each specimen was illuminated with two flashlights (Yongnuo Photographic Equipment, Shenzhen, China) fitted to a transparent cylinder for even and soft light. For the documentation of tiny structures on the head capsule, we used a Canon EOS 70D camera attached to an Olympus BX40 Microscope. The microscope was equipped with lateral lights and a diffuser. Zerene Stacker (Zerene Systems LLC, Richland, USA) was used to process stacks of images with different focus.

Scanning electron microscopy (SEM)

Dried female cephalothoraces glued to card points were mounted on a rotatable specimen holder (Pohl 2010). Each specimen was sputter coated with gold with an Emitech K 500 (Sample preparation division, Quorum Technologies Ltd., Ashford, England). The SEM micrographs were taken with an ESEM XL30 (Philips, Amsterdam, Netherlands) equipped with Scandium FIVE (Olympus, Münster, Germany).

Image processing

All images were processed and arranged into plates with Adobe Photoshop CS5 (Adobe System Incorporated, San Jose, USA) software. CorelDraw X8 (CorelDraw Corporation, Ottawa, ON, Canada) was used for the lettering of the plates.

Terminology and description style

The terminology used for the female cephalothorax and male cephalotheca is based on Richter et al. (2017), Löwe et al. (2016), and Kinzelbach (1971b). Appropriate terminology was developed for morphological characters without specific names. In the

diagnoses emphasis was placed on a distinction between apomorphic and plesiomorphic features within Xenidae in regard to the sister family Stylopidae. Cephalothoraces and cephalothecae were displayed in morphological orientation in figures although their functional orientation in the host body is inverted. Genera are listed in the order based on the phylogeny in Benda et al. (2021), species alphabetically.

Results

General description of the female cephalothorax of Xenidae

Cephalothorax size. Generally quite variable within species and depending on the host identity. Species with the smallest cephalothorax belong to the genera *Brasixenos* (smallest specimen: 0.76 mm long, 0.72 mm broad) and *Macroxenos* (0.84 mm long, 0.64 mm broad). The species with the maximum length are *Deltoxenos* sp. (2.83 mm long, 2.43 mm broad) and *Xenos moutoni* Buysson (2.7 mm long, 2.43 mm broad), while the broadest cephalothorax was recorded for *Paraxenos hungaricus* (Székessy) (1.87 mm long, 2.57 mm broad).

Cephalothorax shape. Compact and ovoid, tapering anteriorly, usually longer than broad, but distinctly broader than long in several species (e.g., *Paraxenos hungaricus*); in cross-section it appears more or less flattened, elliptic, bent dorsad along its lateral margins (Richter et al. 2017).

Cephalothorax coloration. Variable, often pale, sometimes dark, or with multiple brown shades forming distinct patterns.

Head capsule. Prognathous, dorsoventrally more or less strongly flattened. Head length including lateral extensions of head capsule making up ~ ¼ ~ ½ the length of entire cephalothorax (Figs 1A, 2A). Posterior part almost completely fused to prothorax but still distinctly separated from it by birth opening (opening of the brood canal) medially, and by a suture laterally (Fig. 1A); completely separated by birth opening over the entire width of the ventral side only in *Paragioxenos* Ogloblin (Fig. 8A). Compound eyes and cephalic sutures missing. Labrum not present as a defined cephalic element. Clypeus poorly separated from frontal region, epistomal suture (frontoclypeal transverse strengthening ridge) missing, both cephalic areas thus fused; clypeal area tentatively marked by several sensilla; central part of clypeal area often forming a clypeal lobe; if present, then clypeal lobe well visible on head apex and protruding beyond the anterior edge of head capsule (Figs 1B, 2A, 2C); sensilla evenly dispersed over entire clypeal area or more concentrated on clypeal lobe (Fig. 3); lateral clypeal areas forming a mandibular capsule, also beset with sensilla (Fig. 3B). Frontal region not present as a delimited cephalic element, with variable microsculpture: smooth and shiny or rough, often forming reticulate structures or papillae (Fig. 25F). Border between head and thorax obsolete dorsally, but in some species with an interrupted suture and strongly pigmented (Figs 1B, 4A).

Supra-antennal sensillary field. Paired rounded areas, probably of frontal origin, present dorsomedially, with variable microsculpture and many sensilla, close to vestigial antennae (Fig. 4B).

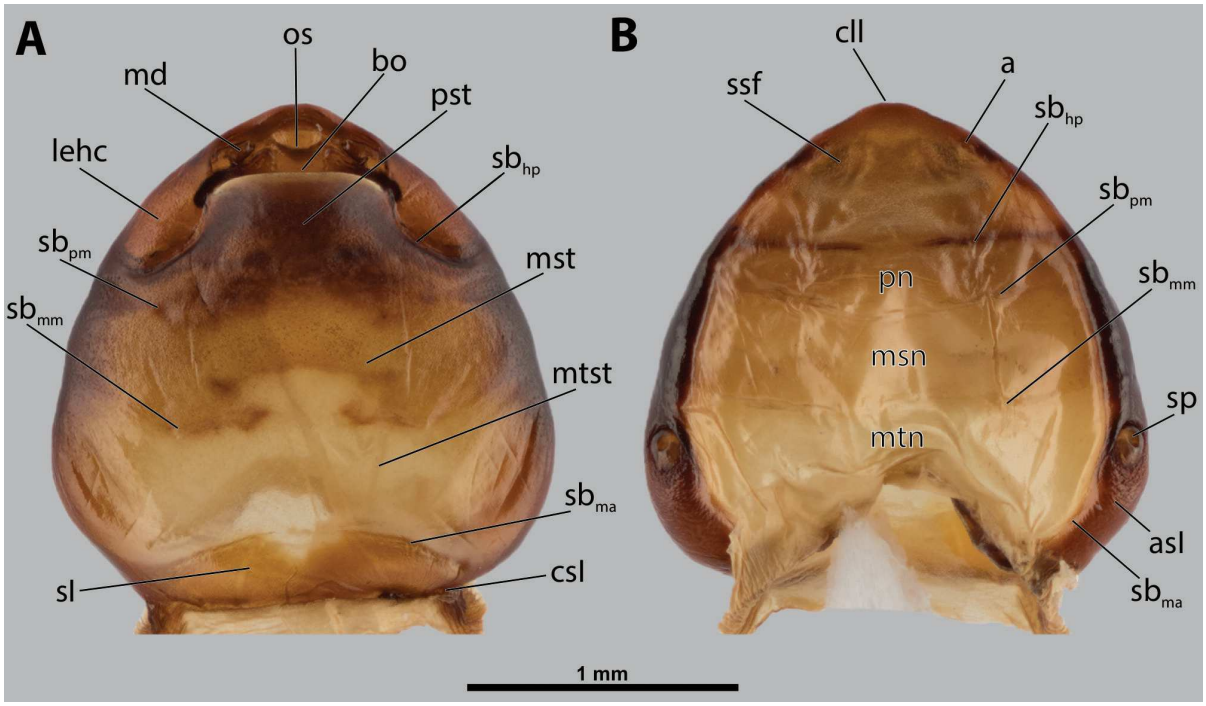

Figure 1. *Deltoxenos* cf. *bequaerti*, female, cephalothorax, photomicrographs **A** ventral side **B** dorsal side. Abbreviations: a – vestigial antenna, asI – abdominal segment I, bo – birth opening, cll – clypeal lobe, csI – constriction of abdominal segment I, lehc – lateral extension of head capsule, md – mandible, msn – mesonotum, mst – mesosternum, mtn – metanotum, mtst – metasternum, os – mouth opening, pn – pronotum, pst – prosternum (prosternal extension), sI – abdominal sternite I, sbhp – segmental border between head and prothorax, sbma – segmental border between metathorax and abdomen, sbmm – segmental border between mesothorax and metathorax, sbpm – segmental border between prothorax and mesothorax, sp – spiracle, ssf – supra-antennal sensillary field.

Antenna. Vestigial, located dorsally on the head, close to the lateral margin, at the same level as maxillary vestige, either preserved as a groove, or as a cavity, or as a poorly defined area with several small, rounded plates and sensilla or setae (Fig. 4B); in some cases, antennal cavity also bearing plates and sensilla. Complete and distinct antennal torulus always missing, but incomplete vestigial torulus visible in some species. Periantennal area present close to vestigial antennae, lacking sensilla and defining the mesal border between antenna and supra-antennal sensillar field.

Labrum. Fused with head capsule, but still defined as oval area anterior to mouth opening; divided into dorsal labral field, likely corresponding with dorsal labral surface, and ventral labral field (Figs 2C, 3A), likely homologous to anterior epipharynx; dorsal field usually bearing several to many setae inserted in cavities, presumably of labral origin, varying in number from 10 to ~ 41; these setae cannot be clearly recognized in some cases. Lower margin of ventral field delimited by mouth opening; ventral field semicircular or oval shaped.

Mandible. Anteromedially directed, usually with hook-shaped apex directed anteriad, anteromesad, or anteroventrad; the angle varies between 20° and 75°. Anteriorly, mandibles partially enclosed by mandibular capsule, probably of clypeal origin (Fig. 3B). Anterior mandibular part bearing serrate tooth, directed distally and more or

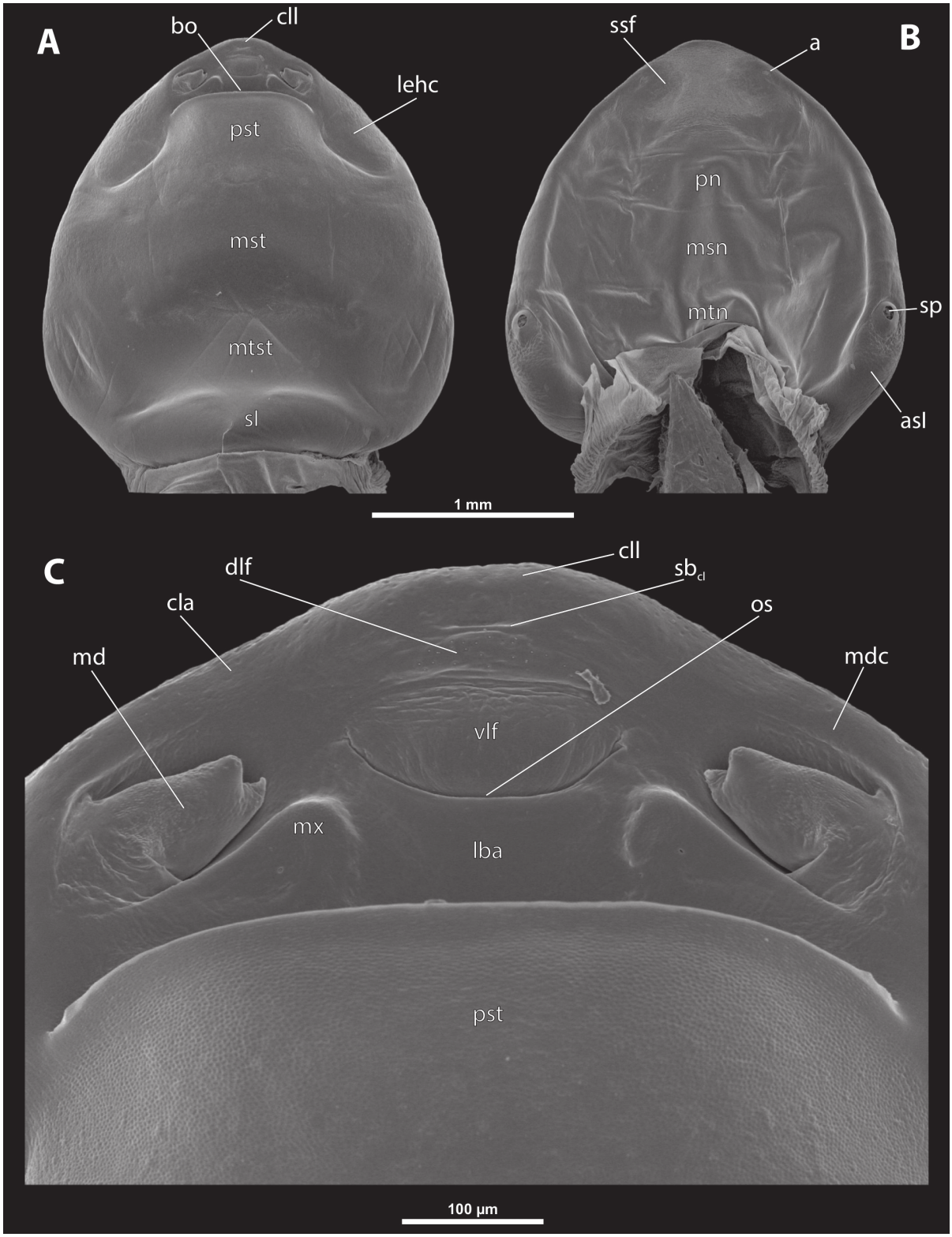

Figure 2. *Deltoxenos* cf. *bequaerti*, female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** mouthparts and base of prosternum, ventral side. Abbreviations: a – vestigial antenna, asI – abdominal segment I, bo – birth opening, cla – clypeal area, cll – clypeal lobe, dlf – dorsal field of labral area, lba – labial area, lehc – lateral extension of head capsule, md – mandible, mdc – mandibular capsule (clypeal origin), msn – mesonotum, mst – mesosternum, mtn – metanotum, mtst – metasternum, mx – vestige of maxilla (maxilla), os – mouth opening, pn – pronotum, pst – prosternum (prosternal extension), sI – abdominal sternite I, sbcl – segmental border between clypeus and labrum, ssf – supra-antennal sensillary field, sp – spiracle, vlf – ventral field of labral area.

less covered with small spines; protuberant mandibular bulge sometimes present laterally, usually bearing several sensilla; cuticle of mandible variously sculptured, reticulate, covered by longitudinal grooves, or completely smooth. Laterally, mandible connected with head capsule by sclerotized mandibular membrane.

Maxilla. Highly variable, inserted posteromesad of mandibles; well-developed, reduced, or completely fused with labial area, placed ventromedially between mandibles (Fig. 2C); connected medially in some taxa. Maxillary base placed below mandible close to its articulatory area (Fig. 3B); anterior maxillary region reaching beyond mandibular tip in some species. Maxillary endite lobes and well-defined maxillary palp missing; variously placed concavity likely representing a rudiment of the latter. Maxillary surface smooth or sculptured, for instance reticulate. Maxillary bases usually continuous with submaxillary groove, which is not part of maxilla; adjacent to border between head and prothorax. In species with a distinctly produced submaxillary groove, this area is visible between the submaxillary groove and the ventrolateral cephalo-prothoracic suture (Figs 18A, 34A, 37A).

Labium and hypopharynx. Labium not recognizable as a separate structure, probably fused to anteroventral cephalic capsule; the well-delimited area between maxillae is probably of labial origin, anteriorly delimited by the mouth opening and posteriorly by the birth opening (Fig. 2C). Labial area raised anteriorly in some taxa as a small spine projecting beyond the mouth opening, or laterally as paired labial corners (Fig. 3B). Hypopharynx absent or rarely present as inconspicuous protuberance.

Mouth opening. Present as narrow transverse cleft between mandibles, maxillae, and labium (Fig. 2C); semicircular to shallowly U-shaped, sometimes arcuate or biarcuate; usually sclerotized marginally, mainly on the labial side.

Salivarium. Not developed.

Birth opening. Present as narrow cleft on ventral side of cephalothorax, indicating border between head and prothorax (Figs 1A, 2A); usually continuous with a suture posterolaterally, but extending over the entire width of the ventral side in *Paragioxenos* (Fig. 8A). In virgin females, the birth opening is closed by larval cuticle (brood canal membrane, Fig. 47C), which is very thin there, translucent, and nearly invisible under an optical microscope (Fig. 45C); remnants of ruptured membrane visible in mated females (Fig. 14C).

Thorax and abdominal segment I. Three thoracic segments completely fused with each other and also with abdominal segment I. Cephalothorax broadest at level of abdominal spiracles I. Thoracic segmental borders and thoraco-abdominal border distinct to different degrees, well visible, in distinct to almost completely invisible; segmental borders less distinct dorsally; in many cases only some of them visible (differentiation of thoracic segments varies even within species, not only between species and genera). Thoracic segments usually separated by mesal furrows combined with pigmented stripes or spots (Fig. 1A, 1B); pigmented areas sometimes without furrows and with changed cuticular sculpture. Cuticle on ventral side of thoracic segments displaying reticulate pattern, with scattered inconspicuous or more distinct pigmented papillae usually forming specific pattern (Figs 10A, 28C); cuticular surface on thorax dorsally smooth or slightly wrinkled. Border between metathorax

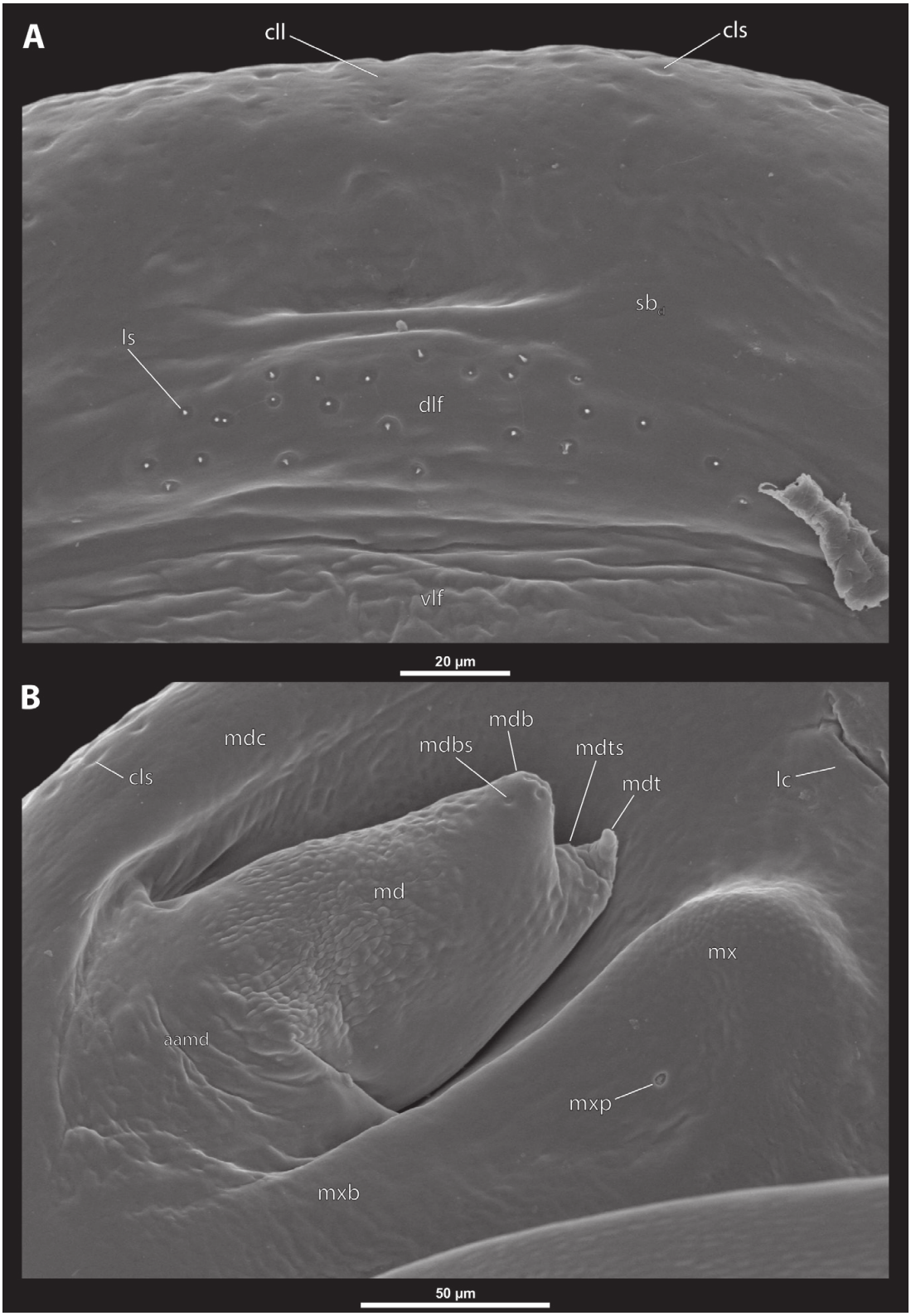

Figure 3. *Deltoxenos* cf. *bequaerti*, female, cephalothorax, SEM micrographs **A** clypeus and labrum, detail, ventral side **B** right mandible and maxilla, ventral side. Abbreviations: aamd – sclerotized mandibular membrane, cll – clypeal lobe, cls – clypeal sensillum, dlf – dorsal field of labral area, lc – labial corner, ls – labral seta in cavity (spine-shaped sensilla), md – mandible, mdb – mandibular bulge, mdbs – sensillum of mandibular bulge, mdc – mandibular capsule (clypeal origin), mdt – mandibular tooth, mdts – spine of mandibular tooth, mx – vestige of maxilla (maxilla), mxb – maxillary base (at mandible base), mxp – vestige of maxillary palp, sbcl – segmental border between clypeus and labrum, vlf – ventral field of labral area.

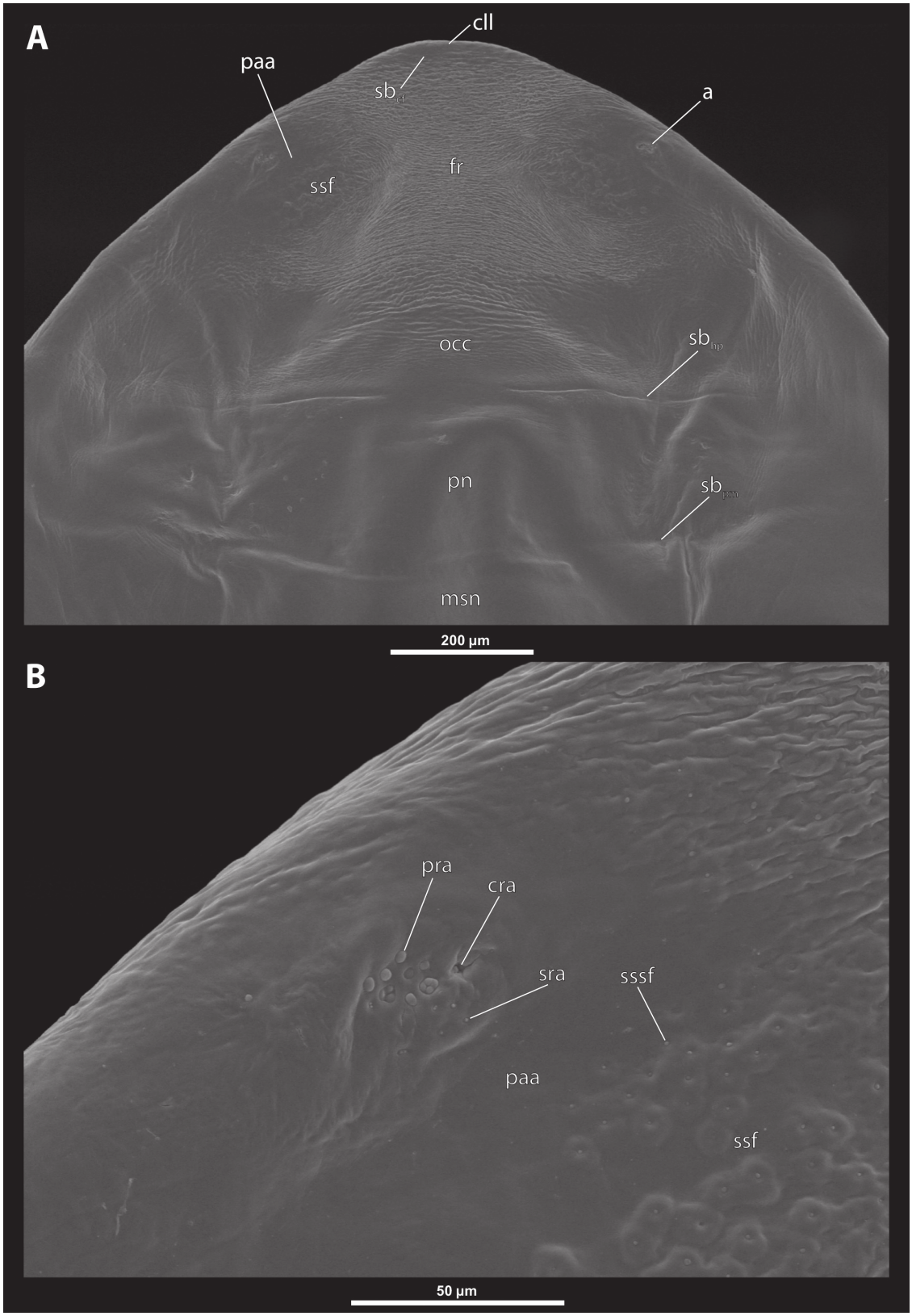

Figure 4. *Deltoxenos* cf. *bequaerti*, female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, dorsal side **B** vestigial antenna, dorsal side. Abbreviations: a – vestigial antenna, cll – clypeal lobe, cra – cavity of vestigial antenna, fr – frontal region, msn – mesonotum, occ – occipital area, paa – periantennal area, pn – pronotum, pra – plate of vestigial antenna, sbhp – segmental border between head and prothorax, sbpm – segmental border between prothorax and mesothorax, sra – sensillum of vestigial antenna, ssf – supra-antennal sensillary field, sssf – sensillum of supra-antennal sensillary field.

and abdomen usually indicated by an edge, color change, or change of cuticular microsculpture (Fig. 1A). Prothorax with prosternal extension reaching towards head capsule (Fig. 1A). Cephalothoracic tergites, pleurites and sternites fused. Legs missing. Transverse medial constriction of abdominal segment I in direct contact with host intersegmental membrane, forming posterior border of cephalothorax (Fig. 1A); posterior part of abdominal segment I and remaining abdominal segments located in body cavity of host. Spiracles of abdominal segment I functional; setae, and cuticular spines present on this segment laterally, below spiracles (Figs 17E, 21E); this area is distinctly wrinkled in some species (Fig. 13E) and sometimes extruding as spiracular corner (Fig. 28D).

Spiracles. Paired, annular or semicircular, located laterally or dorsolaterally on posterior most part of cephalothorax; surrounding cuticle forming distinct ring-shaped microstructure but only slightly elevated (Figs 1B, 2B). Spiracle orientation variable, but in most species anterolateral.

General description of the cephalotheca of the male puparium in Xenidae

Cephalotheca shape. Rounded to elliptic in frontal view; always broader than long, distinctly flattened or almost circular in cross section; rounded or pointed apically in lateral view.

Cephalothecal capsule. Compound eyes present (Figs 5A, 6A); individual ommatidia usually visible as dark sclerotized impressions on pale background of ocular area except for some *Xenos* spp. with ocular area completely dark. Clypeus (**cl**) well developed, flattened, and elongated, with epistomal suture separating it from frontal region; shape variable, more or less curved or nearly straight, usually medially protruding from cephalotheca as clypeal lobe. Clypeal sensilla (Fig. 6B) distributed over entire surface, evenly dispersed, or mainly concentrated on clypeal lobe medially. Lateral clypeal portions forming mandibular capsule. Frontal region well-delimited against clypeus, usually with frontal impression or furrows. Genal regions visible but not clearly delimited. Occipital bulge more or less distinctly developed or absent; usually with coarser microsculpture (Fig. 5).

Supra-antennal sensillary field. Paired kidney-shaped and bulging supra-antennal sensillary fields, probably of frontal origin, located mesad of vestigial antennae; with numerous sensilla; on its mesal side often delimited by a more or less distinct furrow (Figs 15A, D, 19A, D) which also delimits the mesal part of the frontal region connected with the clypeal lobe.

Antenna. Vestigial, inserted between compound eye and supra-antennal sensillary field; rounded and blunt; surrounding area well-defined, equipped with sensilla and delimited by a distinct antennal torulus (Fig. 6B), which is interrupted in some cases. A periantennal area is present close to the vestigial antennae; it lacks sensilla and separates the antenna from supra-antennal sensillary field mesally.

Labrum. Fused with head capsule, but still defined as oval area anterior to mouth opening; divided into dorsal and ventral labral fields (Figs 5A, B, 6B), the

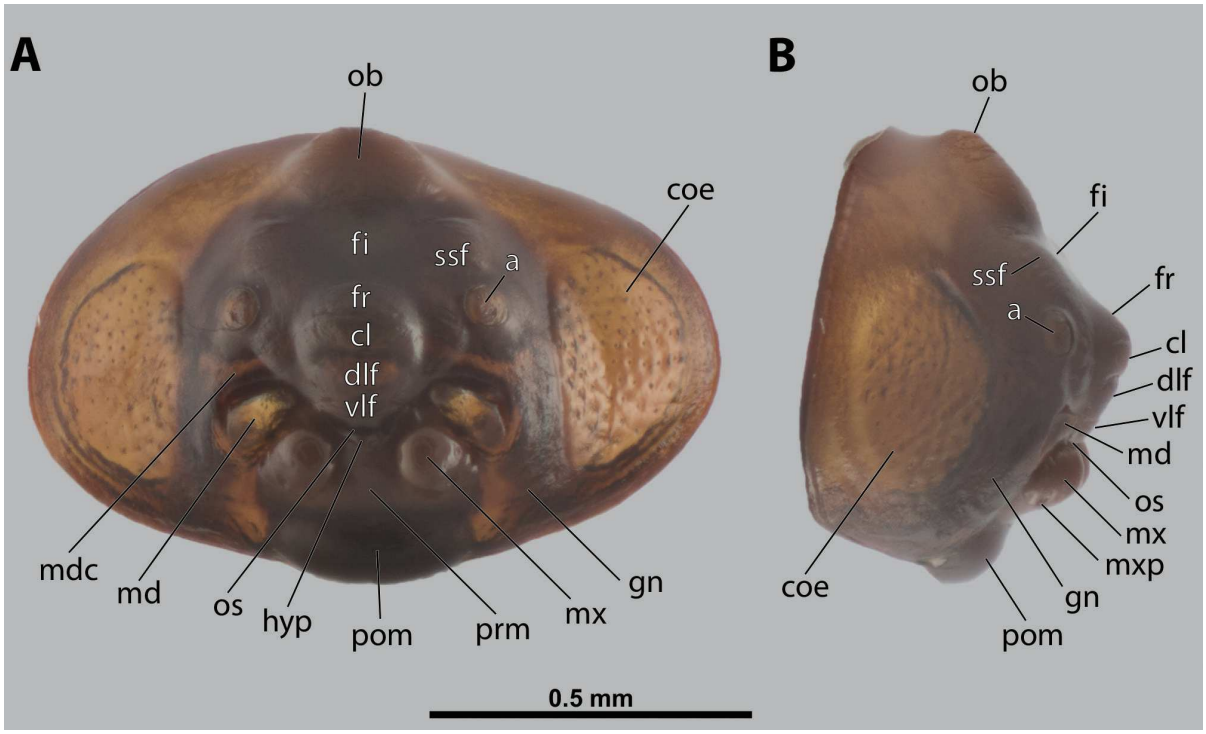

Figure 5. *Deltoxenos* cf. *bequaerti*, male, cephalotheca, photomicrographs **A** frontal view **B** lateral view. Abbreviations: a – vestigial antenna, cl – clypeus, coe – compound eye, dlf – dorsal field of labral area, fi – frontal impression, fr – frontal region, gn – gena, hyp – hypopharynx, md – mandible, mdc – mandibular capsule (clypeal origin), mx – vestige of maxilla (maxilla), ob – occipital bulge, os – mouth opening, pom – postmentum, prm – praementum, ssf – supra-antennal sensillary field, vlf – ventral labral field of area.

former equipped with variable number of setae inserted in cavities. Dorsal field likely homologous with upper labral surface, ventral field with anterior epipharynx.

Mandible. Directed anteromesally, enclosed by mandibular capsule located anterolaterally (Fig. 6B); with small, anteriorly directed serrate tooth anteromesally, bearing dense field of minute spines. A protuberant mandibular bulge present anterolaterally, usually bearing several sensilla.

Maxilla. Inserted posteromesad of mandibles, well-developed as separate structures or completely fused with labial area, which is medially enclosed between the maxillae (Fig. 6B). Vestigial maxillary palp present on maxillary base.

Labium and hypopharynx. Labium distinctly recognizable between and below maxillae, usually clearly subdivided into praementum and postmentum (Figs 5A, 6A). Small median external protuberance (Fig. 6B), possibly homologous with the distal hypopharyngeal region, often present below mouth opening.

Mouth opening. Present as narrow transverse cleft between mandibles and maxillae (Figs 5A, 6B), semicircular to U-shaped, and covered by ventral labral field in some taxa.

Salivarium. Not developed.

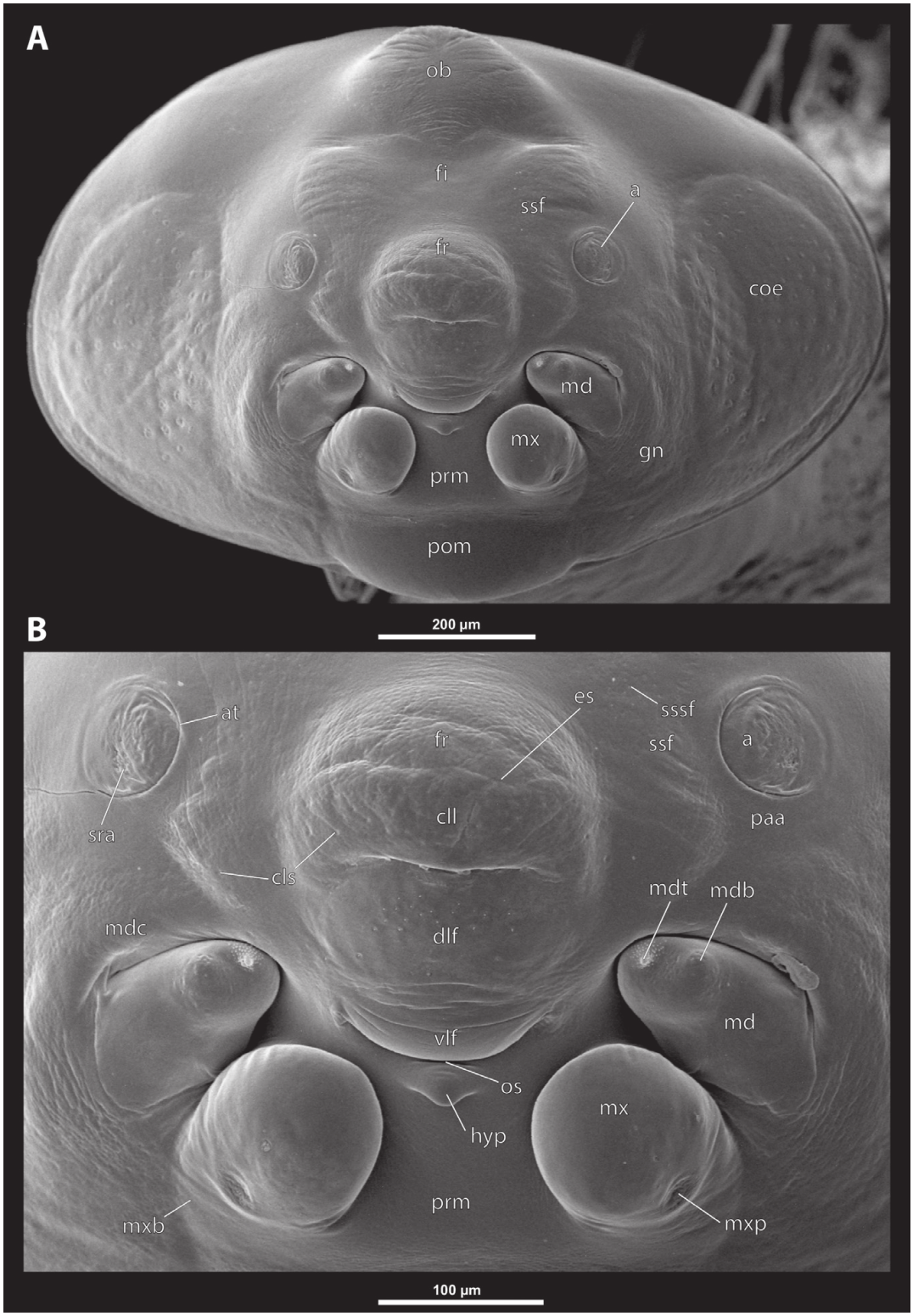

Figure 6. *Deltoxenos* cf. *bequaerti*, male, cephalotheca, SEM micrographs **A** frontal view **B** mouthparts. Abbreviations: a – vestigial antenna, at – antennal torulus (rudiments of antennal torulus), cll – clypeal lobe, cls – clypeal sensillum, coe – compound eye, dlf – dorsal field of labral area, es – epistomal suture, fi – frontal impression, fr – frontal region, gn – gena, hyp – hypopharynxgeal protuberance, md – mandible, mdb – mandibular bulge, mdc – mandibular capsule (clypeal origin), mdt – mandibular tooth, mx – vestige of maxilla (maxilla), mxb – maxillary base (at mandible base), ob – occipital bulge, os – mouth opening, paa – periantennal area, pom – postmentum, prm – praementum, sra – sensillum of vestigial antenna, ssf – supra-antennal sensillary field, sssf – sensillum of supra-antennal sensillary field, vlf – ventral field of labral area.

Review of genera of Xenidae

Paragioxenos Ogloblin, 1923

Paragioxenos Ogloblin, 1923: 46. Type species: *Paragioxenos brachypterus* Ogloblin, 1923, by original designation.

Diagnosis of female cephalothorax. Differing from other Xenidae in following characters. Head and prothorax completely separated by birth opening on ventral side (Fig. 8A). Mandibles distinctly protruding from mandibular capsule; angle of mandibles 75°. Dorsal labral field elliptic, ~ 2× wider than long in midline, distinctly protuberant, straight (Fig. 8A). Conspicuous swelling present on prosternum (Fig. 8A), similar to some *Paraxenos* spp.

Description of female cephalothorax. Shape and coloration. Nearly triangular, slightly wider than long, length 1.68 mm, width 1.82 mm. Anterior cephalic margin very slightly protruding anteriorly. Thorax distinctly widening posteriorly. Coloration comprising multiple brown shades forming distinct pattern, mostly dark (Fig. 7C, D).

Head capsule. Approximately ⅓ as long as entire cephalothorax including lateral cephalic extensions. Coloration mostly brown, including sclerotized labial area and strongly sclerotized mandible; dorsal labral field pale. Clypeal and labral area separated, the former slightly protruding anteriorly, forming inconspicuous clypeal lobe; surface of clypeal area slightly wrinkled; sensilla present. Border between clypeal and frontal regions quite indistinct. Cuticle of frontal region slightly wrinkled. Segmental border between head and prothorax indistinct dorsally; on ventral side completely separated by birth opening (Fig. 8A).

Supra-antennal sensillary field. More or less distinctly delimited by furrow on mesal side (Fig. 8B).

Antenna. Presence or absence of vestige of antennae not verified.

Labrum. Ventral labral field elliptic, not protruding; dorsal field elliptic, ~ 2× wider than long in midline, distinctly protuberant, straight (Fig. 8A). Presence or absence of setae not verified.

Mandible. Anteroventrally directed, distinctly protruding from mandibular capsule, nearly reaching or projecting slightly beyond anterior edge of head (Fig. 8A). Mandibular bulge distinctly raised, with sensilla. Mandibular tooth conspicuous.

Maxilla. Anteriorly directed, distinctly prominent, strongly sclerotized. Bases wide, connected in midline. Apical portion not projecting beyond mandible. Presence or absence of vestige of palp not verified. Submaxillary groove absent.

Labium. Triangular, sclerotized, and flat, located between maxillae, delimited anteriorly by mouth opening and posteriorly by connected maxillae.

Mouth opening. Fissure-shaped, straight medially, curved laterally, with sclerotized margin.

Thorax and abdominal segment I. Two longitudinal ventral furrows present mesally over whole length of thorax, slightly widening posteriorly. Pro-mesothoracic and meso-metathoracic borders indistinct. Border between metathorax and abdomen

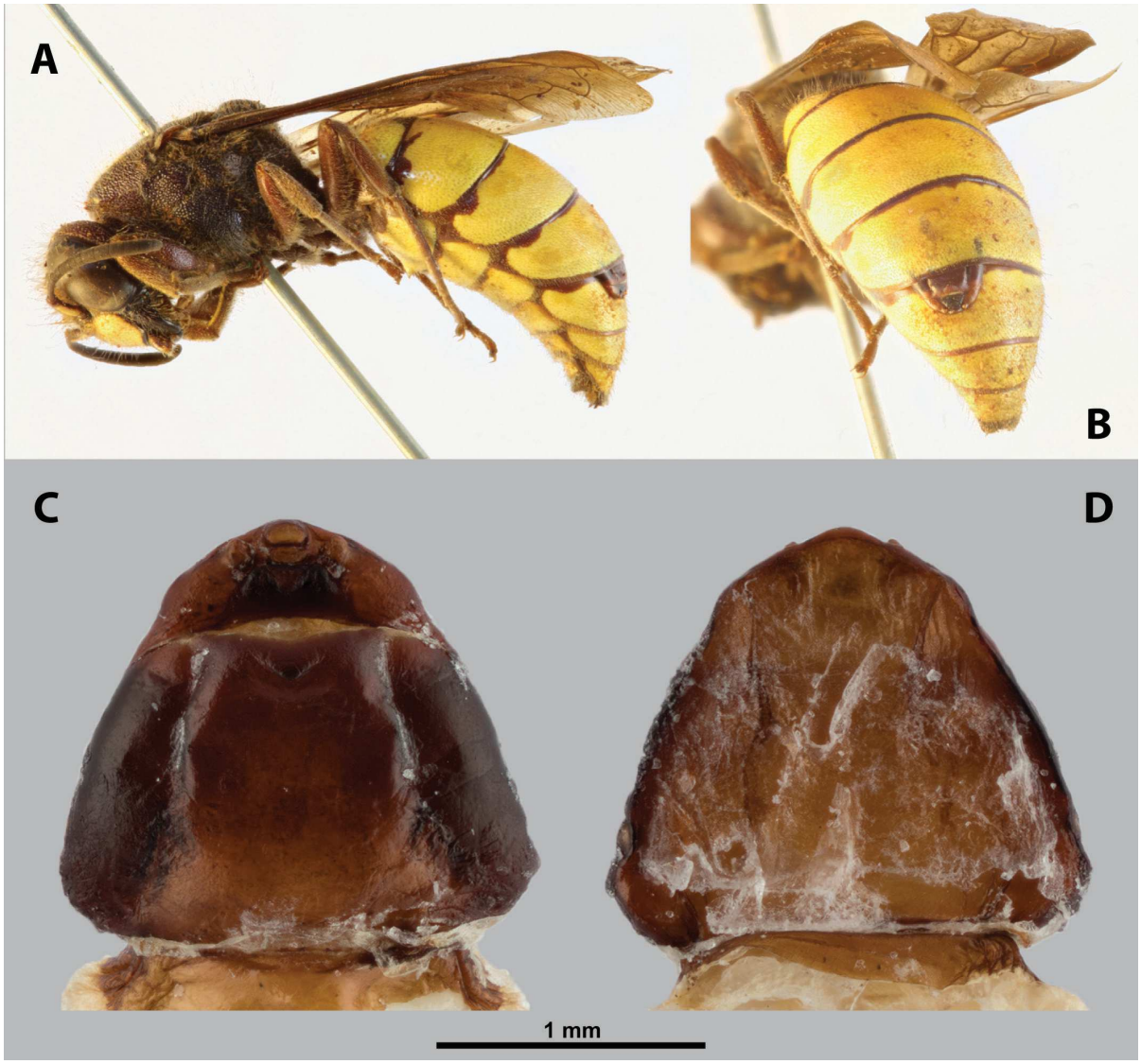

Figure 7. *Paragioxenos brachypterus* Ogloblin, host, female, cephalothorax, photomicrographs **A** *Paragia* cf. *decipiens* Shuckard stylopized by female of *P. brachypterus*, lateral view **B** detail of host abdomen with adult female inside **C** ventral side of cephalothorax **D** dorsal side of cephalothorax.

formed by ridge on dorsal side, indistinct on ventral side. Cuticle of thoracic segments dark laterally, less pigmented mesally between longitudinal furrows. Dorsal surface mostly with uniformly brown coloration except for lateral most region. Prosternum with pointed swelling but lacking extension (Fig. 8A). Setae and cuticular spines on lateral parts of abdominal segment I not examined.

Spiracles. Situated on posterior third of cephalothorax, slightly elevated, with anterolateral orientation.

Diagnosis of male cephalotheca. No male cephalotheca was examined (absent in Ogloblin's type material in NMPC).

Phylogenetic relationships. Unknown.

Diversity and distribution. Monotypic, restricted to Australia.

Host. *Paragia* spp. (Vespidae: Masarinae).

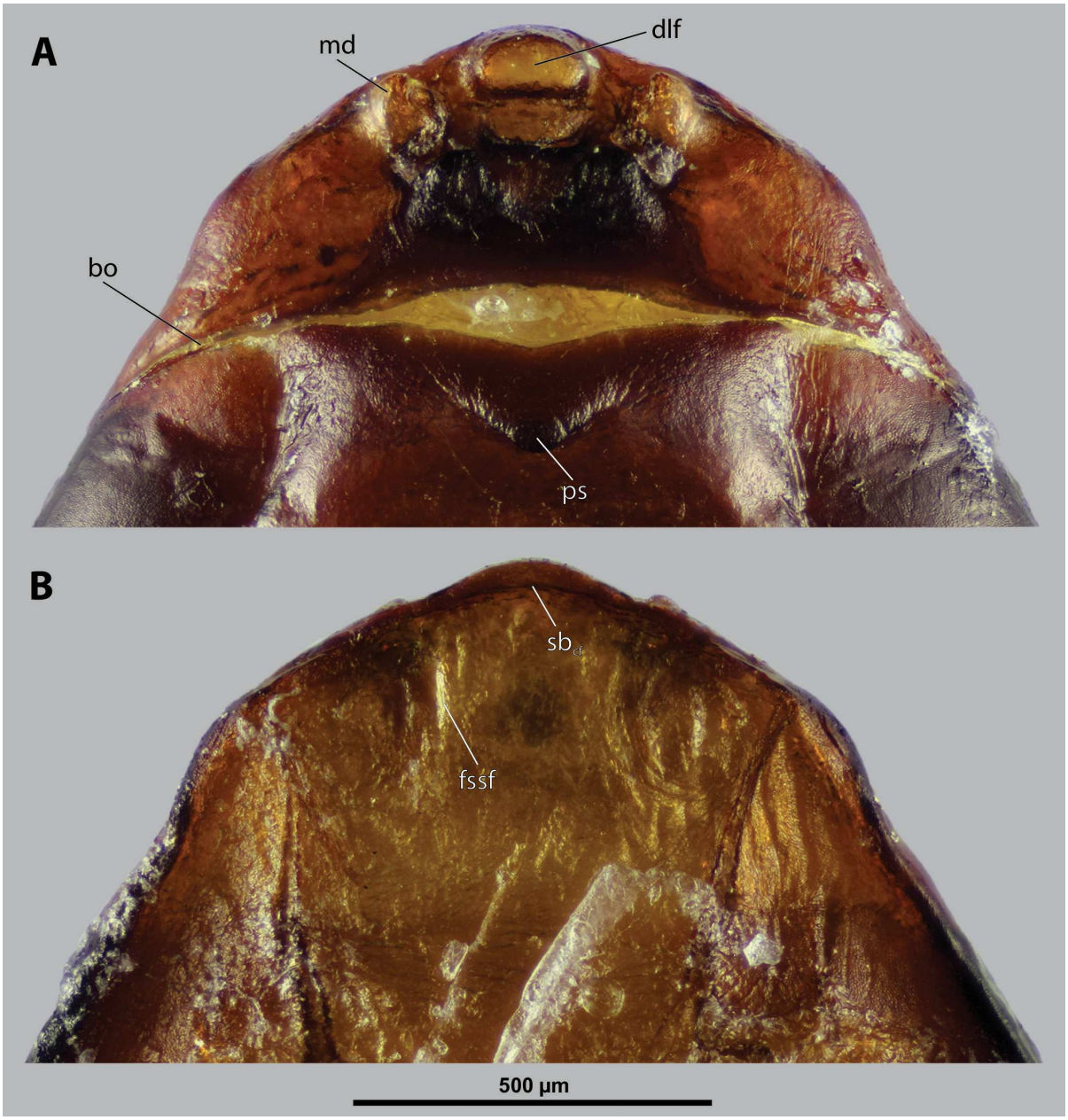

Figure 8. *Paragioxenos brachypterus* Ogloblin, anterior part of female cephalothorax, photomicrographs **A** anterior part of cephalothorax, ventral side **B** Anterior part of cephalothorax, dorsal side. Abbreviations: bo – birth opening, dlf – dorsal field of labral area, fssf – furrow of supra-antennal sensillary field, md – mandible, ps – prosternal swelling, sbcf – segmental border between clypeus and frontal region.

List of species

Paragioxenos brachypterus Ogloblin, 1923

Paragioxenos brachypterus Ogloblin, 1923: 46.

Hosts. *Paragia* cf. *decipiens* Shuckard, 1837 (Ogloblin 1923); *Paragia decipiens* Shuckard, 1837; *Paragia tricolor* Smith, 1850 (Hofeneder 1928).

Distribution. South Australia: Gawler (Ogloblin 1923; Hofeneder 1928).

Nipponoxenos Kifune & Maeta, 1975, stat. res.

Nipponoxenos Kifune & Maeta, 1975: 446 (as a subgenus of *Xenos* Rossi). Type species: *Xenos* (*Nipponoxenos*) *vespularum* Kifune & Maeta, 1975, by original designation.

Diagnosis of female cephalothorax. Differing from most genera in following combination of characters. Mandibles protruding distinctly from mandibular capsule, reaching or slightly projecting beyond cephalic edge (Fig. 10A). Maxilla anteriorly directed, strongly sclerotized. Maxillary bases conspicuously wide, connected in midline along birth opening. Anterior part of maxilla pointed (Fig. 10A). In contrast to *Paragioxenos*, head and prothorax ventrally delimited by birth opening medially and by suture laterally. Cephalothorax mostly pale.

Description of female cephalothorax. Shape and coloration. Cephalothorax distinctly longer than wide, length 2.0 mm, maximum width 1.76 mm. Anterior head margin not protruding. Thorax nearly straight. Meso-metathoracic border slightly constricted (Fig. 9C). Coloration with distinct pattern of different pale brown shades; usually medially pale and slighter darker laterally in ventral and dorsal view.

Head capsule. Almost ⅓ as long as entire cephalothorax including lateral cephalic extensions. Coloration mostly pale brown, but darker on lateral extensions and on distinctly sclerotized maxillae (Fig. 10A). Clypeal area delimited from labral area, slightly protruding anteriorly, forming inconspicuous, slightly pigmented clypeal lobe (Fig. 10A); clypeal sensilla present. Border between clypeal and frontal region distinct. Cuticle of frontal region slightly wrinkled. Segmental border between head and prothorax indistinct dorsally but indicated by coloration; on ventral side separated by birth opening medially and by suture laterally.

Supra-antennal sensillary field. Not delimited by furrow mesally.

Antenna. Presence or absence of antennal vestige not verified.

Labrum. Ventral labral field elliptic, not protruding but slightly convex. Dorsal labral field elliptic, ~ 5× wider than long, slightly arcuate. Presence or absence of labral sensilla not verified.

Mandible. Anteromedially directed at angle of 60°, distinctly protruding from mandibular capsule, reaching or slightly projecting beyond anterior edge of head (Fig. 10A). Bulge not distinctly raised. Sensilla not examined. Mandibular tooth narrow or moderately widened, pointed apically.

Maxilla. Anteriorly directed, pointed, strongly sclerotized. Bases wide, connected medially. Apical region not projecting beyond mandible anteriorly. Presence of palp vestige not verified. Submaxillary groove slightly produced.

Labium. Labial area inserted between maxillae, slightly pigmented medially; anteriorly delimited by mouth opening and posteriorly by connected maxillary bases.

Mouth opening. Mouth opening slightly curved, sclerotized along margin.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders vaguely indicated ventrally by pigmented stripes with specific cuticular surface, but nor recognizable on dorsal side (Fig. 9C, D). Border between metathorax and

abdomen marked by ridge and change of cuticular sculpture and pigmentation. Entire abdominal segment I darker than thorax. Cuticle of thoracic segments on ventral side wrinkled or reticulate, with several small, pigmented papillae on prothorax. Prosternal extension undifferentiated, evenly arched. Dorsal side of thorax mostly smooth. Meso- and metathorax unmodified in shape, transverse. Setae and cuticular spines on lateral region of abdominal segment I not examined.

Spiracles. Situated on posterior ⅓ of cephalothorax, slightly elevated, with anterolateral orientation.

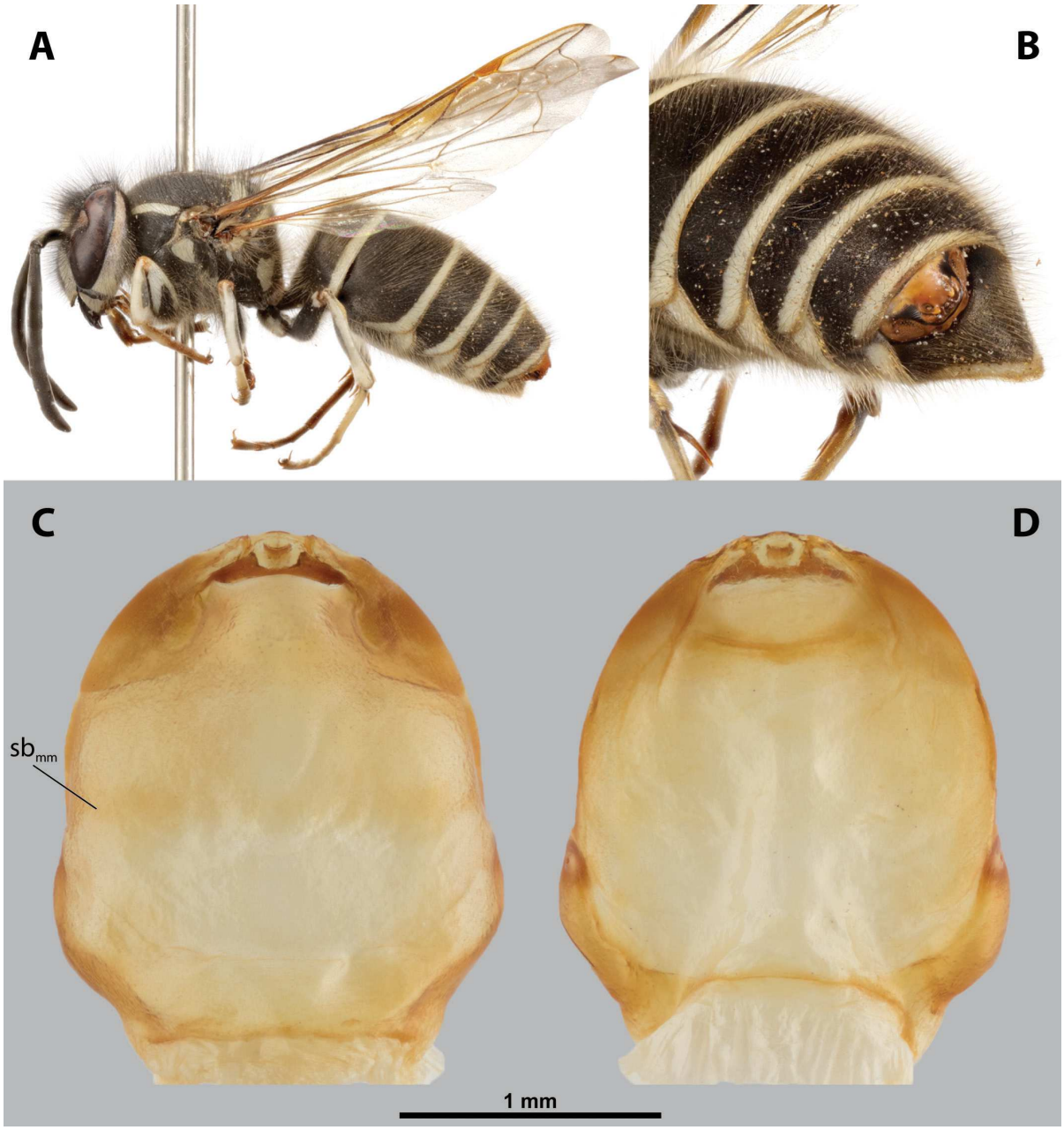

Figure 9. *Nipponoxenos vespularum* Kifune & Maeta, host, male, female, cephalothorax, photomicrographs **A** *Vespula shidai* Ishikawa, Sk. Yamanne & Wagner stylopized by male of *N. vespularum*, lateral view **B** detail of host abdomen with male puparium inside **C** ventral side of female cephalothorax **D** dorsal side of female cephalothorax. Abbreviation: sbmm – segmental border between mesothorax and metathorax.

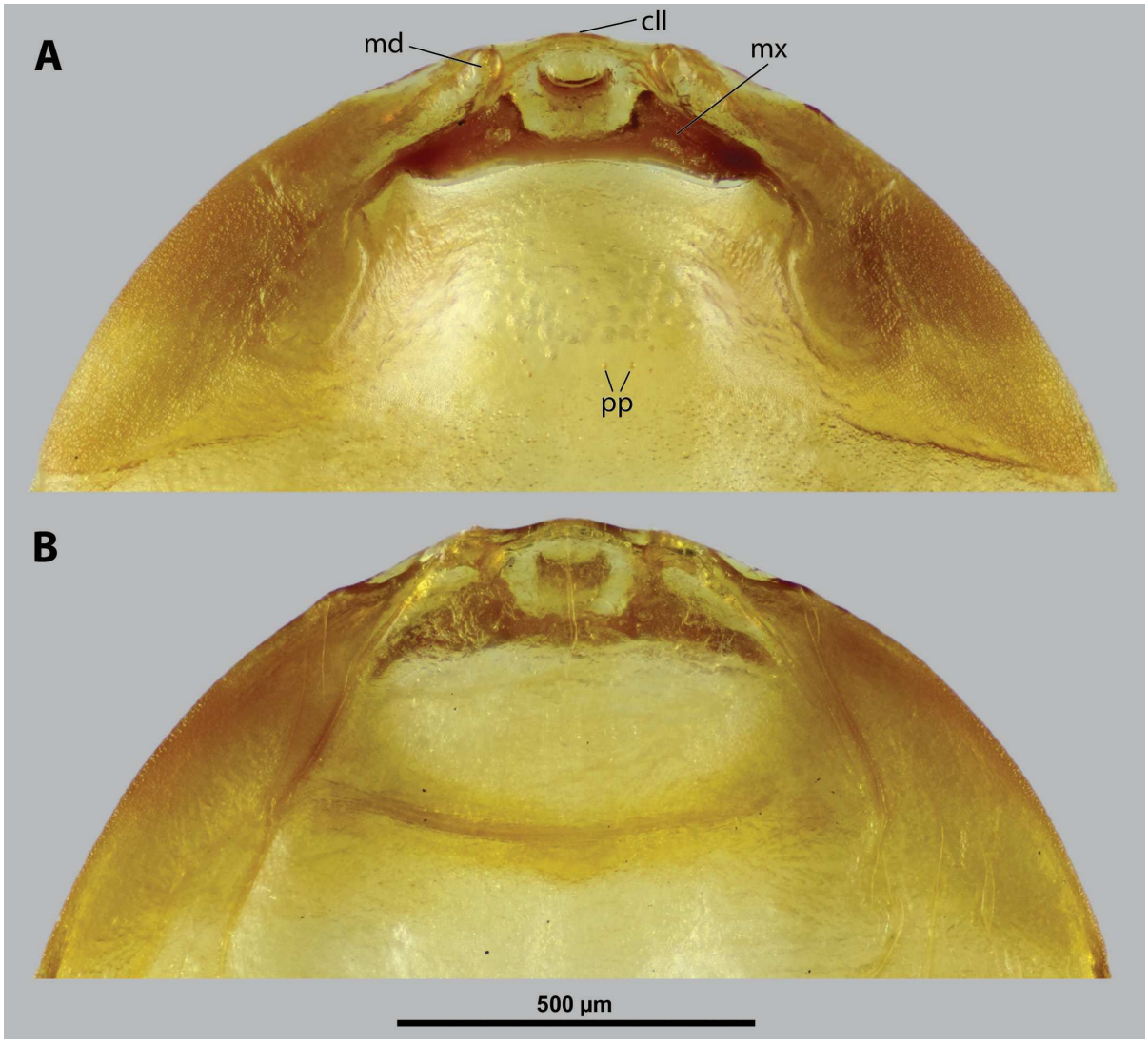

Figure 10. *Nipponoxenos vespularum* Kifune & Maeta, anterior part of female cephalothorax, photomicrographs **A** anterior part of cephalothorax, ventral side **B** Anterior part of cephalothorax, dorsal side. Abbreviations: cll – clypeal lobe, md – mandible, mx – vestige of maxilla (maxilla), pp – pigmented papillae.

Diagnosis of male cephalotheca. Less pigmented than in other genera of Xenidae. With conspicuous, nearly black clypeus and very short and black genae, very distinct on lightly colored surrounding areas of cephalotheca (Fig. 11). Antennal vestige very large (Fig. 11A).

Description of male cephalotheca. Shape and coloration. Rounded laterally in frontal view, widely elliptic (Fig. 11A); rounded in lateral view (Fig. 11B). Coloration pale except for clypeus and genae (Fig. 11).

Cephalothecal capsule. Compound eyes with individual ommatidia well visible. Clypeus black colored; inconspicuous clypeal lobe straight in frontal view; sensilla mainly concentrated on clypeal lobe and on lateral parts of clypeus. Frontal region not deformed, lacking frontal impression. Occipital bulge rather indistinct. Diameter of genae (black) between maxillary base and compound eye very small, subequal to antennal diameter (Fig. 11A). Occipital bulge absent.

Supra-antennal sensillary field. Kidney-shaped and bulging, delimited medially by quite indistinct furrow.

Antenna. Antennal vestige very large, with complete torulus. Periantennal area distinctly delimited.

Labrum. Labral area distinct. Setae of dorsal field present.

Mandible. Anteromedially directed. Coloration darker anteriorly and less pigmented posteriorly. Bulge pointed.

Maxilla. Distinct, prominent. Coloration darker anteriorly, posterior part around vestige of palp less pigmented.

Labium and hypopharynx. Located between and below maxillae. Praementum and postmentum distinct, separated by slightly paler coloration of postmentum. Hypopharyngeal protuberance inconspicuous.

Mouth opening. Mouth opening distinctly arcuate, nearly U-shaped.

Phylogenetic relationships. One of the earliest diverging lineages of Xenidae with a Palearctic origin (Benda et al. 2019). Placed either as sister to *Tachytixenos* Pierce + *Paraxenos* Saunders or as the earliest diverging group, sister to all other Xenidae (Benda et al. 2021).

Diversity and distribution. Monotypic, restricted to East Asia.

Hosts. *Vespula* spp. (Vespidae: Vespinae).

Comments. The monotypic *Nipponoxenos* was originally described as a subgenus of *Xenos* by Kifune and Maeta (1975). We classify it as a valid genus, based on a molecular phylogeny (Benda et al. 2019) and morphological characters newly reported here.

Figure 11. *Nipponoxenos vespularum* Kifune & Maeta, male, cephalotheca, photomicrographs **A** frontal view **B** lateral view. Abbreviations: a – vestigial antenna, cl – clypeus, coe – compound eye, gn – gena, mxb – maxillary base.

List of species

Nipponoxenos vespularum Kifune & Maeta, 1975

Xenos (*Nipponoxenos*) *vespularum* Kifune & Maeta, 1975: 447.

Hosts. *Vespula flaviceps* (Smith, 1870) (as *Vespula lewisi* Cameron, 1903) (Kifune and Maeta 1975); *Vespula flaviceps flaviceps* (Smith, 1870) (Kifune and Yamane 1991), *Vespula shidai* Ishikawa, Sk. Yamanne & Wagner, 1980 (Nakase and Kato 2013).

Distribution. Japan: Honshu; Russia: Primorskij Kraj, Ussurijsk (Kifune and Yamane 1991).

Note. This species was described under the monotypic subgenus *Nipponoxenos* Kifune and Maeta 1975.

Tachytixenos Pierce, 1911, stat. res.

Tachytixenos Pierce, 1911: 501. Type species: *Tachytixenos indicus* Pierce, 1911, by original designation.
Pseudoxenos Saunders, 1872 (partim!) (synonymy proposed by Hofeneder 1949: 148).
Paraxenos Saunders, 1872 (partim!) (synonymy proposed by Kinzelbach 1971b: 162).

Diagnosis of female cephalothorax. Differing from the other genera by a specific shape of the mandibular tooth, which is very wide basally and reaches the area of mandibular bulge. Tooth with pointed, ventrally directed apex. Base of tooth ventrally covered with small depressions continuous with several rows of spines (Fig. 14E). Prosternal extension undifferentiated (compared to similar genus *Paraxenos*), evenly arched, without any swelling or color differentiation. Maxillae distinctly prominent as in *Pseudoxenos*, *Tuberoxenos*, and some *Paraxenos* species. Mandible not protruding from capsule. In contrast to *Paragioxenos*, head and prothorax ventrally delimited by birth opening medially and by suture laterally.

Description of female cephalothorax. Shape and coloration. Cephalothorax compact, ca. as long as wide, or slightly wider than long, or vice versa. Size varying strongly within genus, length 0.94–1.82 mm, width 0.88–1.88 mm. Anterior head margin evenly rounded or projecting. Thorax slightly widening posteriorly. Coloration comprising multiple brown shades and distinct patterns (Fig. 12C, D).

Head capsule. Approximately ¼ ~ ½ as long as entire cephalothorax including lateral extensions. Coloration variable, pale, completely dark brown, or forming specific color pattern. Clypeal area well delimited from labral area, arcuate, or slightly protruding anteriorly forming clypeal lobe. Surface of clypeal area smooth or slightly wrinkled. Sensilla (~ 40–55) regularly dispersed over clypeal surface or mainly concentrated on clypeal lobe. Border between clypeal and frontal region present but indistinct. Frontal

region smooth or slightly wrinkled. Dorsal segmental border between head and prothorax distinct or only recognizable.

Supra-antennal sensillary field. Smooth with dispersed sensilla, delimited by distinct furrow on medial side (Fig. 13B).

Antenna. Preserved as poorly defined area with several minute rounded plates, antennal sensilla, or cavity, in some cases all three combined. Periantennal area smooth, flat, or forming incomplete elliptic wall between antenna and supra-antennal sensillary field (Fig. 13C, D).

Labrum. Ventral field wider than long, elliptic. Dorsal field slightly arcuate, at least 3× wider than long in midline. Dorsal field bearing ~ 15–30 setae inserted in cavities.

Mandible. Anteromedially directed at angle of 40–65°, enclosed in mandibular capsule. Mandibular bulge not distinctly raised, with several sensilla. Cuticle completely smooth to slightly sculptured. Mandibular tooth very wide on its base, reaching area of mandibular bulge. Tooth ventrally directed and pointed apically. Base with small depressions continuous with several rows of spines (Fig. 14E).

Maxilla. Well-developed, prominent, and clearly separated from labial area, strongly sclerotized, directed anteriorly or anteromedially. Not or very slightly overlapping with mandible proximally, not projecting beyond mandibular apex anteriorly. Cuticle usually smooth, rarely wrinkled. Vestige of palp distinct, forming small bulge with more or less distinct plates, situated medially on ventral side of maxilla. Submaxillary groove slightly produced posterolaterally.

Labium. Labial area between maxillae distinct, delimited anteriorly by mouth opening and posteriorly by birth opening. Wider than long in midline and flat or convex. Cuticular surface smooth or slightly reticulated.

Mouth opening. Mouth opening arcuate, sclerotized along margin.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders more or less distinct, usually separated by mesal furrows, often combined with pigmented stripes or spots on dorsal side. Border between metathorax and abdomen usually formed by ridge. Cuticle of thoracic segments on ventral side reticulate, with small scattered pigmented papillae. Dorsal side of thorax smooth or slightly reticulated. Prosternal extension undifferentiated, evenly arched. Shape of meso- and metathorax unmodified, transverse. Setae present on lateral region of abdominal segment I. Cuticular surface distinctly sculptured in cases with sparse setation (Fig. 13E).

Spiracles. Located on posterior ~ ⅓ of cephalothorax, slightly elevated, with lateral, anterolateral, or dorsal orientation.

Diagnosis of male cephalotheca. Genus characterized by combination of distinct paired furrow of supra-antennal sensillary field (Fig. 15A, D) and shape of mandibular tooth. Mandibular tooth very wide on its base and reaching area of mandibular bulge. Tooth base with small depressions continuous with several rows of spines (Fig. 15E, see also 14E). Diameter of genae between maxillary base and compound eye at least 2× as large as diameter of vestigial antenna.

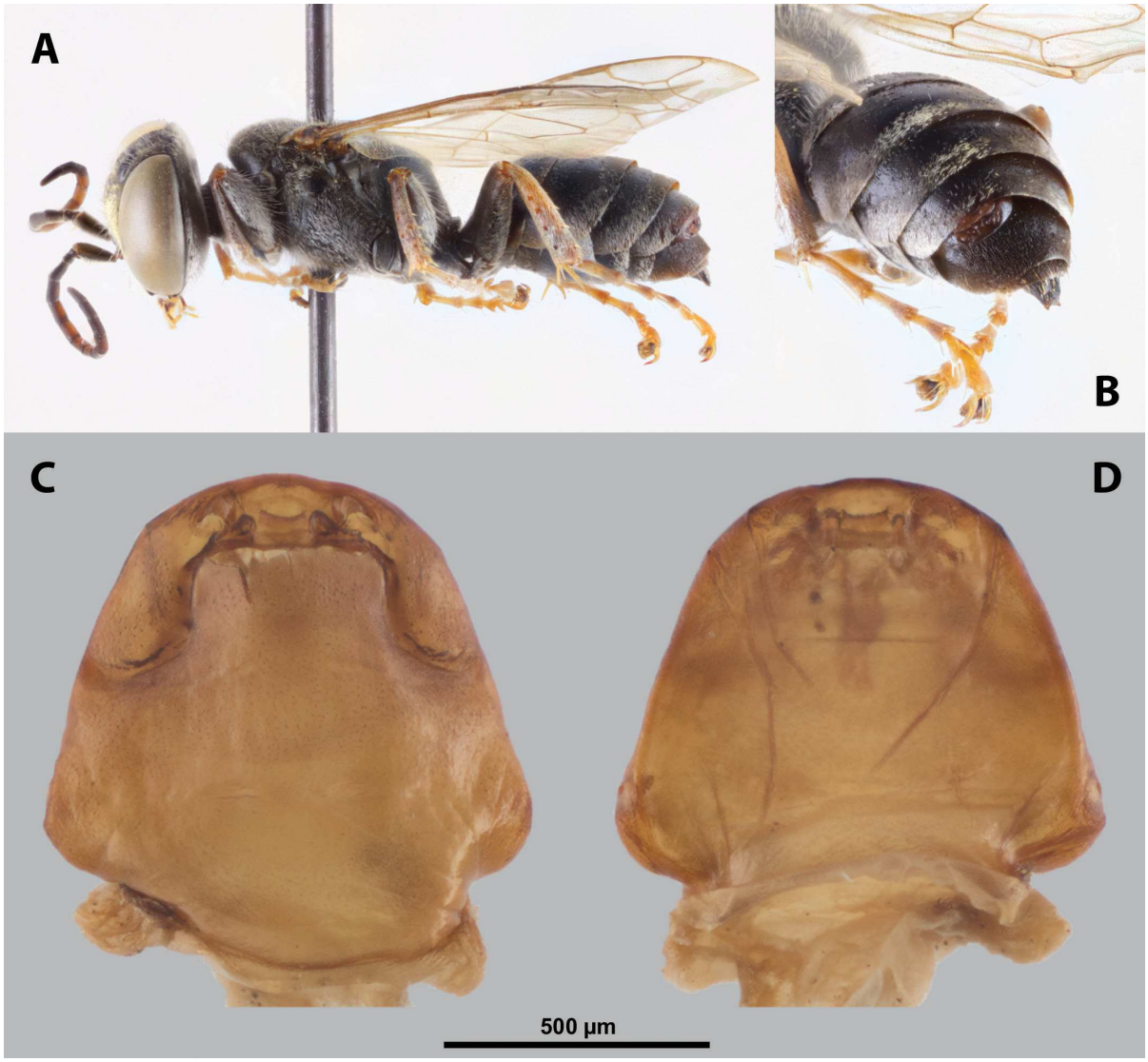

Figure 12. *Tachytixenos* cf. *indicus* Pierce, host, female, cephalothorax, photomicrographs **A** *Tachytes* sp. stylopized by female of *T*. cf. *indicus*, lateral view **B** detail of host abdomen with adult female inside **C** ventral side of cephalothorax **D** dorsal side of cephalothorax.

Description of male cephalotheca. Shape and coloration. Shape of cephalotheca rounded laterally in frontal view, widely elliptic. Anteriorly pointed in lateral view. Coloration forming pattern of pale and dark shades.

Cephalothecal capsule. Compound eyes with darker individual ommatidia well visible on pale background. Clypeal lobe straight in frontal view, distinctly prominent in lateral view. Sensilla mainly concentrated on clypeal lobe. Frontal region with paired furrow of supra-antennal sensillary field, lacking frontal impression. Diameter of genae between maxillary base and compound eye large, ~ 3× as large as diameter of vestigial antenna. Occipital bulge absent.

Supra-antennal sensillary field. Kidney-shaped and bulging, delimited medially by distinct furrow. Furrows relatively wide and not interconnected anteriorly (Fig. 15A, D).

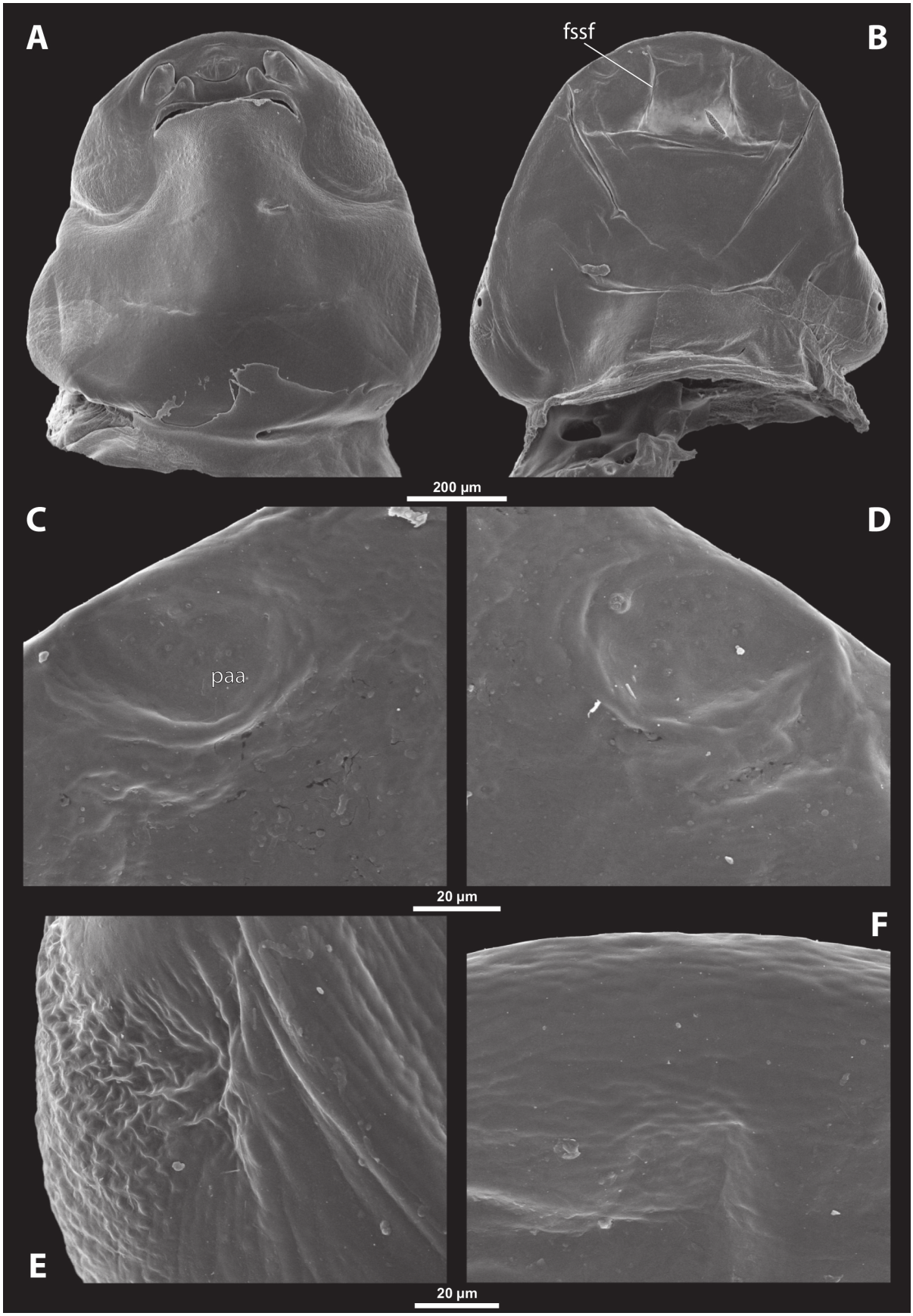

Figure 13. *Tachytixenos* cf. *indicus* Pierce, female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** left vestigial antenna, dorsal side **D** right vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** detail of anterior border of cephalothorax, dorsal side. Abbreviations: fssf – furrow of supra-antennal sensillary field, paa – periantennal area.

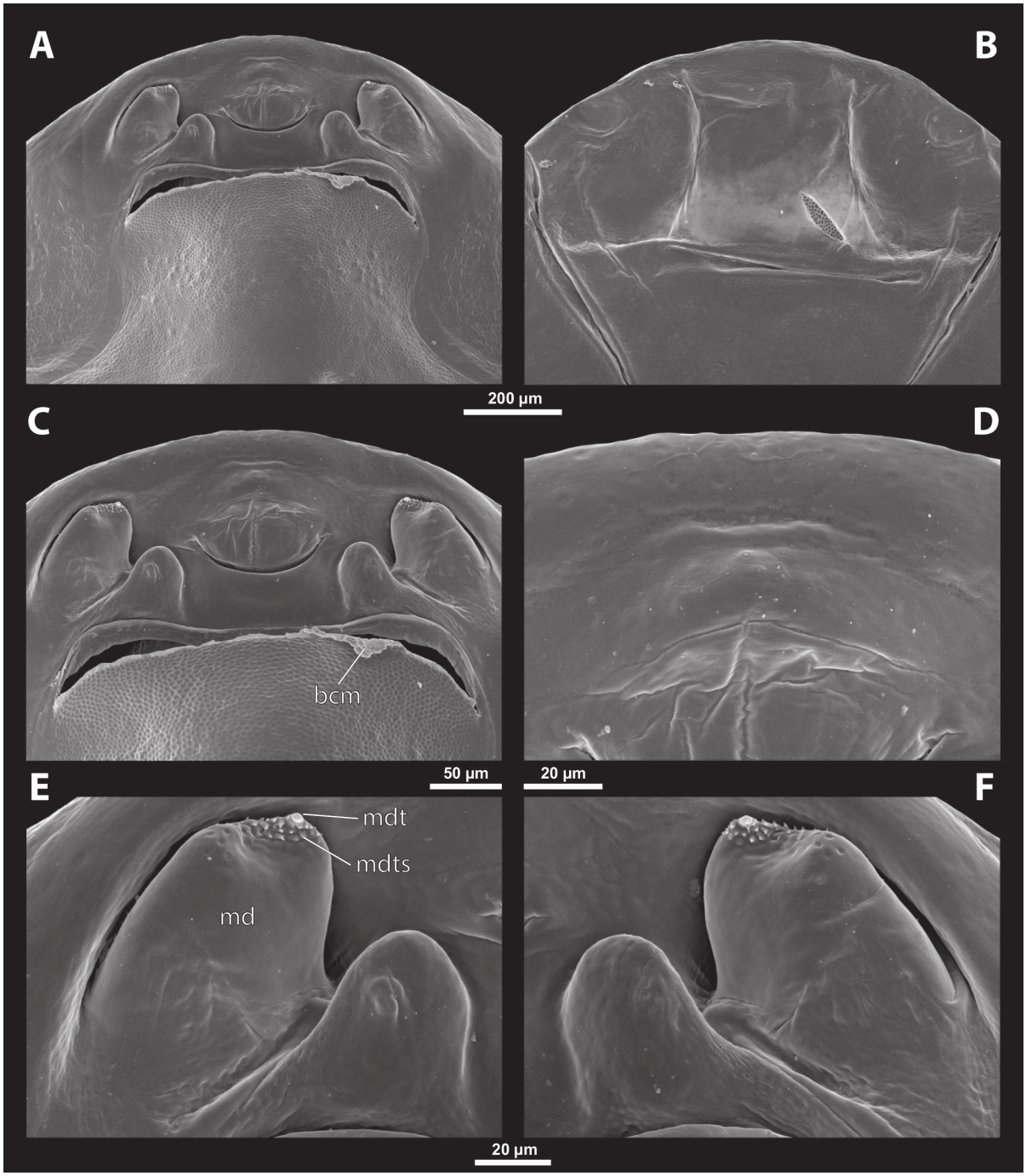

Figure 14. *Tachytixenos* cf. *indicus* Pierce, female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: bcm – brood canal membrane, md – mandible, mdt – mandibular tooth, mdts – spine of mandibular tooth.

Antenna. Of standard shape, small, with complete torulus. Periantennal area not distinctly delimited. Sensilla present (Fig. 15C).

Labrum. Labral area distinct. Setae on dorsal field present.

Mandible. Mandible anteromedially directed. Mandibular tooth very wide on its base and reaches area of mandibular bulge. Tooth base with small depressions continuing in several rows of spines (Fig. 15E). Mandibular bulge bears several sensilla.

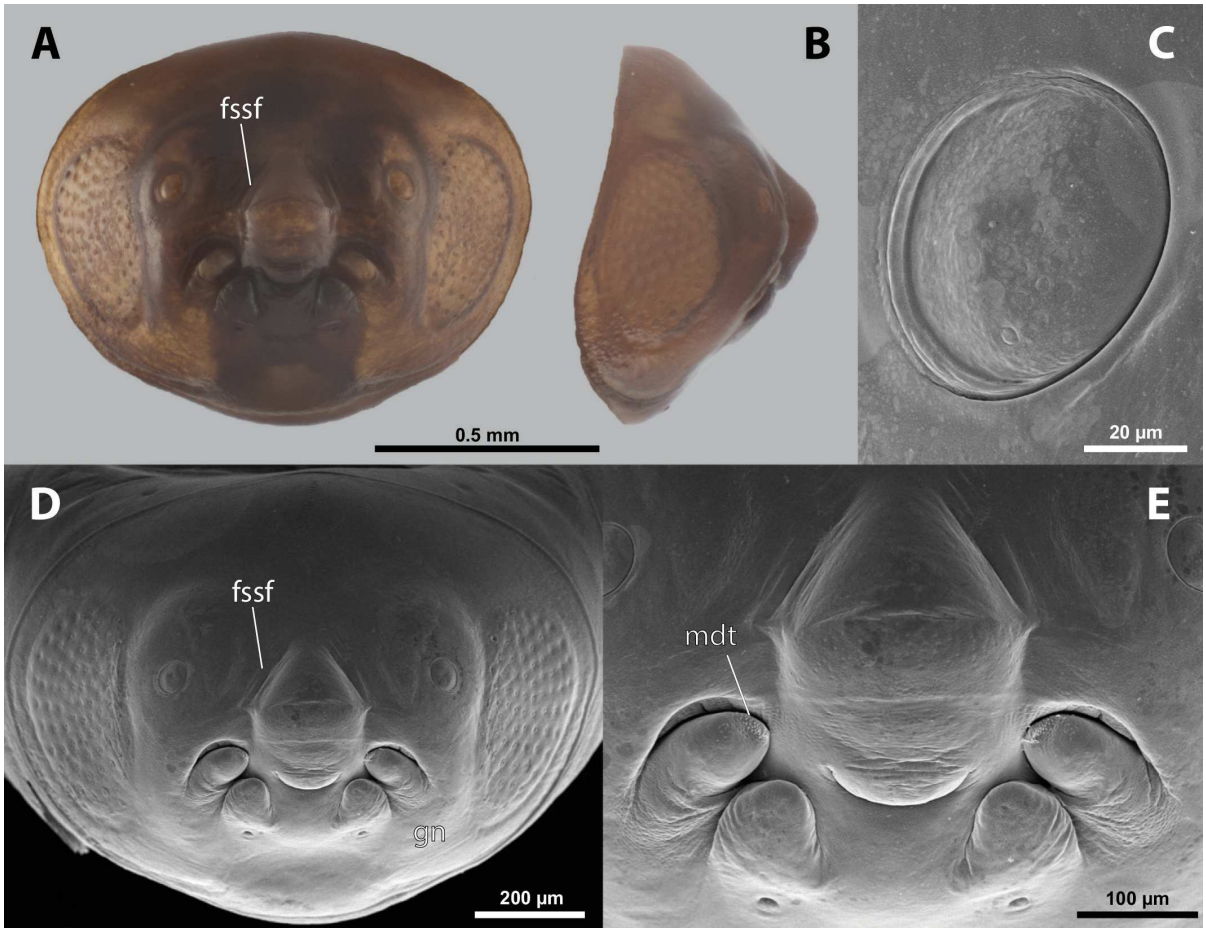

Figure 15. *Tachytixenos* cf. *indicus* Pierce, male, cephalotheca, photomicrographs, SEM micrographs **A** frontal view **B** lateral view **C** vestigial antenna **D** frontal view **E** mouthparts. Abbreviations: fssf – furrow of supra-antennal sensillary field, gn – gena, mdt – mandibular tooth.

Maxilla. Maxilla distinct, prominent, completely dark. Vestige of maxillary palp distinct.

Labium and hypopharynx. Well-developed between and below maxillae, completely dark. Praementum and postmentum slightly separated by furrow. Hypopharyngeal protuberance absent.

Mouth opening. Mouth opening well visible, not covered by ventral labral field, slightly arcuate.

Phylogenetic relationships. One of the earliest diverging lineages of Xenidae. Forming a clade of Palearctic origin with its sister genus *Paraxenos* (Benda et al. 2019).

Diversity and distribution. Monotypic, restricted to the Old World.

Hosts. *Tachytes* spp. (Crabronidae: Crabroninae).

Comments. The monotypic genus *Tachytixenos* was described by Pierce (1911) but only superficial descriptions of the female and male without illustrations were provided. Hofeneder (1949) synonymized it with *Pseudoxenos*, but it was later classified as *Paraxenos* by Kinzelbach (1971b). We restored *Tachytixenos* from synonymy and classify it as a valid genus based on monophyly revealed by the molecular phylogeny (Benda et al. 2019, 2021) and based on morphological characters newly reported here

Note. Cook (1919) noted that Bohart synonymized *Tachytixenos* with *Pseudoxenos* but it was done laterally by Hofeneder (1949).

List of species

Tachytixenos indicus Pierce, 1911

Tachytixenos indicus Pierce, 1911: 502.
Pseudoxenos indicus (Pierce, 1911) (new combination by Hofeneder 1949).
Paraxenos indicus (Pierce, 1911) (new combination by Kinzelbach 1971b).

Hosts. *Tachytes xenoferus* Rohwer, 1911; *T. maculicornis* Saunders, 1910; *T. modestus* Smith, 1856 (Pierce 1911; Kinzelbach 1978; Kifune and Hirashima 1980); *T. vischnu* Cameron (Cook 2019).

Distribution. Algeria; India: Deesa; Thailand: Peninsular Siam; China; Sri Lanka (Pierce 1911; Kinzelbach 1978; Kifune and Hirashima 1980); Denmark? (Cook 2019).

Note. Benda et al. (2021) reported two lineages possibly representing separate species. A more comprehensive sampling and a detailed study are necessary for a taxonomic revision of this genus.

Paraxenos Saunders, 1872

Paraxenos Saunders, 1872: 45. Type species: *Paraxenos erberi* Saunders, 1872, subsequent designation by Pierce (1908).
Paraxenos (*Bembicixenos*) (Székessy, 1955: 280) (considered as subgenus by Kinzelbach 1971b: 162).
Bembicixenos Székessy, 1955: 280 (synonymized by Kinzelbach 1978: 82). Type species: *Pseudoxenos* (*Bembicixenos*) *hungaricus* Székessy, 1955, by original designation.

Diagnosis of female cephalothorax. Differing from *Tachytixenos* by a narrower mandibular tooth and a differentiated prosternal extension. Prosternum with anterior swelling (Fig. 18A) similar to *Paragioxenos*, or with distinct color pattern. Clypeal sensilla well visible, extending to ventral side of clypeal area. Vestige of antenna preserved as cavity (Fig. 17D), additional rounded plates rarely present. Maxillae of two types, fused with labial area or distinctly separated and prominent as in *Tachytixenos*, *Pseudoxenos*, and *Tuberoxenos*. In contrast to *Paragioxenos*, head and prothorax ventrally delimited by birth opening medially and by suture laterally.

Description of female cephalothorax. Shape and coloration. Compact, very variable in shape, distinctly longer than wide, or wider than long. Size very variable, length 0.94–1.9 mm, maximum width 0.8–2.57 mm. Anterior head margin distinctly protruding. Thorax slightly widening posteriorly, sometimes subparallel. Coloration varying from light to dark brown. Cephalothorax displaying multiple brown shades forming distinct patterns (Fig. 16C, D).

Head capsule. Ca. ⅓–½ as long as entire cephalothorax including lateral extensions. Coloration pale to dark, always with species specific patterns. Clypeal area not delimited or well separated from labral area, protruding anteriorly, always forming

clypeal lobe. Surface smooth or very slightly wrinkled. Very distinct sensilla mainly concentrated on clypeal lobe and extending to ventral side of clypeal area. Border between clypeal and frontal region usually not clearly recognizable but present, rarely more distinct. Frontal region distinctly wrinkled or covered by papillae. Segmental border between head and prothorax very indistinct on dorsal side, in most specimens virtually unrecognizable.

Supra-antennal sensillary field. Smooth or slightly wrinkled, with dispersed sensilla, delimited by distinct furrow on medial side (Fig. 18B).

Antenna. Preserved as cavity (Fig. 17D), rarely combined with rounded plates. Antennal sensilla or vestigial setae missing. Periantennal area smooth, sometimes reduced when supra-antennal sensillary field almost reaches vestige of antennae.

Labrum. Ventral field distinctly wider than long, elliptical or semicircular. Dorsal field arcuate to nearly straight, > 3× wider than long in midline. Dorsal field with ~ 20–25 setae inserted in cavities.

Mandible. Anteromedially directed at an angle of 30–65°, enclosed in mandibular capsule or rarely protruding from it. Mandibular bulge not distinctly raised, with ~ 5–18 sensilla. Cuticle completely smooth, or partially sculptured on articulatory area. Mandibular tooth narrow or slightly widened, pointed or blunt, armed with distinct spines.

Maxilla. Very variable, well-developed and separated from labial area, or fused with it and strongly reduced. Cuticle always smooth. Prominent, anteriorly or anteromedially directed, in some cases partially overlapping with mandible proximally. Distal maxillary region not projecting beyond mandible anteriorly. Vestige of palp distinct, forming cavity or small bulge with more or less distinct plate. Located anteriorly or medially on ventral side of maxilla. Submaxillary groove distinctly produced posterolaterally (Fig. 18A).

Labium. Labial area between maxillae distinct, delimited anteriorly by mouth opening and posteriorly by birth opening. Wider than long in midline and flat. Cuticular surface smooth or slightly reticulated.

Mouth opening. Distinctly arcuate to straight, sclerotized around margin.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders more or less distinct, usually separated by mesal furrows on ventral side, rarely combined with pigmented stripes or spots on dorsal side, but not recognizable dorsally in most specimens. Border between metathorax and abdomen usually formed by ridge. Cuticle of thoracic segments reticulate on ventral side, often with small, scattered pigmented papillae. Dorsal side of thorax smooth or slightly reticulated. Prosternal extension anteriorly with arcuate to semicircular swelling in most species, or lacking swelling but with distinct color pattern. Meso- and metathorax unmodified in shape, transverse. Setae and cuticular spines present on lateral region of abdominal segment I (Fig. 17E).

Spiracles. On posterior third of cephalothorax, slightly elevated, with anterolateral or anterodorsal orientation.

Diagnosis of male cephalotheca. Characterized by distinct and relatively wide furrow of supra-antennal sensillary field (Fig. 19A, D). Differing from sister genus *Tachytixenos* in shape of the mandibular tooth, which is conspicuously pointed and not

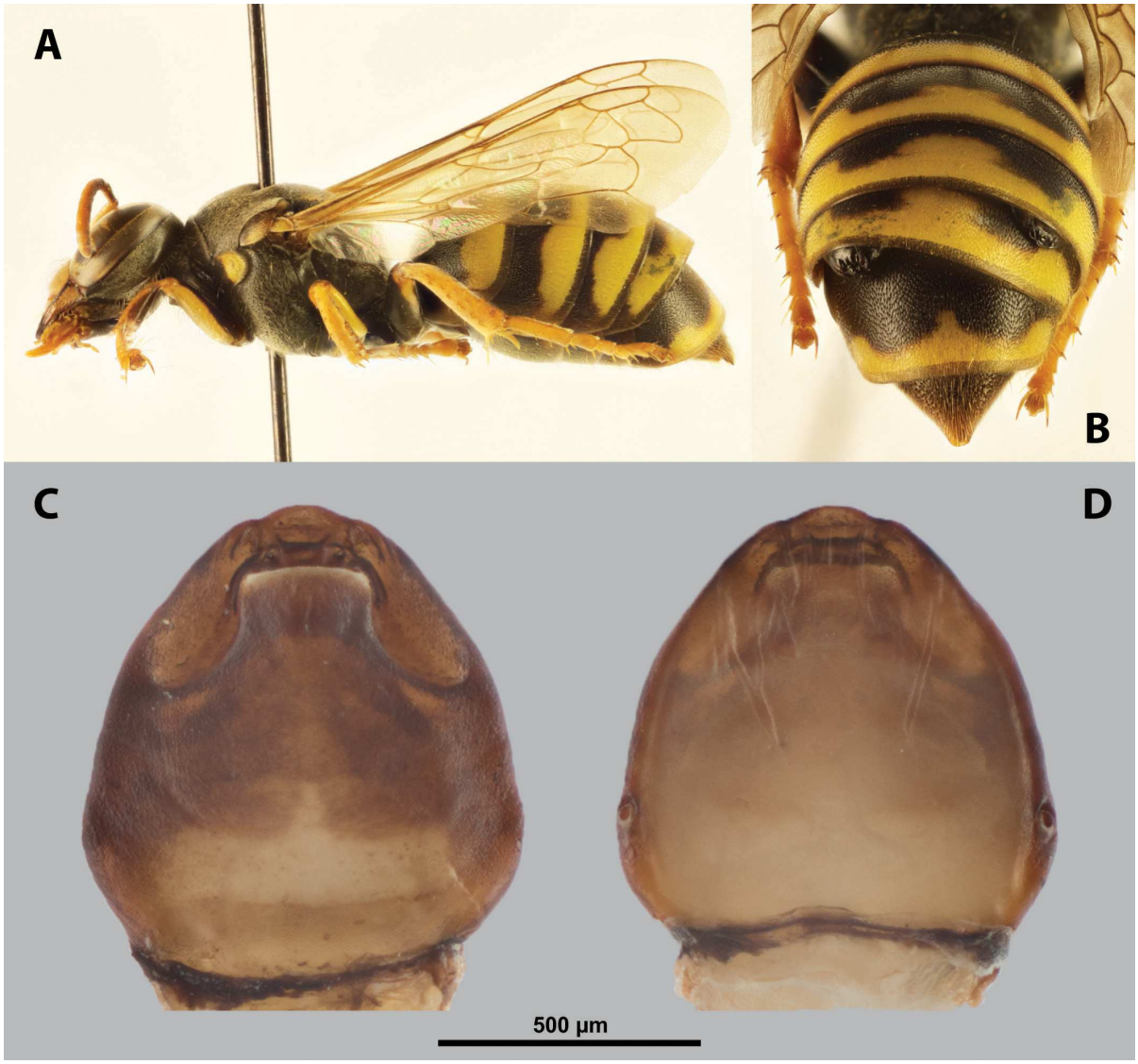

Figure 16. *Paraxenos erberi* Saunders, host, male, female, cephalothorax, photomicrographs **A** *Bembecinus peregrinus* (Smith) stylopized by *P. erberi*, lateral view **B** detail of host abdomen with female under third tergite and male puparium under fourth tergite **C** ventral side of female cephalothorax **D** dorsal side of female cephalothorax.

in contact with mandibular bulge. Diameter of genae between maxillary base and compound eye 2× or several times larger than diameter of vestigial antenna. Cephalotheca of elliptic shape in frontal view.

Description of male cephalotheca. Shape and coloration. Elliptic and rounded laterally in frontal view, also almost rounded in lateral view. Coloration forming pattern of pale and dark shades.

Cephalothecal capsule. Compound eyes with darker individual ommatidia well visible on pale background. Clypeal lobe straight in frontal view, not prominent in lateral view. Sensilla dispersed on clypeal surface. Frontal region with paired furrow of supraantennal sensillary field, lacking impression or occipital bulge. Diameter of genae between maxillary base and compound eye very large, > 3× as large as diameter of vestigial antenna.

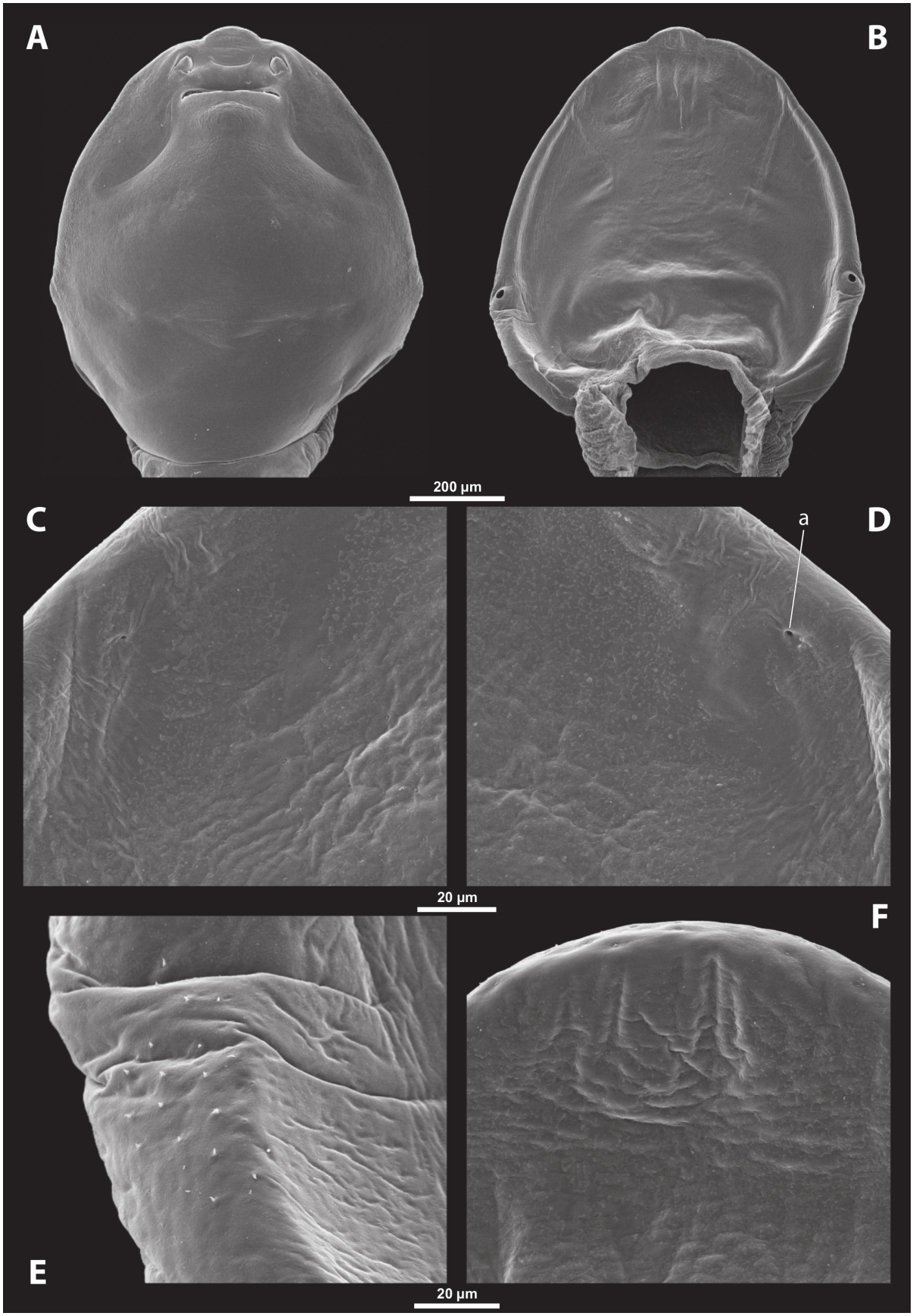

Figure 17. *Paraxenos* sp., female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** left vestigial antenna, dorsal side **D** right vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** detail of anterior border of cephalothorax, dorsal side. Abbreviation: a – vestigial antenna.

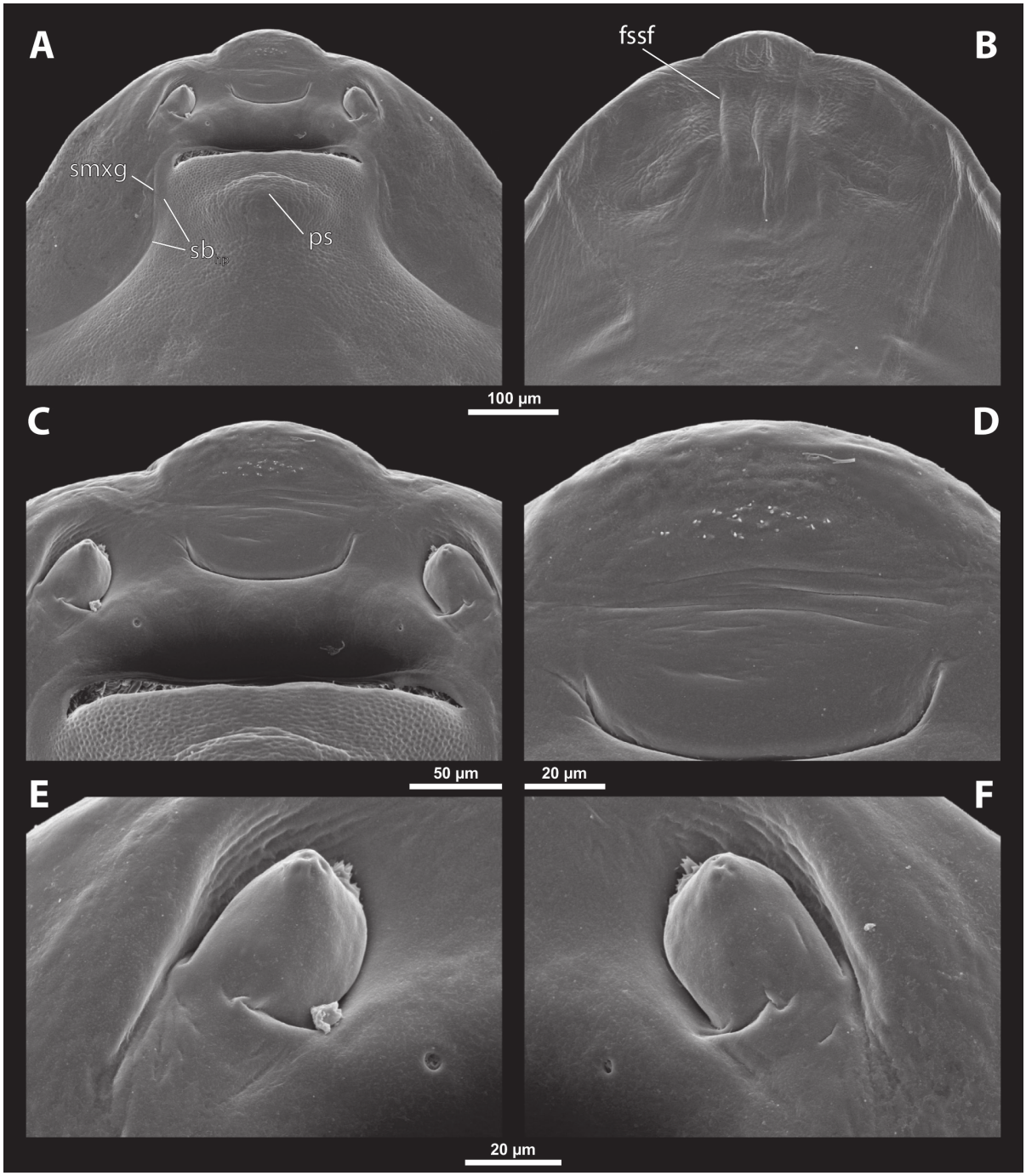

Figure 18. *Paraxenos* sp., female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: fssf – furrow of supra-antennal sensillary field, ps – prosternal swelling, sbhp – segmental border between head and prothorax, smxg – submaxillary groove.

Supra-antennal sensillary field. Kidney-shaped and bulging, delimited medially by distinct furrow. Furrows relatively wide, not connected anteriorly (Fig. 19A, D).

Antenna. Of standard shape, small, with small plates and cavity (Fig. 19C), torulus interrupted. Periantennal area not clearly delimited from supra-antennal sensillary field.

Labrum. Labral area distinct. Setae present on dorsal field.

Mandible. Anteromedially directed. Tooth apically pointed, not very wide basally, not reaching area of mandibular bulge (Fig. 19E), which bears sensilla.

Maxilla. Distinct, prominent. Coloration pale centrally and dark laterally. Vestige of palp distinct, dark.

Labium and hypopharynx. Labium distinct between and below maxillae, dark. Praementum and postmentum indistinctly separated by furrow. Hypopharyngeal protuberance present or not.

Mouth opening. Well visible, not covered by ventral labral field, slightly arcuate.

Phylogenetic relationships. Forming a clade of Palearctic origin with *Tachytixenos* (Benda et al. 2019).

Diversity and distribution. Thirteen described species, distributed in the Old World and Australia.

Hosts. *Bembecinus*, *Bembix* and *Stizus* spp. (Bembicidae: Bembicinae).

Comments. *Paraxenos* was described by Saunders (1872) but only a superficial description of the male was provided. Kinzelbach (1971b) synonymized several additional genera with *Paraxenos*, all of them described by Pierce (1908, 1909, 1911, 1919) from the New World (*Eupathocera*, *Opthalmochlus*, *Homilops*, *Sceliphronechthrus*)

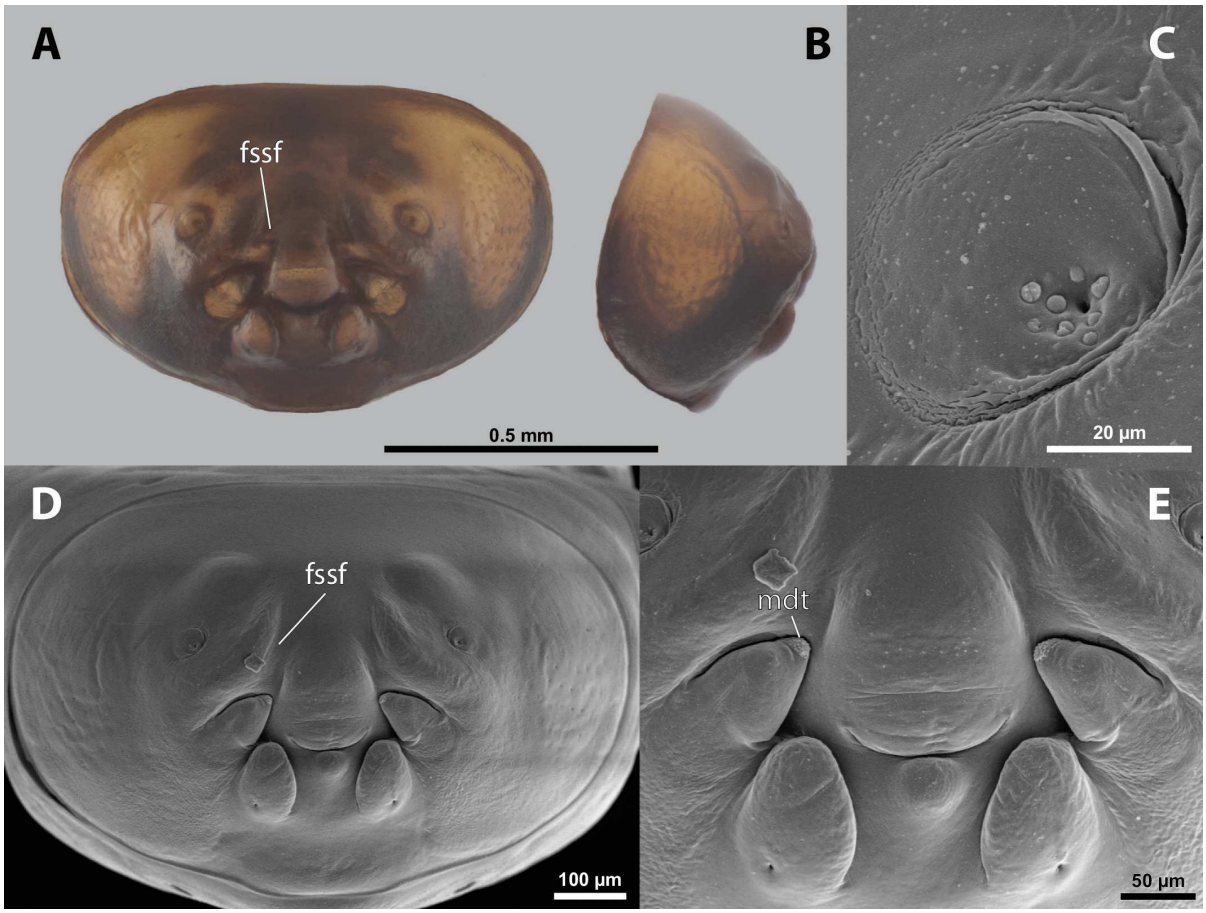

Figure 19. *Paraxenos erberi* Saunders, male, cephalotheca, photomicrographs, SEM micrographs **A** frontal view **B** lateral view **C** vestigial antenna **D** frontal view **E** mouthparts. Abbreviations: fssf – furrow of supra-antennal sensillary field, mdt – mandibular tooth.

and Old World (*Tachytixenos*). He also classified *Bembicixenos* described by Székessy (1955) as subgenus of *Paraxenos*, but later considered it a synonym of *Paraxenos* (Kinzelbach 1978). We classify *Paraxenos* as a valid genus based on monophyly revealed by a molecular phylogeny (Benda et al. 2019, 2021) and based on morphological characters newly reported here.

List of species

***Paraxenos australiensis* Kifune & Hirashima, 1987**

Paraxenos australiensis Kifune & Hirashima, 1987: 157.

Host. *Bembix musca* (Handlirsch, 1893) (Kifune and Hirashima 1987).
Distribution. Australia: Queensland (Kifune and Hirashima 1987).

***Paraxenos beaumonti* (Pasteels, 1951)**

Pseudoxenos beaumonti Pasteels, 1951: 76.
Paraxenos beaumonti (Pasteels, 1951) (new combination by Kinzelbach 1971b).

Host. *Stizus marthae* Handlirsch, 1892 (Pasteels 1951).
Distribution. Algeria (Pasteels 1951).

***Paraxenos biroi* (Székessy, 1956)**

Pseudoxenos biroi Székessy, 1956: 147.
Paraxenos biroi (Székessy, 1956) (new combination by Kinzelbach 1971b).

Host. *Bembecinus antipodum* (Handlirsch, 1892) (Székessy 1956).
Distribution. New Guinea (Székessy 1956).

***Paraxenos erberi* Saunders, 1872**

Paraxenos erberi Saunders, 1872: 46.
Pseudoxenos crassidens Pasteels, 1954 (synonymized by Kinzelbach 1978).

Hosts. *Bembecinus hungaricus* (Frivaldsky, 1876); *Bembecinus peregrinus* (Smith, 1856); *Bembecinus tridens* (Fabricius, 1781) (Saunders 1872; Kinzelbach 1978).
Distribution. Algeria; Europe (Kinzelbach 1978).

Paraxenos hofenederi (Pasteels, 1956)

Pseudoxenos hofenederi Pasteels, 1956: 111.
Paraxenos hofenederi (Pasteels, 1956) (new combination by Kinzelbach 1971b).

Hosts. *Sphecius nigricornis* (Dufour, 1838), *Stizus biclypeatus* (Christ, 1791), *Stizus bizonatus* Spinola, 1839, *Stizus pubescens* (Klug, 1835), *Stizus ruficornis* (Fabricius, 1787) (Pasteels 1956; Kinzelbach 1978).

Distribution. Algeria; Cyprus; Egypt; Greece; India; Jordan; Tajikistan (Kinzelbach 1978; Batelka and Straka 2005); Senegal? (Kinzelbach 1978).

Paraxenos hofenederianus Luna de Carvalho, 1978

Paraxenos hofenederianus Luna de Carvalho, 1978: 95.

Host. *Stizus ruficornis* (J. Förster, 1771) (as *Stizus distinguendus* Handlirsch, 1901) (Luna de Carvalho 1978a).

Distribution. Senegal (Luna de Carvalho 1978a).

Paraxenos hungaricus (Székessy, 1955)

Pseudoxenos (*Bembicixenos*) *hungaricus* Székessy, 1955: 281.
Paraxenos hungaricus (Székessy, 1955) (new combination by Kinzelbach 1971b).

Hosts. *Bembix oculata* Panzer, 1801, *Bembix rostrata* (Linnaeus, 1758), *Bembix* sp. (Kinzelbach 1978).

Distribution. Czech Republic; Germany; Hungary; Italy; Mongolia; Spain (Székessy 1955; Kinzelbach 1978; Benda et al. 2021); Turkey (this study).

Paraxenos krombeini Kifune & Hirashima, 1987

Paraxenos krombeini Kifune & Hirashima, 1987: 155.

Host. *Bembix orientalis* (Handlirsch, 1893) (Kifune and Hirashima 1987).

Distribution. Sri Lanka (Kifune and Hirashima 1987).

Paraxenos nagatomii Kifune, 1985

Paraxenos nagatomii Kifune & Yamane, 1985: 49.

Host. *Bembecinus bimaculatus* (Matsumura & Uchida, 1926) (Kifune and Yamane 1985).
Distribution. Japan (Kifune and Yamane 1985).

Paraxenos novaeguineae (Székessy, 1956)

Pseudoxenos novaeguineae Székessy, 1956: 147.
Paraxenos novaeguineae (Székessy, 1956) (new combination by Kinzelbach 1971b).

Host. *Bembecinus gazagnairei* (Handlirsch, 1892) (Székessy 1956).
Distribution. New Guinea (Székessy 1956).

Paraxenos occidentalis Kifune & Hirashima, 1987

Paraxenos occidentalis Kifune & Hirashima, 1987: 156.

Host. *Bembix atrifrons* (F. Smith, 1956) (Kifune and Hirashima 1987).
Distribution. Australia: Western Australia (Kifune and Hirashima 1987).

Paraxenos polli (Pasteels, 1956)

Pseudoxenos polli Pasteels, 1956: 109.
Paraxenos polli (Pasteels, 1956) (new combination by Kinzelbach 1971b).

Host. *Bembecinus braunsii* (Handlirsch, 1894) (as *Sphecius fraunsi* Handlirsch, 1894) (Pasteels 1956).
Distribution. Democratic Republic of Congo (Pasteels 1956).

Paraxenos rieki (Pasteels, 1956)

Pseudoxenos rieki Pasteels, 1956: 113.
Paraxenos rieki (Pasteels, 1956) (new combination by Kinzelbach 1971b).

Host. *Stizus basalis* Guérin-Méneville, 1844 (Pasteels 1956).
Distribution. Mali: Djenné (Pasteels 1956).

Brasixenos Kogan & Oliveira, 1966, stat. res.

Brasixenos Kogan & Oliveira, 1966: 358. Type species: *Brasixenos fluminensis* Kogan & Oliveria, 1966, by original designation.

Xenos Rossi, 1793 (partim!) (synonymy proposed by Kinzelbach 1971b: 160).
Brasixenos Kogan & Oliveira, 1966 (restored from synonymy by Trois 1988: 268).
Xenos Rossi, 1793 (partim!) (synonymy proposed by Cook 2019: 232).

Diagnosis of female cephalothorax. Maxilla distinctly reduced, flattened, anteriorly rounded, not distinctly prominent; fused to labial area but well defined by its strong sclerotization, conspicuous compared to usually pale cephalothorax as in *Nipponoxenos* and some species of *Xenos*. Maxillary bases appear connected and fused to each other. Vestigial palps differ from those of all other genera, preserved only as inconspicuous concavity on wrinkled maxillary surface, without any vestigial plate. Located anteriorly on ventral side of maxilla, at level of mandibles (Fig. 22E). Clypeal area not delimited from labral area, apparently more or less fused (Fig. 22D). Mandible nested in capsule. In contrast to *Paragioxenos*, head and prothorax ventrally delimited by birth opening medially and by suture laterally.

Description of female cephalothorax. Shape and coloration. Compact and usually ovoid, ca. as long as wide, or slightly wider, rarely longer than wide. Abdominal segment I of some species extruded laterally, forming corner below abdominal spiracles. Species relatively variable in size, length 0.76–1.62 mm, maximum width 0.72–1.74 mm. Anterior head margin evenly rounded or protruding. Thorax slightly to strongly widening posteriorly, sometimes subparallel. Coloration mostly pale, with light shadows of brown dominating. Some parts of cephalothorax, especially maxillae, dark and sclerotized.

Head capsule. Including lateral extensions ~ ⅓–½ as long as entire cephalothorax. Color pattern formed by shades of pale and dark brown, with maxillae always dark. Clypeal area not delimited from labral area, apparently more or less fused, slightly or distinctly protruding anteriorly, always forming clypeal lobe (Fig. 22D). Surface wrinkled apically on clypeal lobe (sometimes with lamellar structures), smooth ventrolaterally and dorsally. Clypeal surface with ~ 50–70 sensilla or more. Border between clypeal and frontal region indistinguishable. Frontal area smooth. Segmental border between head and prothorax difficult to recognize on dorsal side in some specimens.

Supra-antennal sensillary field. Smooth or slightly wrinkled, with dispersed sensilla. Not delimited or indistinctly by furrow on medial side.

Antenna. Preserved only as elongated depression or inconspicuous furrow (Fig. 21C). Rounded plate, small cavity or sensilla missing. Periantennal area slightly wrinkled or smooth.

Labrum. Ventral field slightly wider than long, nearly circular. Dorsal field anterior to mouth opening slightly arcuate, at least 4× wider than long at midline, with setae inserted in cavities on surface.

Mandible. Anteriorly to anteromedially directed at angle of 40–70°, enclosed in capsule. Mandibular bulge sometimes indistinct, with up to ten spine-shaped or blunt sensilla, or lacking these structures. Cuticle completely sculptured or partially smooth. Tooth narrow, armed with several rows of spines.

Maxilla. Reduced and not protruding, fused to labium but clearly indentifiable by distinct sclerotization; appearing connected and fused medially, with sclerotization continuous along birth opening. Cuticle distinctly wrinkled. Apical maxillary region almost reaching upper edge of mandible in some species. Vestige of palp present as inconspicuous cavity on wrinkled maxillary surface, lacking vestigial plate. Located anteriorly on ventral side, at level of mandibles. Maxillary base slightly raised and less sclerotized than anterior region (Fig. 20C). Submaxillary groove slightly produced posterolaterally.

Labium. Labial area recognizable between maxillae but fused with them, anteriorly delimited by mouth opening; convex, wider than long in midline, pale laterally, strongly sclerotized medially and around mouth opening. Cuticular surface smooth or wrinkled, with wrinkles indistinct on well sclerotized areas.

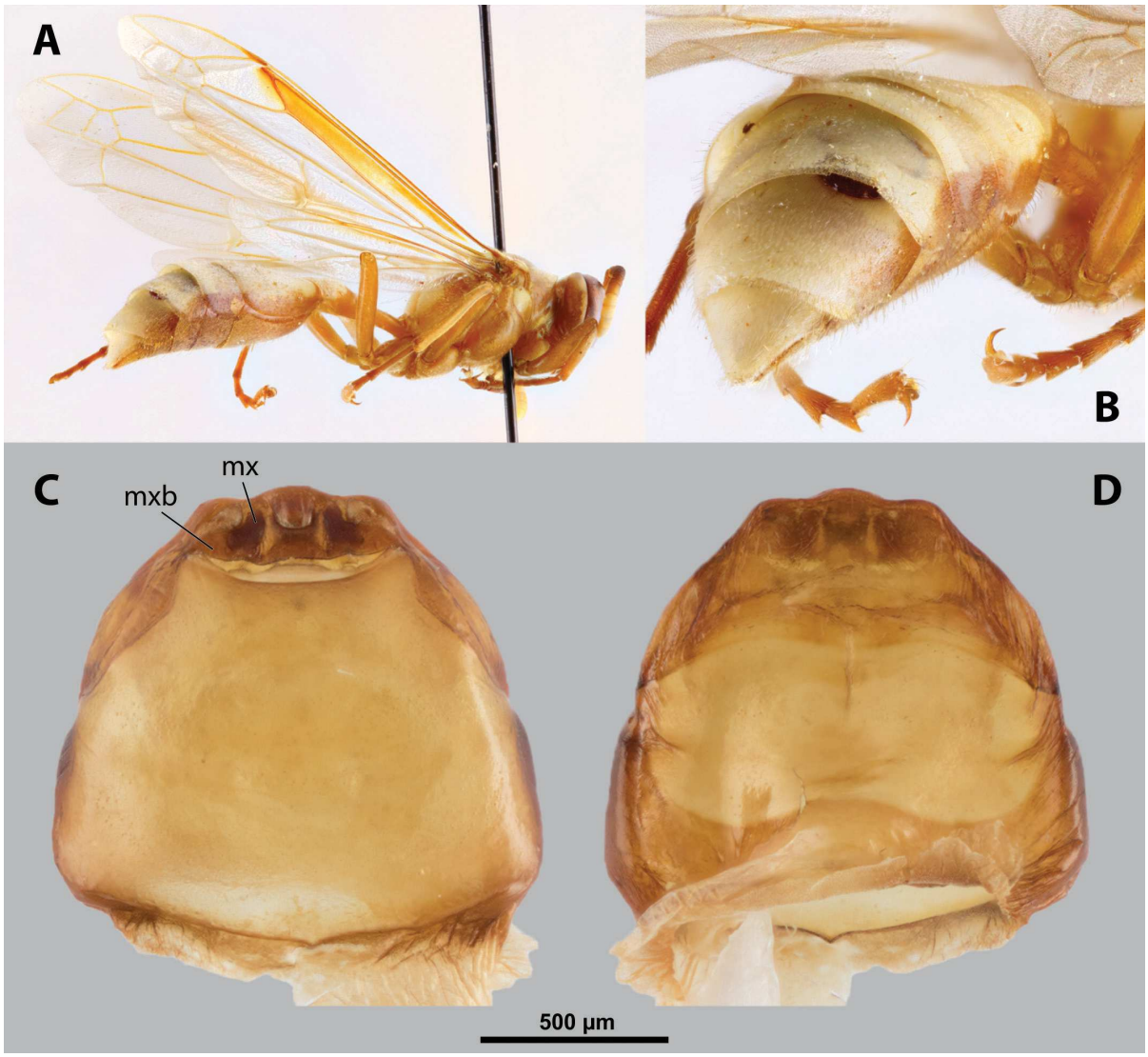

Figure 20. *Brasixenos araujoi* (Oliveira & Kogan), host, female, cephalothorax, photomicrographs **A** *Apoica pallens* (Fabricius) stylopized by female of *B. araujoi*, lateral view **B** detail of host abdomen with adult female inside **C** ventral side of cephalothorax **D** dorsal side of cephalothorax. Abbreviations: mx – vestige of maxilla, mxb – maxillary base.

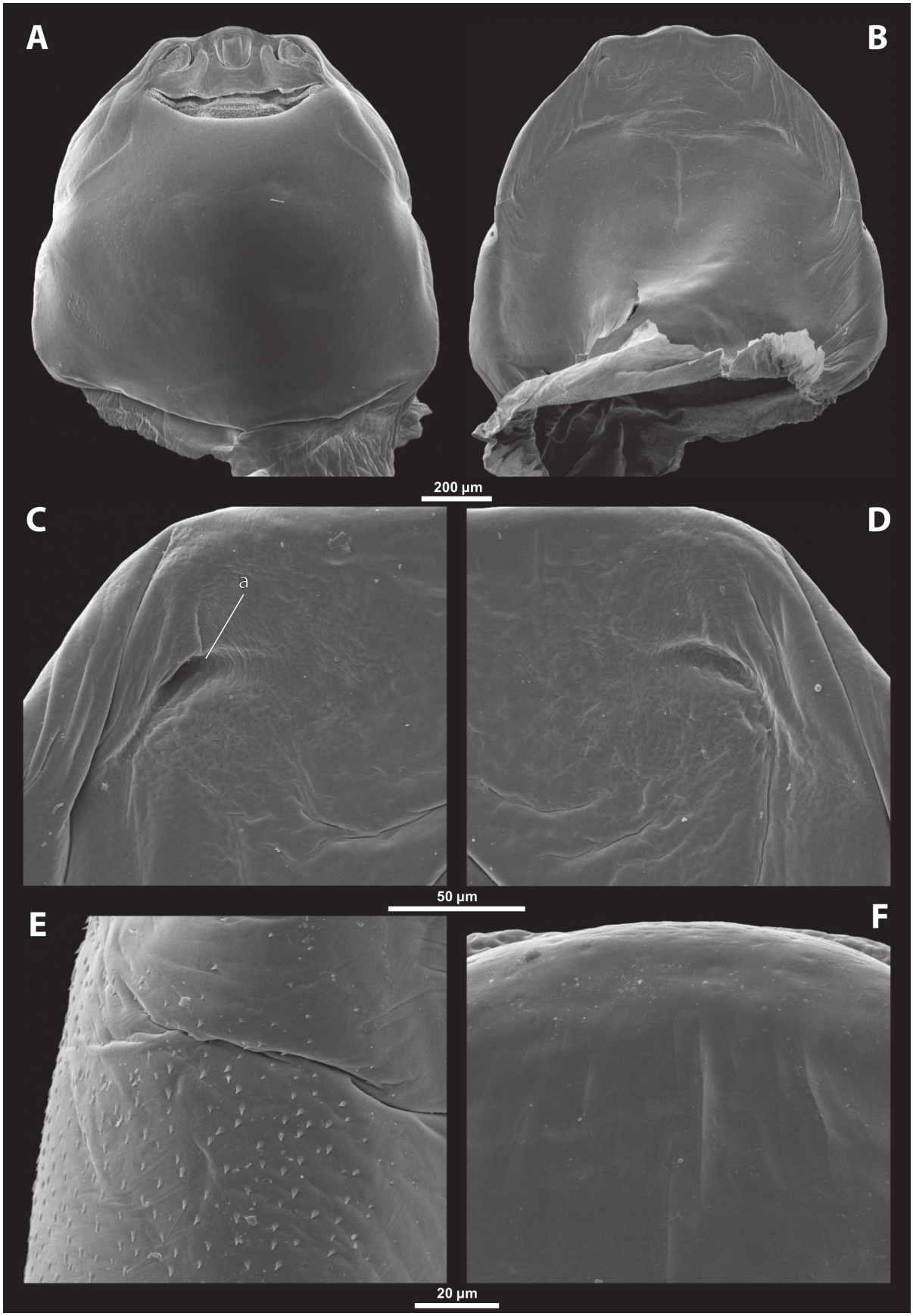

Figure 21. *Brasixenos araujoi* (Oliveira & Kogan), female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** left vestigial antenna, dorsal side **D** right vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** detail of anterior border of cephalothorax, dorsal side. Abbreviation: a – vestigial antenna.

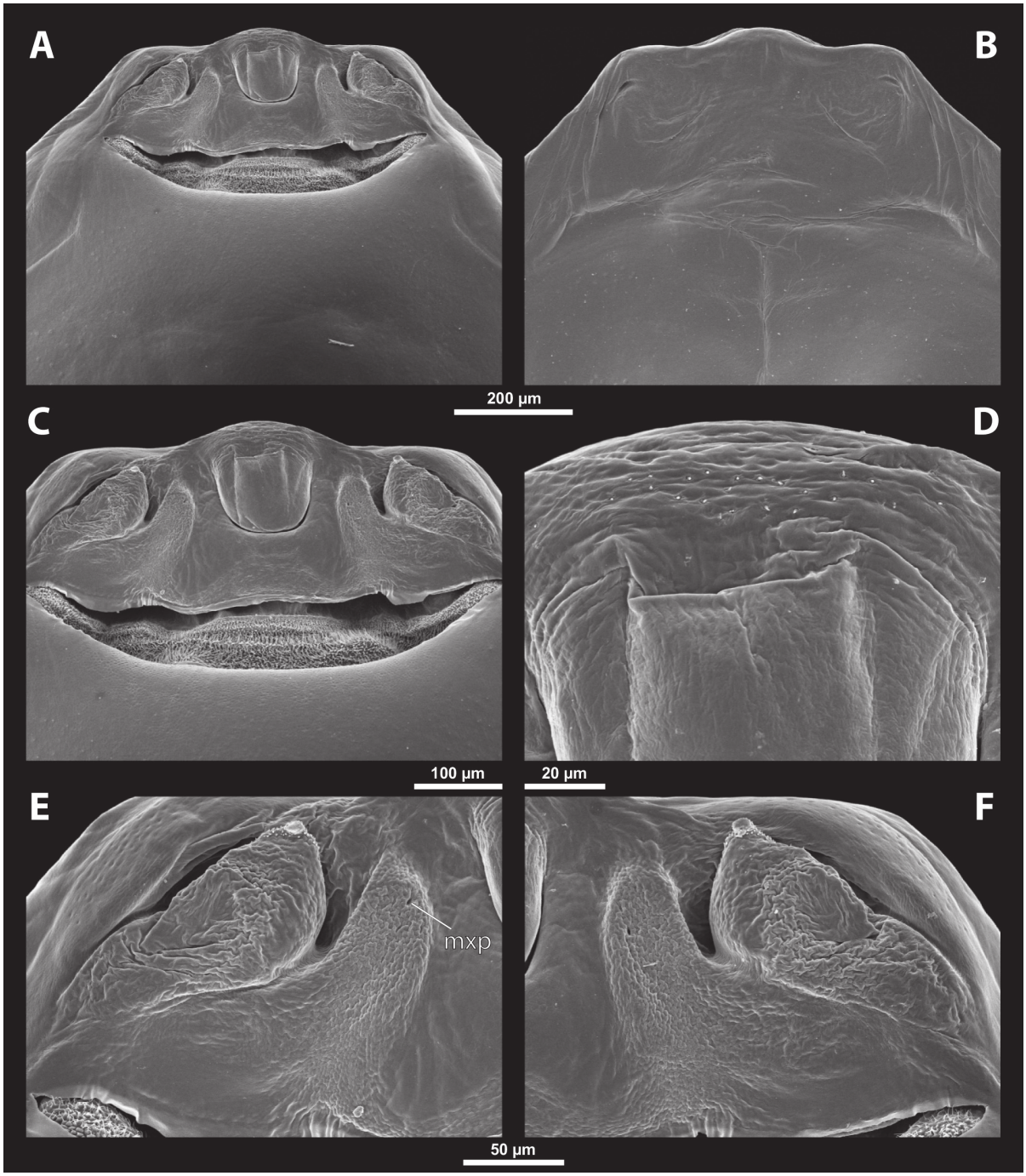

Figure 22. *Brasixenos araujoi* (Oliveira & Kogan), female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviation: mxp – vestige of maxillary palp.

Mouth opening. Arcuate to distinctly U-shaped, sclerotized around margin.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders more or less distinct, usually indicated by pigmented stripes or changed coloration on dorsal side. Mesal furrows absent. Border between metathorax and abdomen usually indicated by change in coloration or cuticular sculpture, separating ridge indistinct. Cuticle of thoracic segments with smooth surface on the ventral side, in some

cases with small scattered pigmented papillae. Dorsal side of thorax usually completely smooth. Prosternal extension not very distinctly prolonged, usually evenly arched. Thoracic segments constricted laterally, distance between lateral extensions of head and spiracles thus reduced (Fig. 20D). Setae and cuticular spines present on lateral region of abdominal segment I (Fig. 21E).

Spiracles. Spiracles situated on posterior half or posterior third of cephalothorax, slightly elevated, with anterolateral orientation.

Diagnosis of male cephalotheca. Differing from other genera by fusion of maxilla with cephalotheca. Maxillary cuticular surface with longitudinal grooves (Fig. 23E). Vestige of maxillary palp visible (distinct in optical microscope, very inconspicuous on SEM micrographs) (Fig. 23A, D).

Description of male cephalotheca. Shape and coloration. Laterally rounded in frontal view, elliptic, in lateral view pointed anteriorly. Coloration mostly dark, but with some lighter areas such as ocular region or surroundings of maxillary palps (Fig. 23A).

Cephalothecal capsule. Compound eyes with darker individual ommatidia well visible on pale ocular background. Clypeus with longitudinal pale line (Fig. 23A). Clypeal lobe arcuate or straight in frontal view, prominent in lateral view; with sensilla evenly dispersed. Frontal region with conspicuous impression (Fig. 23D). Diameter of genae between maxillary base and compound eye large, > 2× as large as diameter of vestigial antenna. Occipital bulge absent.

Supra-antennal sensillary field. Kidney-shaped and bulging, medially delimited by frontal impression, with visible but indistinct furrows.

Antenna. Of standard shape, small, with complete torulus. Periantennal area indistinct but present. Sensilla usually absent.

Labrum. Labral area well visible but dorsal field not clearly separated from clypeus. Setae on dorsal field present.

Mandible. Anteromedially directed, pale centrally and dark laterally. Mandibular bulge not conspicuous, with several sensilla.

Maxilla. Not recognizable as separate structure, fused with cephalotheca. Cuticular surface of maxillary area sculptured, with longitudinal grooves (Fig. 23E). Vestige of palp well visible (with light microscope, very indistinct on SEM micrographs) (Fig. 23A, D).

Labium and hypopharynx. Distinct, inserted between and below maxillae, completely dark. Praementum and postmentum very indistinctly separated. Hypopharyngeal protuberance recognizable, not well delimited.

Mouth opening. Well visible, U-shaped, partially covered by ventral labral field.

Phylogenetic relationships. Sister to a large clade containing representatives of genera previously known as *Pseudoxenos*, *Paraxenos*, and *Xenos* (Benda et al. 2019).

Diversity and distribution. Group of Xenidae with origin in the New World and restricted to this region. Comprising seven species, all of which are known from Brazil.

Hosts. Various genera of Epiponini (Vespidae: Polistinae).

Comments. The genus *Brasixenos* was described and differentiated from *Xenos* by Kogan and Oliveira (1966), but the description of the female cephalothorax was super-

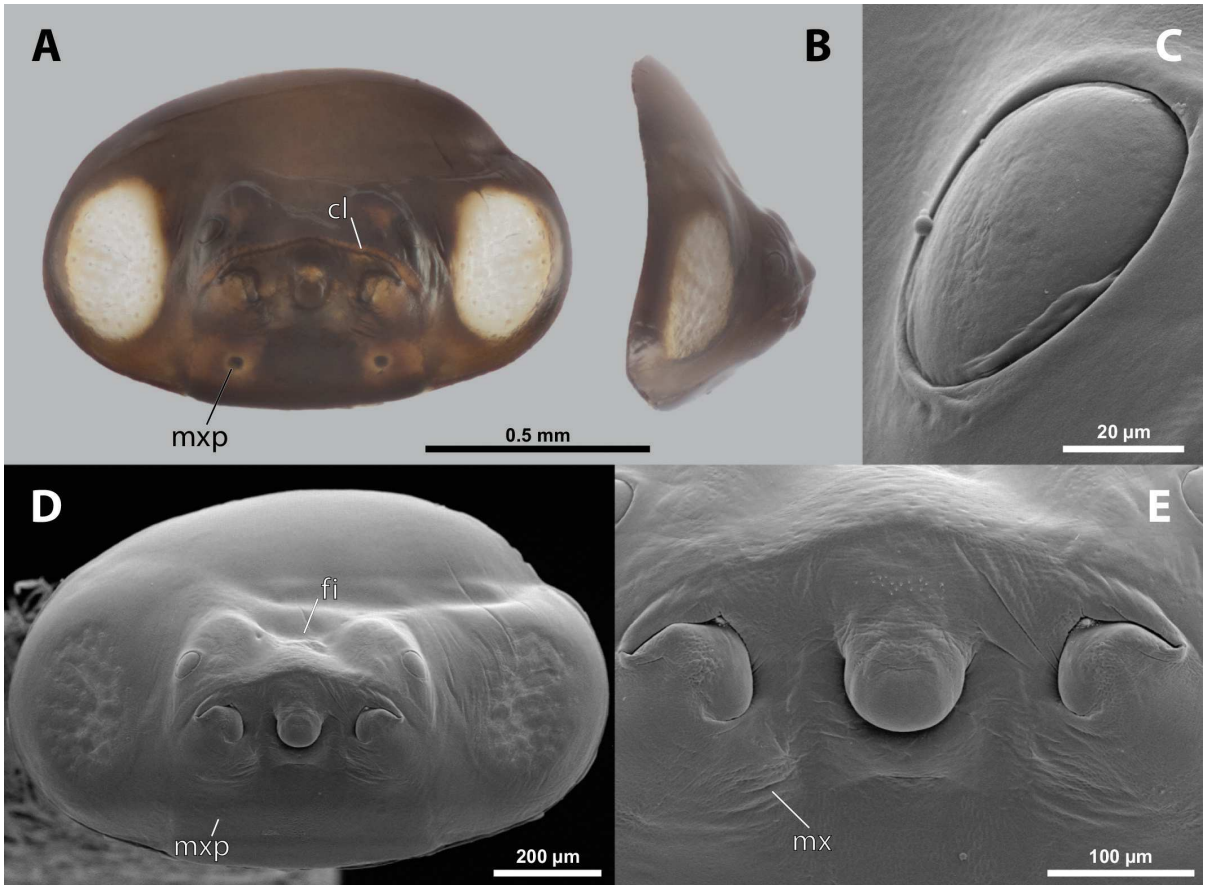

Figure 23. *Brasixenos* sp., male, cephalotheca, photomicrographs, SEM micrographs **A** frontal view **B** lateral view **C** vestigial antenna **D** frontal view **E** mouthparts. Abbreviations: cl – clypeus, fi – frontal impression, mx – vestige of maxilla, mxp – vestige of maxillary palp.

ficial. Although Kinzelbach (1971b) treated *Brasixenos* as a junior synonym of *Xenos*, Trois (1988) attempted to reinstate *Brasixenos* as a valid genus. Nevertheless, no author has followed this opinion (Cook 2019). Although Kogan and Oliveira (1966) expected a close relationship of *Xenos* with *Brasixenos* in their description, Benda et al. (2019) revealed the group as a separate lineage unrelated to *Xenos*. We classify *Brasixenos* as a valid genus, based on a molecular phylogeny (Benda et al. 2019, 2021) and morphological characters newly reported here.

List of species

***Brasixenos acinctus* Kogan & Oliveira, 1966, stat. res.**

Brasixenos acinctus Kogan & Oliveira, 1966: 356.
Xenos acinctus (Kogan & Oliveira, 1966) (synonymy proposed by Kinzelbach 1971b).

Host. *Polybia* sp., close to *Polybia sericea* (Olivier, 1792).

Distribution. Brazil: Rio de Janeiro (Kogan and Oliveira 1966).

Brasixenos araujoi **(Oliveira & Kogan, 1962), stat. res.**

Xenos araujoi Oliveira & Kogan, 1962: 6 (combination restored by Kinzelbach 1971b and Cook 2019).
Brasixenos araujoi (Oliveira & Kogan, 1962) (new combination by Kogan and Oliveira 1966 and Trois 1988).

Hosts. *Apoica pallens* (Fabricius, 1804) (Oliveira and Kogan 1962); *Apoica flavissima* Vecht, 1973; *Apoica thoracica* Buysson, 1906 (this study).

Distribution. Brazil: Amazonas (Oliveira and Kogan 1962).

Brasixenos bahiensis **Kogan & Oliveira, 1966, stat. res.**

Brasixenos bahiensis Kogan & Oliveira, 1966: 353.
Xenos bahiensis (Kogan & Oliveira, 1966) (new combination by Kinzelbach 1971b).

Host. *Polybia ignobilis* (Haliday, 1836).

Distribution. Brazil: Bahia (Kogan and Oliveira 1966).

Brasixenos brasiliensis **Kogan & Oliveira, 1966, stat. res.**

Brasixenos brasiliensis Kogan & Oliveira, 1966: 355.
Xenos brasiliensis (Kogan & Oliveira, 1966) (new combination by Kinzelbach 1971b).

Host. *Polybia sericea* (Olivier, 1792).

Distribution. Brazil: Rio de Janeiro, Pará (Kogan and Oliveira 1966).

Brasixenos fluminensis **Kogan & Oliveira, 1966, stat. res.**

Brasixenos fluminensis Kogan & Oliveira, 1966: 347.
Xenos fluminensis (Kogan & Oliveira, 1966) (new combination by Kinzelbach 1971b).

Host. *Polybia ignobilis* (Haliday, 1836) (as *Polybia atra* Saussure, 1854).

Distribution. Brazil: Rio de Janeiro (Kogan and Oliveira 1966).

Brasixenos myrapetrus **Trois, 1988, stat. res.**

Brasixenos myrapetrus Trois, 1988: 277.
Xenos myrapetrus (Trois, 1988) (new combination by Cook, 2019).

Host. *Polybia* (*Myrapetra*) *paulista* Ihering, 1896 (Trois 1988).

Distribution. Brazil (Trois 1988).

Brasixenos zikani Kogan & Oliveira, 1966, stat. res.

Brasixenos zikani Kogan & Oliveira, 1966: 350.
Xenos zikani (Kogan & Oliveira, 1966) (new combination by Kinzelbach 1971b).

Host. *Polybia tinctipennis* Fox, 1898 (as *Polybia ypiranguensis* Ihering, 1904) (Kogan and Oliveira 1966).

Distribution. Brazil: Rio de Janeiro (Kogan and Oliveira 1966).

Leionotoxenos Pierce, 1909, stat. res.

Leionotoxenos Pierce, 1909: 137. Type species: *Leionotoxenos jonesi* Pierce, 1909, by original designation.
Pseudoxenos Saunders, 1872 (partim!) (synonymy proposed by Bohart, 1937: 133). *Paraxenos* Saunders, 1872 (partim!) (synonymy proposed by Kinzelbach 1971b: 162).
Monobiaphila Pierce, 1909: 139 (syn. nov.). Type species: *Monobiaphila bishoppi* Pierce, 1909, by original designation.
Montezumiaphila Brèthes, 1923: 45 (syn. nov.). Type species: *Montezumiaphila vigili* Brèthes 1923, by monotypy.

Diagnosis of female cephalothorax. Differing from its sister genus *Eupathocera* in the following characters. Frontal region with conspicuous coverage of papillae (Fig. 25F). Supra-antennal sensillary field with wrinkled surface, which almost reaches vestigial antenna. Periantennal area small and indistinct (Fig. 25C). Prothorax ventrally connected to head on same plane, versus usually elevated in *Eupathocera* (Fig. 25A). Position of sensilla on clypeal lobe not extended onto ventral side of clypeal area as in *Xenos* or *Paraxenos*. Rudiments of torulus usually preserved (Figs 25C, 29D). Mandible not protruding from mandibular capsule. In contrast to *Paragioxenos*, head and prothorax ventrally delimited by birth opening medially and by suture laterally.

Description of female cephalothorax. Shape and coloration. Cephalothorax compact and usually ovoid, varying distinctly in shape, longer than wide to distinctly wider than long. Species relatively variable in size, length 0.88–1.7 mm, maximum width 0.72–1.68. Anterior head margin evenly rounded or slightly protruding anteriorly. Thorax slightly to strongly widening posteriorly, sometimes subparallel. Coloration of cephalothorax with multiple dark and light brown shades forming distinct pattern (Fig. 24C, D).

Head capsule. Ca. ⅓ to nearly ½ as long as entire cephalothorax including lateral cephalic extensions. Coloration variable, pale to dark brown or forming specific patterns. Clypeal area well delimited from labral region, clypeal lobe

indistinct or slightly protruding anteriorly. Surface more or less wrinkled, in some cases with reticulated pattern (Fig. 26D), with 12–26 (or more) sensilla distributed anteriorly. Border between clypeal and frontal region indistinct but still recognizable. Frontal region with conspicuous coverage of papillae (Fig. 25F). Dorsal border between head and prothorax indicated by interrupted suture, distinct coloration, or largely obliterated.

Supra-antennal sensillary field. Conspicuously wrinkled or reticulated. Usually delimited by indistinct furrow on medial side, but otherwise by change in cuticular sculpture, with wrinkled surface of supra-antennal sensillary field versus papillae on frontal region (Fig. 26B).

Antenna. Preserved as more or less defined area, with several rounded plates and setae (Fig. 25C). Torulus largely reduced or absent, rudiment usually recognizable as interrupted furrow (Fig. 25C). Periantennal area small and indistinct, supra-antennal sensillary field with wrinkled surface almost reaching antennal vestige (Fig. 25C).

Labrum. Ventral field wider than long, elliptic. Dorsal field slightly arcuate, at least 3× to 4× wider than medially along midline. Dorsal field with several inconspicuous setae (10 to 20) inserted in cavities.

Mandible. Anteromedially directed at an angle of 40–55° and enclosed in capsule. Mandibular bulge more or less distinctly raised, with 5–7 sensilla. Cuticle smooth to slightly sculptured or with longitudinal grooves. Mandibular tooth narrow or slightly widened, with or without spines.

Maxilla. Reduced and not distinctly protruding, fused to labium, often not clearly separated from labial area. Cuticle smooth or slightly wrinkled. Maxillary apex not projecting beyond mandible anteriorly. Vestige of palp inconspicuous, forming small bulge, sometimes very indistinct, located medially on ventral side of maxilla. Submaxillary groove more or less distinctly produced posteriorly to maxillary base.

Labium. Labial area flat, wider than long in midline or as wide as long, usually recognizable between maxillae but sometimes fused with them. Anteriorly delimited by mouth opening and posteriorly by birth opening. Cuticular surface smooth or slightly reticulated.

Mouth opening. Distinctly arcuate to nearly straight, sclerotized marginally.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders distinct or indistinct, usually indicated by mesal furrows, often combined with pigmented stripes. Border between metathorax and abdomen usually formed by indistinct ridge or change in cuticular surface. Cuticle of thoracic segments on ventral side reticulate, often with scattered small and pigmented papillae. Dorsal side smooth or slightly wrinkled or reticulated. Prosternal extension either undifferentiated or indicated anteriorly by color pattern, in which case a swelling can be present or absent. Region of prosternal extension evenly connected to head on same plane (Fig. 25A). Meso- and metathorax unmodified in shape, transverse. Setae or cuticular spines present on lateral region of abdominal segment I (Fig. 25E).

Spiracles. Located on posterior ~ ⅓ of cephalothorax, slightly elevated, with anterolateral or anterodorsal orientation.

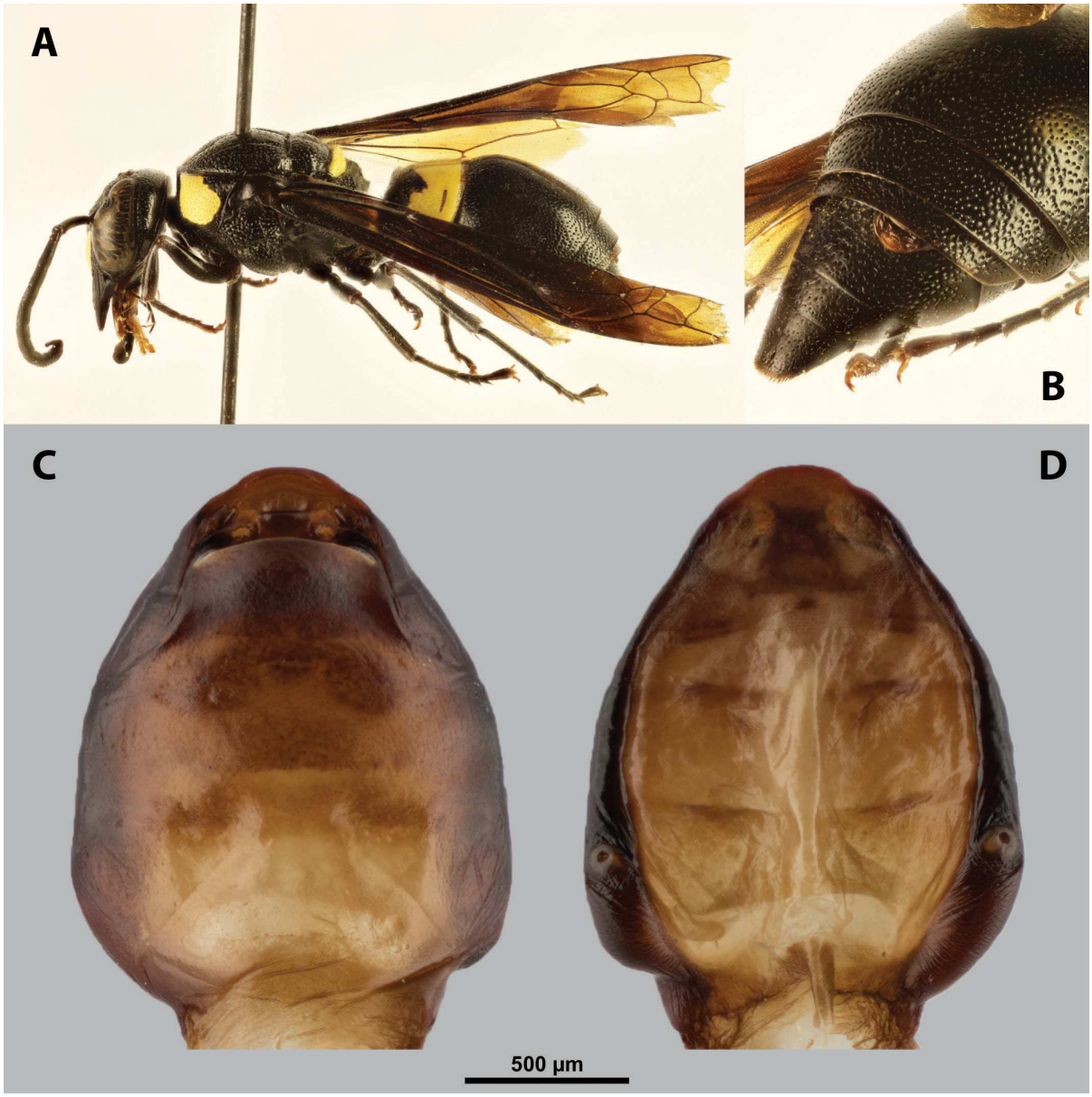

Figure 24. *Leionotoxenos bishoppi* (Pierce), host, female, cephalothorax, photomicrographs **A** *Monobia quadridens* (Linnaeus) stylopized by female of *L. bishoppi*, lateral view **B** detail of host abdomen with adult female inside **C** ventral side of cephalothorax **D** dorsal side of cephalothorax.

Diagnosis of male cephalotheca. Differing from other genera in the following characters. Diameter of genae between maxillary base and compound eye at least 2× as large as diameter of vestigial antenna. Distinct paired furrow of supra-antennal sensillary field absent. Cephalotheca always of elliptic shape (Fig. 27A). Frontal fissure very distinct (Fig. 27D). Maxilla prominent, at least 1.5× longer than basally wide (Fig. 27E).

Description of male cephalotheca. Shape and coloration. In frontal view rounded laterally, elliptic, in lateral view pointed anteriorly. Coloration with pattern of pale and dark shades.

Cephalothecal capsule. Compound eyes with darker individual ommatidia well visible on pale ocular background. Clypeal lobe arcuate in frontal view, prominent in lateral view. Clypeal sensilla mainly concentrated medially on clypeus. Frontal region slightly deformed by frontal impression (Fig. 27D). Occipital bulge present

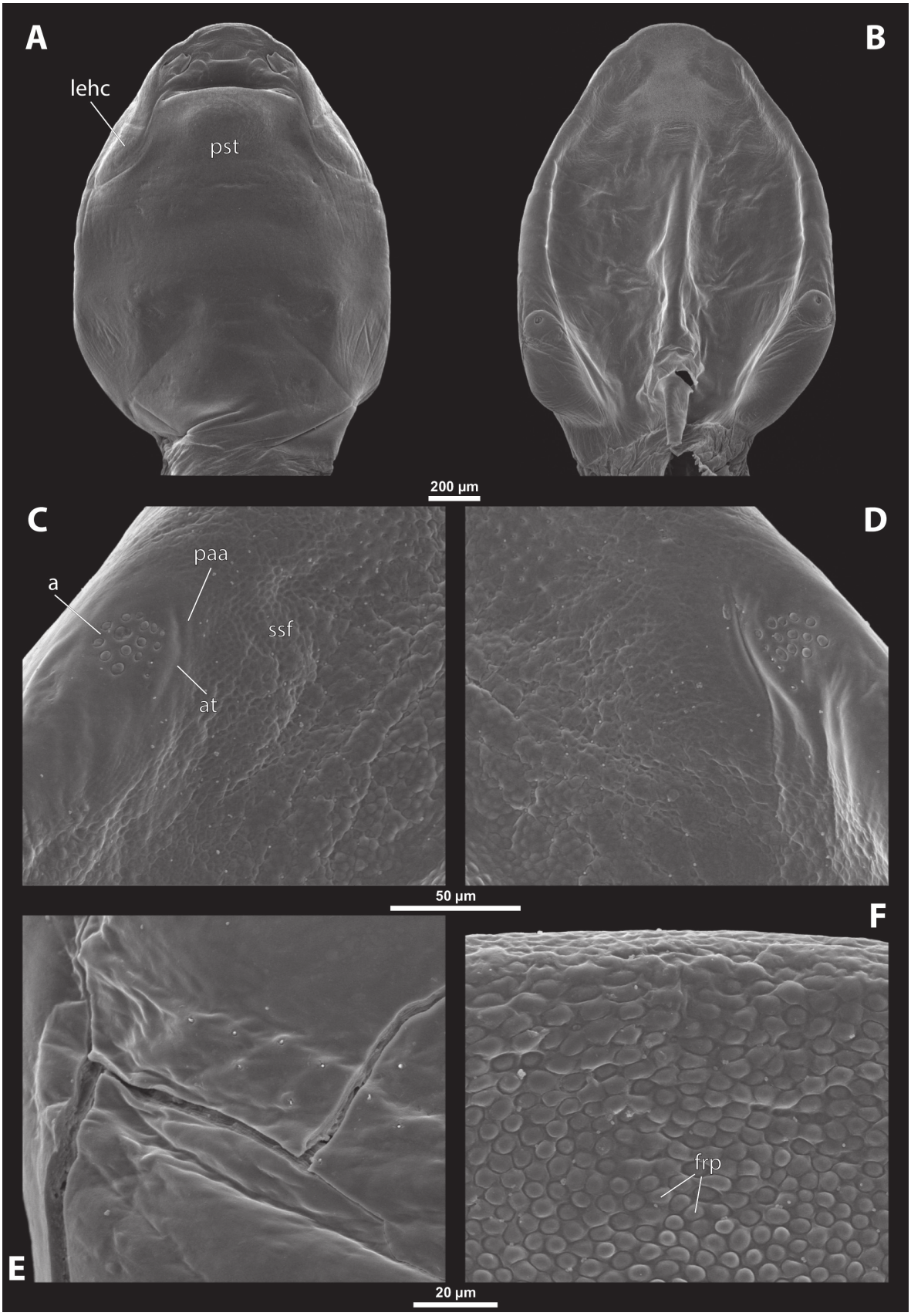

Figure 25. *Leionotoxenos bishoppi* (Pierce), female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** left vestigial antenna, dorsal side **D** right vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** detail of anterior border of cephalothorax, dorsal side. Abbreviations: a – vestigial antenna, at – antennal torulus, frp – frontal papillae, lehc – lateral extension of head capsule, paa – periantennal area, pst – prosternum (prosternal extension), ssf – supra-antennal sensillary field.

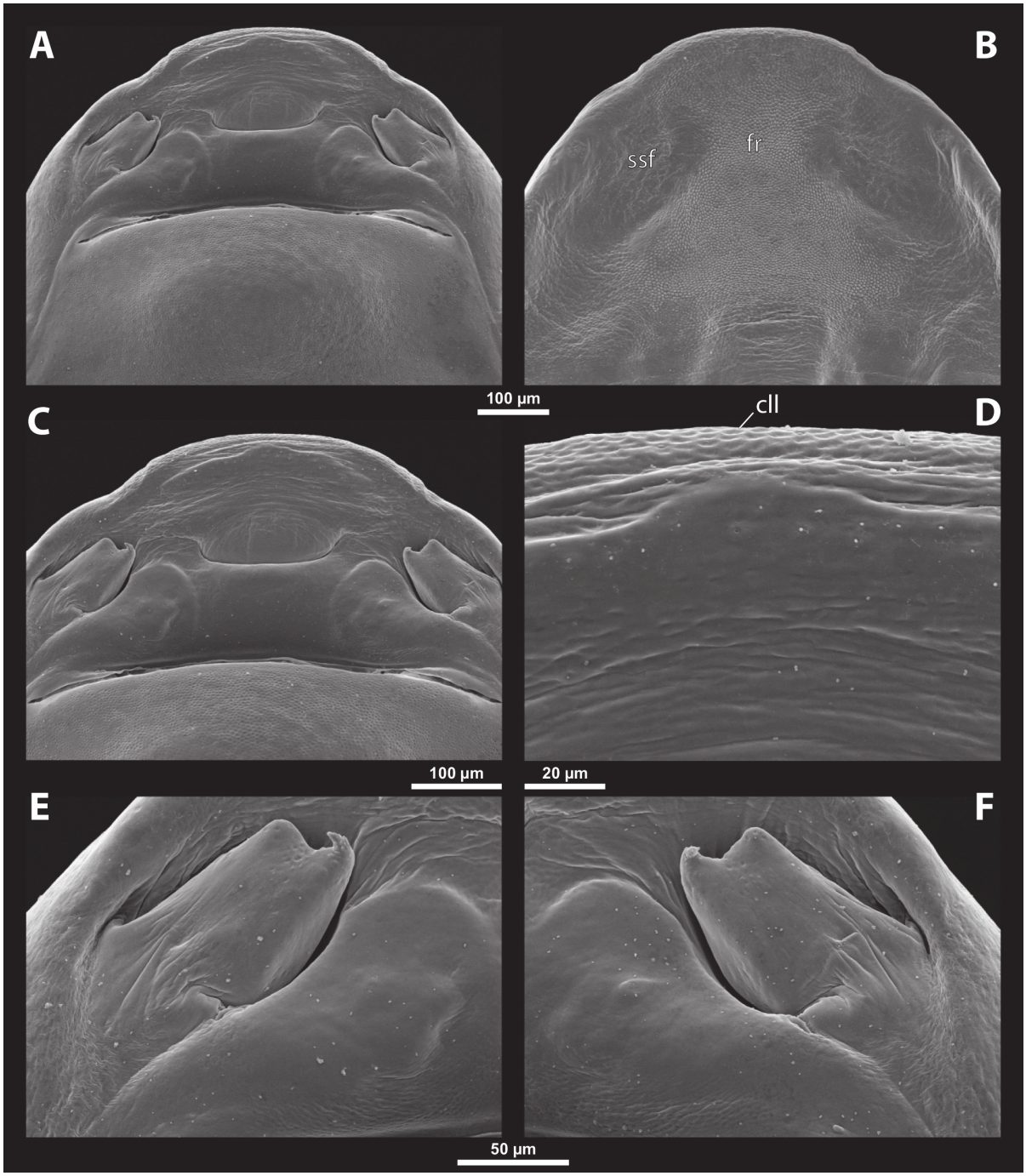

Figure 26. *Leionotoxenos bishoppi* (Pierce), female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: cll – clypeal lobe, fr – frontal region, ssf – supra-antennal sensillary field.

(Fig. 27D). Diameter of genae between maxillary base and compound eye very large, ~ > 3× as large as diameter of vestigial antenna.

Supra-antennal sensillary field. Kidney-shaped and bulging, medially delimited by frontal impression, lacking distinctly visible furrows.

Antenna. Of standard shape, small, with complete torulus and small plates (Fig. 27C). Periantennal area not clearly delimited from supra-antennal sensillary field.

Labrum. Labral area distinct. Setae on dorsal field present.

Mandible. Anteromedially directed. Tooth pointed apically, not reaching area of mandibular bulge basally. Bulge set with sensilla.

Maxilla. Distinct, prominent, entirely dark. Vestige of palp distinct.

Labium and hypopharynx. Distinct, dark, inserted between and below maxillae. Praementum and postmentum clearly separated by furrow. Hypopharyngeal protuberance not present.

Mouth opening. Distinctly arcuate but not well visible, covered by ventral labral field.

Phylogenetic relationships. According to Benda et al. (2019) part of a clade of a New World origin, with *Eupathocera* Pierce as sister group.

Diversity and distribution. Fourteen described species, restricted to the New World.

Hosts. Various genera of Odynerini (Vespidae: Eumeninae).

Comments. The genus *Leionotoxenos* was described by Pierce (1909) based on his suggestion that a new genus of Strepsiptera should be established if it utilizes a different host genus. No diagnosis or description was presented. It was later synonymized with *Pseudoxenos* (Bohart 1937) and then with *Paraxenos* (Kinzelbach 1971b). We restore *Leionotoxenos* from synonymy and classify it as a valid genus, based on the molecular phylogeny (Benda et al. 2019, 2021) and morphological characters newly reported here. We classify the names *Monobiaphila* and *Montezumiaphila* as synonyms of *Leionotoxenos*.

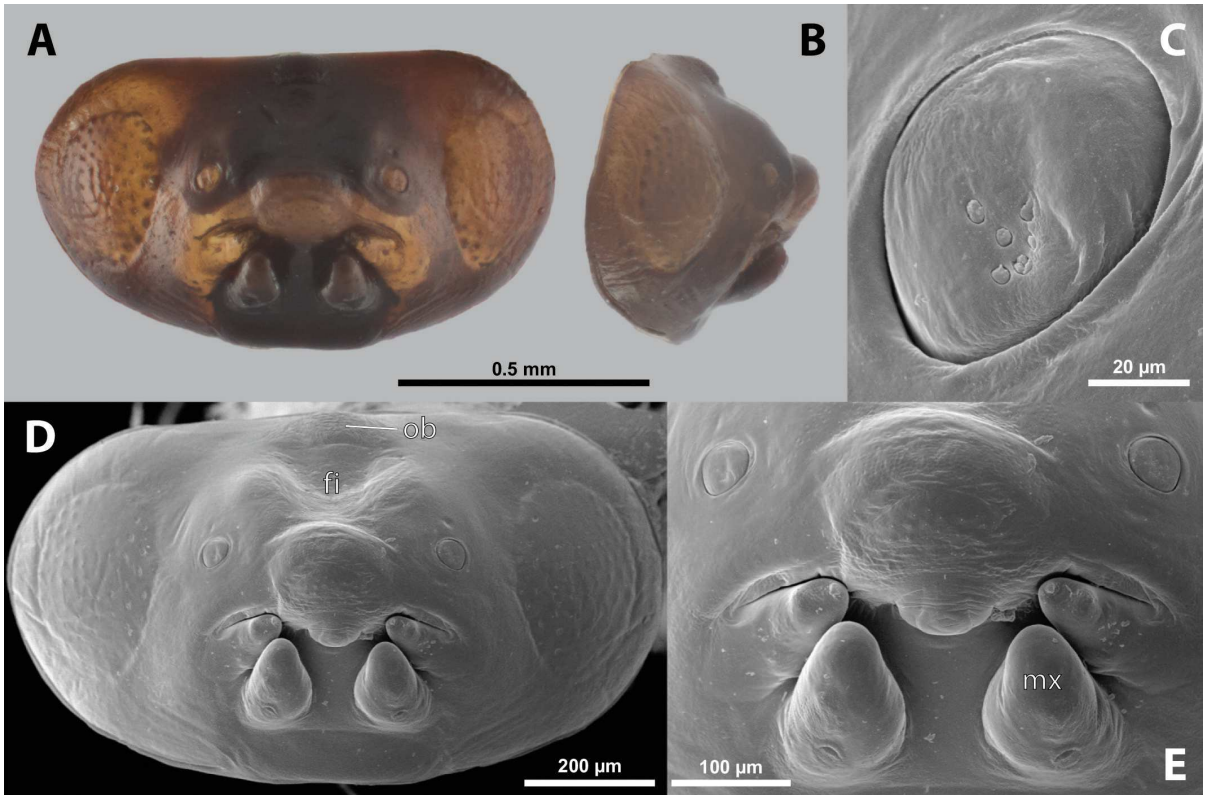

Figure 27. *Leionotoxenos* sp., male, cephalotheca, photomicrographs, SEM micrographs **A** frontal view **B** lateral view **C** vestigial antenna **D** frontal view **E** mouthparts. Abbreviations: fi – frontal impression, mx – vestige of maxilla, ob – occipital bulge.

List of species

***Leionotoxenos arvensidis* (Pierce, 1911), comb. nov.**

Pseudoxenos arvensidis Pierce, 1911: 499.

Hosts. *Euodynerus annulatus arvensis* (Saussure, 1869) (as *Odynerus* (*Leionotus*) *arvensis* Saussure, 1869) (Pierce 1911), *Euodynerus annulatus sulphureus* (Saussure, 1858) (Kinzelbach 1971b).

Distribution. USA: Illinois (Pierce 1911).

***Leionotoxenos bishoppi* (Pierce, 1909), comb. nov.**

Monobiaphila bishoppi Pierce, 1909: 139.
Pseudoxenos bishoppi (Pierce, 1909) (new combination by Bohart 1941).

Host. *Monobia quadridens* (Linnaeus, 1763) (Pierce 1909).

Distribution. USA: Texas (Pierce 1909), Kansas, Pennsylvania (this study).

***Leionotoxenos foraminati* (Pierce, 1911), comb. nov.**

Pseudoxenos foraminati Pierce, 1911: 499.

Host. *Euodynerus foraminatus* (Saussure, 1853) (as *Odynerus foraminatus* Saussure, 1853) (Pierce 1911).

Distribution. USA: New Jersey (Pierce 1911).

***Leionotoxenos fundati* (Pierce, 1911), comb. nov.**

Pseudoxenos fundati Pierce, 1911: 500.

Host. *Stenodynerus proquinquus* (Saussure, 1870) (as *Odynerus* (*Leionotus*) *fundatus* Cresson, 1872) (Pierce 1911).

Distribution. USA: Ilinois (Pierce 1911).

***Leionotoxenos hookeri* Pierce, 1909, stat. res.**

Leionotoxenos hookeri Pierce, 1909: 139.
Pseudoxenos hookeri (Pierce, 1909) (new combination by Bohart 1937).

Hosts. *Euodynerus annulatus* (Say, 1824) (as *Leionotus verus* (Cresson, 1872)) (Pierce 1909), *Euodynerus foraminatus* (Saussure, 1853) (Krombein 1967).

Distribution. USA: Texas (Pierce 1909).

***Leionotoxenos huastecae* (Székessy, 1965), comb. nov.**

Pseudoxenos huastecae Székessy, 1965: 477.

Host. *Montezumia centralis* Zavattari, 1912 (as *Montezumia huasteca* var. *centralis* Zavattari, 1912) (Székessy 1965).

Distribution. Honduras (Székessy 1965).

***Leionotoxenos itatiaiae* (Trois, 1984b), comb. nov.**

Pseudoxenos itatiaiae Trois, 1984b: 25.

Host. *Eumenes* sp. (Trois 1984b).

Distribution. Brazil, Rio de Janeiro (Trois 1984b).

Note. Probably misidentification of host. *Eumenes* does not occur in South America.

***Leionotoxenos jonesi* Pierce, 1909, stat. res.**

Leionotoxenos jonesi Pierce, 1909: 138.
Pseudoxenos jonesi (Pierce, 1909) (new combination by Bohart 1937).

Host. *Parancistrocerus vagus* (Saussure, 1857) (as *Leionotus colon* (Cresson, 1872)) (Pierce 1909).

Distribution. USA: Louisiana, Texas (Pierce 1909).

***Leionotoxenos louisianae* Pierce, 1909, stat. res.**

Leionotoxenos louisianae Pierce, 1909: 138.
Pseudoxenos louisianae (Pierce, 1909) (new combination by Bohart 1937).
Pseudoxenos histrionis Pierce, 1911: 500 (synonymized by Bohart 1941).
Pseudoxenos pedestridis Pierce, 1911: 500 (synonymized by Bohart 1941).

Hosts. *Parancistrocerus vagus* (Saussure, 1857) (as *Leionotus vagans* Saussure, 1857); *Parancistrocerus histrio* (Lepeletier, 1841) (as *Odynerus* (*Ancistrocerus*) *histrio* Lepeletier, 1841); *Parancistrocerus pedestris* (Saussure, 1855) (as *Odynerus* (*Leionotus*) *pedestris* Saussure, 1855) (Pierce 1909, 1911).

Distribution. USA: Florida, Illinois, Louisiana, Nebraska (Pierce 1909, 1911).

***Leionotoxenos neomexicanus* (Pierce, 1919), comb. nov.**

Pseudoxenos neomexicanus Pierce, 1919: 463.

Host. *Stenodynerus toas* (Cresson, 1867) (as *Odynerus taos* Cresson, 1867) (Pierce 1919).
Distribution. USA: New Mexico (Pierce 1919).

***Leionotoxenos prolificum* (Teson & Remes Lenicov, 1979), comb. nov.**

Pseudoxenos prolificum Teson & Remes Lenicov, 1979: 115.

Hosts. *Hypodynerus vespiformis* (Haliday, 1837), *Hypodynerus coarctatus* (Saussure, 1852), *Monobia cingulata* Brèthes, 1903 (Teson and Remes Lenicov 1979).
Distribution. Chile; Argentina: Salta (Teson and Remes Lenicov 1979).

***Leionotoxenos robertsoni* (Pierce, 1911), comb. nov.**

Pseudoxenos robertsoni Pierce, 1911: 501.

Host. *Stenodynerus histrionalis* (Robertson, 1901) (as *Odynerus* (*Ancistrocerus*) *histrionalis* Robertson, 1901) (Pierce 1911).
Distribution. USA: Illinois (Pierce 1911).

***Leionotoxenos tigridis* (Pierce, 1911), comb. nov.**

Pseudoxenos tigridis Pierce, 1911: 501.

Host. *Ancistrocerus adiabatus* (Saussure, 1853) (as *Odynerus* (*Ancistrocerus*) *tigris* Saussure, 1853) (Pierce 1911).
Distribution. USA: Illinois (Pierce 1911).

***Leionotoxenos vigili* (Brèthes, 1923), comb. nov.**

Montezumiaphila vigili Brèthes, 1923: 45.
Pseudoxenos vigili (Brèthes, 1923) (new combination by Kinzelbach 1971b).

Host. *Montezumia bruchii* Brèthes, 1903 (as *Montezumia vigilii* Brèthes, 1910) (Brèthes 1923).
Distribution. Argentina: Córdoba (Brèthes 1923); Venezuela (this study).

Eupathocera Pierce, 1908, stat. res.

Eupathocera Pierce, 1908: 79. Type species: *Eupathocera lugubris* Pierce, 1908, by original designation.
Pseudoxenos Saunders, 1872 (partim!) (synonymy proposed by Bohart 1937: 133).
Paraxenos Saunders, 1872 (partim!) (synonymy proposed by Kinzelbach 1971b: 162).
Homilops Pierce, 1908: 80 (syn. nov.). Type species: *Xenos westwoodii* Templeton, 1838, by subsequent designation.
Sceliphronechthrus Pierce, 1909: 141 (syn. nov.). Type species: *Sceliphronechthrus fasciati* Pierce, 1909, by original designation.
Ophthalmochlus Pierce, 1909: 142 (syn. nov.). Type species: *Ophthalmochlus duryi* Pierce, 1909, by original designation.
Ophthalmochlus (*Isodontiphila*) Pierce, 1919: 465 (syn. nov.). Type species: *Ophthalmochlus auripedis* Pierce, 1911.

Diagnosis of female cephalothorax. Differing from its sister genus *Leionotoxenos* by the shape of the periantennal area and the microstructure of the frontal area. Periantennal area expanded, sometimes raised, smooth (Fig. 29C). Distance between antennal area and supra-antennal sensillary field relatively large. Frontal region smooth or indistinctly wrinkled (Fig. 30B). Prosternum of most species of *Eupathocera* distinctly elevated above head medially and laterally, but apparently flat in *Leionotoxenos* (Fig. 29A). Rudiments of antennal torulus usually preserved (Fig. 29D). Sensilla restricted to clypeal lobe, not extended to ventral side of clypeal area. Mandible not protruding from capsule. In contrast to *Paragioxenos*, head and prothorax ventrally delimited by birth opening medially and by suture laterally.

Description of female cephalothorax. Shape and coloration. Compact, variable in shape, longer than wide to nearly as long as wide. Abdominal segment I sometimes protruding laterally, forming corner below spiracles (Fig. 28D). Very variable in size, length 1.02–2.47 mm, maximum width 0.88–2.5 mm. Anterior head margin evenly rounded or slightly protruding. Thorax slightly widening posteriorly. Coloration variable, with mostly dark or light brown pattern, but also patterns of multiple brown shades.

Head capsule. Ca. ¼ ~ ⅖ as long as entire cephalothorax including lateral cephalic extensions. Coloration rather pale to dark or forming specific patterns. Clypeal area well defined or not well delimited from labral area, with indistinct or slightly protruding clypeal lobe. Surface varying from wrinkled, lamellar, with scarcely visible sensilla, to completely smooth with distinctly exposed sensilla. Number of clypeal sensilla 20–80 or even more. Border between clypeal and frontal region clearly recognizable or indistinct but still present. Frontal region smooth or indistinctly wrinkled (Fig. 30B). Segmental border between head and prothorax distinct or only faintly recognizable on dorsal side.

Supra-antennal sensillary field. Smooth or slightly wrinkled, with dispersed sensilla (Fig. 29D). Not distinctly delimited by furrow medially, but border marked by different surface structure of supra-antennal sensillary field and smooth frontal region (Fig. 30B).

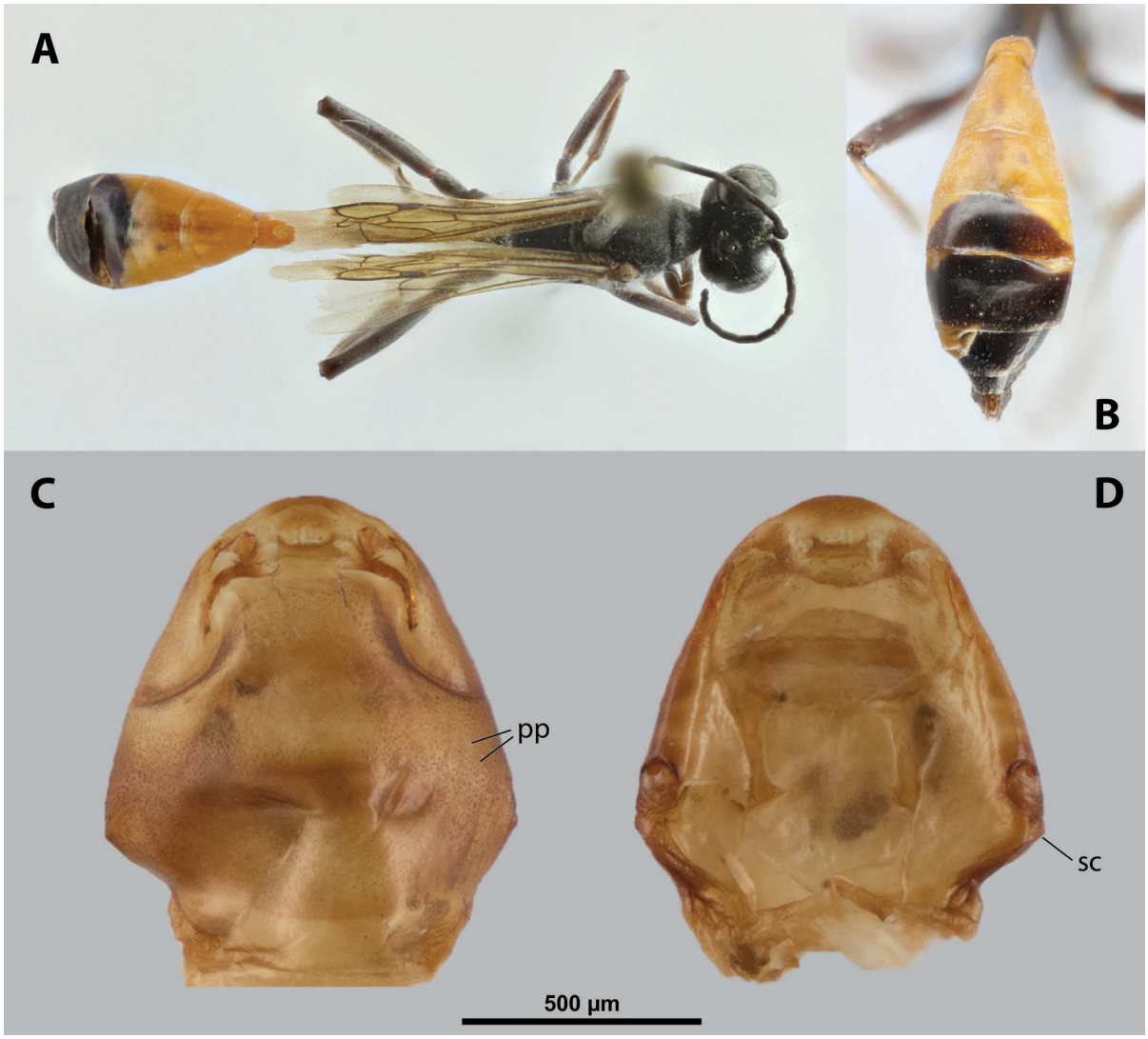

Figure 28. *Eupathocera luctuosae* (Pierce), host, female, cephalothorax, photomicrographs **A** *Ammophila* sp. stylopized by female of *E. luctuosae*, lateral view **B** detail of host abdomen with adult female inside **C** ventral side of cephalothorax **D** dorsal side of cephalothorax. Abbreviations: pp – pigmented papillae, sc – spiracular corner.

Antenna. Preserved as more or less clearly defined area. Antennal torulus usually reduced, preserved as interrupted furrow (Fig. 29D). Periantennal area expanded, sometimes raised, smooth (Fig. 29C). Distance between antennal area and supra-antennal sensillary field relatively large.

Labrum. Ventral field wider than long, elliptic to nearly circular. Dorsal labral field slightly arcuate, at least 4× wider than long in midline. Setae on dorsal field conspicuous, ~ 10–22.

Mandible. Anteromedially directed at an angle of 30–55°, enclosed in mandibular capsule. Mandibular bulge more or less distinctly raised, with ~ 5 indistinct sensilla. Cuticle of mandible smooth with longitudinal grooves or sculptured. Mandibular tooth narrow or slightly widened, with or without spines.

Maxilla. Reduced and not distinctly protruding, not projecting beyond mandible anteriorly. Partially fused to labial area, both regions often not clearly separated.

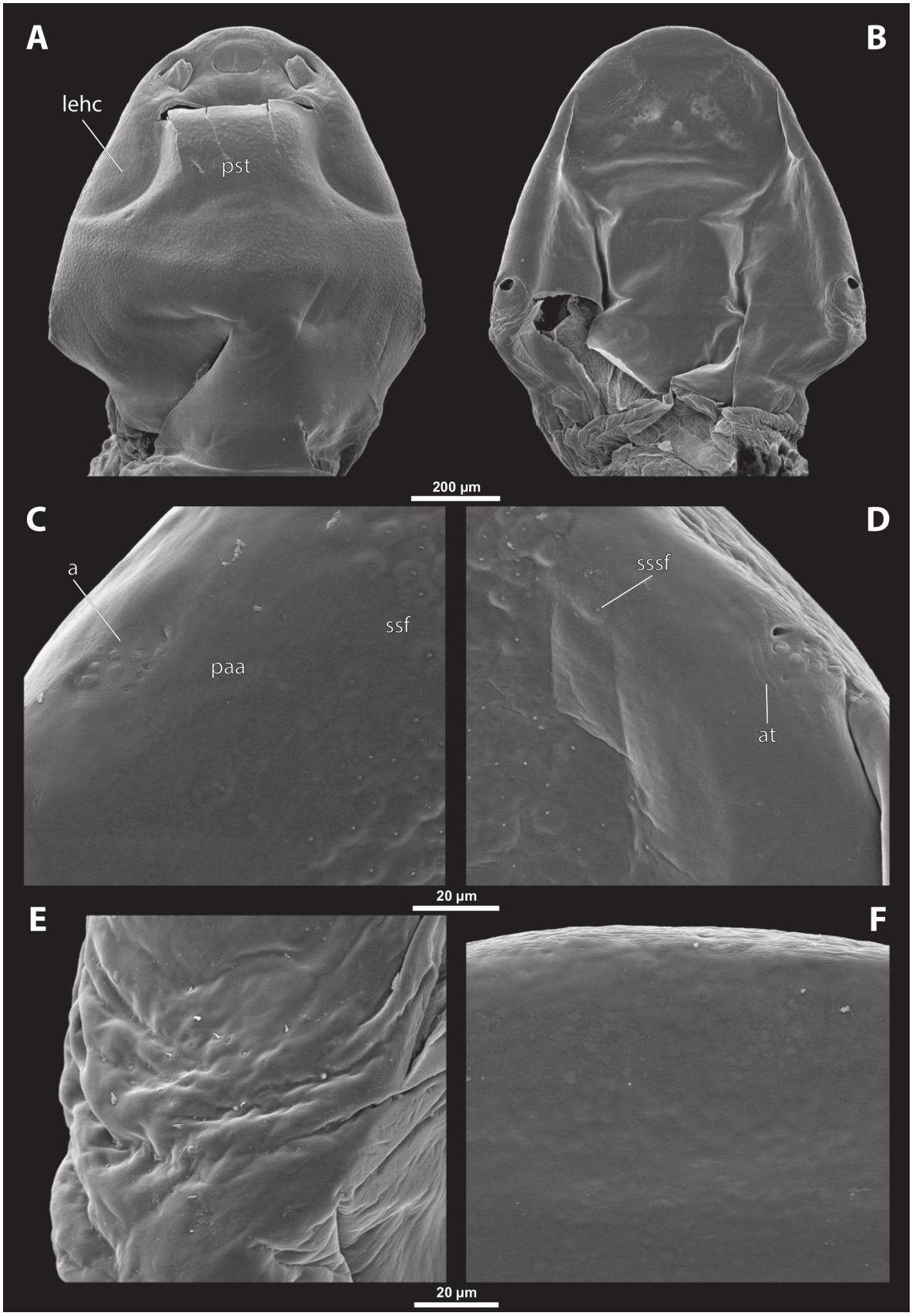

Figure 29. *Eupathocera luctuosae* (Pierce), female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** left vestigial antenna, dorsal side **D** right vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** detail of anterior border of cephalothorax, dorsal side. Abbreviations: a – vestigial antenna, at – antennal torulus, lehc – lateral extension of head capsule, paa – periantennal area, pst – prosternum, ssf – supra-antennal sensillary field, sssf – sensillum of supra-antennal sensillary field.

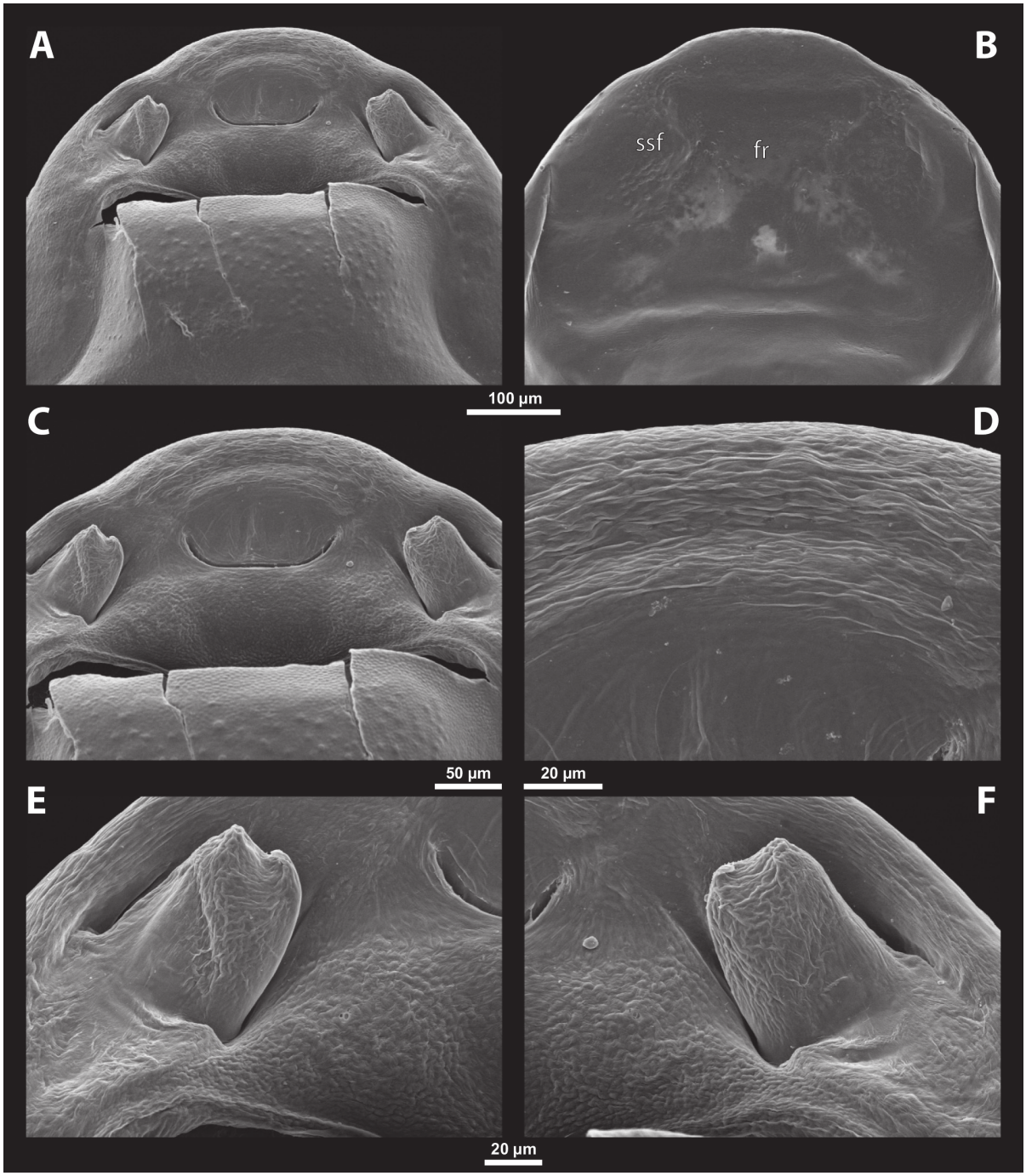

Figure 30. *Eupathocera luctuosae* (Pierce), female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: fr – frontal region, ssf – supra-antennal sensillary field.

Cuticle wrinkled or reticulated, in some cases with smooth areas. Vestige of palp inconspicuous, forming small bulge, sometimes very indistinct, located anteriorly or medially on ventral side of maxilla. Submaxillary groove indistinctly produced posteriorly to maxillary base.

Labium. Labial area more or less distinctly recognizable between maxillae, flat, longer than wide in midline or as long as wide. Anteriorly delimited by mouth opening, posteriorly by birth opening. Cuticular surface smooth or slightly reticulated.

Mouth opening. More or less arcuate, sclerotized along margin.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders variable, distinct or indistinct, usually indicated by mesal furrows, often combined with pigmented stripes. Border between metathorax and abdomen usually marked by change in cuticular surface structure or pigmentation. Cuticle of thoracic segments reticulate on ventral side, often with scattered small, pigmented papillae. Dorsal side of thorax smooth or slightly wrinkled. Prosternal extension undifferentiated, or anteriorly with specific color pattern. Prosternum distinctly elevated above head medially and laterally in most species (Fig. 29A). Shape of meso- and metathorax unmodified, transverse. Setae and cuticular spines present on lateral region of abdominal segment I (Fig. 29E).

Spiracles. Spiracles on posterior ~ ⅓ of cephalothorax slightly elevated, with anterolateral or anterodorsal orientation.

Diagnosis of male cephalotheca. Differing from other genera in the following characters. Diameter of genae between maxillary base and compound eye at least 2× as large as diameter of vestigial antenna. Paired furrow of supra-antennal sensillary field indistinct or absent. Cephalotheca usually of nearly circular shape (Fig. 31A). Antennal diameter ca. as long as width of mandible (Fig. 31E). Mandible directed anteromedially.

Description of male cephalotheca. Shape and coloration. In frontal view rounded, nearly circular, in lateral view pointed anteriorly. Coloration with a pattern of dark and slightly paler shades.

Cephalothecal capsule. Compound eyes with dark individual ommatidia well visible on paler ocular background. Very conspicuous clypeal lobe straight in frontal view, prominent in lateral view, bulging. Sensilla mainly concentrated on clypeal lobe. Frontal impression indistinct. Occipital bulge absent. Diameter of genae between maxillary base and compound eye large, > 2× as large as diameter of vestigial antenna.

Supra-antennal sensillary field. Kidney-shaped and bulging, delimited medially by weakly developed frontal impression. Distinct furrows not visible.

Vestigial antenna. Of standard shape, small, sometimes with incomplete torulus, and with small plates or cavities (Fig. 31C). Periantennal area not clearly delimited from supra-antennal sensillary field.

Labrum. Labral area distinct, with setae on dorsal field.

Mandible. Anteromedially directed. Tooth pointed, not reaching area of mandibular bulge basally. Bulge with sensilla.

Maxilla. Distinct, prominent, completely dark. Vestige of palp distinct.

Labium and hypopharynx. Labium distinct between and below maxillae, dark. Praementum and postmentum indistinctly separated by furrow. Hypopharyngeal protuberance absent.

Mouth opening. Poorly visible, partially covered by ventral labral field, arcuate.

Phylogenetic relationships. According to Benda et al. (2019) part of a clade of a New World origin, also containing *Leionotoxenos* Pierce.

Diversity and distribution. Including 16 valid species, restricted to the New World.

Hosts. Various wasps from three families, but mostly sphecids (Sphecidae: Sphecinae, Ammophilinae), rarely *Tachytes* (Crabronidae: Crabroninae) and *Zethus* (Vespidae: Zethinae).

Comments. The genus *Eupathocera* was described by Pierce (1908) based on his concept that a new genus of Strepsiptera should be established if it utilizes a different host genus. The description of the male was too short and superficial. It was later synonymized with *Pseudoxenos* (Bohart 1937) and then with *Paraxenos* (Kinzelbach 1971b). We restore *Eupathocera* from synonymy and classify it as a valid genus, based on the molecular phylogeny (Benda et al. 2019, 2021) and morphological characters newly reported here. We classify the names *Ophthalmochlus*, *Ophthalmochlus* (*Isodontiphila*), *Homilops*, and *Sceliphronechthrus* as synonyms of *Eupathocera*. Based on morphological characters, species parasitising *Pachodynerus* (Vespidae) were assigned to *Eupathocera*.

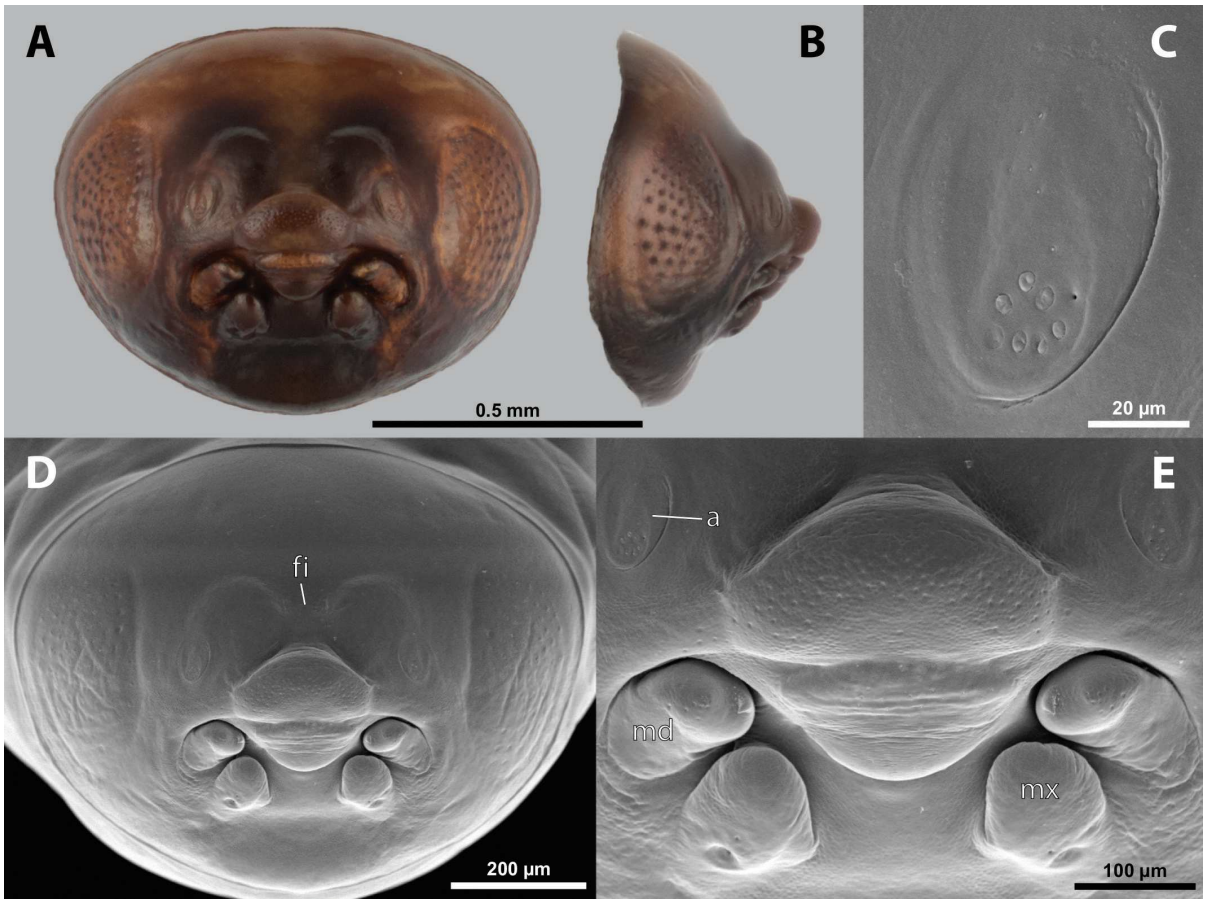

Figure 31. *Eupathocera* cf. *inclusa* (Oliveira & Kogan), male, cephalotheca, photomicrographs, SEM micrographs **A** frontal view **B** lateral view **C** vestigial antenna **D** frontal view **E** mouthparts. Abbreviations: a – vestigial antenna, fi – frontal impression, md – mandible, mx – vestige of maxilla.

List of species

***Eupathocera argentina* (Brèthes, 1923), comb. nov.**

Ophthalmochlus (*Homilops*) *argentinus* Brèthes, 1923: 52.
Pseudoxenos argentinus (Brèthes, 1923) (new combination by Bohart 1937).
Paraxenos argentinus (Brèthes, 1923) (new combination by Kinzelbach 1971b).

Host. *Prionyx thomae* (Fabricius, 1775) (as *Proterosphex platensis* Brèthes, 1908) (Brèthes 1923).

Distribution. Argentina: Buenos Aires (Brèthes 1923).

***Eupathocera auripedis* (Pierce, 1911), comb. nov.**

Ophthalmochlus auripedis Pierce, 1911: 503.
Pseudoxenos auripedis (Pierce, 1911) (new combination by Bohart 1937).
Paraxenos auripedis (Pierce, 1911) (new combination by Kinzelbach 1971b).

Hosts. *Isodontia auripes* (Fernald, 1906) (Pierce 1911); *Isodontia mexicana* (Saussure, 1867) (Benda et al. 2021).

Distribution. USA: Maryland (Pierce 1911).

***Eupathocera bucki* (Trois, 1984a), comb. nov.**

Paraxenos bucki Trois, 1984a: 16.

Host. *Ammophila* sp. (Trois 1984a).

Distribution. Brazil (Trois 1984a).

***Eupathocera duryi* (Pierce, 1909), comb. nov.**

Ophthalmochlus duryi Pierce, 1909: 142.
Ophthalmochlus duryi Pierce, 1908: nomen nudum.
Pseudoxenos duryi (Pierce, 1909) (new combination by Bohart 1937).
Paraxenos duryi (Pierce, 1909) (new combination by Kinzelbach 1971b).

Host. *Prionyx atratus* (Lepeletier, 1845) (as *Priononyx atrata* Lepeletier, 1845) (Pierce 1909).

Distribution. USA: Ohio (Pierce 1909).

***Eupathocera erynnidis* (Pierce, 1911), comb. nov.**

Pseudoxenos erynnidis Pierce, 1911: 499.

Host. *Pachodynerus erynnis* (Lepeletier, 1941) (as *Odynerus erynnys* Lepeletier, 1941) (Pierce 1911).

Distribution. USA: Florida (Pierce 1911), Colorado (this study).

Note. This species has an lineage with unclear phylogenetic position (Benda et al. 2021). It is provisionally assigned to *Eupathocera* based on morphological characters. A more comprehensive sampling and a detailed study are necessary for a reliable classification of this taxon.

***Eupathocera fasciati* (Pierce, 1909), comb. nov.**

Sceliphronechthrus fasciati Pierce, 1909: 141.
Pseudoxenos fasciati (Pierce, 1909) (new combination by Bohart 1937).
Paraxenos fasciati (Pierce, 1909) (new combination by Kinzelbach 1971b).

Host. *Sceliphron fasciatum* (Lepeletier, 1845) (as *Sceliphron* (*Pelopaeus*) *fasciatus* Lepeletier, 1845) (Pierce 1909).

Distribution. Dominican Republic: Santo Domingo (Pierce 1909).

***Eupathocera fuliginosi* (Brèthes, 1923), comb. nov.**

Ophthalmochlus (*Homilops*) *fuliginosi* Brèthes, 1923: 49.
Pseudoxenos fuliginosi (Brèthes, 1923) (synonymy proposed by Bohart 1937).
Paraxenos fuliginosi (Brèthes, 1923) (synonymy proposed by Kinzelbach 1971b).

Hosts. *Sphex servillei* Lepeletier, 1845 (as *Proterosphex fuliginosus* Dahlbom, 1843) (Brèthes 1923); *Sphex argentinus* Taschenberg, 1869 (Benda et al. 2021).

Distribution. Argentina: Tucumán (Brèthes 1923).

***Eupathocera inclusa* (Oliveira & Kogan, 1963), comb. nov.**

Pseudoxenus inclusus Oliveira & Kogan, 1963: 351.
Paraxenos inclusus (Brèthes, 1923) (new combination by Kinzelbach 1971b).

Host. *Ammophila* sp. (Oliveira and Kogan 1963).

Distribution. Brazil: Espírito Santo (Oliveira and Kogan 1963).

***Eupathocera insularis* (Kifune, 1983), comb. nov.**

Pseudoxenos insularis Kifune, 1983: 335.

Host. *Pachodynerus cinerascens* (Fabricius, 1775) (Kifune 1983).

Distribution. Virgin Islands (Kifune 1983).

Note. As *Eupathocera erynnidis* this species has an unclear phylogenetic position (Benda et al. 2021). It is also provisionally included in the genus *Eupathocera* Pierce, 1908, stat. res. based on morphological evidence.

***Eupathocera luctuosae* Pierce, 1911, stat. res.**

Eupathocera luctuosae Pierce, 1911: 502.
Pseudoxenos luctuosae (Brèthes, 1923) (new combination by Bohart 1937).
Paraxenos luctuosae (Brèthes, 1923) (new combination by Kinzelbach 1971b).

Hosts. *Podalonia luctuosa* (F. Smith, 1856) (as *Sphex* (*Psammophila*) *luctuosa* F. Smith, 1856) (Pierce 1911); *Podalonia argentifrons* (Cresson, 1865); *Podalonia violaceipennis* (Lepeletier, 1845) (Kinzelbach 1971b).

Distribution. USA: Idaho, Colorado (Pierce 1911).

***Eupathocera lugubris* Pierce, 1909, stat. res.**

Eupathocera lugubris Pierce, 1909: 143.
Eupathocera lugubris Pierce, 1908: nomen nudum
Paraxenos lugubris (Pierce, 1908) (new combination by Kinzelbach 1971b).
Eupathocera pruinosae Pierce, 1909 (synonymized by Bohart 1941).
Eupathocera pictipennidis Pierce, 1911 (synonymized by Bohart 1941).
Eupathocera vulgaridis Pierce, 1911 (synonymized by Bohart 1941).

Hosts. *Ammophila aberti* Haldeman, 1852 (as *Sphex transversus* Ferdanand, 1934); *Ammophila arvensis* Lepeletier, 1845 (as *Sphex arvensis* (Dahlbom, 1843)); *Ammophila breviceps* F. Smith, 1856 (= *Sphex breviceps* (F. Smith, 1856)); *Ammophila extremitata* Cresson, 1865; *Ammophila fernaldi* (Murray, 1938); *Ammophila gracilis* Lepeletier, 1845 (as *Sphex* (*Ammophila*) *fragilis* (F. Smith, 1856)); *Ammophila kennedyi* (Murray, 1938) (as *Sphex* (*Ammophila*) *vulgaris* (Cresson, 1865)); *Ammophila nasalis* Provancher, 1895 (as *Sphex craspedotus* Fernald, 1934 and *S. nasalis* (Provancher, 1895)); *Ammophila pictipennis* Walsh, 1869 (as *Sphex* (*Ammophila*) *pictipennis* (Walsh, 1869)); *Ammophila pruinosa* Cresson, 1865 (as *Sphex* (*Ammophila*) *pruinosa* (Cresson, 1865)); *Ammophila urnaria* Dahlbom, 1843 (as *Sphex urnarius* (Dahlbom, 1843)); *Eremnophila aureonotata*

(Cameron, 1888) (as *Sphex aureonotatus* (Cameron, 1888)) (Pierce 1909; Bohart 1941; Kathirithamby et al. 2012; Cook 2019).

Distribution. USA: Ohio, Colorado, Illinois, Iowa (Pierce 1909; Bohart 1941; Cook 2019).

Eupathocera mendozae (Brèthes, 1923), comb. nov.

Ophthalmochlus (*Homilops*) *mendozae* Brèthes, 1923: 51.
Pseudoxenos mendozae (Brèthes, 1923) (new combination by Bohart 1937).
Paraxenos mendozae (Brèthes, 1923) (new combination by Kinzelbach 1971b).

Host. *Prionyx neoxenus* (Kohl, 1890) (as *Priononyx neoxenus*, var. *melanogaster* Brèthes, 1910) (Brèthes 1923).

Distribution. Argentina: Mendoza (Brèthes 1923).

Eupathocera piercei (Brèthes, 1923), comb. nov.

Ophthalmochlus (*Homilops*) *piercei* Brèthes, 1923: 50.
Pseudoxenos piercei (Brèthes, 1923) (new combination by Bohart 1937).
Paraxenos piercei (Brèthes, 1923) (new combination by Kinzelbach 1971b).

Host. *Isodontia costipennis* (Spinola, 1851) (Brèthes 1923).

Distribution. Argentina: La Rioja (Brèthes 1923).

Eupathocera striati (Brèthes, 1923), comb. nov.

Ophthalmochlus (*Homilops*) Brèthes, 1923: 48.
Pseudoxenos striati (Brèthes, 1923) (new combination by Bohart 1937).
Paraxenos striati (Brèthes, 1923) (new combination by Kinzelbach 1971b).

Host. *Prionyx fervens* (Linnaeus, 1758) (as *Priononyx striatus* F. Smith, 1856) (Brèthes 1923).

Distribution. Argentina: Córdoba (Brèthes 1923).

Eupathocera taschenbergi (Brèthes, 1923), comb. nov.

Ophthalmochlus (*Homilops*) *taschenbergi* Brèthes, 1923: 47.
Pseudoxenos taschenbergi (Brèthes, 1923) (new combination by Bohart 1937).
Paraxenos taschenbergi (Brèthes, 1923) (new combination by Kinzelbach 1971b).

Host. *Prionyx pumilio* (Taschenberg, 1869) (as *Neosphex pumilio* (Taschenberg, 1869) (Brèthes 1923).

Distribution. Argentina: Mendoza (Brèthes 1923).

Eupathocera westwoodii (Templeton, 1841), comb. nov.

Xenos westwoodii Templeton, 1841: 53.
Pseudoxenos westwoodii (Templeton, 1841) (new combination by Bohart 1937).
Paraxenos westwoodii (Templeton, 1841) (new combination by Kinzelbach 1971b).
Paraxenos westwoodi (incorrect subsequent spelling): Kinzelbach (1971b).
Xenos smithii Heyden, 1867 (synonymized by Kinzelbach 1971b).
Homilops ashmeadi Pierce, 1909 (synonymized by Kinzelbach 1971b).
Pseudoxenos ashmeadi (Pierce, 1909) (new combination by Bohart 1937).
Homilops bishoppi Pierce, 1909 (synonymized by Kinzelbach 1971b).
Pseudoxenos bishoppi (Pierce, 1909) (new combination by Bohart 1937).

Hosts. *Sphex ichneumoneus* (Linnaeus, 1758) (as *Sphex aurocapillus* Templeton, 1841; *Sphex ichneumoneus aurifluus* Perty, 1838; *Proterosphex* (*Sphex*) *ichneumoneus* Linnaeus, 1758); unknown name (*Proterosphex* (*Sphex*) *pernanus* Kohl) (Pierce 1909); *Sphex pensylvanicus* Linnaeus, 1763 (Miller et al. 2010); *Tachytes* sp. (this study).

Distribution. Brazil: Rio de Janeiro (Templeton 1841); Dominican Republic: Santo Domingo; USA: Texas, Montana (Pierce 1909; Miller et al. 2010); Mexico (this study).

Macroxenos Schultze, 1925, stat. res.

Macroxenos Schultze, 1925: 238. Type species: *Macroxenos piercei* Schultze, 1925, by original designation.
Pseudoxenos Saunders, 1872 (partim!) (synonymy proposed by Bohart 1937).

Diagnosis of female cephalothorax. Maxilla reduced, not distinctly prominent (Fig. 34E). Two distinct dark spots present mesally on border between head and prothorax (Fig. 32D). Thoracic segments conspicuously sclerotized laterally from dorsal side (Fig. 32D). Lateral parts of abdomen posterior to spiracles always pale (Fig. 32D). Clypeal region bulging, very distinctly separated from labral area (Fig. 34D). Mandible not protruding from capsule. In contrast to *Paragioxenos*, head and prothorax ventrally delimited by birth opening medially and by suture laterally.

Description of female cephalothorax. Shape and coloration. Nearly as long as wide, or as long as or distinctly longer than wide. Very variable in size, length 0.8–1.82 mm, width 0.64–1.9 mm in midline. Anterior head margin evenly rounded or protruding. Thorax slightly or distinctly widening posteriorly. Cephalothorax with multiple brown shades forming distinct pattern.

Head capsule. Between ⅓ and > ½ × as long as entire cephalothorax including the lateral cephalic extensions. Coloration forming specific pattern with pale and dark shades. Clypeal region very distinctly delimited from labral area (Fig. 34D), arcuate, or protruding and forming clypeal lobe. Surface smooth or distinctly wrinkled. Sensilla mainly concentrated on clypeal lobe. Border between clypeal area and frontal region clearly indicated by change in cuticular surface. Cuticle of frontal area variable, distinctly wrinkled or covered with papillae. Border between head and prothorax usually distinct on dorsal side, delimited by transverse stripe of distinctive coloration and two distinct dark spots on mesal region (Fig. 32D).

Supra-antennal sensillary field. Smooth, with dispersed sensilla. Furrow between supra-antennal sensillary field and frontal region absent, or very indistinct and only indicated by change in cuticular sculpture (Fig. 34B).

Antenna. Preserved as poorly defined area, with several small, rounded plates, antennal sensilla, or cavity, in some cases all three combined. Periantennal area smooth or slightly wrinkled, sometimes indistinct.

Labrum. Ventral field wider than long, elliptic to nearly circular. Dorsal field arcuate, distinctly raised (Fig. 34D), sometimes very wide and narrow, ~ 5–8× wider than long in midline. Dorsal field with 14–41 (or more) setae or sensilla inserted in cavities.

Mandible. Anteromedially directed at angle of 30–35° and enclosed in mandibular capsule. Mandibular bulge distinctly raised, with several sensilla. Cuticle smooth or slightly sculptured, sometimes with longitudinal grooves (Fig. 34E). Tooth narrow or slightly widened, pointed apically or ventrally, more or less distinctly armed with spines.

Maxilla. Almost completely fused with labial area, or slightly raised (Fig. 34E), not projecting beyond mandible. Cuticle smooth or wrinkled. Vestige of palp present as cavity or poorly defined area; usually located medially on ventral side of maxilla (Fig. 34E). Submaxillary groove more or less distinctly produced anterolaterally to maxillary base.

Labium. Labial area between maxillae usually more or less distinct, delimited anteriorly by mouth opening and posteriorly by birth opening. Labial area wider than long in midline, flat or convex. Cuticular surface smooth or reticulated.

Mouth opening. Widely arcuate, sclerotized marginally.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders more or less distinct, usually separated by mesal furrows, rarely combined with pigmented stripes or spots on dorsal and ventral side. Border between metathorax and abdomen usually formed by ridge or indicated by change in cuticular sculpture. Cuticle of thoracic segments on ventral side reticulate, with scattered inconspicuous or more distinct pigmented papillae. Dorsal surface of thorax smooth or slightly reticulated. Prosternal extension undifferentiated or distinct, in some cases extremely elongated. Thoracic segments conspicuously sclerotized laterally from dorsal side (Fig. 32D). Shape of meso- and metathorax unmodified, transverse, or narrowed laterally in species with elongated head. Lateral parts of abdomen posterior to spiracles always pale (Fig. 32D). Setae present on lateral region of abdominal segment I (Fig. 33E, F).

Spiracles. Spiracles on posterior ~ ⅓ of cephalothorax slightly elevated, with anterodorsal and anterolateral orientation.

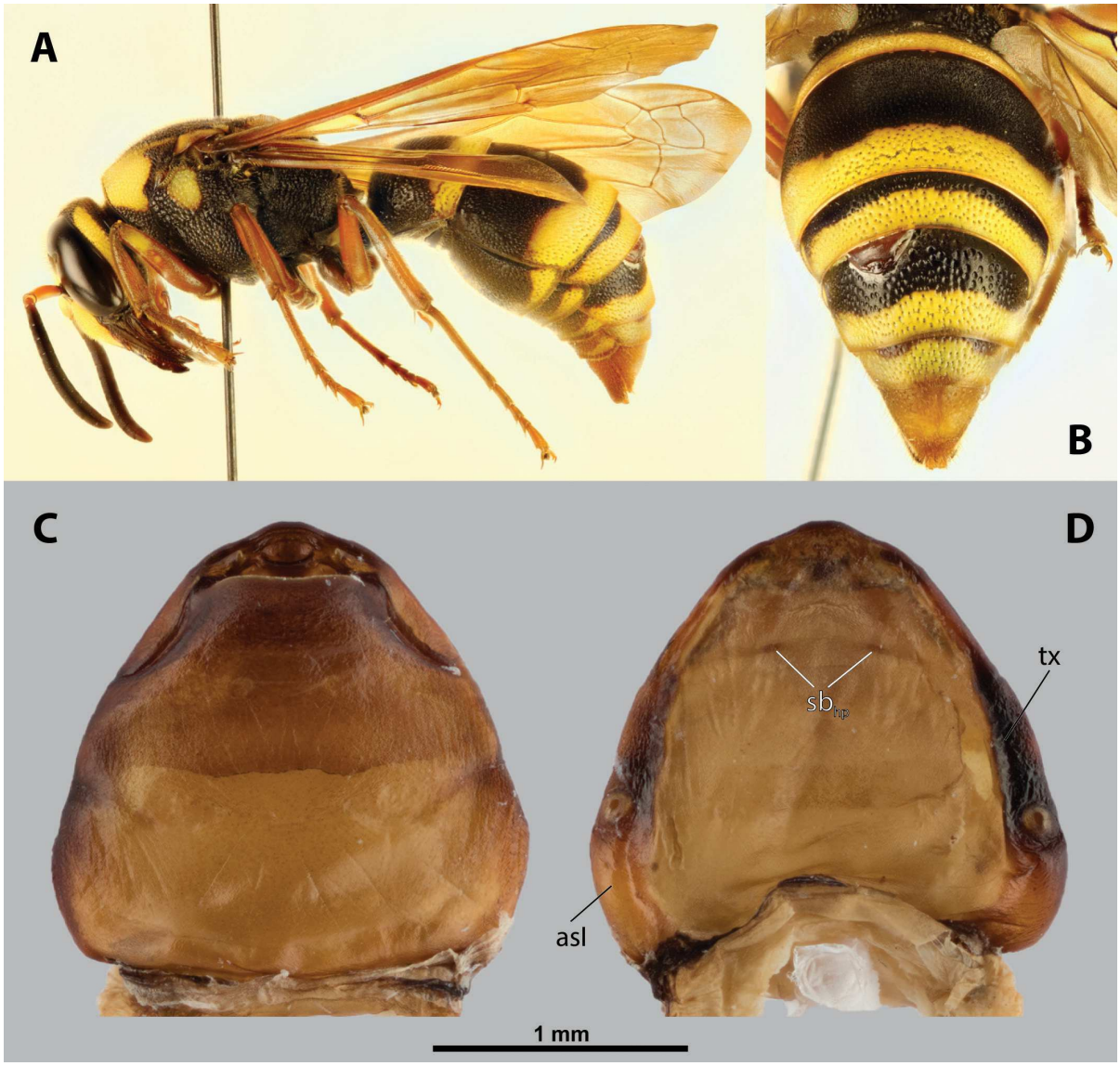

Figure 32. *Macroxenos* cf. *piercei*, host, female, cephalothorax, photomicrographs **A** *Anterhynchium flavomarginatum* stylopized by female of *M.* cf. *piercei*, lateral view **B** detail of host abdomen with adult female inside **C** ventral side of cephalothorax **D** dorsal side of cephalothorax. Abbreviations: asI – abdominal segment I, sbhp – segmental border between head and prothorax, tx – thorax.

Diagnosis of male cephalotheca. Male cephalotheca unknown.

Phylogenetic relationships. The phylogenetic position is unstable. Benda et al. (2019) revealed it as sister to a lineage including *Sphecixenos*, *Tuberoxenos*, and *Pseudoxenos* in our concept. In contrast, Benda et al. (2021) resolved its position as sister to a clade including *Sphecixenos*, *Tuberoxenos*, *Pseudoxenos*, *Deltoxenos*, and *Xenos*. In both cases, the support was very weak. Further phylogenomic investigations with robust data are needed to resolve the intergeneric relationships.

Diversity and distribution. A lineage of Australasian origin, with dispersion into the Indomalayan region (Benda et al. 2019). The two currently known species are restricted to these two biogeographic regions.

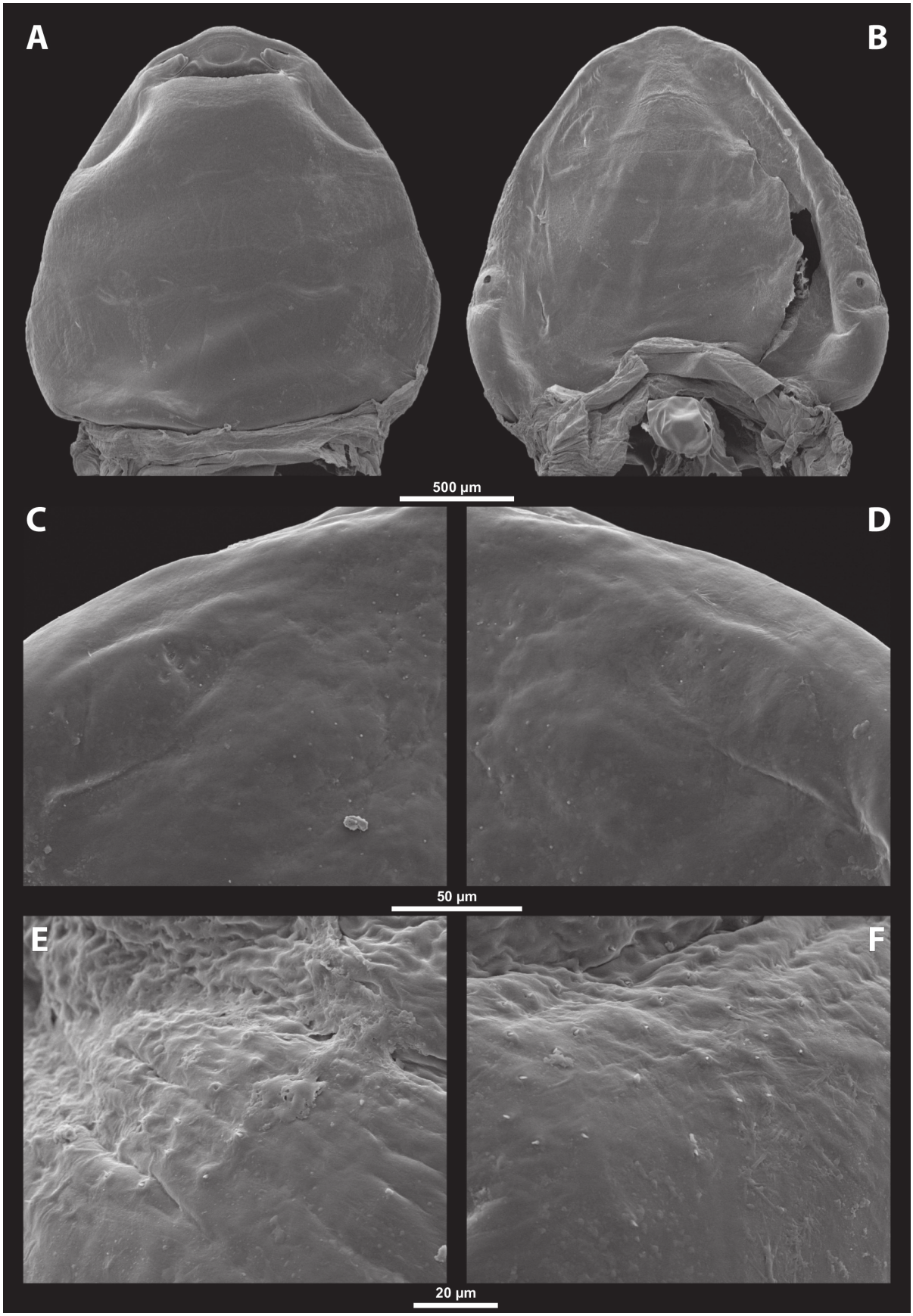

Figure 33. *Macroxenos* cf. *piercei*, female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** left vestigial antenna, dorsal side **D** right vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** right lateral border of abdominal segment I below spiracle, dorsal side.

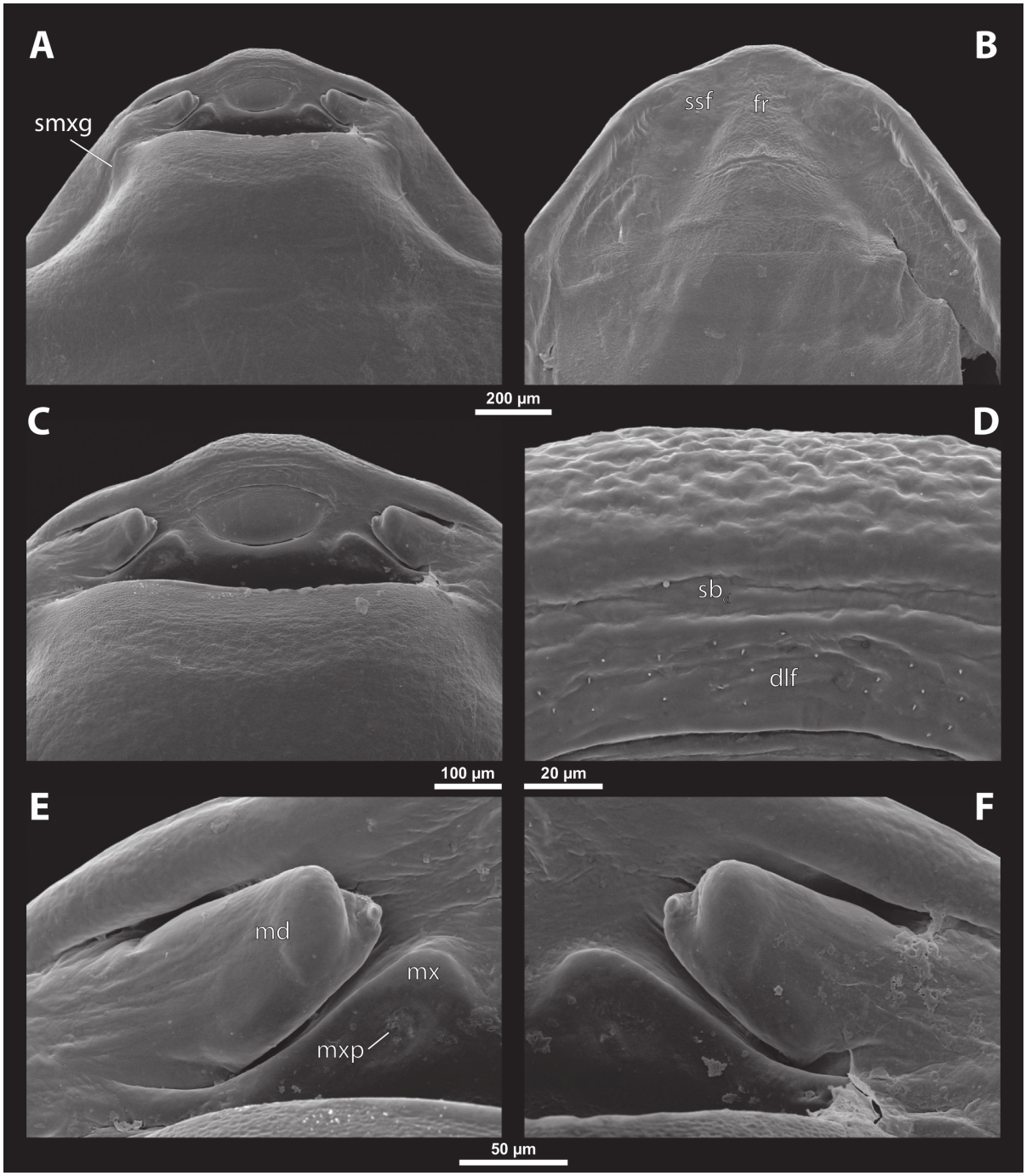

Figure 34. *Macroxenos* cf. *piercei*, female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: dlf – dorsal field of labral area, fr – frontal region, md – mandible, mx – vestige of maxilla, mxp – vestige of maxillary palp, sbcl – segmental border between clypeus and labrum, smxg – submaxillary groove, ssf – supra-antennal sensillary field.

Hosts. Various genera of Odynerini (Vespidae: Eumeninae).

Comments. The genus *Macroxenos* was described by Schultze (1925) but the descriptions of male and female was superficial. Later, Bohart (1937) synonymized it with *Pseudoxenos*. We classify this lineage as a separate genus, based on molecular phylogenies (Benda et al. 2019, 2021) and morphological characters newly reported here.

However, this genus is quite complicated to diagnose because of a high morphological variability of species. More samples are still needed for a better characterization and recognition of this formerly overlooked group.

List of species

***Macroxenos papuanus* (Székessy, 1956), comb. nov.**

Pseudoxenos papuanus Székessy, 1956: 149.

Host. *Allodynerus floricola* (Saussure, 1852) (as *Odynerus floricola* Saussure) (Székessy 1956).

Distribution. New Guinea (Székessy 1956).

Note. The occurrence of *Allodynerus* in New Guinea is unlikely. Host identity thus requires a confirmation. Although only *Macroxenos* is known from the Australasian region as parasitic lineage of Odynerini wasps, we decided to assign this species to this genus preliminarily, pending a more detailed study in the future.

***Macroxenos piercei* Schultze, 1925, stat. res.**

Macroxenos piercei Schultze, 1925: 238.
Pseudoxenos piercei (Schultze, 1925) (new combination by Bohart 1937).
Pseudoxenos schultzei Kifune & Maeta, 1965: 7 (synonymized by Kinzalbach 1971a).

Host. *Rhynchium atrum* Saussure, 1852 (Schultze 1925); *Rhynchium atrissimum* Vecht, 1968 (Kifune and Tano 1991).

Distribution. Philippines: Luzon (Schultze 1925), Mindanao (Kifune and Tano 1991).

Note. Kifune and Maeta (1965) proposed a new replacement name for *Macroxenos piercei* Schultze, 1925, a secondary homonym of *Ophthalmochlus piercei* Brèthes, 1923 (now *Eupathocera piercei* (Brèthes, 1923), comb. nov.) when both were placed in the same genus *Pseudoxenos*. *Macroxenos piercei* is reinstated here as a valid name following the Article 59.4 of ICZN (1999).

***Sphecixenos* gen. nov.**
http://zoobank.org/B5D80275-0542-40D3-B4F4-DB229A6DDDDD

Type species. *Paraxenos orientalis* Kifune, 1985, here designated.

Diagnosis of female cephalothorax. Differing from all other genera of Xenidae by very distinct prosternal features: prosternal extension anteriorly with very conspicuous, extensive pale spot, sometimes associated with cuticular impression (Figs 35C, 37A). A feature linked with the maxillae is shared with *Paraxenos* or *Tuberoxenos*: submaxillary groove distinctly produced posterolaterally to maxillary base (Fig. 37A), extend-

ing along cephalic border distally and then connected to border between head and prothorax. In contrast to *Paragioxenos*, head and prothorax ventrally delimited by birth opening medially and by suture laterally.

Description of female cephalothorax. Shape and coloration. Compact, ca. as long as wide, or slightly longer. Size variable, length 0.96–1.64 mm, maximum width 0.9–1.8 mm. Anterior head margin rounded, not protruding. Thorax slightly widening posteriorly. Abdominal segment I sometimes protruding laterally, forming rounded corner below spiracles. Coloration never completely pale, comprising multiple brown shades forming distinct patterns.

Head capsule. ~ ⅓ ~ ⅖ as long as entire cephalothorax including lateral cephalic extensions. Combination of pale and dark brown shades resulting in specific color pattern. Clypeal region well delimited from labral area, arcuate, without or with slightly protruding clypeal lobe. Surface smooth or slightly wrinkled. Sensilla (> 30) better visible in dorsal view than ventrally, concentrated mainly on anterior clypeal area. Border between clypeal region and frontal area indistinctly recognizable. Frontal area smooth or slightly reticulated. Dorsal border between head and prothorax indicated by interrupted suture or distinctive coloration, or scarcely recognizable.

Supra-antennal sensillary field. Smooth or slightly wrinkled, with evenly dispersed sensilla, not delimited or indistinctly delimited by furrow medially (Fig. 37B).

Antenna. Preserved as poorly defined area with several small, rounded plates, cavity, or sensilla. Periantennal area smooth (Fig. 36C).

Labrum. Ventral field wider than long, elliptic. Dorsal field slightly arcuate, 3–4× wider than long in midline. Dorsal field with several inconspicuous setae, usually blunt, not pointed.

Mandible. Mandibles anteromedially directed at angle of 35–55°, enclosed in mandibular capsule. Mandibular bulge rounded or pointed, with several sensilla. Cuticle smooth, with longitudinal grooves. Tooth narrow, armed with spines.

Maxilla. Variable in shape, in some cases reduced and fused to labium, otherwise well-developed, separated from labial area, anteriorly directed, prominent but not projecting beyond mandible. Cuticle finely reticulated. Vestige of palp present as cavity with accessory plates or reduced. Submaxillary groove distinctly produced posterolaterally to maxillary base extending along cephalic border (Fig. 37A).

Labium. Labial area between maxillae flat but distinct, delimited anteriorly by mouth opening and posteriorly by birth opening. Wider than long in midline or as long as wide. Cuticular surface smooth or reticulated.

Mouth opening. Distinctly arcuate to nearly straight, sclerotized marginally.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders relatively distinct, indicated by mesal furrows combined with stripes of specific coloration. Border between metathorax and abdomen usually indicated by change in cuticular surface structure or pigmentation. Cuticle of thoracic segments on ventral side reticulate with scattered small and pigmented papillae. Cuticle of dorsal side of thorax indistinctly reticulated. Prosternal extension differentiated anteriorly, with very conspicuous extensive pale spot, sometimes associated with cuticular impression (Figs 35C, 37A). Shape of meso- and metathorax unmodified, transverse. Setae

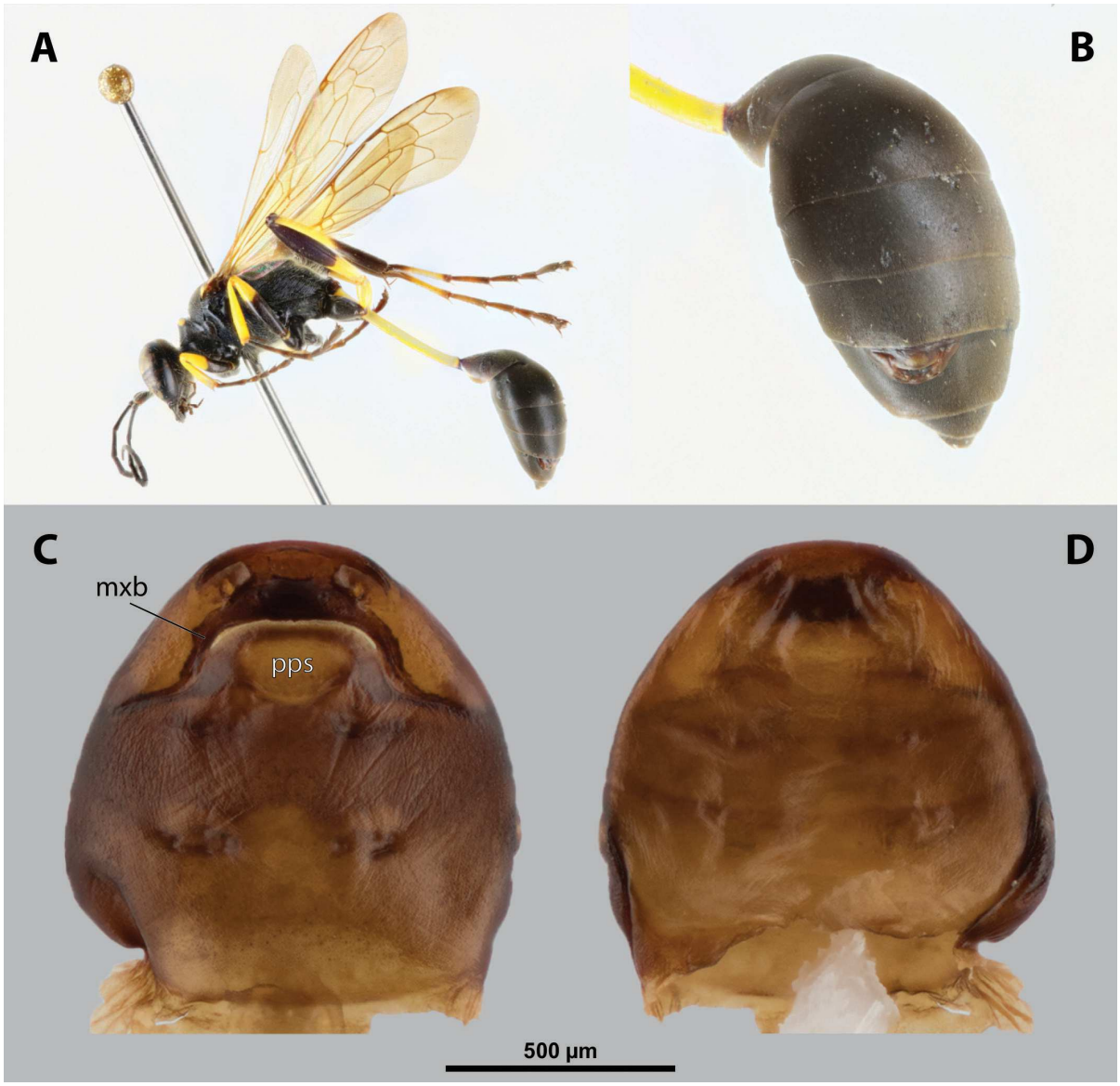

Figure 35. *Sphecixenos orientalis*, host, female, cephalothorax, photomicrographs **A** *Sceliphron madraspatanum* stylopized by female of *S. orientalis*, lateral view **B** detail of host abdomen with adult female inside **C** ventral side of cephalothorax **D** dorsal side of cephalothorax. Abbreviations: mxb – maxillary base, pps – prosternal pale spot.

on lateral region of abdominal segment I (Fig. 36E, F) present, or cuticular surface distinctly sculptured.

Spiracles. Spiracles on posterior third of cephalothorax slightly elevated, with lateral or anterolateral orientation.

Diagnosis of male cephalotheca. Differing from other genera by large diameter of genae between maxillary base and compound eye, at least 2× as large as diameter of vestigial antenna. Distinct paired furrow of supra-antennal sensillary field absent. Cephalotheca nearly circular in frontal view (Fig. 38A). Diameter of vestigial antennae smaller than width of medially directed mandible (Fig. 38E).

Description of male cephalotheca. Shape and coloration. In frontal view rounded, nearly circular, in lateral view rounded or slightly pointed anteriorly. With pattern of multiple shades of brown.

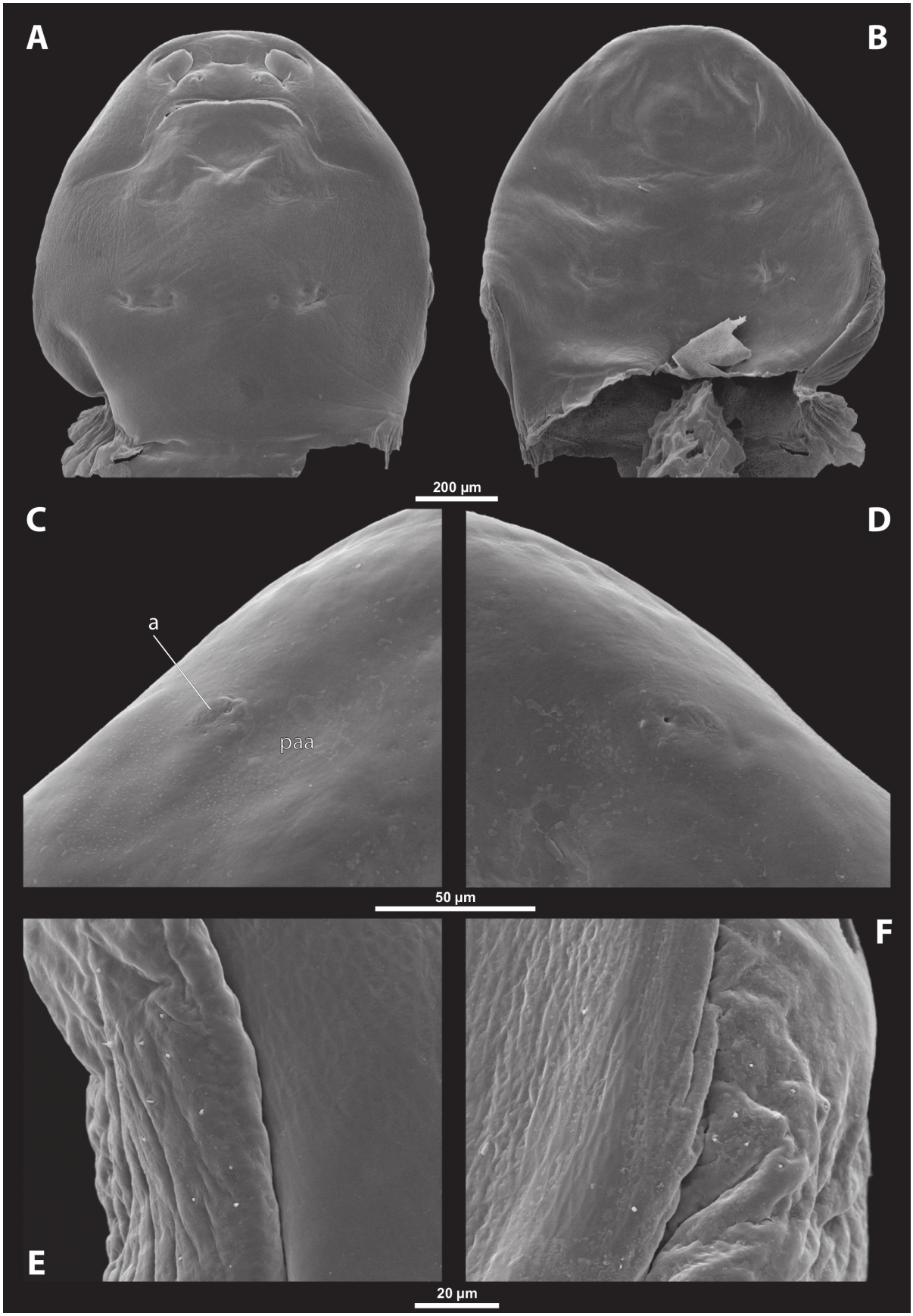

Figure 36. *Sphecixenos orientalis*, female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** left vestigial antenna, dorsal side **D** right vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** right lateral border of abdominal segment I below spiracle, dorsal side. Abbreviations: a – vestigial antenna, paa – periantennal area.

Cephalothecal capsule. Compound eyes with dark individual ommatidia well visible on paler ocular background. Clypeal lobe straight in frontal view, slightly protruding in lateral view. Sensilla mainly concentrated on medial clypeal region. Frontal impression indistinct. Occipital bulge absent. Diameter of genae between maxillary base and compound eye large, > 2× as large as diameter of vestigial antenna.

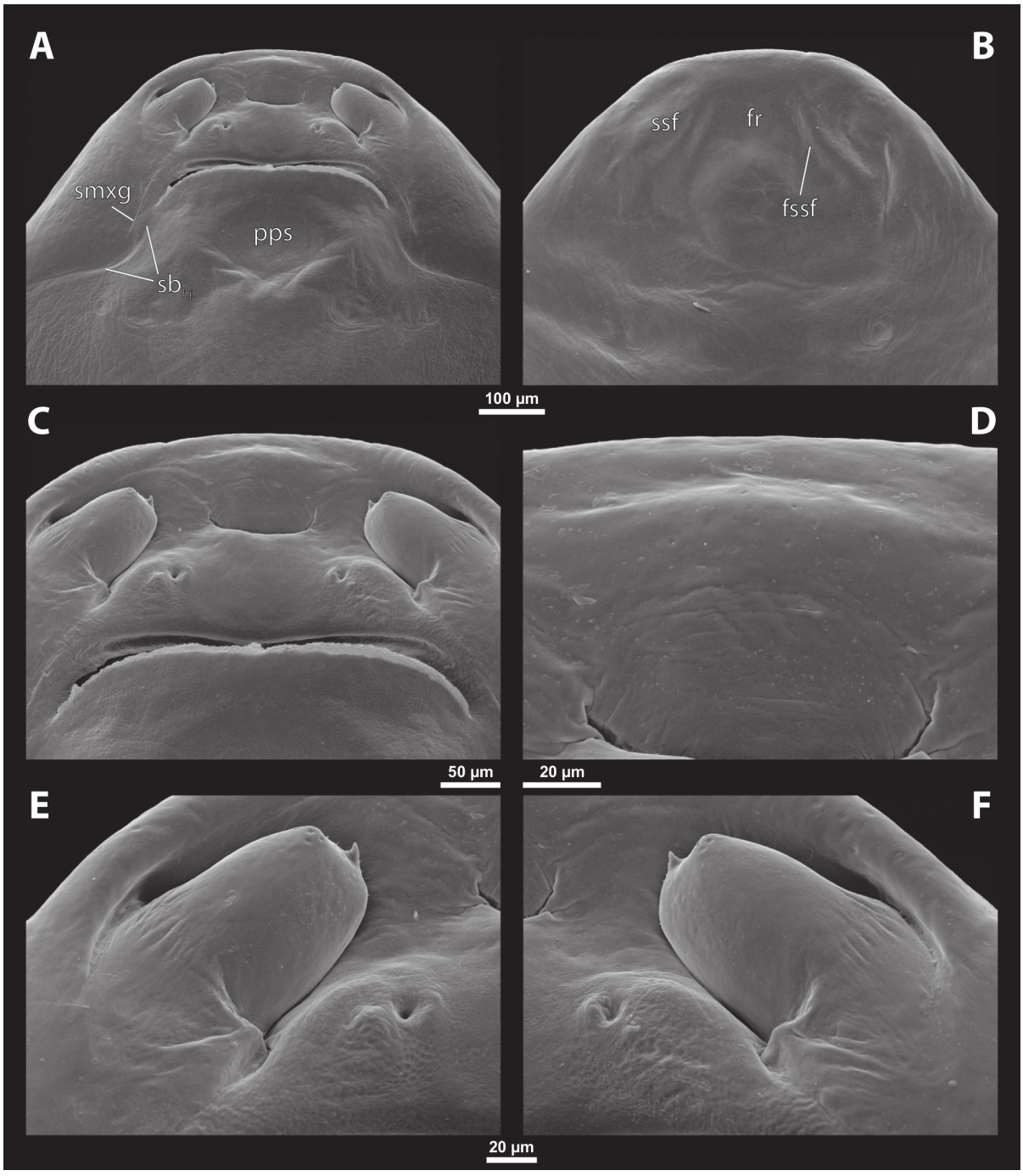

Figure 37. *Sphecixenos orientalis*, female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: fr – frontal region, fssf – furrow of supra-antennal sensillary field, pps – prosternal pale spot, sbhp – segmental border between head and prothorax, smxg – submaxillary groove, ssf – supra-antennal sensillary field.

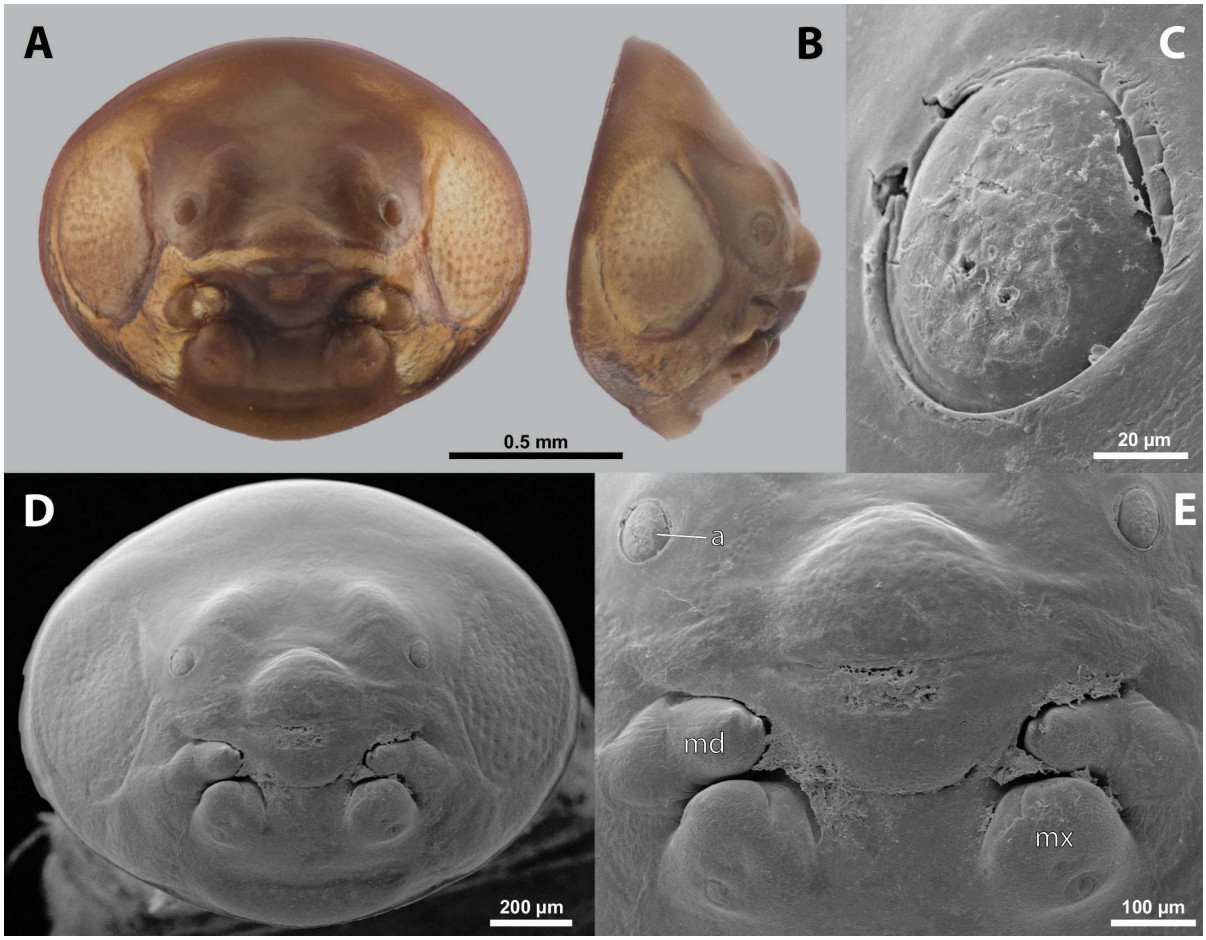

Figure 38. *Sphecixenos* cf. *gigas*, male, cephalotheca, photomicrographs, SEM micrographs **A** frontal view **B** lateral view **C** vestigial antenna **D** frontal view **E** mouthparts. Abbreviations: a – vestigial antenna, md – mandible, mx – vestige of maxilla.

Supra-antennal sensillary field. Kidney-shaped and bulging, distinctly developed. Lacking distinct furrows medially.

Antenna. Of standard shape but very small, with small plates or cavities and complete torulus (Fig. 38C). Periantennal area not clearly delimited from supra-antennal sensillary field.

Labrum. Labral area distinct.

Mandible. Rather medially directed than anteromedially. Mandibular tooth pointed, not reaching area of mandibular bulge basally.

Maxilla. Distinct, prominent, with entirely dark coloration. Vestige of palp distinct.

Labium and hypopharynx. Dark labium distinct between and below maxillae. Praementum and postmentum separated by furrow. Hypopharyngeal protuberance not present.

Mouth opening. Clearly visible, not covered by ventral labral field, slightly arcuate.

Phylogenetic relationships. According to Benda et al. (2019, 2021) sister to a monophyletic lineage containing *Pseudoxenos* and *Tuberoxenos* gen. nov.

Diversity and distribution. This genus represents a lineage of Afrotropical origin which dispersed to Australia (Benda et al. 2019). It currently comprises 12

species, distributed in the Old World (mainly Afrotropical and Oriental regions) and Australian region.

Hosts. *Sphex*, *Isodontia* (Sphecidae: Sphecinae), *Sceliphron* (Sphecidae: Sceliphrinae), and *Chlorion* (Sphecidae: Chloriontinae).

Etymology. The name is derived from the family Sphecidae, the only known host family of this genus. The ending *-xenos* is used in several generic names, mainly in the family Xenidae. It is from a Greek substantive meaning enemy or stranger. Gender masculine.

Comments. All described species of *Sphecixenos* gen. nov. were previously placed in *Paraxenos* based on parasitising digger wasps (Kinzelbach 1971b). Despite this concept, this group is morphologically well defined. We classify it as a separate genus, based on the molecular phylogeny (Benda et al. 2019, 2021) and morphological characters newly reported here.

List of species

***Sphecixenos abbotti* (Pierce, 1909), comb. nov.**

Homilops abbotti Pierce, 1909: 147.
Pseudoxenos abbotti (Pierce, 1909) (new combination by Bohart 1937).
Paraxenos abbotti (Pierce, 1909) (new combination by Kinzelbach 1971b).

Host. *Sphex* sp. (as *Proterosphex* sp.) (Pierce 1909).

Distribution. Thailand: Trang (Pierce 1909).

***Sphecixenos astrolabensis* (Székessy, 1956), comb. nov.**

Pseudoxenos astrolabensis Székessy, 1956: 144.
Paraxenos astrolabensis (Székessy, 1956) (new combination by Kinzelbach 1971b).

Host. *Sphex cognatus* F. Smith, 1856 (as *Sphex formosus* F. Smith, 1856) (Székessy 1956).

Distribution. New Guinea: New Britain (Székessy 1956).

***Sphecixenos dorae* (Luna de Carvalho, 1956), comb. nov.**

Pseudoxenos dorae Luna de Carvalho, 1956: 41.
Paraxenos dorae (Luna de Carvalho, 1956) (new combination by Kinzelbach 1971b).

Hosts. *Chlorion* sp. (Luna de Carvalho 1956); *Sphex nigrohirtus* Kohl, 1895 (Kinzelbach 1971b).

Distribution. Angola (Luna de Carvalho 1956).

***Sphecixenos erimae* (Székessy, 1956), comb. nov.**

Pseudoxenos erimae Székessy, 1956: 146.
Paraxenos erimae (Saunders, 1872) (new combination by Kinzelbach 1971b).

Host. *Sphex fumicatus* Christ, 1791 (as *Sphex metallicus* Taschenberg, 1869) (Székessy 1956).

Distribution. New Guinea (Székessy 1956).

***Sphecixenos esakii* (Hirashima & Kifune, 1962), comb. nov.**

Pseudoxenos esakii Hirashima & Kifune, 1962: 175.
Paraxenos esakii (Hirashima & Kifune, 1962) (new combination by Kinzelbach 1971b).

Hosts. *Isodontia maidli* (Yasumatsu, 1938) (Kifune and Tano 1985); *Isodontia nigella* (F. Smith, 1856) (as *Sphex nigellus* F. Smith, 1856) (Hirashima and Kifune 1962).

Distribution. Japan (Hirashima and Kifune 1962).

***Sphecixenos gigas* (Pasteels, 1950), comb. nov.**

Pseudoxenos gigas Pasteels, 1950: 290.
Paraxenos gigas (Pasteels, 1950) (new combination by Kinzelbach 1971b).

Hosts. *Sphex lanatus* Mocsáry, 1883; *Sphex argentatus* Fabricius, 1787 (as *Sphex umbrosus* Christ, 1791); *Sphex fumicatus* Christ, 1791 (as *Sphex metallicus* Taschenberg, 1869); *Sphex schoutedeni* Kohl, 1913 (as *Isodontia* (*Proterosphex*) *schoutedeni* Kohl, 1913); *Isodontia stanleyi* (Kohl, 1890) (as *Sphex stanleyi* Kohl, 1890) (Pasteels 1950; Kinzelbach 1971b).

Distribution. Democratic Republic of Congo (Pasteels 1950).

***Sphecixenos kurosawai* (Kifune, 1984), comb. nov.**

Paraxenos kurosawai Kifune, 1984: 87.

Host. *Sphex madasummae* Vecht, 1973 (Kifune 1984).

Distribution. Philippines: Palawan (Kifune 1984).

***Sphecixenos laetus* (Ogloblin, 1926), comb. nov.**

Sceliphronechthrus laetum Ogloblin, 1926: 133.
Pseudoxenos laetum (Saunders, 1872) (new combination by Bohart, 1937).

Paraxenos laetum (Saunders, 1872) (new combination by Kinzelbach 1971b).

Host. *Sceliphron laetum* (Smith, 1856).

Distribution. New Guinea; Australia: Queensland (Ogloblin 1926).

Note. According to the article 34.2.1 of ICZN (1999), the ending of species name was adjusted to the grammatical gender of the new genus.

***Sphecixenos orientalis* (Kifune, 1985), comb. nov.**

Paraxenos orientalis Kifune in Kifune & Yamane, 1985: 52.

Host. *Sceliphron madraspatanum formosanum* Vecht, 1968 (Kifune and Yamane 1985).

Distribution. Japan: Iriomote and Ishigaki islands (Kifune and Yamane 1985); Laos; Thailand (this study).

***Sphecixenos reticulatus* (Luna de Carvalho, 1972), comb. nov.**

Paraxenos reticulatus Luna de Carvalho, 1972: 136.

Host. *Sphex tomentosus* Fabricius, 1787 (as *Sphex tuberculatum* F. Smith, 1873) (Luna de Carvalho 1972).

Distribution. Angola: Dundo (Luna de Carvalho 1972).

***Sphecixenos simplex* (Székessy, 1956), comb. nov.**

Pseudoxenos simplex Székessy, 1956: 145.
Paraxenos simplex (Székessy, 1956) (new combination by Kinzelbach 1971b).

Host. *Isodontia praslinia* (Guérin-Méneville, 1831) (as *Sphex simplex* Kohl, 1898) (Székessy 1956).

Distribution. New Guinea (Székessy 1956).

***Sphecixenos vanderiisti* (Pasteels, 1952), comb. nov.**

Pseudoxenos vanderiisti Pasteels, 1952: 252.
Paraxenos vanderiisti (Pasteels, 1952) (new combination by Kinzelbach 1971b).

Host. *Isodontia pelopoeiformis* (Dahlbom, 1845) (as *Chlorion* (*Isodontia*) *pelopaeiformis*, Gerstaecker) (Pasteels 1952).

Distribution. Democratic Republic of Congo (Pasteels 1952).

Note. Pasteels (1952) probably misspelled the host name and the author of its description. Kinzelbach (1971b) probably overlooked these mistakes. We adjust it in accordance with Cook (2019).

Pseudoxenos Saunders, 1872

Pseudoxenos Saunders, 1872: 44. Type species: *Pseudoxenos schaumii* Saunders, 1872, by original designation.

Diagnosis of female cephalothorax. Differs from *Tuberoxenos* by flat dorsal field of labrum (Fig. 41C) and more flattened cephalothorax, with more or less even shape (Fig. 39C), appearing flattened-elliptical in cross section. Distinguished from *Deltoxenos* by dorsal labral field laterally as long as along midline (Fig. 41C, D), and meso-metathoracic segmental border not constricted laterally. In contrast to *Macroxenos* lateral parts of abdomen posterior to spiracles with dark coloration (Fig. 39D). Mandible nested in mandibular capsule. In contrast to *Paragioxenos*, head and prothorax ventrally delimited by the birth opening in middle region and laterally by a suture.

Description of female cephalothorax. Shape and coloration. Compact, longer than wide, elliptic in cross-section. Meso-metathoracic segmental border not constricted laterally. Size fairly constant, length 1.08–1.44 mm, maximum width 1.02–1.4 mm. Anterior head margin rounded or protruding. Thorax slightly widening posteriorly. Coloration with multiple brown shades forming pattern.

Head capsule. Ca. ⅖ as long as entire cephalothorax including lateral extensions. Coloration mostly dark brown, often with specific patterns. Clypeal region delimited from labral area (Fig. 41D), arcuate, or protruding and forming clypeal lobe. Surface smooth or slightly wrinkled. Approximately 35–56 sensilla mainly concentrated anteriorly but dispersed over entire clypeal area. Border between clypeal area and frontal region hardly distinct but still recognizable. Frontal surface smooth (Fig. 40F). Segmental border between head and prothorax clearly recognizable or indistinct on dorsal side, often indicated by dark brown stripes, and in some cases with two distinct dark spots on mesal region (Fig. 39D).

Supra-antennal sensillary field. Smooth or slightly wrinkled, with dispersed sensilla. Furrow forming border on medial side more or less distinct (Fig. 41B).

Antenna. Preserved as poorly defined area, sometimes raised, usually with several small, rounded plates, rarely with additional sensilla or cavity (Fig. 40C). Periantennal area smooth.

Labrum. Ventral field distinctly wider than long, elliptic. Dorsal field nearly straight, slightly arcuate, at least 4–5× wider than long in midline, flat and smooth, with 15–21 clearly visible setae inserted in cavities (Fig. 41C, D). Dorsal field laterally as long as medially, in some cases almost merging with head capsule.

Mandible. Mandibles anteromedially directed at an angle of 35–45° and nested in mandibular capsule. Mandibular bulge not or slightly raised, bears several sensilla.

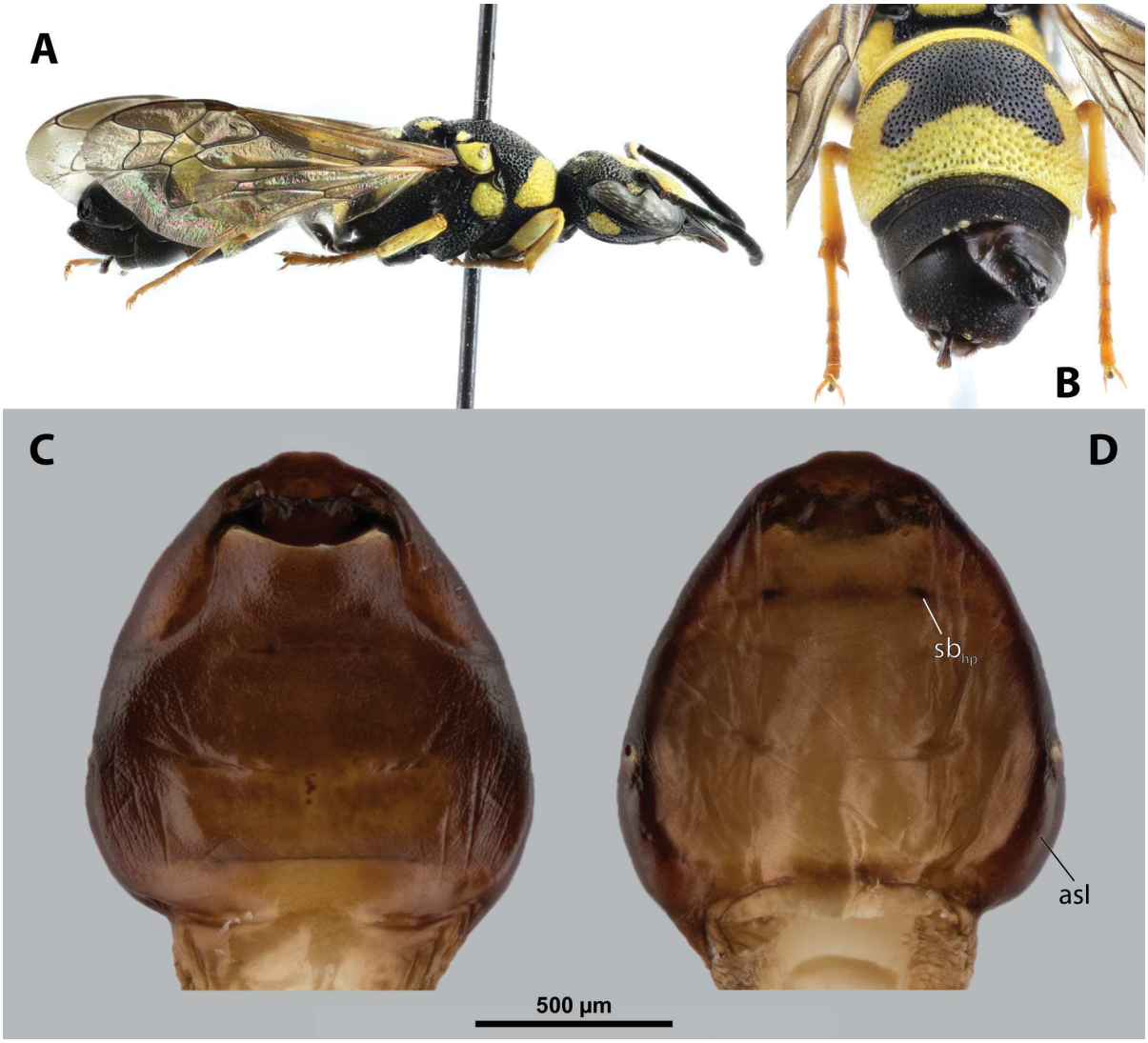

Figure 39. *Pseudoxenos* sp., host, male, female, cephalothorax, photomicrographs **A** *Paradontodynerus* sp. stylopized by male of *Pseudoxenos* sp., lateral view **B** detail of host abdomen with male puparium inside **C** ventral side of female cephalothorax **D** dorsal side of female cephalothorax. Abbreviations: asI – abdominal segment I, sbhp – segmental border between head and prothorax.

Cuticle of mandible sculptured to nearly smooth. Mandibular tooth narrow, pointed, straight or hook-shaped, armed with spines.

Maxilla. Separated from labial area, slightly or distinctly protruding, prominent portion directed anteriorly or anterolaterally, maxilla slightly overlapping with mandible proximally (Fig. 41F), but not projecting beyond it anteriorly. Cuticle usually smooth, rarely wrinkled. Vestige of palp very distinct, with more or less distinct plates or cavity, located medially on ventral side of maxilla. Submaxillary groove more or less distinctly produced posterolaterally to maxillary base.

Labium. Labial area between maxillae flat but distinct, relatively large, delimited anteriorly by mouth opening and posteriorly by birth opening. As long as wide or longer than wide. Cuticular surface in most cases largely smooth and shiny, or faintly and uniformly sculptured.

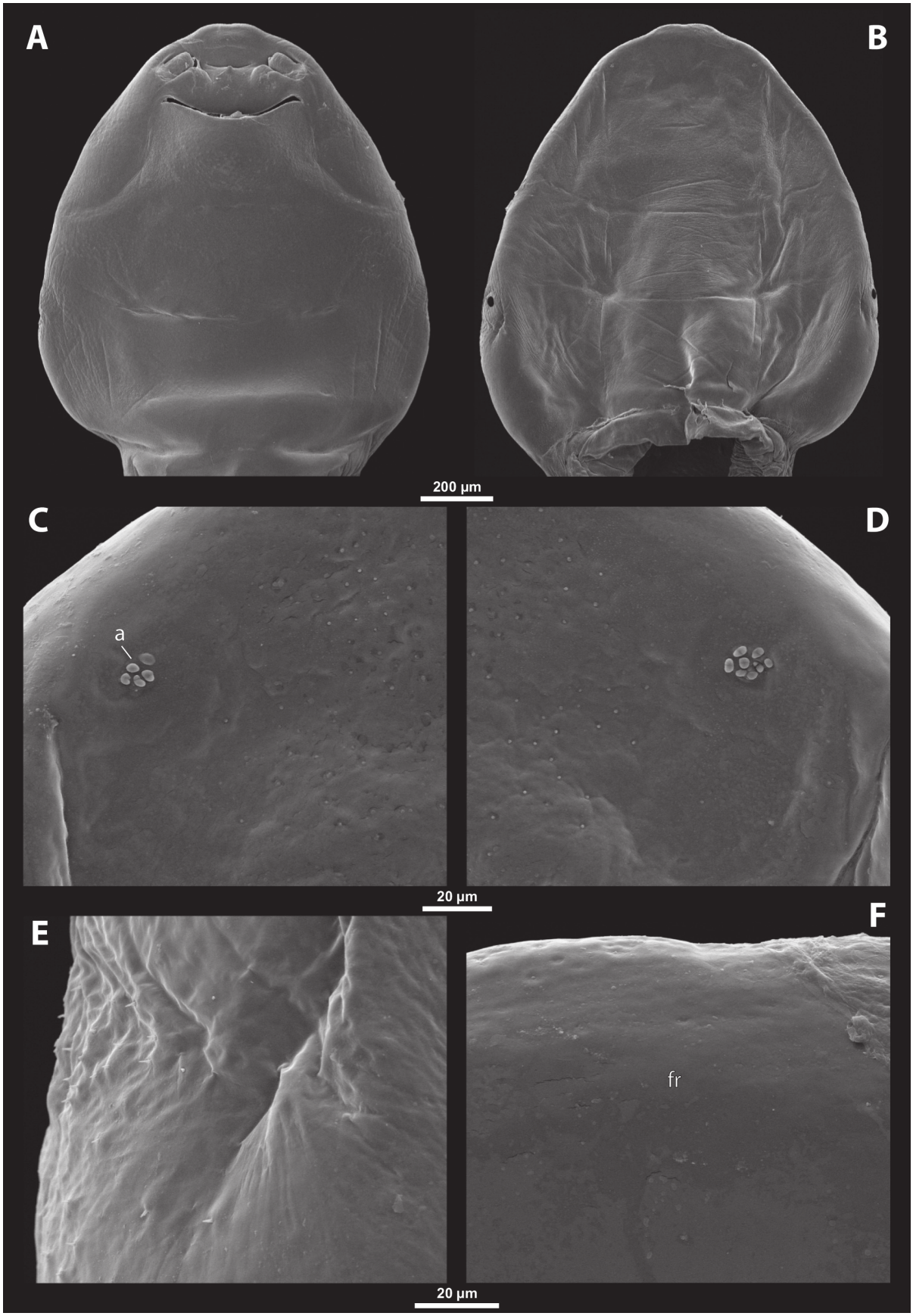

Figure 40. *Pseudoxenos* sp., female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** left vestigial antenna, dorsal side **D** right vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** detail of anterior border of cephalothorax, dorsal side. Abbreviations: a – vestigial antenna, fr – frontal region.

Mouth opening. Mouth opening arcuate, nearly straight, or bi-arcuate, sclerotized marginally.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders more or less distinct, separated by mesal furrows. Border between metathorax and abdomen formed by ridge. Cuticle of thoracic segments on ventral side reticulate with scattered small and pigmented papillae. Cuticle of dorsal side of thorax smooth or slightly wrinkled. Prosternal extension undifferentiated, anterior margin evenly arched. Meso- and metathorax transverse. Lateral parts of abdomen posterior to spiracle dark (Fig. 39D). Setae present on lateral region of abdominal segment I.

Spiracles. Spiracles on posterior ⅓ of cephalothorax slightly elevated, with anterolateral or lateral orientation.

Diagnosis of male cephalotheca. Diameter of genae between maxillary base and compound eye ~ 1.5× as large as diameter of vestigial antenna. Occipital bulge present (Fig. 42D). Frontal region very distinctly deformed by frontal impression (Fig. 42D). Distinct paired furrows of supra-antennal sensillary field absent.

Description of male cephalotheca. Shape and coloration. In frontal view rounded laterally, flattened, elliptical, in lateral view pointed anteriorly. Coloration with pattern of pale and dark shades.

Cephalothecal capsule. Compound eyes with darker individual ommatidia well visible on pale ocular background. Clypeal lobe straight or slightly arcuate in frontal view, prominent in lateral view. Sensilla mainly concentrated medially. Frontal impression distinctly present (Fig. 42D). Occipital bulge present (Fig. 42D). Diameter of genae between maxillary base and compound eye small, ~ 1.5× diameter of vestigial antenna.

Supra-antennal sensillary field. Kidney-shaped and bulging, delimited medially by frontal impression, without distinct furrows.

Antenna. Vestiges large, with complete torulus. Periantennal area not clearly delimited from supra-antennal sensillary field. Small plates or sensilla present (Fig. 27C).

Labrum. Labral area distinct, with setae on dorsal field.

Mandible. Anteromedially directed. Mandibular bulge with sensilla, separated from pointed tooth.

Maxilla. Distinct, prominent. Coloration completely dark. Vestige of palp distinct.

Labium and hypopharynx. Labium distinct between and below maxillae, dark. Praementum and postmentum distinctly separated by furrow. Hypopharyngeal protuberance present.

Mouth opening. Well visible, not covered by ventral labral field, slightly arcuate.

Phylogenetic relationships. Deeply nested within Xenidae (Benda et al. 2019, 2021), part of a clade of an Old Word origin, with *Tuberoxenos* gen. nov. as sister group.

Diversity and distribution. A group of Palearctic origin (Benda et al. 2019), comprising seven currently valid species restricted to this region.

Hosts. Various genera of Odynerini (Vespidae: Eumeninae).

Comments. *Pseudoxenos* was described by Saunders (1872) but only a superficial description of the male was provided. Bohart (1937) synonymized many names previously

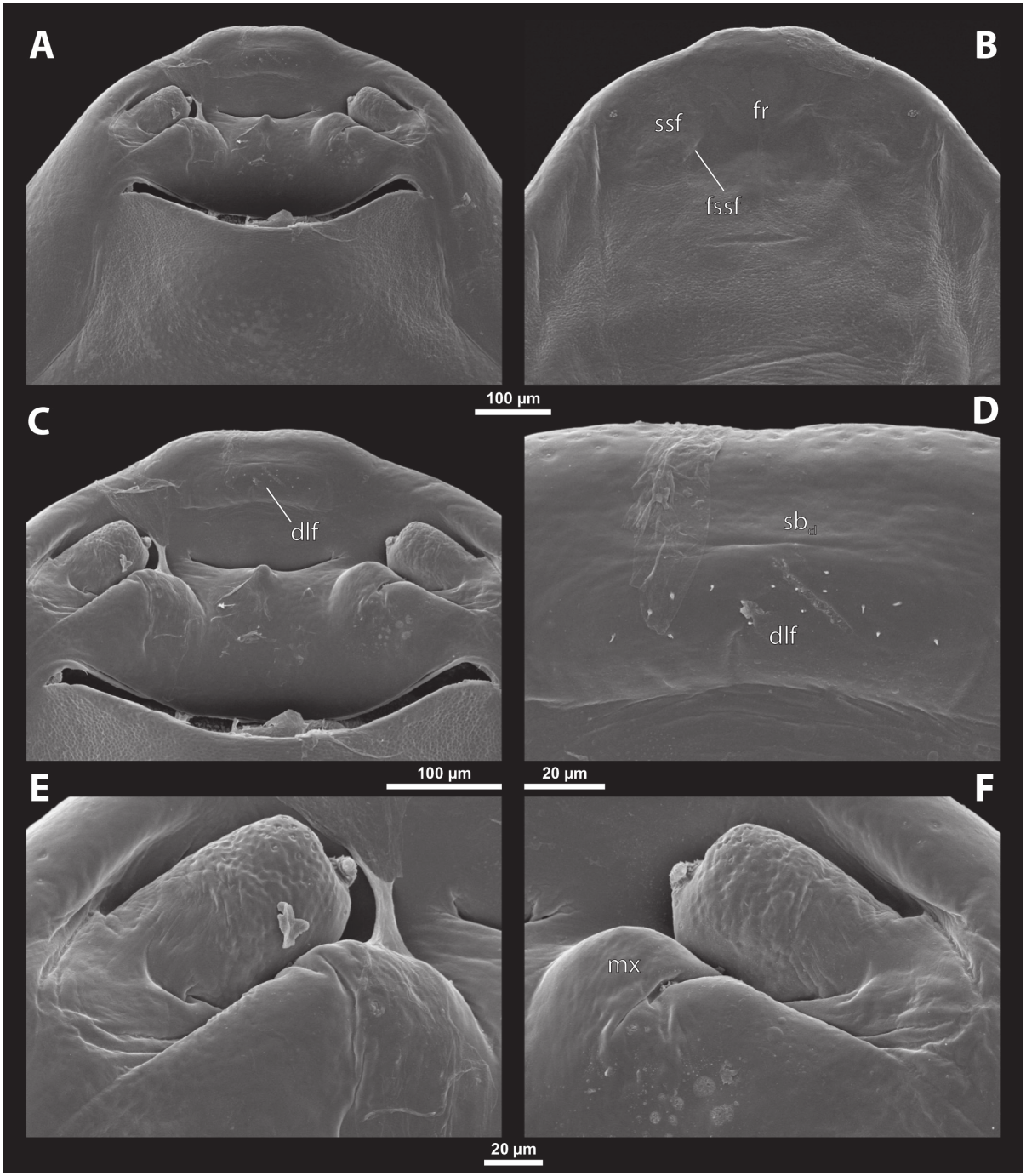

Figure 41. *Pseudoxenos* sp., female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: dlf – dorsal field of labral area, fr – frontal region, fssf – furrow of supra-antennal sensillary field, mx – vestige of maxilla, sbhp – segmental border between head and prothorax, ssf – supra-antennal sensillary field.

designed (*Eupathocera*, *Ophthalmochlus*, *Homilops*, *Leionotoxenos Sceliphronecthrus*, *Macroxenos*) with *Pseudoxenos*. Although later Kinzelbach (1971b) used *Pseudoxenos* for all xenids parasitising solitary Vespidae worldwide, the genus corresponds to a Palearctic clade utilizing Odynerini according to the molecular phylogeny of Benda et al. (2019, 2021).

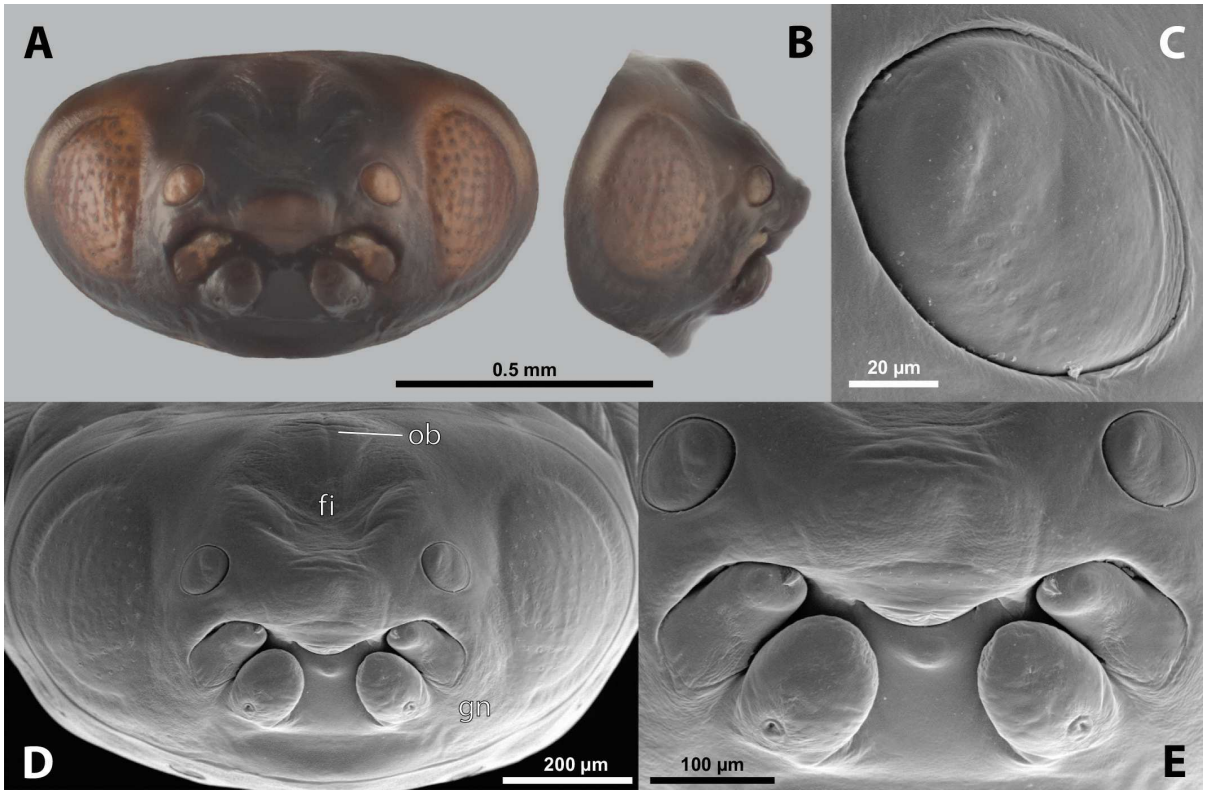

Figure 42. *Pseudoxenos* sp., male, cephalotheca, photomicrographs, SEM micrographs **A** frontal view **B** lateral view **C** vestigial antenna **D** frontal view **E** mouthparts. Abbreviations: fi – frontal impression, ob – occipital bulge.

We classify this lineage as a separate genus, based on these molecular phylogenic works and morphological characters newly reported here.

List of species

Only hosts from original descriptions are included. As the phylogeny of this genus is not clarified we do not present any other host species from later studies. The actual extent of morphological variation within and between species in Europe has not been assessed yet (Cook 2019). A more comprehensive sampling and a detailed taxonomic revision are necessary for a clarification of interspecific relationships and individual species concepts.

Pseudoxenos andradei Luna de Carvalho, 1953

Pseudoxenos andradei Luna de Carvalho, 1953: 3.
Pseudoxenos heydenii (Saunders, 1852) (partim!) (synonymy proposed by Kinzelbach 1978).

Host. *Ancistrocerus triphaleratus* (Saussure, 1855) (Luna de Carvalho 1953).
Distribution. Portugal: Vale do Gaio (Luna de Carvalho 1953).

Pseudoxenos atlanticus Luna de Carvalho, 1969

Pseudoxenos atlanticus Luna de Carvalho, 1969: 9.
Pseudoxenos heydenii (Saunders, 1852) (partim!) (synonymy proposed by Kinzelbach 1978).

Host. *Odynerus* sp. (Luna de Carvalho 1969).
Distribution. Portugal: Madeira isl., Funchal (Luna de Carvalho 1969).

Pseudoxenos corcyricus (Saunders, 1872)

Paraxenos corcyricus Saunders, 1872: 46.
Pseudoxenos corcyricus (Saunders, 1872) (new combination by Pierce 1909).
Pseudoxenos heydenii (Saunders, 1852) (partim!) (synonymy proposed by Kinzelbach 1978).

Host. *Odynerus spinipes* (Linaeus, 1758) (Saunders 1872).
Distribution. Greece: Corfu (Saunders 1872).

Pseudoxenos heydenii (Saunders, 1852)

Xenos heydenii Saunders, 1852: 141.
Pseudoxenos heydenii (Saunders, 1852) (new combination by Saunders 1872).
Pseudoxenos heydeni (incorrect subsequent spelling): Kinzelbach (1971b).
Pseudoxenos heydeni (incorrect subsequent spelling): Kinzelbach (1978).

Hosts. *Antepipona deflenda* (Saunders, 1853) (as *Ancistrocerus deflendus*, Saunders, 1853).
Distribution. Greece: Preveza, Epirus reg., Ambracian Gulf (Saunders 1852).

Pseudoxenos klugii (Saunders, 1852)

Xenos klugii Saunders, 1852: 142.
Pseudoxenos klugii (Saunders, 1852) (new combination by Saunders 1872).
Pseudoxenos klugi (incorrect subsequent spelling): Kinzelbach (1971b).
Pseudoxenos heydenii (Saunders, 1852) (partim!) (synonymy proposed by Kinzelbach 1978).

Host. *Gymnomerus laevipes* (Shuckard, 1837) (as *Odynerus rubicola* Dufour, 1839) (Saunders 1852).
Distribution. Greece: Preveza (Saunders 1852).

Pseudoxenos seyrigi Monod, 1925

Pseudoxenos seyrigi Monod, 1925: 230.
Pseudoxenos heydenii (Saunders, 1852) (partim!) (synonymy proposed by Kinzelbach 1978).

Host. *Euodynerus variegatus* (Fabricius, 1793) (as *Odynerus crenatus* Lepeletier, 1841) (Monod 1925).

Distribution. Spain: Sierra Morena (Monod 1925).

Pseudoxenos schaumii Saunders, 1872

Pseudoxenos schaumii Saunders, 1872: 44.
Pseudoxenos schaumi (incorrect subsequent spelling): Kinzelbach (1971b).
Pseudoxenos heydenii (Saunders, 1852) (partim!) (synonymy proposed by Kinzelbach 1978).

Host. *Ancistrocerus parietum* (Linnaeus, 1758) (as *Odynerus parietum* Linnaeus, 1758) (Saunders 1872).

Distribution. Greece: Corfu (Saunders 1872).

Tuberoxenos gen. nov.

http://zoobank.org/99152C5A-B0FE-47A3-85B7-2A3F5ED548DA

Type species. *Xenos sphecidarum* Siebold, 1839, here designated.

Diagnosis of female cephalothorax. Distinguished from *Pseudoxenos* by conspicuously convex, round cephalothorax (Fig. 43C), and distinctly raised, anteriorly protruding dorsal labral field (Fig. 45D). Differring from other genera by the following combination of characters. Maxilla well-developed and clearly separated from labial area, prominent and directed anteriorly (Fig. 45E). Mandibular tooth narrow or slightly widened. Prosternal extension undifferentiated, evenly arched but in some cases protruding and overlapping with maxillolabial area and posterior part of mandibles. Differing from *Nipponoxenos* by mandible nested in capsule. In contrast to *Paragioxenos*, head and prothorax ventrally delimited by birth opening medially and by suture laterally.

Description of female cephalothorax. Shape and coloration. Compact, ca. as long as wide or longer than wide. In ventral view appearing conspicuously convex, rotund (Fig. 43C), high-elliptic in cross-section. Species rather constant in size, length 1.06–1.34 mm, maximum width 0.94–1.4 mm. Anterior head margin evenly rounded or very slightly protruding. Thorax slightly or distinctly widening posteriorly. Coloration with multiple brown shades forming distinct pattern, mostly dark.

Head capsule. Ca. ⅓ – ⅖ as long as entire cephalothorax including lateral cephalic extension. Coloration of head dominantly pale or brown, forming specific

Figure 43. *Tuberoxenos sphecidarum*, host, male, female, cephalothorax, photomicrographs **A** *Podalonia tydei* stylopized by females of *T. sphecidarum*, lateral view **B** detail of host abdomen with three adult females inside **C** ventral side of female cephalothorax **D** dorsal side of female cephalothorax.

color pattern. Clypeal region well delimited from labral area, arcuate, or very slightly protruding and forming clypeal lobe. Surface smooth or slightly wrinkled. Ca. 50–95 sensilla regularly dispersed on clypeal area. Border between clypeal area and frontal region clearly recognizable or indistinct. Frontal region smooth or slightly wrinkled. Segmental border between head and prothorax quite distinct on dorsal side, indicated by furrow, change in cuticular sculpture or coloration.

Supra-antennal sensillary field. Slightly wrinkled or reticulated, delimited by more or less distinct furrow on medial side (Fig. 45B).

Antenna. Preserved as poorly defined area, in some cases indistinct (Fig. 44C). With cavities, several small, rounded plates, or sensilla, the latter combined in some cases. Periantennal area smooth or slightly wrinkled (Fig. 44C).

Labrum. Ventral field at least slightly wider than long, elliptical or semicircular. Dorsal field widely arcuate, ~ 5× wider than long in midline, distinctly raised (Fig. 45D). Dorsal field with ~ 17–28 pointed or blunt setae on its surface.

Mandible. Anteromedially directed at angle of 20–40°, enclosed in mandibular capsule. Mandibular bulge slightly or distinctly raised, with several sensilla. Cuticle

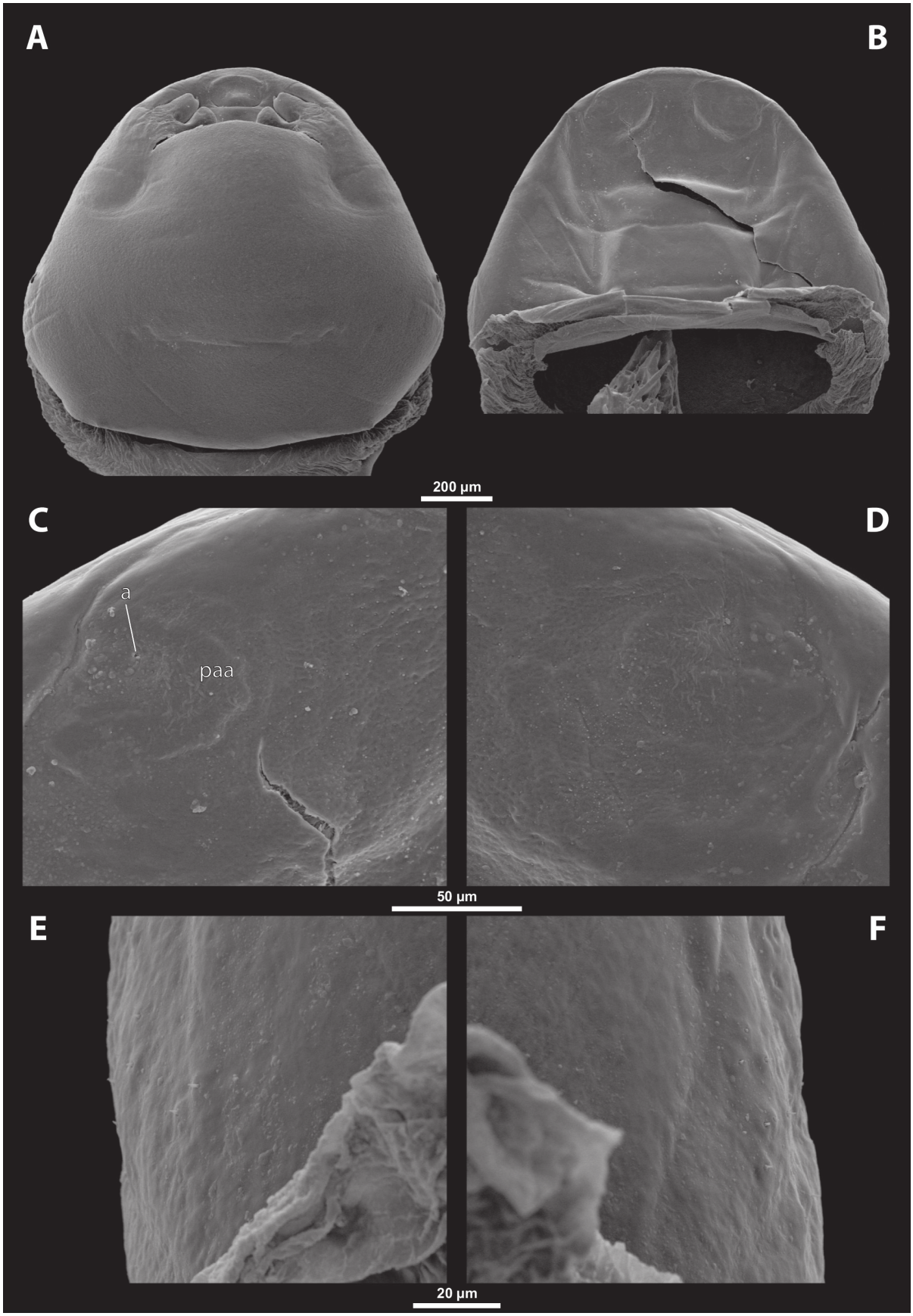

Figure 44. *Tuberoxenos sphecidarum*, female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** left vestigial antenna, dorsal side **D** right vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** right lateral border of abdominal segment I below spiracle, dorsal side. Abbreviations: a – vestigial antenna, paa – periantennal area.

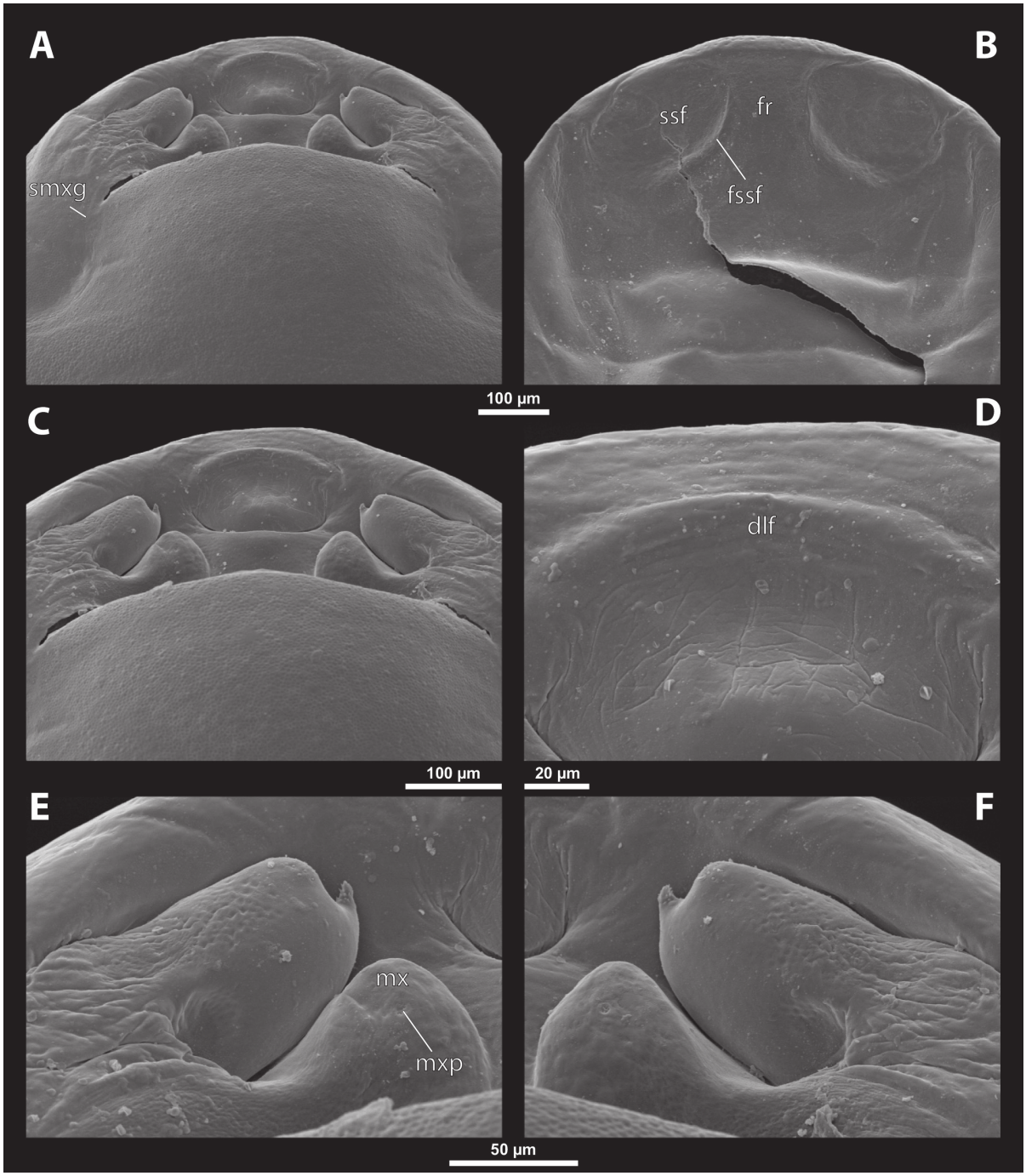

Figure 45. *Tuberoxenos sphecidarum*, female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: dlf – dorsal field of labral area, fr – frontal region, fssf – furrow of supra-antennal sensillary field, mx – vestige of maxilla, mxp – vestige of maxillary palp, smxg – submaxillary groove, ssf – supra-antennal sensillary field.

smooth, slightly sculptured or reticulated. Longitudinal grooves on articular area present. Tooth narrow, pointed, more or less armed with spines.

Maxilla. Well developed and clearly separated from labial area, prominent and anteriorly directed. Protruding maxillary part usually slightly overlapping with proxi-

mal portion of mandible (Fig. 45E), but not projecting beyond mandible anteriorly. Cuticle smooth or very slightly wrinkled. Vestige of palp inconspicuous, preserved as small bulge with indistinct plates, located anteromedially on ventral side of maxilla (Fig. 45E). Maxillary base distinctly produced anterolaterally as submaxillary groove.

Labium. Labial area distinct between maxillae, delimited anteriorly by mouth opening and posteriorly by birth opening. Labial area wider than long in midline, flat or slightly convex. Cuticular surface smooth or slightly reticulated.

Mouth opening. Arcuate, nearly straight, or bi-arcuate, sclerotized marginally.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders distinct, usually separated by mesal furrows, often combined with color stripes or spots on dorsal and ventral sides. Border between metathorax and abdomen usually indicated by change of cuticular sculpture and very indistinct ridge. Cuticle of thoracic segments on ventral side reticulate with scattered small and pigmented papillae. Dorsal side of thorax smooth or slightly reticulated. Prosternal extension undifferentiated, prosternal margin evenly arched but in some cases protruding and overlapping with maxillolabial area and posterior part of mandibles. Meso- and metathorax of standard transverse shape. Setae present on lateral region of abdominal segment I (Fig. 44 E, F).

Spiracles. Spiracles on posterior half or third of cephalothorax slightly elevated, with lateral or anterolateral orientation.

Diagnosis of male cephalotheca. Differing from other genera by the following combination of characters. Diameter of genae between maxillary base and compound eye ~ 1.5× larger than diameter of vestigial antenna. Occipital bulge absent and frontal impression indistinct or missing. Distinct paired furrows of supra-antennal sensillary field present (Fig. 46A, D). Cephalotheca always appearing rotund.

Description of male cephalotheca. Shape and coloration. In frontal view rounded, almost circular (Fig. 46A), in lateral view pointed anteriorly. Coloration forming pattern of pale and dark shades.

Cephalothecal capsule. Compound eyes with darker individual ommatidia well visible on pale ocular background. Conspicuous clypeal lobe arcuate in frontal view, prominent in lateral view. Sensilla dispersed over entire clypeal area. Paired furrows of supra-antennal sensillary field distinctly presented but impression lacking on frontal region. Occipital bulge absent. Diameter of genae between maxillary base and compound eye small, ~ 1.5× larger than diameter of vestigial antenna.

Supra-antennal sensillary field. Kidney-shaped and bulging, medially delimited by distinct furrow (Fig. 46A, D).

Antenna. Large, with complete torulus. Periantennal area not clearly delimited from supra-antennal sensillary field. Small plates, cavities and sensilla present (Fig. 46C).

Labrum. Labral area distinct. Setae on dorsal field present.

Mandible. Anteromedially directed. Tooth pointed, not reaching area of mandibular bulge basally. Bulge with sensilla.

Maxilla. Distinct, prominent. Coloration completely dark or brighter around distinct vestige of maxillary palp.

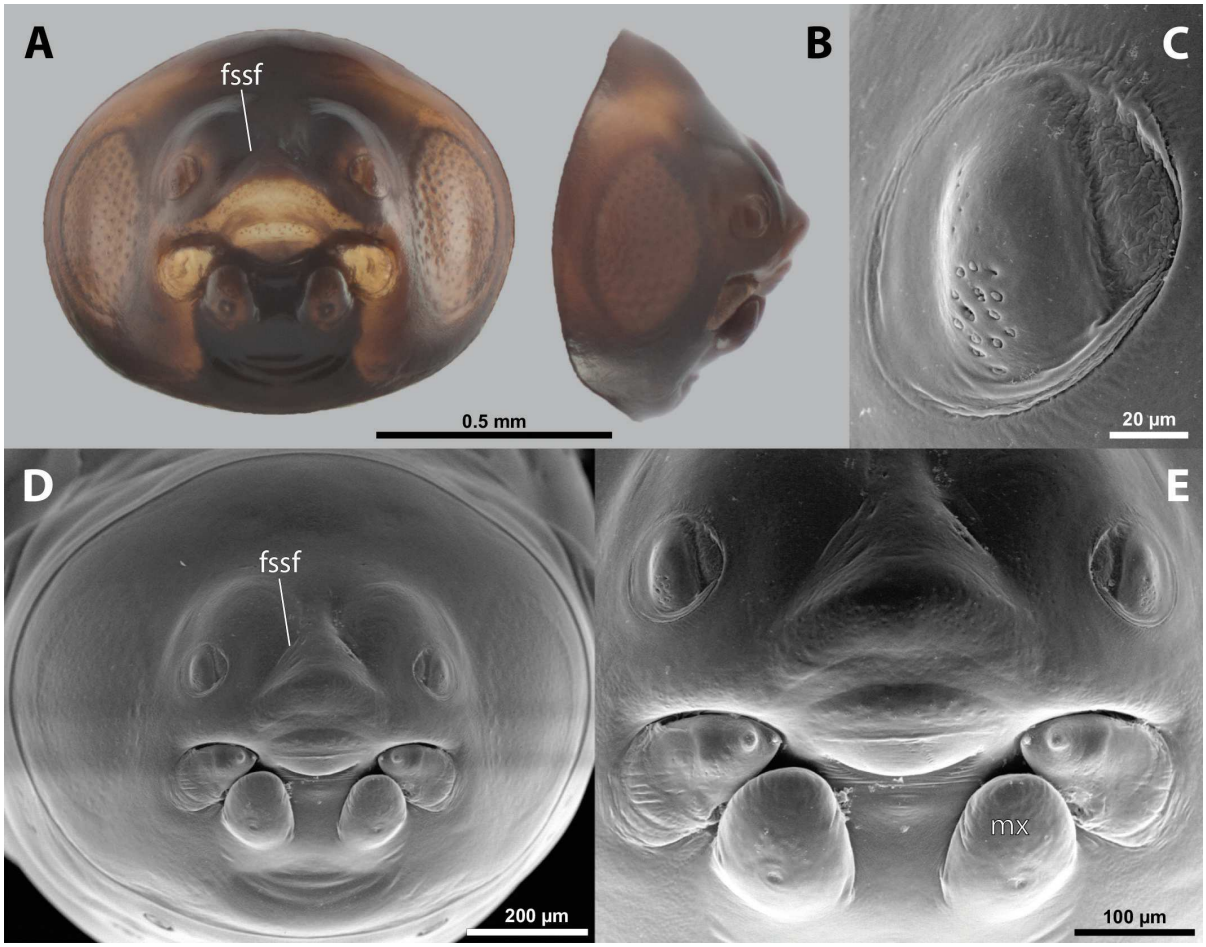

Figure 46. *Tuberoxenos sphecidarum*, male, cephalotheca, photomicrographs, SEM micrographs **A** frontal view **B** lateral view **C** vestigial antenna **D** frontal view **E** mouthparts. Abbreviations: fssf – furrow of supra-antennal sensillary field, mx – vestige of maxilla.

Labium and hypopharynx. Labium distinct between and below maxillae, dark. Praementum and postmentum separated by furrow. Hypopharyngeal protuberance indistinct or absent.

Mouth opening. Well visible, not covered by ventral labral field, slightly arcuate.

Phylogenetic relationships. Deeply nested within Xenidae (Benda et al. 2019, 2021), part of a clade of an Old Word origin, with *Pseudoxenos* Saunders as sister group.

Diversity and distribution. A lineage of Afrotropical-Palearctic origin, comprising 5 currently valid species, restricted to these regions. It is an example of connectivity between both biogeographic regions (Benda et al. 2019).

Hosts. *Ammophila* and *Podalonia* spp. (Sphecidae: Ammophilinae), rarely *Prionyx* spp. (Sphecidae: Sphecinae).

Etymology. From the Latin substantive *tuber*, meaning a swelling. The name refers to conspicuous swellings on the host abdomen caused by protruded xenid specimens under tergites or sternites. Gender masculine.

Comments. All described species of *Tuberoxenos* gen. nov. were previously placed in *Paraxenos* based on parasitising Sphecidae (Kinzelbach 1971b). Despite this concept, this group is morphologically well defined. We classify it as a separate genus,

based on molecular phylogenies (Benda et al. 2019, 2021) and morphological characters newly reported in this paper.

List of species

***Tuberoxenos altozambeziensis* (Luna de Carvalho, 1959), comb. nov.**

Pseudoxenos altozambeziensis Luna de Carvalho, 1959: 136.
Paraxenos altozambeziensis (Luna de Carvalho, 1959) (new combination by Kinzelbach 1971b).

Hosts. *Ammophila* sp. (Luna de Carvalho 1959); *Ammophila rubripes* Spinola, 1839 (Benda et al. 2021).

Distribution. Angola (Luna de Carvalho 1959); Tanzania (Benda et al. 2021).

***Tuberoxenos sinuatus* (Pasteels, 1956), comb. nov.**

Pseudoxenos sinuatus Pasteels, 1956: 115.
Paraxenos sinuatus (Pasteels, 1956) (new combination by Kinzelbach 1971b).

Hosts. *Ammophila punctaticeps* (Arnold, 1920); *Podalonia tydei* (Le Guillou, 1841) (as *Ammophila tydei* Le Guillou, 1841) (Pasteels 1956; Kinzelbach 1971b); *Ammophila argyrocephala* Arnold, 1951 (Benda et al. 2021).

Distribution. Democratic Republic of Congo (Pasteels 1956); Tanzania (Benda et al. 2021).

***Tuberoxenos sphecidarum* (Siebold, 1839), comb. nov.**

Xenos sphecidarum Siebold, 1839: 72.
Eupathocera sphecidarum (Dufour, 1837) (new combination by Pierce, 1908, incorrectly assigned authorship).
Paraxenos sieboldii Saunders, 1872 (synonymized by Pierce, 1909).
Paraxenos sieboldii (Dufour, 1837) (new combination by Pierce 1919, incorrectly assigned authorship).
Pseudoxenos sphecidarum (Dufour, 1837) (new combination by Bohart 1937, incorrectly assigned authorship).
Paraxenos sphecidarum (Dufour, 1837) (new combination by Kinzelbach 1971b, incorrectly assigned authorship).

Hosts. *Ammophila apicalis* Guérin-Méneville, 1835 (as *Ammophila apicalis* Brullé, 1839); *A. campestris* Latreille, 1809; *A. heydeni* Dahlbom, 1845 (as *Ammophila heydeni* Dahlberg?); *A. holosericea* (Fabricius, 1793); *A. nasuta* Lepeletier, 1845;

A. pubescens Curtis, 1836; *A. sabulosa* (Linnaeus, 1758); *Podalonia affinis* (Kirby, 1798) (as *Ammophila affinis* Kirby, 1798); *P. dispar* (Taschenberg, 1869) (as *Ammophila dispar* Taschenberg, 1869); *P. ebenina* (Spinola, 1839) (as *Ammophila ebenina* Spinola, 1839); *P. hirsuta* (Scopoli, 1763) (as *Ammophila hirsuta* Scopoli); *P. nigrohirta* (Kohl, 1888) (as *Ammophila nigrohirta* Kohl, 1888); *P. tydei* (Le Guillou, 1841) (as *Ammophila tydei* Le Guillou, 1841); *Eremochares dives* (Brullé, 1833) (as *Ammophila dives* Brullé, 1833); *Prionyx kirbii* (Vander Linden, 1827) (as *Sphex albisectus* Lep. & Serv., 1828); *P. viduatus* (Christ, 1791) (as *Sphex viduatus* Christ, 1791); *P. niveatus* (Dufour, 1854) (as *Sphex niveatus* Dufour, 1854) (Kinzelbach 1978); *Ammophila dupla* Kohl, 1901; *Podalonia chalybea* (Kohl, 1906); *Podalonia flavida* (Kohl, 1901) (Benda et al. 2021).

Distribution. Poland: Gdańsk (Siebold 1839); Palearctic (Kinzelbach 1978).

Note. Benda et al. (2021) proposed at least four distinctive *T. sphecidarum* lineages possibly representing separate species. More comprehensive sampling and detailed study are necessary.

Tuberoxenos teres (Pasteels, 1950), comb. nov.

Pseudoxenos teres Pasteels, 1950: 289.
Paraxenos teres (Pasteels, 1950) (new combination by Kinzelbach 1971b).

Hosts. *Ammophila beniniensis* (Palisot de Beauvois, 1806) (as *Sphex beniniensis* Palisot de Beauvois, 1806); *Ammophila beniniensis tomentosa* (Arnold, 1920) (as *Sphex beniniensis tomentosus* Arnold, 1920) (Kinzelbach 1971); *Ammophila ferrugineipes* Lepeletier, 1845 (as *Sphex bonaespei ferrugineipes* Lepeletier, 1845) (Kinzelbach 1971b, Pasteels 1950).

Distribution. Democratic Republic of Congo (Pasteels 1950).

Tuberoxenos tibetanus (Yang, 1981), comb. nov.

Paraxenos tibetanus Yang, 1981: 572.

Hosts. *Ammophila* sp.

Distribution. China.

Note. The article from Yang (1981) could not be found despite of great effort and the citation is not available.

Deltoxenos gen. nov.

http://zoobank.org/78A7DB5E-AA8B-4DCE-9F60-2001D2B218CB

Type species. *Pseudoxenos bidentatus* Pasteels, 1950, here designated.

Diagnosis of female cephalothorax. Maxilla not prominent, only slightly raised or nearly fused to labial area. Meso-metathoracic segmental border slightly or distinctly

constricted laterally (Fig. 47C, D), especially in species with elongated cephalothorax. Pro-mesothoracic segmental border rarely constricted. Dorsal labral field slightly or distinctly arcuate, raised or flat, in the latter case narrower laterally than medially (Fig. 3A). Lateral parts of abdomen posterior to spiracles not pale (Figs 1B, 47D). Mandible not protruding from capsule. In contrast to *Paragioxenos*, head and prothorax ventrally delimited by birth opening medially and by suture laterally.

Description of female cephalothorax. Shape and coloration. Very variable, ca. as long as wide, slightly wider than long, or distinctly longer than wide. Meso-metathoracic segmental border slightly or distinctly constricted laterally (Fig. 47C, D), especially in species with elongated cephalothorax. Pro-mesothoracic segmental border rarely constricted. Extremely variable in size, length 0.9–2.83 mm, maximum width 0.74–2.43 mm. Anterior head margin evenly rounded or protruding. Thorax slightly or distinctly widening posteriorly, sometimes nearly parallel-sided. Cephalothorax with conspicuous color pattern. Coloration comprising multiple brown and orange shades forming distinct pattern.

Head capsule. Ca. ¼ ~ ½ as long as entire cephalothorax including lateral cephalic extension. Coloration of head forming specific color pattern with pale and dark combined. Clypeal area well delimited from labral area, arcuate, or protruding and forming clypeal lobe. Surface smooth or slightly wrinkled. Sensilla (24 to 45 or more) regularly distributed on clypeal area or mainly concentrated on clypeal lobe. Border between clypeal region and frontal area not clearly distinguishable but border still recognizable. Cuticle of frontal region very variable, from distinctly wrinkled, slightly wrinkled to nearly smooth, or covered with distinct papillae. Border between head and prothorax well visible or faintly recognizable on dorsal side, often indicated by colored transverse stripe (Fig. 1B).

Supra-antennal sensillary field. Smooth, wrinkled or reticulated, with dispersed sensilla. Not delimited or indistinctly delimited by furrow on medial side, but border of field still distinctly visible (Figs 4A, 49B).

Antenna. Preserved as poorly defined area, with several small, rounded plates, antennal sensilla, or cavity, often combined (Figs 4B, 48C). Periantennal area smooth or wrinkled.

Labrum. Ventral field wider than long, elliptic to nearly circular. Dorsal field slightly or distinctly arcuate, raised, or flat and laterally narrower than medially (Fig. 3A). Ca. 4–6× wider than long in midline. Dorsal field with ~ 10–25 setae or sensilla inserted in cavities.

Mandible. Mandibles anteromedially directed at an angle of 25–65° and nested in mandibular capsule. Mandibular bulge distinctly raised, with several sensilla. Cuticle of mandible completely smooth to partially sculptured (Fig. 49E). Mandibular tooth narrow or slightly widened, pointed apically or ventrally, armed with spines.

Maxilla. Very variable in shape, distinctly reduced and almost fused with labial area, or slightly raised but not distinctly prominent (Figs 3B, 49E). Cuticle smooth or wrinkled. Apical maxillary region not or slightly projecting beyond mandibular apex. Basal portion firmly connected with labium and not overlapping with mandible, or in some cases elevated and overlapping with mandible very slightly. Vestige of palp inconspicuous, forming cavity or poorly defined area with indistinct plate. Usually located medially on ventral side of maxilla (Fig. 3B). Maxillary base more or less distinctly produced anterolaterally as submaxillary groove.

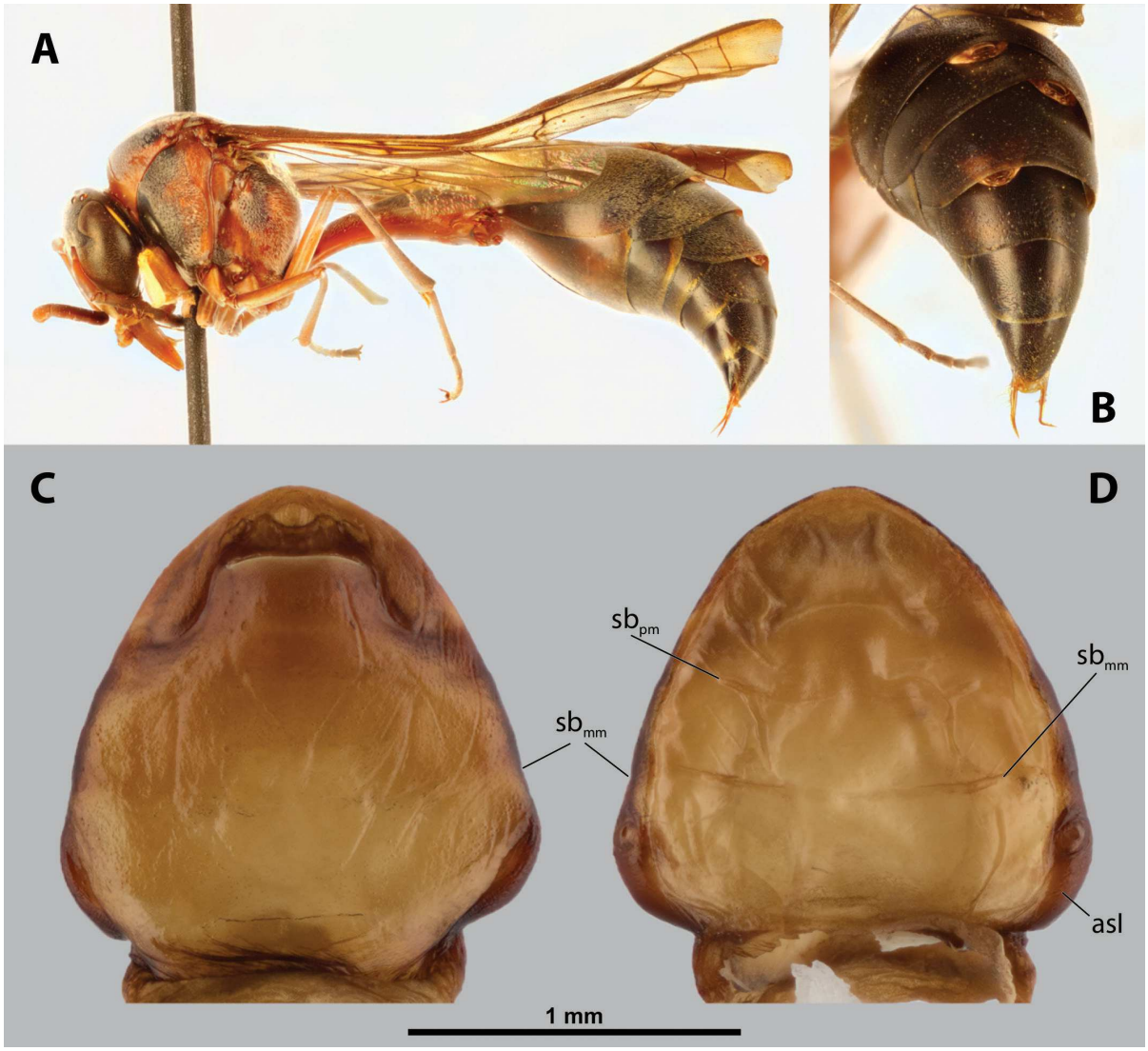

Figure 47. *Deltoxenos bidentatus*, host, female, cephalothorax, photomicrographs **A** *Afreumenes* cf. *aethiopicus* stylopized by female of *D. bidentatus*, lateral view **B** detail of host abdomen with adult female **C** ventral side of cephalothorax **D** dorsal side of cephalothorax. Abbreviations: asI – abdominal segment I, sbmm – segmental border between mesothorax and metathorax, sbpm – segmental border between prothorax and mesothorax.

Labium. Labial area usually distinct between maxillae, delimited anteriorly by mouth opening and posteriorly by birth opening. Flat, longer than wide or wider than long. Cuticular surface smooth or reticulated.

Mouth opening. Widely arcuate to nearly straight or bisinuate, sclerotized along margin.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders more or less distinct, usually separated by mesal furrows, combined with pigmented stripes or spots on dorsal and ventral side (Figs 1A, B, 47C, D). Border between metathorax and abdomen usually formed by ridge or indicated by change of cuticular sculpture (Fig. 1A). Cuticle of thoracic segments on ventral side reticulate with scattered inconspicuous or more distinct pigmented papillae. Dorsal side of thorax smooth or slightly reticulated. Prosternal extension undifferentiated, evenly arched.

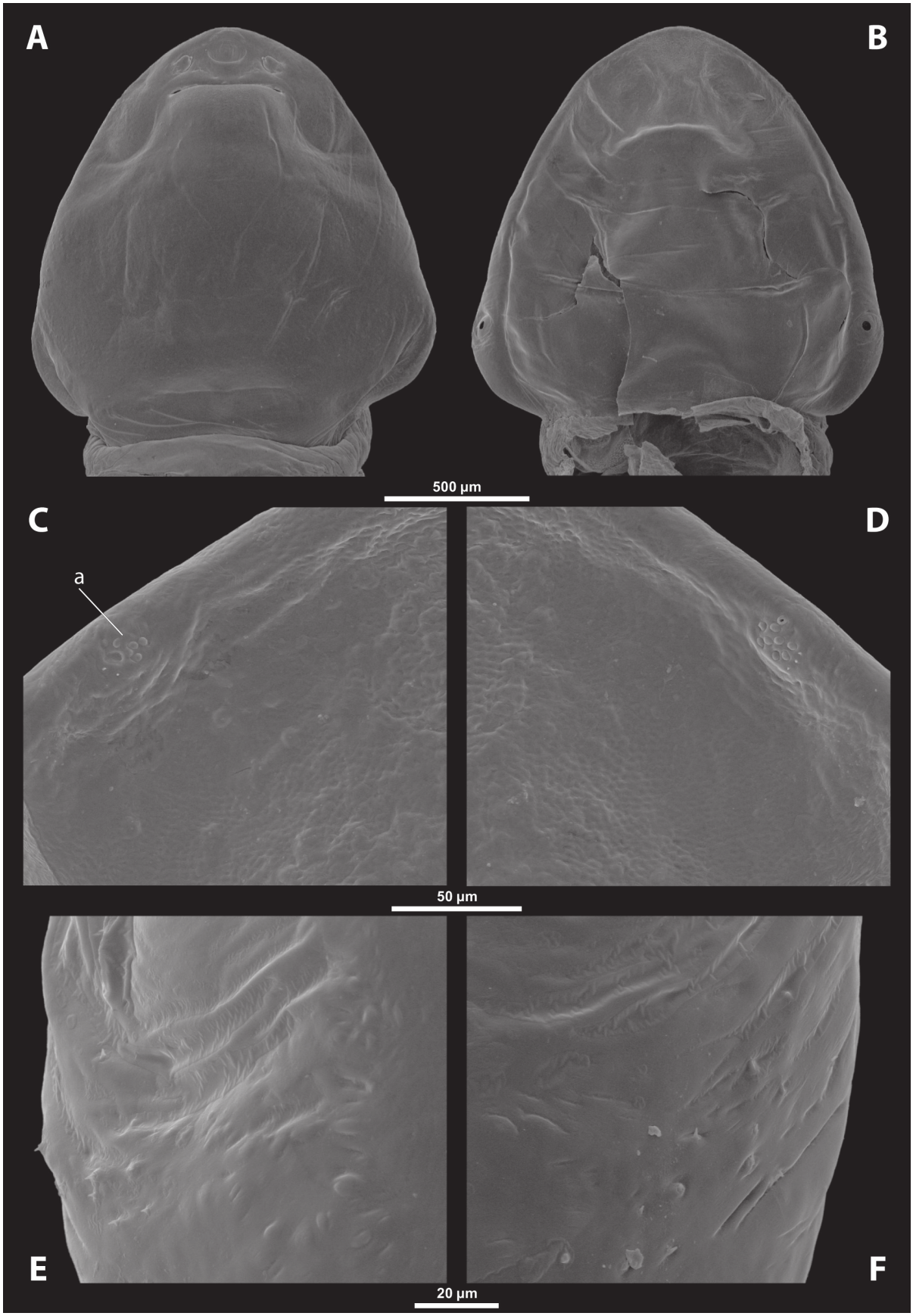

Figure 48. *Deltoxenos bidentatus*, female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** left vestigial antenna, dorsal side **D** right vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** right lateral border of abdominal segment I below spiracle, dorsal side. Abbreviations: a – vestigial antenna.

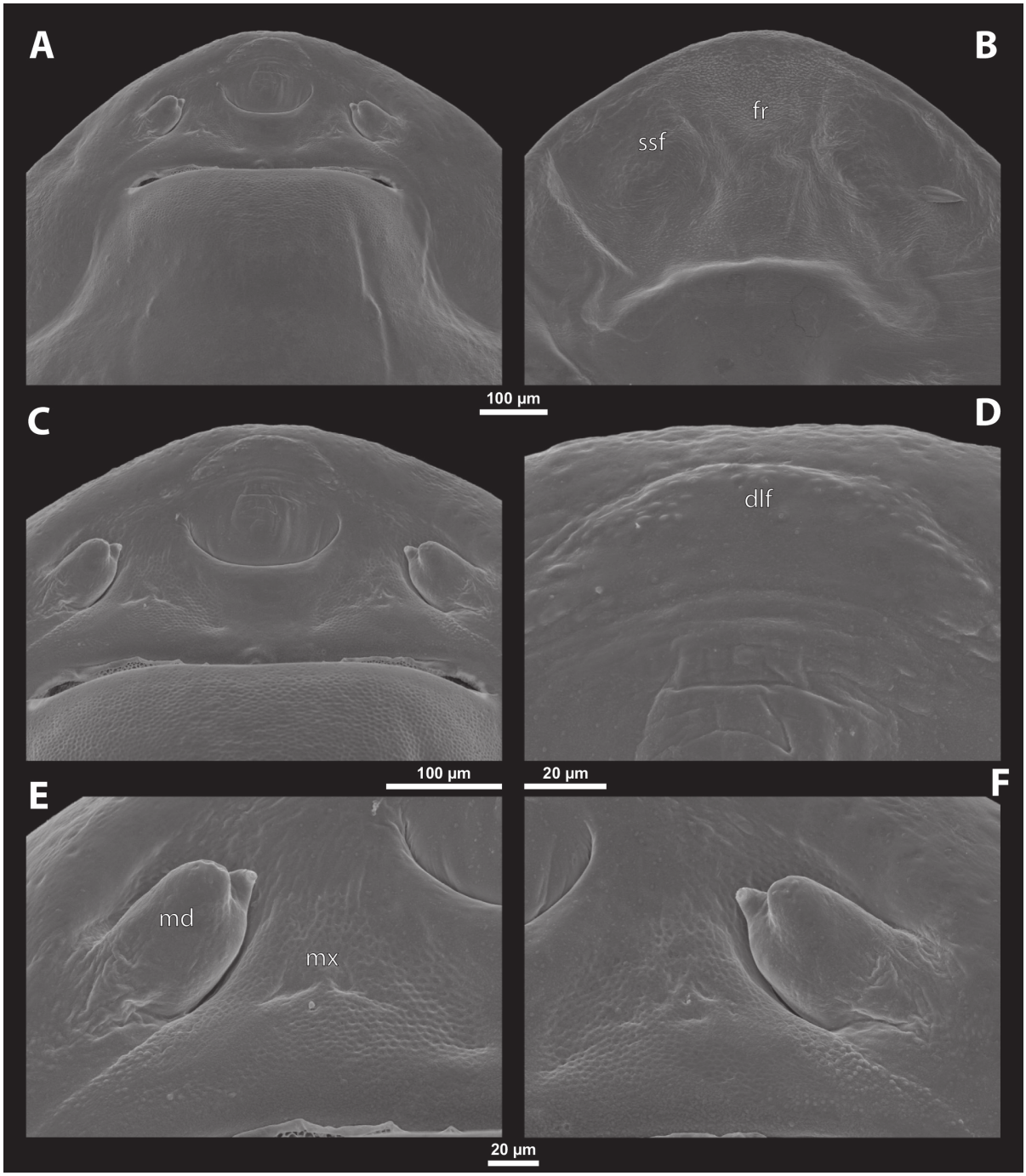

Figure 49. *Deltoxenos bidentatus*, female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: dlf – dorsal labral field of labral area, fr – frontal region, md – mandible, mx – vestige of maxilla, ssf – supra-antennal sensillary field.

Meso- and metathorax usually transverse or elongated in some cases. Lateral parts of abdomen posterior to spiracles dark (Figs 1B, 47D). Setae and cuticular spines present on lateral region of abdominal segment I (Fig. 48E, F).

Spiracles. Located on posterior third of cephalothorax, slightly elevated with anterodorsal and anterolateral orientation.

Diagnosis of male cephalotheca. Differing from other genera by the following combination of characters. Diameter of genae between maxillary base and compound eye at least 2× as large as diameter of vestigial antenna. Distinct paired furrow of supra-antennal sensillary field absent. Cephalotheca always elliptic (Figs 5A, 50A). Frontal fissure hardly distinct of nearly absent (Figs 6A, 50D). Maxilla not distinctly elongated, at most 1.5× longer than basally wide (Fig. 50E). Occipital bulge well developed (Figs 6A, 50D). Coloration forming pattern of pale and dark shades (Figs 5A, 50A).

Description of male cephalotheca. Shape and coloration. In frontal view rounded laterally, elliptic, in lateral view pointed anteriorly. Coloration forming pattern of pale and dark shades.

Cephalothecal capsule. Compound eyes with darker individual ommatidia well visible on pale ocular background. Clypeal lobe straight or slightly arcuate in frontal view, prominent in lateral view, in some cases bulging (Figs 6B, 50D). Sensilla mainly concentrated on clypeal lobe. Frontal impression more or less distinct (Figs 5A, 6A). Occipital bulge distinct (Figs 5A, 50D). Diameter of genae between maxillary base and compound eye smaller, > 2× larger than diameter of vestigial antenna.

Supra-antennal sensillary field. Kidney-shaped and bulging, medially delimited by more or less distinct frontal impression, lacking furrows.

Antenna. Of standard shape, with recognizable complete torulus. Periantennal area not clearly delimited from supra-antennal sensillary field. Small plates, cavities or sensilla present.

Labrum. Labral area distinct, with setae on dorsal field.

Mandible. Anteromedially directed. Mandibular bulge with sensilla, separated from pointed tooth.

Maxilla. Distinct, prominent, completely dark. Vestige of palp distinct.

Labium and hypopharynx. Labium distinct between and below maxillae, dark. Praementum and postmentum separated by furrow. Hypopharyngeal protuberance present or not.

Mouth opening. Well visible, not covered by ventral labral field, distinctly arcuate.

Phylogenetic relationships. Deeply nested within Xenidae, with *Xenos* as sister group (Benda et al. 2019; Straka and Benda unpubl. results).

Diversity and distribution. A lineage of Afrotropical origin with later expansion to the Palearctic and Indomalayan regions (Benda et al. 2019). Present distribution of 7 species comprising the Old World and Australasian region.

Hosts. Various genera of Eumenini and Odynerini (Vespidae: Eumeninae).

Etymology. Name derived from the generic name *Delta* Saussure, one of the most common host genera. Gender masculine.

Comments. All described species of *Deltoxenos* gen. nov. were previously placed in *Pseudoxenos* based on parasitism in solitary wasps (Kinzelbach 1971b). Despite this concept, this group is morphologically well defined. Although this group was not recognized in Kinzelbach's concept, we classify it as a separate genus based on molecular phylogenies (Benda et al. 2019, 2021) and morphological characters newly reported here.

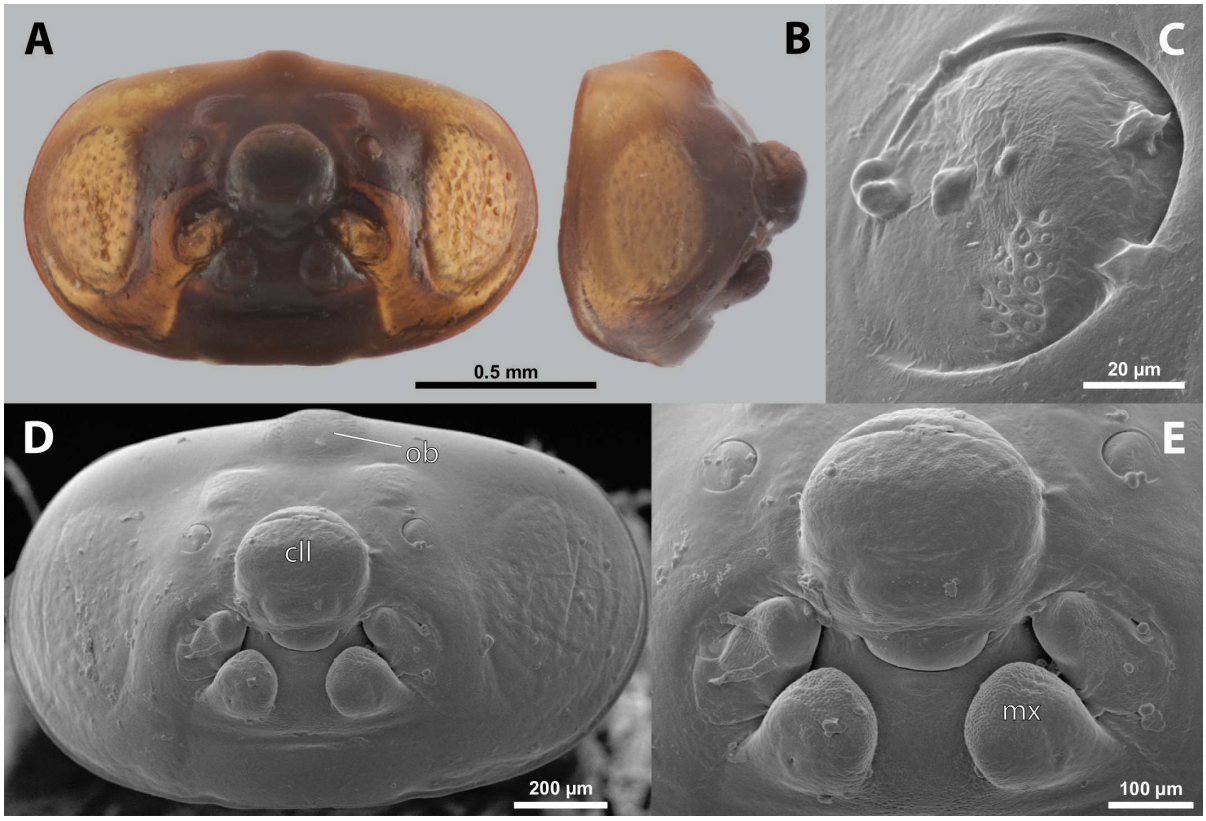

Figure 50. *Deltoxenos rueppelli*, male, cephalotheca, photomicrographs, SEM micrographs **A** frontal view **B** lateral view **C** vestigial antenna **D** frontal view **E** mouthparts. Abbreviations: cll – clypeal lobe, mx – vestige of maxilla, ob – occipital bulge.

List of species

***Deltoxenos bequaerti* (Luna de Carvalho, 1956), comb. nov.**

Pseudoxenos bequaerti Luna de Carvalho, 1956: 40.

Host. *Antepipona tropicalis* (Saussure, 1853) (as *Rygchium tropicale* Saussure, 1853) (Luna de Carvalho 1956).

Distribution. Angola: Dundo (Luna de Carvalho 1956).

***Deltoxenos bidentatus* (Pasteels, 1950), comb. nov.**

Pseudoxenos bidentatus Pasteels, 1950: 288.

Hosts. *Afreumenes melanosoma* (Saussure, 1852) (as *Eumenes melanosoma decipiens* Kirby, 1896); *Delta tropicale* (Saussure, 1852) (Benda et al. 2021); *Afreumenes* cf. *aethiopicus* (Saussure, 1852) (this study).

Distribution. Democratic Republic of Congo; Liberia (Pasteels 1950; Luna de Carvalho 1978a); Central African Republic (Benda et al. 2021); Malawi (this study).

***Deltoxenos hirokoae* (Kifune & Yamane, 1992), comb. nov.**

Pseudoxenos hirokoae Kifune & Yamane, 1992: 343.

Host. *Stenodynerus rufomaculatus* Sk. Yamane & Gusenleitner, 1982.

Distribution. Japan: Amami Oshima (Kifune and Yamane 1992).

Note. No DNA sequences from Xenidae parasitizing *Stenodynerus* Saussure in East Asia have been available. Strepsipterans parasitizing *Stenodynerus* in Japan are preliminarily included in *Deltoxenos* gen. nov. here based on their morphology, which, however, should be supported by future molecular phylogenetic analyses.

***Deltoxenos iwatai* (Esaki, 1931), comb. nov.**

Pseudoxenos iwatai Esaki, 1931: 63.

Host. *Oreumenes decoratus* (Smith, 1852) (as *Eumenes japonica* Saussure, 1858) (Esaki 1931).

Distribution. Japan (Esaki 1931).

***Deltoxenos lusitanicus* (Luna de Carvalho, 1960), comb. nov.**

Pseudoxenos lusitanicus Luna de Carvalho, 1960: 2.

Host. *Ancistrocerus renimacula* Lepeletier, 1841 (as *Ancistrocerus recinula* Lepeletier, 1841) (Kinzelbach 1971).

Distribution. Portugal (Luna de Carvalho 1960); Palearctic (Benda et al. 2021).

Note. This species corresponds to a lineage widely distributed from Portugal to Mongolia (Benda et al. 2021). Although its phylogenetic position is still unclear, it is provisionally included into *Deltoxenos* gen. nov. here based on morphology.

***Deltoxenos minor* (Kifune & Maeta, 1978), comb. nov.**

Pseudoxenos minor Kifune & Maeta, 1978: 416.

Host. *Stenodynerus frauenfeldi* (Saussure, 1867).

Distribution. Japan: Nagano Pref., Fukuoka Pref. (Kifune and Maeta 1978).
Note. See the comment under *D. hirokoae*.

Deltoxenos rueppelli (Kinzelbach, 1971a), comb. nov.

Pseudoxenos rueppelli Kinzelbach 1971a: 272.

Hosts. *Delta fenestrale* (Saussure, 1852) (as *Delta fenestralis* Saussure, 1852), *Delta emarginatum* (Linnaeus, 1758) (as *Eumenes tinctor* Christ, 1791 = *E. maxillosus* (De Geer, 1783)); *Delta caffrum* (Linnaeus, 1767) (Benda et al. 2021).

Distribution. Ethiopia (Kinzelbach 1971a); Tanzania (Benda et al. 2021); Kenya; Namibia; Yemen (this study).

Xenos Rossi, 1794

Xenos Rossi, 1794: 114. Type species: *Xenos vesparum* (Rossi, 1793), by monotypy.
Acroschismus Pierce, 1908: 79 (synonymized by Bohart 1941). Type species: *Acroschismus hubbardi* Pierce, 1908.
Schistosiphon Pierce, 1908: 80 (synonymized by Bohart 1941). Type species: *Xenos peckii* Kirby, 1813.
Vespaexenos Pierce, 1909: 133 (synonymized by Bohart 1941). Type species: *Vespaexenos crabronis* Pierce, 1909.
Belonogastechthrus Pierce, 1911: 498 (synonymized by Bohart 1941). Type species: *Belonogastechthrus zavattarii* Pierce 1911.
Clypoxenos Brèthes, 1923: 46 (synonymized by Bohart 1941). Type species: *Clypoxenos americanus* Brèthes, 1923.

Diagnosis of female cephalothorax. Differing from other genera by the combination of following characters. Clypeal sensilla distinct, position on clypeal lobe extended onto ventral side, often present near clypeo-labral border (Fig. 53D). Maxilla variable in shape, almost fused with labial area, or raised from it, but not distinctly prominent anteriorly (Fig. 53E, F). Reduced forms of maxilla often indistinctly separated from labial area. Cuticle of maxilla in some cases strongly sclerotized like in *Brasixenos*, but border between clypeus and labrum always distinct (Fig. 53D). Prosternal extension not differentiated. Mandible not protruding from capsule. In contrast to *Paragioxenos*, head and prothorax ventrally delimited by birth opening medially and by suture laterally.

Description of female cephalothorax. Shape and coloration. Extremely variable, ca. as long as wide, slightly wider than long, or distinctly longer than wide. Meso-metathoracic segmental border in some cases distinctly constricted laterally. Extremely variable in size, length 0.8–2.7 mm, maximum width 0.84–2.43 mm. Anterior head

Figure 51. *Xenos peckii*, host, female, cephalothorax, photomicrographs **A** *Polistes fuscatus* stylopized by two females of *X. peckii*, lateral view **B** detail of host abdomen with two adult females inside **C** ventral side of cephalothorax **D** dorsal side of cephalothorax. Abbreviations: bcm – brood canal membrane.

margin evenly rounded, protruding, or strongly protruding. Thorax slightly or distinctly widening posteriorly. Cephalothorax uniformly pale or colorful. Coloration with multiple brown (nearly black) and orange shades forming distinct pattern, often with pale anterior part and dark posterior area (Fig. 51C).

Head capsule. Ca. ⅓ ~ ½ as long as entire cephalothorax including lateral cephalic extension. Coloration forming specific pattern with pale and dark combined. Clypeal region well delimited from labral area, border between clypeus and labrum often distinct (Figs 52F, 53D). Clypeal area variable in shape, apical margin arcuate, nearly flat, or protruding, forming distinct clypeal lobe. Cuticle smooth or slightly wrinkled. Numerous distinct sensilla present on clypeal surface, between 20 and 60 (or more), mainly concentrated anteriorly, rarely also scattered laterally, on clypeal lobe extending to ventral side, often near indistinct clypeo-labral border (Fig. 53D). Cuticle of frontal region slightly wrinkled. Segmental border between head and prothorax often indistinct to almost absent, at most indicated by change of color or transverse colored stripe.

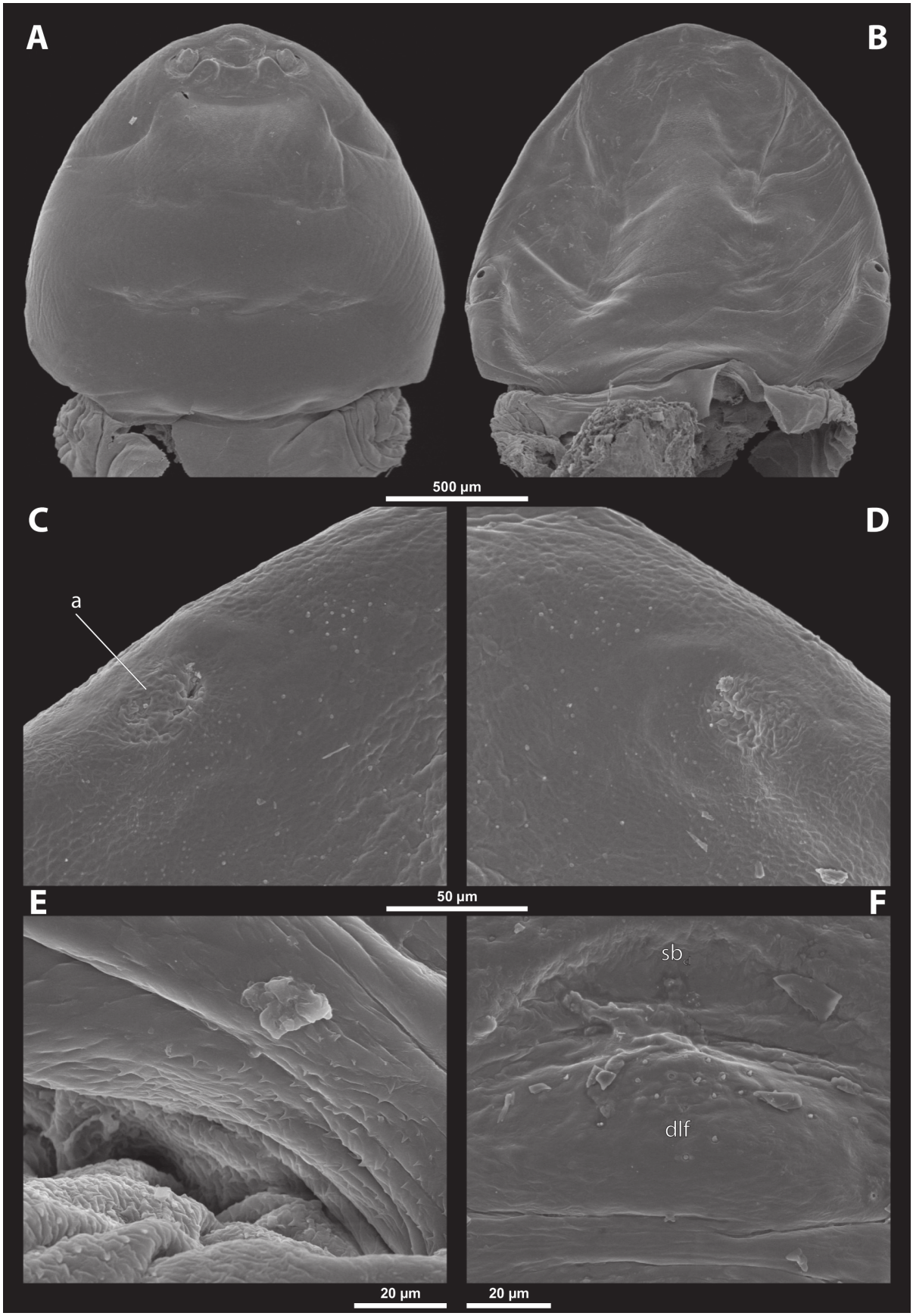

Figure 52. *Xenos peckii*, female, cephalothorax, SEM micrographs **A** ventral side **B** dorsal side **C** left vestigial antenna, dorsal side **D** right vestigial antenna, dorsal side **E** left lateral border of abdominal segment I below spiracle, dorsal side **F** detail of labral area, dorsal side. Abbreviations: a – vestigial antenna, dlf – dorsal labral field of labral area, sbcl – segmental border between clypeus and labrum.

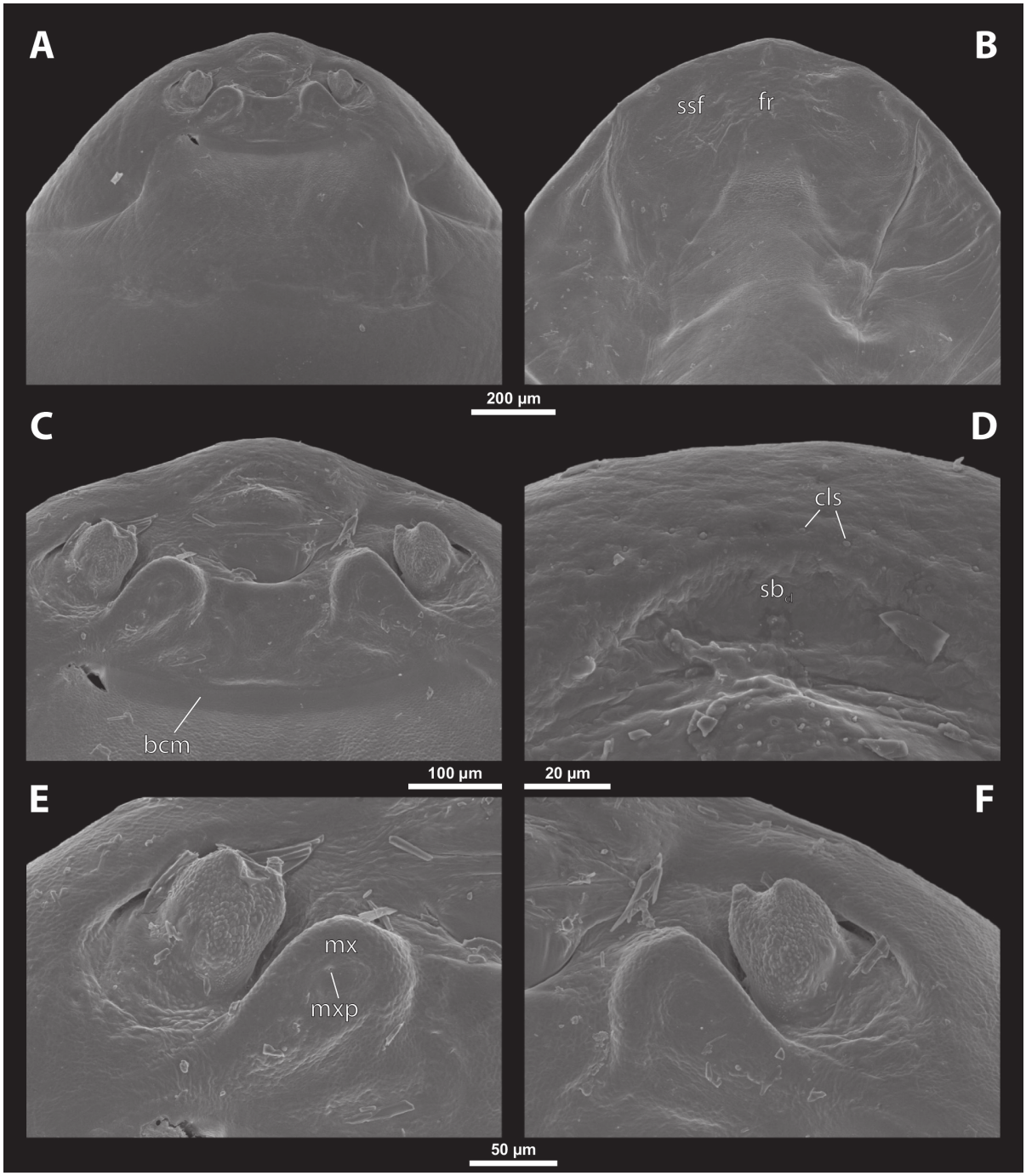

Figure 53. *Xenos peckii*, female, cephalothorax, SEM micrographs **A** anterior part of cephalothorax, ventral side **B** anterior part of cephalothorax, dorsal side **C** mouthparts, ventral side **D** detail of anterior border of cephalothorax, ventral side **E** right mandible and maxilla, ventral side **F** left mandible and maxilla, ventral side. Abbreviations: bcm – brood canal membrane, cls – clypeal sensillum, fr – frontal region, mx – vestige of maxilla, mxp – vestige of maxillary palp, sbcl – segmental border between clypeus and labrum, ssf – supra-antennal sensillary field.

Supra-antennal sensillary field. Slightly wrinkled with dispersed sensilla. Not delimited or indistinctly delimited by furrow medially, but border usually still recognizable (Fig. 53B).

Antenna. Preserved as poorly defined area, usually with several small, rounded plates, antennal sensilla, or cavity (Fig. 52C), in some cases combined, but antennal

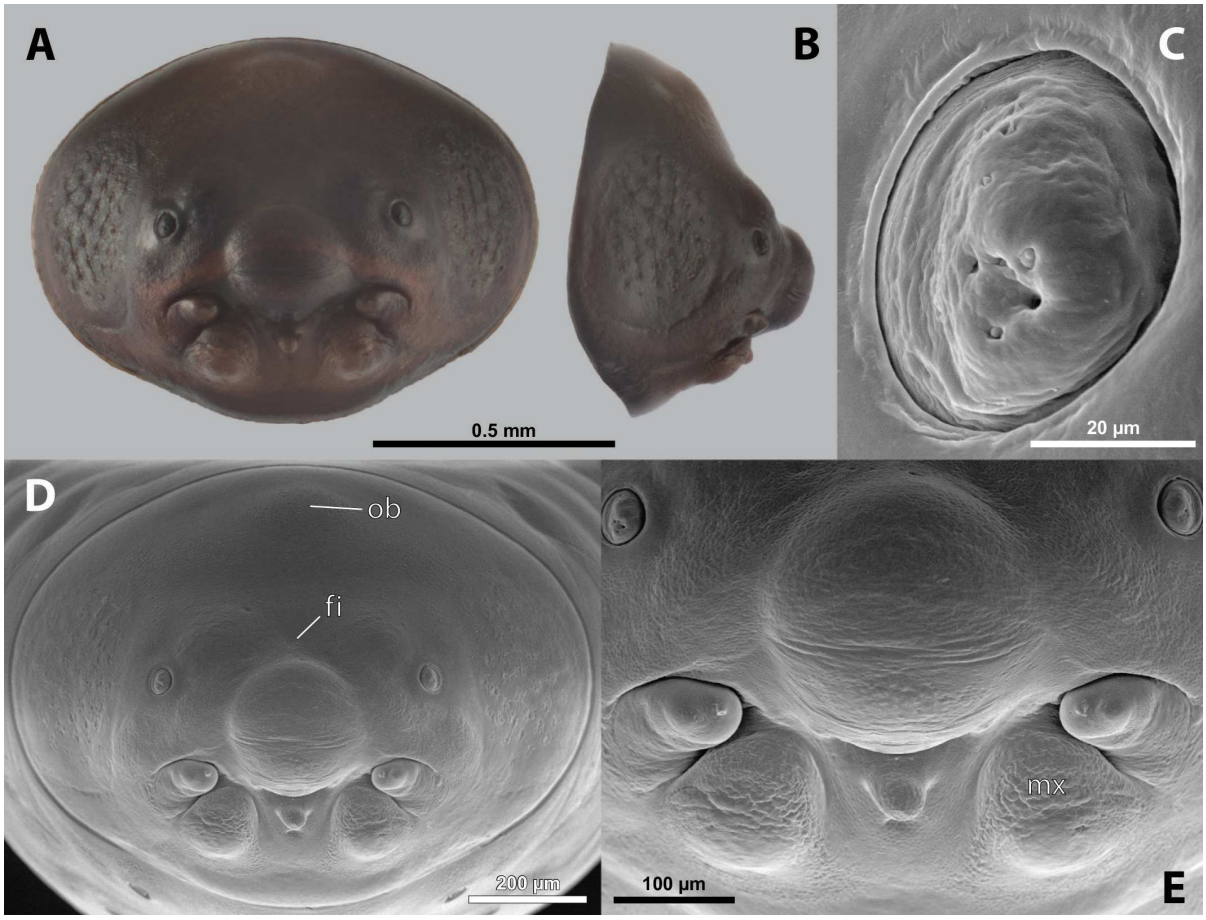

Figure 54. *Xenos peckii*, male, cephalotheca, photomicrographs, SEM micrographs **A** frontal view **B** lateral view **C** vestigial antenna **D** frontal view **E** mouthparts. Abbreviations: fi –frontal impression, mx – vestige of maxilla, ob – occipital bulge.

vestige in some cases only visible as strongly sculptured cuticle, without any plates or sensilla. Periantennal area wrinkled or reticulated.

Labrum. Ventral field variable, semicircular to nearly circular, elliptic, or subtriangular. Dorsal field slightly arcuate to straight, raised, or flat, ~ 4–5× wider than long in midline (Fig. 52F). Dorsal field laterally as long as medially, or laterally narrowed, with ~ 10–20 setae or sensilla inserted in cavities.

Mandible. Anteromedially directed at angle of 30–75° and enclosed in mandibular capsule, exceptionally slightly protruding. Mandibular bulge more or less distinctly raised, with several sensilla. Cuticle of mandible completely or partially sculptured. Tooth narrow or wider, pointed apically, more or less distinctly armed with spines.

Maxilla. Variable in shape, nearly fused with labial area and scarcely distinguishable from it, or raised but not distinctly prominent anteriorly (Fig. 53E, F). Cuticle smooth, wrinkled or reticulated, in some cases strongly sclerotized. Maxillary apex not projecting beyond mandible anteriorly but in some cases elevated maxillary base very slightly overlapping base of mandible. Vestige of palp inconspicuous, very poorly defined, often forming cavity or completely missing. If recognizable usually located medially or slightly apically on ventral side of maxilla (Fig. 53E). Maxillary base usually indistinctly produced anterolaterally as a submaxillary groove.

Labium. Labial area more or less recognizable between maxillae, delimited anteriorly by mouth opening and posteriorly by birth opening. Flat, slightly wider than long, as long as wide, or longer than wide. Cuticular surface smooth or reticulated.

Mouth opening. Widely arcuate to nearly straight or bisinuate, in some cases V-shaped, sclerotized along margin.

Thorax and abdominal segment I. Pro-mesothoracic and meso-metathoracic borders more or less distinct, usually indicated by mesal furrows, combined with pigmented stripes or spots on dorsal side. Border between metathorax and abdomen usually formed by ridge or indicated by change of cuticular sculpture. Cuticle of thoracic segments on ventral side reticulate with scattered small or larger pigmented papillae. Dorsal side of thorax smooth or slightly reticulated. Prosternal extension undifferentiated, evenly arched. Meso- and metathorax of standard transverse shape, in few cases constricted laterally. Setae and cuticular spines present on lateral region of abdominal segment I (Fig. 52E).

Spiracles. Spiracles on posterior third of cephalothorax slightly elevated, with anterodorsal and anterolateral orientation.

Diagnosis of male cephalotheca. Differing from other genera by the following combination of characters. Diameter of genae between maxillary base and compound eye ~ 2–3× larger than diameter of vestigial antenna. Paired furrow of supra-antennal sensillary field slightly distinct or indistinct. Cephalotheca usually elliptic (Fig. 54A). Frontal fissure indistinct or almost absent (Fig. 54D). Maxilla not distinctly elongated, at most 1.5× longer than basally wide (Fig. 54E). Occipital bulge strongly reduced or missing (Fig. 54D). Cephalotheca mostly dark (Fig. 54A).

Description of male cephalotheca. Shape and coloration. In frontal view rounded, elliptic, in lateral view slightly pointed anteriorly or rounded. Coloration with pattern of pale and dark shades but dark color dominant.

Cephalothecal capsule. Compound eyes completely dark or lighter, with dark individual cornea lenses visible. Clypeal lobe straight or slightly arcuate in frontal view, not or slightly prominent in lateral view. Sensilla mainly concentrated on clypeal lobe. Frontal impression inconspicuous or distinct (Fig. 54D). Occipital bulge indistinct (Fig. 54D) or absent. Diameter of genae between maxillary base and compound eye ~ 2–3× larger than diameter of vestigial antenna.

Supra-antennal sensillary field. Kidney-shaped and bulging, without furrows, delimited medially by more or less distinct frontal impression.

Antenna. Of standard shape, with small plates, cavities or sensilla, and complete torulus (Fig. 54C). Periantennal area not clearly delimited from supra-antennal sensillary field.

Labrum. Labral area distinct, with setae on dorsal field.

Mandible. Anteromedially directed. Mandibular bulge with sensilla, separated from pointed tooth.

Maxilla. Distinct, prominent, dark. Vestige of palp distinct.

Labium and hypopharynx. Dark labium distinctly visible between and below maxillae. Praementum and postmentum separated by indistinct transverse furrow. Hypopharyngeal protuberance present.

Mouth opening. Well visible, not covered by ventral labral field, slightly or distinctly arcuate.

Phylogenetic relationships. Deeply nested within Xenidae, representing the largest radiation (Benda et al. 2021), sister to *Deltoxenos* gen. nov. (Benda et al. 2019; Straka and Benda unpubl. results)

Diversity and distribution. The geographic origin is unclear, probably the New World or Afrotropical region (Benda et al. 2019). The present distribution of 33 described species comprising the Old and New World.

Hosts. Several tribes of social Vespidae (Vespini, Polistini, Mischocyttarini, and Ropalidiini).

Comments. The first species of Strepsiptera, *Xenos vesparum*, was superficially described by Rossi (1793), who assigned it to the genus *Ichneumon* in Hymenoptera. The genus *Xenos* was introduced later by Rossi (1794). Pierce (1908, 1909, 1911) described several genera (*Acroschismus*, *Belonogastrechthrus*, *Schistosiphon*, *Vespaexenos*) based on his hypothesis of host specialization. These were later synonymized with *Xenos* by Bohart (1941), and also the genus *Clypoxenos* described by Brèthes (1923). Kinzelbach (1971b) maintained this concept and extended it to *Brasixenos*, and considered representatives of *Xenos* as parasites of social wasps. Benda et al. (2019, 2021) revealed xenids parasitizing social Vespidae as a polyphyletic group. We classify *Xenos* as a valid genus based on the monophyly revealed by molecular phylogenies (Benda et al. 2019, 2021) and based on morphological characters newly reported here.

List of species

Xenos afer Pasteels, 1950

Xenos afer Pasteels, 1950: 284.

Hosts. *Polistes marginalis* (Fabricius, 1775); *P. tristis* Meade-Waldo, 1911 (as *Polistes smithi tristis* Meade-Waldo, 1911); *P. africanus* Palisot de Beuvois, 1818 (as *P. marginalis* v. *africanus* Palisot de Beuvois, 1818) (Pasteels 1950; Luna de Carvalho 1956).

Distribution. Democratic Republic of Congo (Pasteels 1950); Angola (Luna de Carvalho 1956); Central African Republic; Ethiopia; Zanzibar (Benda et al. 2021).

Xenos americanus (Brèthes, 1923)

Clypoxenos americanus Brèthes, 1923: 46.
Xenos americanus (Brèthes, 1923) (new combination by Bohart 1941).

Host. *Mischocyttarus flavicans* (Fabricius, 1804) (as *Clypeopolybia duckei* Brèthes, 1923) (Brèthes 1923).

Distribution. Bolivia (Brèthes 1923).

Xenos argentinus Brèthes, 1923

Xenos argentinus Brèthes, 1923: 43.

Hosts. *Polistes cavapyta* Saussure, 1853 (Brèthes 1923); *Polistes buyssoni* Brethes, 1903 (this study).

Distribution. Argentina: San Luis (Brèthes 1923), Cachi (this study).

Xenos boharti Hofmann, 1965

Xenos boharti Hofmann, 1965: 35.

Host. *Polistes peruvianus* Bequard, 1934 (Hofmann 1965).

Distribution. Chile: Tarapacá (Hofmann 1965).

Xenos bohlsi Hoffmann, 1914

Xenos bohlsi Hoffmann, 1914: 100.

Host. *Polistes canadensis canadensis* (Linnaeus, 1758) (Hoffmann 1914).

Distribution. Argentina; Brazil; Paraguay (Hoffmann 1914; Oliveira and Kogan 1962; Kinzelbach 1971b).

Xenos bonairensis Brèthes, 1923

Xenos bonairensis Brèthes, 1923: 44.

Host. *Polistes versicolor* (Olivier, 1792) (Brèthes 1923).

Distribution. Argentina: Buenos Aires (Brèthes 1923); Brazil (Luna de Carvalho 1978b).

Xenos circularis Kifune & Maeta, 1985

Xenos circularis Kifune & Maeta, 1985: 430.

Host. *Polistes rothneyi gressitti* Vecht, 1968 (Kifune and Maeta 1985).

Distribution. Taiwan (Kifune and Maeta 1985).

Xenos colombiensis Cook, Mayorga-Ch & Sarmiento, 2020

Xenos colombiensis Cook, Mayorga-Ch & Sarmiento, 2020: 332.

Host. *Polistes myersi* Bequaert, 1934 (Cook et al. 2020).
Distribution. Colombia (Cook et al. 2020).

Xenos dianshuiwengi Yang, 1999

Xenos dianshuiwengi Yang, 1999: 186.

Host. *Vespa* sp. (Yang 1999).
Distribution. China: Fujian (Yang 1999).

Xenos formosanus Kifune & Maeta, 1985

Xenos formosanus Kifune & Maeta, 1985: 426.

Host. *Vespa velutina flavitarsus* Sonan, 1939 (Kifune and Maeta 1985).
Distribution. Taiwan (Kifune and Maeta 1985).

Xenos hamiltoni Kathirithamby & Hughes, 2006

Xenos hamiltoni Kathirithamby & Hughes, 2006: 37.

Host. *Polistes carnifex* (Fabricius, 1775) (Kathirithamby and Hughes 2006).
Distribution. Mexico: Veracruz (Kathirithamby and Hughes 2006).

Xenos hebraei Kinzelbach, 1978

Xenos hebraei Kinzelbach, 1978: 69.

Hosts. *Polistes olivaceus* (De Geer, 1773) (as *Polistes hebraeus* Fabricius, 1787) (Kinzelbach 1978); *Polistes wattii* Cameron, 1900 (this study).
Distribution. Iraq; India (Kinzelbach 1978); Oman (this study).

Xenos hospitus Oliveira & Kogan, 1962

Xenos hospitus Oliveira & Kogan, 1962: 7.

Host. *Polistes versicolor* (Olivier, 1791) (as *Polistes versicolor vulgaris* Bequaert, 1934) (Oliveira and Kogan 1962).

Distribution. Brazil: Santa Catarina (Oliveira and Kogan 1962); Ecuador (this study).

Xenos hunteri (Pierce, 1909)

Acroschismus hunteri Pierce, 1909: 130.
Xenos hunteri (Pierce, 1909) (new combination by Bohart 1941).

Host. *Polistes* sp., near *P. minor* Palisot de Beauvois, 1818 (Pierce 1909).

Distribution. USA: Texas (Pierce 1909).

Xenos indespectus Oliveira & Kogan, 1962

Xenos indespectus Oliveira & Kogan, 1962: 10.

Host. *Polistes* sp. (Oliveira and Kogan 1962).

Distribution. Brazil: São Paulo (Oliveira and Kogan 1962).

Xenos iviei Kifune, 1983

Xenos iviei Kifune, 1983: 330.

Host. *Polistes crinitus* (Felton, 1764) (Kifune 1983).

Distribution. Virgin Islands (Kifune 1983).

Xenos kifunei Cook & Mathison, 1997

Xenos kifunei Cook & Mathison, 1997: 246.

Host. *Polistes comanchus navajoe* Cresson, 1868 (Cook and Mathison 1997).

Distribution. USA: Arizona (Cook and Mathison 1997; Garza and Cook 2021).

Xenos moutoni Buysson, 1903

Xenos moutoni Buysson, 1903: 175.
Vespaexenos moutoni (Buysson, 1903) (new combination by Pierce 1909).
Vespaexenos crabronis Pierce, 1909 (synonymized by Bohart 1941).
Vespaexenos buyssoni Pierce, 1909 (synonymized by Bohart 1941).
Vespaexenos matsumarai Szekessy, 1965 (synonymized by Kinzelbach 1971b).

Hosts. *Vespa analis nigrans* Buysson, 1903 (as *Vespa nigrans* Buysson, 1903); *Vespa crabro* Linnaeus, 1758; *Vespa ducalis* Smith, 1852; *Vespa dybowskii* André, 1884; *Vespa mandarinia* Smith, 1852; *Vespa mandarina magnifica* Smith, 1852 (as *Vespa magnifica* Smith, 1852); *Vespa simillima* Smith, 1868 (Buysson 1903; Nakase and Kato 2013).

Distribution. China: Anhui, Yunnan; Taiwan; Japan; Laos (Buysson 1903; Nakase and Kato 2013).

Xenos niger Pasteels, 1950

Xenos niger Pasteels, 1950: 287.

Host. *Polistes tenellus* Buysson, 1905 (Pasteels 1950).

Distribution. Democratic Republic of Congo (Pasteels 1950).

Xenos nigrescens Brues, 1903

Xenos nigrescens Brues, 1903: 247.

Host. *Polistes carolina* (Linneaus, 1767) (as *Polistes rubiginosus* Lepeletier, 1836) (Brues 1903; Cook 2019).

Distribution. USA: Texas (Brues 1903), Georgia (Garza and Cook 2021).

Notes. *Polistes carolina* (Linneaus, 1767) was listed as a host by Cook (2019), because it was a former synonym of *Polistes rubiginosus* Lepeletier, 1836, which does not occur in the USA. Kinzelbach (1971b) incorrectly stated Argentina as a location.

Xenos oxyodontes Nakase & Kato, 2013

Xenos oxyodontes Nakase & Kato, 2013: 333.

Hosts. *Vespa analis* Fabricius, 1775, *Vespa simillima* Smith, 1868 (Nakase and Kato 2013).

Distribution. Japan; South Korea (Nakase and Kato 2013).

Xenos pallidus Brues, 1903

Xenos pallidus Brues, 1903: 246.
Acroschismus hubbardi Pierce, 1908 (synonymized by Bohart 1941).
Acroschismus pallidus texensis Pierce, 1909 (synonymized by Bohart 1941).

Hosts. *Polistes annularis* (Linnaeus, 1763); *Polistes crinitus* (Felton, 1764) (as *Polistes* (*americanus*) *crinitus* (Felton, 1764)); *Polistes carnifex* (Fabricius, 1775), *Polistes bellicosus* Cresson, 1872 (Brues 1903; Cook 2019, misspelt as *P. vellicosus*).

Distribution. USA: Texas, Florida; Mexico (Brues 1903; Dunkle 1979).

Xenos peckii Kirby, 1813

Xenos peckii Kirby, 1813: 116.
Xenos wheeleri Pierce, 1908 (synonymized by Bohart 1941).
Acroschismus bruesi Pierce, 1909 (synonymized by Bohart 1941).
Acroschismus pecosensis Pierce, 1909 (synonymized by Bohart 1941).
Acroschismus bowditchi Pierce, 1909 (synonymized by Bohart 1941).
Acroschismus texani Pierce, 1909 (synonymized by Bohart 1941).
Acroschismus maximus Pierce, 1909 (synonymized by Bohart 1941).
Xenos auriferi Pierce, 1911 (synonymized by Bohart 1941).
Xenos californicus Pierce, 1919 (synonymized by Bohart 1941).
Xenos pecki (incorrect subsequent spelling): Kinzelbach (1971b).

Hosts. *Polistes apachus* Saussure, 1857 (as *Polistes texanus* Cresson, 1872); *Polistes aurifer* Saussure, 1853; *Polistes carolina* (Linnaeus, 1767) (as *Polistes rubiginosus* Lepeletier, 1836); *Polistes fuscatus* (Fabricius, 1793); *Polistes metricus* Say, 1831 (Kirby 1813; Pierce 1908, 1909).

Distribution. USA: Massachusetts (Kirby 1813; Pierce 1909), Connecticut, Michigan, Ohio, Texas, California (Kirby 1813; Pierce 1908, 1909, 1919), New Jersey, New York, Colorado, Wyoming (Garza and Cook 2021).

Xenos peruensis Kifune, 1979

Xenos peruensis Kifune, 1979: 408.

Host. *Polistes lanio* (Fabricius, 1775) (Kifune 1979).

Distribution. Peru (Kifune 1979).

Xenos provesparum Kifune, 1986

Xenos provesparum Kifune, 1986: 84.

Hosts. *Provespa anomala* (Saussure, 1854); *Provespa nocturna* Vecht, 1935 (Kifune 1986).

Distribution. Indonesia: Sumatra, Padang (Kifune 1986); Thailand (Kifune and Yamane 1998).

Xenos ropalidiae (Kinzelbach, 1975), comb. nov.

Pseudoxenos ropalidiae Kinzelbach, 1975: 69.

Hosts. *Ropalidia cincta* (Lepeletier, 1836); *Ropalidia fulvopruinosa* (Cameron, 1906); *Ropalidia marginata* (Lepeletier, 1836) (as *Ropalidia ferruginea* F.); *Ropalidia nobilis* (Gerstäcker, 1857); *Ropalidia variegata* (Smith, 1852) (Kinzelbach 1975; Cook 2019); *Ropalidia malayana* (Cameron 1903) (Benda et al. 2021).

Distribution. Democratic Republic of Congo; India; Indonesia: Java; Papua New Guinea; Philippines (Kinzelbach 1975; Cook 2019); Laos; Nepal; Malaysia (Benda et al. 2021).

Note. Benda et al. (2021) proposed three lineages possibly representing separate species. More comprehensive sampling and detailed study are necessary.

Xenos rostratus Trois, 1984b

Xenos rostratus Trois, 1984b: 24.

Hosts. *Polistes billardieri ruficornis* Saussure, 1853 (as *Polistes ruficornis ruficornis* Saussure, 1853); *Polistes billardieri biglumoides* Ducke, 1904 (as *Polistes ruficornis biglumoides* Ducke, 1904) (Trois 1984b).

Distribution. Brazil, Sao Paulo; Paraguay, Villarcia; Peru, Ayacucho (Trois 1984b); Argentina (Benda et al. 2021).

Xenos rubiginosi (Pierce, 1909)

Acroschismus rubiginosi Pierce, 1909: 132.
Xenos rubiginosi (Pierce, 1909) (new combination by Bohart 1941).

Host. *Polistes carolina* (Linnaeus, 1767) (as *Polistes rubiginosus* Lepeletier) (Pierce 1909).

Distribution. USA: Louisiana (Pierce 1909).

Xenos stuckenbergi Pasteels, 1956

Xenos stuckenbergi Pasteels, 1956: 441.

Host. *Polistes marginalis* (Fabricius, 1775) (Pasteels 1956).
Distribution. RSA: Natal (Pasteels 1956).

Xenos vesparum (Rossi, 1793)

Ichneumon vesparum Rossi, 1793: 49.
Xenos vesparum (Rossi, 1793) (new combination by Rossi 1794).
Xenos rossii Kirby, 1813 (synonymized by Saunders 1872).
Xenos jurinei Saunders, 1872 (synonymized by Kinzelbach 1971b).
Xenos minor Kinzelbach, 1971a, syn. nov.

Hosts. *Polistes albellus* Giordani Soika, 1976; *Polistes associus* (Kohl, 1898); *Polistes biglumis* (Linnaeus, 1758); *Polistes dominula* (Christ, 1791) (as *Vespa gallica* Linnaeus and *Polistes gallicus* Linnaeus); *Polistes gallicus* (Linnaeus, 1767) (as *Polistes foederatus* Kohl, 1898); *Polistes nimpha* (Christ, 1791); *Polistes sulcifer* (Zimmerman, 1930); *Polistes semenowi* (Morawitz, 1889); *Vespula vulgaris* (Linnaeus,1758); *Ropalidia* sp. (Kinzelbach 1971a, 1978; Benda et al. 2021).

Distribution. Italy (Rossi 1793, 1794); Palearctic (Kinzelbach 1978; Benda et al. 2021); India (Benda et al. 2021).

Note. *Xenos minor* is synonymized under *X. vesparum* based on the results of a recent molecular phylogeny of Benda et al. (2021). Specimens morphologically corresponding to *Xenos minor* were nested within the lineage of *Xenos vesparum*. The former taxonomy was probably misled by the large phenotypic variability of *Xenos vesparum*, corresponding to different host taxa (smaller specimens of *X. vesparum* are associated with smaller individuals of *Polistes* spp.).

Xenos yamaneorum Kifune & Maeta, 1985

Xenos yamaneorum Kifune & Maeta, 1985: 430.

Host. *Polistes gigas* Kirby, 1826 (Kifune and Maeta 1985).
Distribution. Taiwan (Kifune and Maeta 1985).

Xenos yangi Dong, Liu & Li, 2022

Xenos yangi Dong, Liu & Li, 2022: 15.

Hosts. *Vespa velutina* Lepeletier, 1836 and *Vespa bicolor* Fabricius, 1787 (Dong et al. 2022).

Distribution. China: Yunnan (Dong et al. 2022).

Xenos zavattarii (Pierce, 1911)

Belonogastechthrus zavattarii Pierce, 1911: 498.
Xenos zavattarii (Pierce, 1911) (new combination by Bohart 1941).

Hosts. *Belonogaster lateritia* Gerstaecker, 1857 (as *Belonogaster elegans* Gerstaecker, 1857); *Belonogaster juncea* (Fabricius, 1781); (Pierce 1911; Kinzelbach 1978).

Distribution. Uganda: Butiti (Pierce 1911); Angola; Democratic Republic of Congo; Liberia; Libya: Tripolis (Pasteels 1950; Luna de Carvalho 1956; Kinzelbach 1978); Central African Republic; Ethiopia; Yemen: Socotra (Benda et al. 2021).

Note. Benda et al. (2021) reported two lineages that could be considered as separate species.

Key to genera of Xenidae based on the female cephalothorax

1 Head and prothorax on ventral side completely separated by birth opening (Fig. 8A). Dorsal labral field elliptic, ~ 2× wider than medially long, distinctly protuberant (dlf, Fig. 8A) ..... ***Paragioxenos*** **Ogloblin (Australia; *Paragia* spp.)**

– Head and prothorax on ventral side separated by birth opening medially and by suture laterally (Fig. 1A). Dorsal labral field at least 3× wider than long in midline (dlf, Fig. 3A) **2**

2 Maxillae strongly sclerotized, partially fused with labial area, not prominent, appearing connected proximally along birth opening (Fig. 10A, 20C). Cephalothorax mostly lightly colored **3**

– Sclerotization of maxillae different. Maxillae partly fused with labium or prominent. Cephalothorax variously colored **5**

3 Mandible distinctly protruding from mandibular capsule, reaching or slightly projecting beyond anterior edge of head (md, Fig. 10A). Anterior part of maxilla pointed (mx, Fig. 10A) ..... ***Nipponoxenos*** **Kifune & Maeta (East Asia; *Vespula* spp.)**

– Mandible not protruding from mandibular capsule, anterior part of maxilla rounded (mx, Fig. 20C) **4**

4 Border between clypeus and labrum always distinct (sbcl, Fig. 47D)..... ***Xenos*** **Rossi, in part (Old and New World; Vespini, Polistini, Mischocyttarini, Ropalidiini)**

– Clypeal region not clearly delimited from labral area, more or less fused (Fig. 22D)..... ***Brasixenos*** **Kogan & Oliveira (New World; Epiponini)**

5 Prosternal extension anteriorly with conspicuous extensive pale spot, sometimes associated with cuticular impression (pps, Figs 35C, 37A). Maxillary base continued anterolaterally as a distinct submaxillary groove (smxg, Fig. 37A) ..... ***Sphecixenos*** **gen. nov. (Old World and Australia; Sphecidae)**

– Prosternal extension different. Submaxillary groove distinct (smxg, Fig. 18A) or indistinct (smxg, Fig. 53A) **6**

6 Maxillae prominent (Figs 14E, 41E) **7**

– Maxillae not prominent, partially or completely fused with head capsule, rarely slightly raised **10**

7 Mandibular tooth very wide basally, reaching area of mandibular bulge. Tooth base ventrally covered with small depressions continuous with several rows of spines (md, mdt, Fig. 14E) .... ***Tachytixenos*** **Pierce (Old World;** ***Tachytes*** **spp.)**

– Mandibular tooth narrow or only slightly widened **8**

8 Vestige of antenna preserved as cavity, additional rounded plates rarely present (a, Fig. 17D)..... ***Paraxenos*** **Saunders, in part (Old World and Australia;** ***Bembix*** **spp.,** ***Stizus*** **spp.)**

– Vestige of antenna different..... **9**

9 Cephalothorax conspicuously convex, round (Fig. 43C), highly elliptic in cross-section. Dorsal labral field raised, protruding anteriorly (dlf, Fig. 45D) ..... ***Tuberoxenos*** **gen. nov. (Afrotropic + Palearctic; Sphecidae)**

– Cephalothorax more flattened, not or indistinctly bulging (Fig. 36C), more flattened in cross-section. Dorsal labral field flat (dlf, Fig. 38D)..... ..... ***Pseudoxenos*** **Saunders, in part (Palearctic; Odynerini)**

10 Vestige of antenna preserved as cavity, additional rounded plates rarely present ... . ***Paraxenos*** **Saunders, in part (Old World and Australia;** ***Bembecinus*** **spp.)**

– Vestige of antenna different..... **11**

11 Two distinct dark spots present mesally on border between head and prothorax (sbhp, Fig. 32D). Thoracic segments conspicuously sclerotized laterally from dorsal side (tx, Fig. 32D). Lateral parts of abdomen posterior to spiracles pale (asI, Fig. 32D). Clypeal area very distinctly delimited from labral area (sbcl, Fig. 34D) ..... ***Macroxenos*** **Schultze (Australasian and Indomalayan regions; Odynerini)**

– Combination of characters different..... **12**

12 Sensilla on clypeal lobe extended to ventral side, often present close to clypeolabral border (cls, Fig. 53D) ..... ***Xenos*** **Rossi, in part (Old and New World; Vespini, Polistini, Mischocyttarini, Ropalidiini)**

– Position of sensilla different **13**

13 Rudiments of antennal torulus only rarely preserved. Distributed in the Old World or Australia **14**

– Rudiments of antennal torulus usually present (at, Figs 25C, 29D). Distributed in the New World distribution..... **15**

14 Meso-metathoracic segmental border constricted laterally (sbmm, Fig. 47C, D). Dorsal labral field raised (dlf, Fig. 49D), when flat, then narrower laterally than medially (dlf, Fig. 3A) ..... ***Deltoxenos* gen. nov. (Old World + Australia; Eumeninae)**

– Meso-metathoracic segmental border not constricted laterally (Fig. 39C, D). Dorsal labral field flat, laterally as long as medially (dlf, Fig. 41C, D) ..... ***Pseudoxenos* Saunders, in part (Palearctic; Eumeninae)**

15 Frontal region conspicuously covered with frontal papillae (frp, Fig. 25F). Periantennal area small, indistinct, suppressed by supra-antennal sensillary field (paa, Fig. 25C). Prosternum connected to head on same plane, but elevated anteriorly (pst, lehc, Fig. 25A)..... ***Leionotoxenos* Pierce (New World; Odynerini)**

– Frontal region smooth or very slightly wrinkled, without papillae (fr, Fig. 30B). Periantennal area expanded, sometimes raised, smooth. Distance between antennal area and supra-antennal sensillary field relatively large (paa, Fig. 29C). Prosternum more elevated above head along entire cephalo-prothoracic border (pst, lehc, Fig. 29A) .....***Eupathocera* Pierce (New World; Sphecidae, Crabronidae, Zethinae, *Pachodynerus* spp.)**

Key to genera of Xenidae based on the cephalotheca of the male puparium

Cephalothecae of *Paragioxenos* and *Macroxenos* unknown.

1 Maxilla scarcely recognizable, fused with cephalotheca (mx, Fig. 23E). Vestige of palp distinct in optical microscope but hardly visible in SEM micrographs (mxp, Fig. 23A, D)..... ***Brasixenos* Kogan & Oliveira (New Word; Epiponini)**

– Maxilla distinct, prominent (e.g., Fig. 6A, B)..... **2**

2 Diameter of genae between maxillary base and compound eye relatively small, ca. as large as diameter of vestigial antenna (gn, Fig. 11A). Vestigial antenna very large (a, Fig. 11A). Cephalotheca always pale, only clypeus and genae dark..... ***Nipponoxenos* Kifune & Maeta (East Asia; *Vespula* spp.)**

– Diameter of genae between maxillary base and compound eye distinctly larger than diameter of vestigial antenna (gn, Fig. 5A). Vestigial antenna smaller, cephalotheca usually darker..... **3**

3 Diameter of genae between maxillary base and compound eye ~ 1.5× larger than diameter of vestigial antenna (gn, Fig. 42D) **4**

– Diameter of genae between maxillary base and compound eye at least 2× larger than diameter of vestigial antenna (gn, e.g., Fig. 15D) **5**

4 Occipital bulge present (ob, Fig. 42D). Frontal region distinctly deformed by frontal impression (fi, Fig. 42D). Paired furrows of supra-antennal sensillary field absent. Cephalotheca elliptic..... ***Pseudoxenos* Saunders (Palearctic; Odynerini)**

– Occipital bulge absent. Frontal impression absent. Paired furrows of supra-antennal sensillary field present (fssf, Fig. 46A, D). Cephalotheca nearly

circular in frontal view ..... ***Tuberoxenos*** **gen. nov., in part (Afrotropic and Palearctic regions; Sphecidae)**

5 Paired furrow of supra-antennal sensillary field present (fssf, Figs 15A, D, 19A, D) **6**

– Paired furrow of supra-antennal sensillary field absent (Figs 5A, 6A) **8**

6 Mandibular tooth wide basally, reaching mandibular bulge (mdt, Fig. 15E). Tooth base with small depressions continuous with several rows of spines (mdts, Fig. 14E) ..... ***Tachytixenos*** **Pierce (Old World;** ***Tachytes*** **spp.)**

– Mandibular tooth narrow or slightly widened (mdt, Fig. 19E) 7

7 Cephalotheca elliptic in frontal view (Fig. 19A) ..... ***Paraxenos*** **Saunders (Old World and Australia;** ***Bembix*** **spp.,** ***Stizus*** **spp.)**

– Cephalotheca nearly circular in frontal view (Fig. 46A) ..... ***Tuberoxenos*** **gen. nov., in part (Afrotropic and Palearctic regions; Sphecidae)**

8 Cephalotheca nearly circular in frontal view (Figs 38A, 46A) **9**

– Cephalotheca elliptic in frontal view (e.g., Figs 50A, 54A) **10**

9 Vestigial antenna with diameter subequal to width of mandible (a, md, Fig. 31E). Mandible directed anteromedially ..... ***Eupathocera*** **Pierce (New World; Sphecidae, Crabronidae, Zethinae,** ***Pachodynerus***)

– Vestigial antenna with diameter smaller than width of mandible (a, md, Fig. 38E). Mandible directed almost medially ..... ***Sphecixenos*** **gen. nov. (Old World + Australia; Sphecidae)**

10 Frontal fissure very distinct (fi, Fig. 27D). Maxilla prominent, at least 1.5× longer than wide at base (mx, Fig. 27E) ..... ***Leionotoxenos*** **Pierce (New World; Odynerini)**

– Frontal fissure quite indistinct or nearly absent (fi, Fig. 6A, 54D). Maxilla not distinctly elongated, at most 1.5× longer than basally wide (mx, Figs 50E, 54E) **11**

11 Occipital bulge present, well- developed (ob, Figs 6A, 50D). Cephalotheca with a pattern of pale and dark shades (Figs 5A, 50A) ..... ***Deltoxenos*** **gen. nov. (Old World + Australia; Eumeninae)**

– Occipital bulge strongly reduced or missing (ob, Fig. 54D). Cephalotheca mostly dark (Fig. 54A) ..... ***Xenos*** **Rossi (Old and New World; Vespini, Polistini, Mischocyttarini, Ropalidiini)**

Discussion

The results of this study are mainly compared with external characters of the cephalothorax of females of *Xenos vesparum* (Richter et al. 2017) and *Stylops ovinae* (Löwe et al. 2016). Characters of the cephalotheca of the male puparium are compared with Kinzelbach (1971b). The morphology of adult males is also potentially valuable for the taxonomy of Xenidae. However, it was not considered here, as only few well-preserved specimens were available. Likewise, the morphology of the first instars can be useful for taxonomy,

especially the well-developed pattern of setae (Pohl 2002; Straka et al. 2014). However, these features were not included in this study due to limited material.

Cephalothorax of the female

A conspicuous autapomorphy of stylopidian females is the secondary tagmosis with an anterior cephalothorax which is protruding from the host and a large, sack-shaped posterior body region which remains hidden in the body lumen of the abdomen (Löwe et al. 2016). This profound structural transformation is closely linked with the endoparasitic lifestyle. This also includes the reduction of antennae, mouthparts, compound eyes, and legs, which are preserved as rudiments or completely lost (Kinzelbach 1971b; Pohl and Beutel 2005). The wings of females are already absent in the ground plan of Strepsiptera (Pohl and Beutel 2005, 2008).

The homology of the cephalothorax was discussed in previous studies. Kinzelbach (1971b) was the first who suggested that it is formed by fusion of the head, thorax, and the anterior part of abdominal segment I, which bears a pair of functional spiracles. This interpretation was later supported by comprehensive treatments based on modern techniques (Pohl and Beutel 2005, 2008; Löwe et al. 2016; Richter et al. 2017). Alternatively, it was suggested that the cephalothorax comprises the head and thorax (e.g., Lauterbach 1954), or even only the head and prothorax (Hrabar et al. 2014; Kathirithamby et al. 2015). Recognizable segmental borders and different cuticular microstructures clearly support the concept proposed by Kinzelbach (1971b) and Richter et al. (2017). The segmental border between the head and prothorax is distinctly visible on the ventral side, demarcated by the birth opening and often by lateral sutures. However, the latter are absent in many members of Stylopidae, as for instance in some *Stylops* (Löwe et al. 2016) or *Halictoxenos* (Straka et al. 2006). An exception among Xenidae is the genus *Paragioxenos*, with the head and prothorax completely separated by the birth opening on the ventral side, as it is also the case in *Rozenia* of Stylopidae (Straka et al. 2014). On the dorsal side, the head and prothorax of females of Xenidae are completely fused, but a border region is still indicated by changes in the cuticular surface or by pigmented stripes. This is in contrast to *Stylops*, where the border is delimited by a distinct furrow (Löwe et al. 2016). The pro-mesothoracic and meso-metathoracic borders are usually more distinct on the ventral side as it was previously shown in *Xenos* and *Stylops* (Löwe et al. 2016; Richter et al. 2017), but with differences among genera or species. The prosternum is very variable in Xenidae. It can be variously modified, with a prosternal swelling present in some cases (*Paragioxenos*, *Paraxenos*) or with a protruding margin overlapping with the maxillolabial area and the posterior part of the mandibles (*Macroxenos*, *Tuberoxenos*, *Xenos*). The shape of the meso- and metathorax is mostly transverse and unmodified, but in some cases constricted laterally, resulting in an unusual proximity of the head and abdominal spiracles (*Brasixenos*, some *Macroxenos*).

The distinct constriction in the middle region of abdominal segment I, the zone of contact with the host cuticle, is distinct in all genera of Xenidae. Functionally this can be explained as an adaptation preventing the exposed anterior body from slipping back into the host body cavity (Löwe et al. 2016). Richter et al. (2017) suggested

that cuticular spines on abdominal segment I have probably the same function. These structures are apparently missing in *Stylops*. Cuticular spines occur in most genera of Xenidae and are functionally replaced by a very roughly sculptured lateral cuticle in cases where they are missing. In some species of *Brasixenos*, *Eupathocera* and *Sphecixenos*, the area below the abdominal spiracles extrudes as a spiracular corner, in some cases very distinct, as in *Rozenia* (Straka et al. 2014). Spiracles are functional in all Xenidae with variable orientation and position.

The more or less flattened ellipsoid shape of the cephalothorax of all species of Xenidae stabilizes its position between the host abdominal segments. Kinzelbach (1971b) interpreted this as an adaptation to a mechanical strain caused by the host cuticle. It is noteworthy in this context that the male puparium is not flattened. Apparently, the adaptation of the female is more advanced, likely due to a stronger selective pressure caused by permanent endoparasitism (Pohl and Beutel 2008).

The function of the fissure-shaped mouth opening is the uptake of the host hemolymph by the secondary larvae (Giusti et al. 2007). It is well-developed and sclerotized along the margin in all examined species of Xenidae, but obviously non-functional after the extrusion of the cephalothorax from the host. The birth opening between the head and prosternum is the site where copulation and the release of the first instar larvae take place (Pohl and Beutel 2008). This structure is an autapomorphy of Stylopiformia (Pohl and Beutel 2005). It was shown that the membranous cuticle of this region is perforated by the penis during copulation in *Stylops* (Peinert et al. 2016). In the case of *Xenos*, Kathirithamby et al. (2015) hypothesized that the brood canal membrane is ruptured during the super-extrusion of the cephalothorax, thereby facilitating the release of pheromones during mate signaling. However, a perforation by the male penis during copulation is also possible (Beani et al. 2005).

Cephalic structures are always distinctly reduced. Richter et al. (2017) interpreted the assemblages of circular fields on the dorsal side of head capsule as vestiges of antennae in *Xenos vesparum*. We confirmed the presence of vestigial antennae across Xenidae in various stages of reduction. They are preserved as a remnant of an antennal torulus in *Leionotoxenos* and *Eupathocera*, with rounded plates and vestigial antennal setae, whereas only a simple groove or cavity is present in *Brasixenos* and *Paraxenos*. Previously, a vestigial antenna was ascribed to the entire Stylopidia (Kinzelbach 1971b, Pohl and Beutel 2005). Even though this is very likely part of the groundplan, the antenna is completely reduced in *Stylops* (Löwe et al. 2016).

The vestigial maxillae are very variable in Xenidae, providing valuable characters for the identification of genera and species. They are variably sculptured, prominent in *Tachytixenos*, *Tuberoxenos* or *Eupathocera*, or completely fused with the labial area in *Brasixenos*. However, any degree of reduction occurring in Xenidae does not match the nearly complete absence in genera of Stylopidae, such as *Stylops* (Löwe et al. 2016), *Rozenia* (Straka et al. 2014) or *Halictoxenos* (Straka et al. 2006). Maxillary bases adjacent with the birth opening can be medially fused as in *Paragioxenos*. Sclerotized and fused maxillae are very conspicuous on a pale head capsule as in *Nipponoxenos* and *Brasixenos*. The presence of a submaxillary groove is probably correlated with a prosternal extension projecting into the head capsule. It is missing in *Paragioxenos* where this structure is

absent, but conspicuously developed in some genera with a well-developed extension of the prosternum. A similar condition was not found in *Stylops* (Löwe et al. 2016).

In contrast to the maxillae, the mandibles are well-developed in all Xenidae. They are the only movable cephalic appendages with a flexible articulatory membrane, and extended and flexed by the two antagonistic craniomandibular muscles. Shortly after the emergence from the host, the entire surface of the cephalothorax is sclerotized, and the mandibles are immobilized (Richter et al. 2017). According to Lauterbach (1954), the mandibles help penetrating the host membrane during the extrusion. They are equipped with a tooth, which is also present in *Stylops* (Löwe et al. 2016). It can be used as a character for distinguishing related genera (*Tachytixenos*, *Paraxenos*) and its shape can also be species-specific in Xenidae (Nakase and Kato 2013). The labrum is not distinctly developed as a separate cephalic appendage, but only preserved as dorsal and ventral labral fields. The latter was described as a "semicircular structure" in *Stylops* (Löwe et al. 2016) or a "semicircular field possibly of labral origin" in *Xenos vesparum* (Richter et al. 2017). This structure is variably shaped in Xenidae, not always semicircular, and arguably formed by an everted epipharynx as hypothesized by Lauterbach (1954). The dorsal field bears several rows of spine-like sensilla (setae) in all genera of Xenidae. They were described by Richter et al. (2017) and are probably also present in *Stylops* (Löwe et al. 2016). Sensilla are also present on a narrow clypeal area and on a supra-antennal field near the vestigial antennae. Clypeal sensilla were mentioned in Richter et al. (2017) for the first time as "sensilla on the anterior head capsule". Possible homologous structures were described as the "field of sensilla" in *Malayaxenos* (Corioxenidae) (Pohl and Beutel 2005). A supra-antennal field of sensilla was described for the first time by Kinzelbach (1971b) as "Pigmentzelle" on the female cephalotheca and female of Mengenillidae. It is conceivable that the sensory function of these organs facilitates the orientation of secondary larvae in the body lumen of the host and the proper extrusion from the host abdomen.

Cephalotheca of the male puparium

The cephalotheca is the anterior part of the puparium, where the male emerges after extrusion from the host and completes its development. The puparium is formed by the sclerotized exuvia of the male secondary larvae. The cephalotheca is homologous to the head capsule of the cephalothorax of the female (Kinzelbach 1971b). Compared to the female cephalothorax and the adult male, the external morphology of the male cephalotheca was very poorly studied previously. Kinzelbach (1971b) presented the first comparison of cephalothecae across the entire Strepsiptera, with descriptions of many features. It turned out that the cephalotheca can provide important and practical characters for species delimitation (Nakase and Kato 2013). In Xenidae, it is apparently more convenient to work with cephalothecae than with males enclosed in the puparium, as the latter are often immature, unsclerotized, or poorly preserved, especially in older museum material. The cephalothecal characters are also well visible externally on the puparium extruding from the host abdomen, without prior dissection.

The most striking feature of the cephalotheca, in contrast to the female cephalothorax, is the presence of compound eyes. Individual ommatidia are usually visible on the pale background of the ocular area as in *Halictoxenos* or *Myrmecolax* (Soon et al. 2012; Nakase et al. 2014). A completely dark ocular area occurs only in some species of *Xenos*. In lateral view, the cephalotheca appears rounded or pointed, with the clypeus and its sensilla placed apically. In contrast to the apical region of the female cephalothorax, the clypeus of the male cephalotheca is distinctly developed, with an epistomal suture separating it from the frontal region. The cephalothecal supra-antennal sensillary field is more conspicuous and usually bulging in contrast to the flat one on the female cephalothorax. We assume that these structural elements are homologous in both sexes, that they have a sensorial function, and that they facilitate the orientation of the male and female secondary larvae in the host body lumen.

The vestigial antennae are less reduced than in the female cephalothorax. They vary mainly in size, whereas the shape is variable in females. An antennal torulus is always distinctly developed, but in some cases interrupted. A scapus and pedicellus can be distinguished in the genus *Myrmecolax* (Myrmecolacidae) according to Kathirithamby et al. (2010) and Nakase et al. (2014). However, the homology of these basal antennal segments of immature stages is highly uncertain in Holometabola (e.g., Beutel et al. 2011). The mandibles are well developed, with homologous features in secondary larvae of both sexes. Nakase and Kato (2013) found the same shape of mandibular tooth on the male and female secondary larvae of *Xenos*, which is constant intraspecifically and could be easily used for species identification. We found a specific shape of the mandibular tooth characteristic for the genus *Tachytixenos*. The maxillae of male cephalotheca have not undergone such diverse changes and modifications as in female cephalothorax in Xenidae. In most genera, they are well developed except for *Brasixenos* with maxillae completely fused to the head capsule. Kinzelbach (1971b) even presented that some genera of Halictophagidae could have preserved the articulation of maxillae on the male cephalotheca.

Taxonomy and host specialization of Xenidae

The monophyly of Xenidae is well supported by morphological and molecular data (Pohl and Beutel 2005; McMahon et al. 2011). We have newly delimited 13 genera of this family with a total of 119 described species. Although we did not deal with a precise species delimitation of all material available, we approximately estimated at least 70 undescribed species, which represents more than half of the known diversity (Table 1). This estimation is very conservative. It is based on a comprehensive phylogenetic analysis (Benda et al. 2021) and material examined by the authors in various collections. Part of this material is prepared for species descriptions in subsequent publications. However, small genera with many autapomorphies, which would render other genera paraphyletic, have not been found.

The monotypic genus *Paragioxenos* was described by Ogloblin (1923) from Australia and has never been reported since. Although an early divergence was assumed,

its phylogenetic position is still unknown. The male was characterized by a specific shape of the penis. The characterization of the female was based on the condition of the border between the head and prothorax, described by Ogloblin (1923: 46) as a "transversal slit, which separates front part of cephalothorax not curved, but simply rounded". Additionally, Kinzelbach (1971b) pointed out to the unique shape of the maxillae. Our own study of the type material suggests a clear delimitation of this genus by the shape of the birth opening, features of the mandibles and dorsal labral field. Fresh material for extraction of DNA sequences is urgently required. Analyses of molecular data would likely reveal the phylogenetic position of this enigmatic genus with a unique specialization on pollen wasps.

The monotypic *Nipponoxenos* was originally described as a subgenus of *Xenos* from the genus *Vespula* Thomson in East Asia (Kifune and Maeta 1975). The female was characterized by almost straight and anteriorly tapering lateral margins of the cephalothorax, slightly constricted just anterior to the spiracles. The defining feature of the male was a typical penis with prominent dorsal spine, pickaxe-shaped in lateral view.

Table 1. Overview of Xenidae genera with general information on distribution, hosts, and the number of described species; a conservative estimate of the number of undescribed species is also provided.

Genus	Distribution	Hosts	Number of species	Number of undescribed species
Paragioxenos Ogloblin, 1923	Australia	*Paragia* (Vespidae: Masarinae)	1	0
Nipponoxenos (Kifune & Maeta, 1975), stat. res.	East Asia	*Vespula* (Vespidae: Vespinae)	1	0
Tachytixenos Pierce, 1911, stat. res.	Old World	*Tachytes* (Crabronidae: Crabroninae)	1	4
Paraxenos Saunders, 1872	Old World, Australasian	*Bembecinus*, *Bembix*, and *Stizus* (Bembicidae: Bembicinae)	13	7
Brasixenos Kogan & Oliveira, 1966, stat. res.	New World	Epiponini (Vespidae: Polistinae)	7	7
Leionotoxenos Pierce, 1909, stat. res.	New World	Odynerini (Vespidae: Eumeninae)	14	3
Eupathocera Pierce, 1908, stat. res.	New World	Sphecinae, Ammophilinae (Sphecidae); *Tachytes* (Crabronidae: Crabroninae); *Zethus* (Vespidae: Zethinae); *Pachodynerus* (Vespidae: Eumeninae)	16	8
Macroxenos Schultze, 1925, stat. res.	Australasian, Indomalayan	Odynerini (Vespidae: Eumeninae)	2	3
Sphecixenos gen. nov.	Old World, Australasian	*Sphex*, *Isodontia* (Sphecidae: Sphecinae); *Sceliphron* (Sphecidae: Sceliphrinae); *Chlorion* (Sphecidae: Chloriontinae)	12	1
Pseudoxenos Saunders, 1872	Palearctic	Odynerini (Vespidae: Eumeninae)	7	2
Tuberoxenos gen. nov.	Afrotropical, Palearctic	*Ammophila*, *Podalonia* (Sphecidae: Ammophilinae); *Prionyx* (Sphecidae: Sphecinae)	5	8
Deltoxenos gen. nov.	Old World, Australasian	Eumenini, Odynerini (Vespidae: Eumeninae)	7	17
Xenos Rossi, 1793	Old and New World	Vespini (Vespidae: Vespinae); Polistini, Mischocyttarini, Ropalidiini (Vespidae: Polistinae)	33	11

Benda et al. (2021) found *Nipponoxenos* as the earliest diverging group, sister to all other Xenidae or sister to *Tachytixenos* and *Paraxenos*.

The monotypic genus *Tachytixenos* was described by Pierce (1911) from India by a unique association with wasp hosts of the genus *Tachytes*. Later Kinzelbach (1978) cited supplementary records of stylopized *Tachytes* from the Palearctic and Indomalayan regions. We re-establish *Tachytixenos* as a genus with a wider distribution than expected, and estimate existence of at least four undescribed species. Apart from *Tachytixenos*, Kinzelbach (1971b, 1978) synonymized several additional genera with *Paraxenos*. In contrast, Benda et al. (2019, 2021) delimited *Paraxenos* as a lineage with a distribution in the Old World and the Australasian region, and parasitizing exclusively species of Bembicinae. We provide a redescription of *Paraxenos* based on new characters, and report at least seven undescribed species. The genus *Brasixenos* was expected as closely related to *Xenos*, but Benda et al. (2019) revealed the group as a separate lineage parasitizing social Epiponini and unrelated to *Xenos*. *Brasixenos* is well delimited by the female cephalothorax and male cephalotheca. A revision of adult males is needed as well as an evaluation of male diagnoses provided by Kogan and Oliveira (1966). We expected the diversity within the genus *Brasixenos* to be at least twice higher than the number of described species.

Previously, several genera were described by Pierce (1908, 1909, 1911, 1919), mainly from the New World. He suggested that a new genus of Strepsiptera should be established if it utilizes a different host genus. We restored the genera *Leionotoxenos* and *Eupathocera* for two sister clades from the New World, revealed by Benda et al. (2019, 2021). Although *Leionotoxenos* is specialized on solitary wasps of the tribe Odynerini, *Eupathocera* is more generalist utilizing mainly species of Sphecidae but rarely the subfamilies Crabroninae, Zethinae or Eumeninae.

The genus *Macroxenos* was described by from the Philipines as a parasite of potter wasps of *Anterhynchium* (Schultze 1925). Although Bohart (1937) synonymized it with *Pseudoxenos*, Benda et al. (2019) found a remarkable lineage with an Australasian origin that dispersed to the Indomalayan region. We classify it as *Macroxenos* and report at least three undescribed species. Nevertheless, we assume that diversity of this genus is much higher because of a high morphological variability of species, especially in Australasian region. The lineage named here as *Sphecixenos* gen. nov. was revealed by Benda et al. (2019, 2021) who found it as a separate clade with an Afrotropical origin, dispersed into the Indomalayan and Australasian regions. Its main hosts are wasps of the genus *Sphex*, less often *Isodontia*, *Sceliphron*, and *Chlorion*.

Pseudoxenos was described by Saunders (1872) with the description of several species parasitizing Odynerini in European Mediterranean. The taxonomic validity of some described species within *Pseudoxenos* from the West Palearctic region is questionable and a more detailed study is necessary for the clarification of interspecific relationships (Cook 2019; Benda et al. 2021). In the phylogenetic tree from Benda et al. (2021) a sister-group relationship between a lineage parasitizing *Pachodynerus* and another Palearctic lineage parasitizing Eumenini was suggested, but the branch support values were very low, and this relationship is not supported by morphology. The latter lineage

is provisionally included here in *Deltoxenos* gen. nov. and the lineage from *Pachodynerus* is provisionally included in *Eupathocera* based on morphology. More comprehensive sampling and a robust genomic analysis are necessary for the clarification of systematics and phylogeny of these taxa. *Tuberoxenos* gen. nov. is described here as the sister genus to *Pseudoxenos*, restricted to the Afrotropical and Palearctic regions and associated mainly with *Ammophila* and *Podalonia*, very rarely *Prionyx*.

Deltoxenos gen. nov. utilizes a diverse range of hosts from Odynerini and Eumenini (Vespidae: Eumeninae) (Benda et al. 2021). Only few species were described from the Afrotropical and Palearctic regions, but we estimate more than twice as many species than currently described. The distribution of the genus is wider, spanning over the Old World and the Australasian region. Benda et al. (2019) suggested a unique evolution of this lineage including a dispersion of the group from the Afrotropics through the Palearctic and Indomalayan regions to Australasia. This dispersion was probably initialized by the switch from Odynerini to Eumenini that provided an opening of a new host niche and an opportunity to utilize a wide range of host taxa.

Xenos was the first named genus in Strepsiptera, although it took some time before the order was formally introduced (Cook 2019; Rossius 1794). We have redescribed *Xenos* by a combination of characters as parasites of four tribes of social Vespidae. In comparison to other xenid genera, *Xenos* is the only genus distributed both in the Old and the New World, but its origin and expansion is not well clarified (Benda et al. 2019). It represents the most species-rich genus of Xenidae with 32 described species and at least 11 undescribed species.

The previous classification of genera of Xenidae by Pierce (1908, 1909, 1911, 1919) implied a specialization on the level of host genus, while the classification by Kinzelbach (1971b) suggested a specialization on the level of host family or subfamily. Our generic concept combines both approaches and is more complex. Some representatives of the current genera parasitize only one host genus (e.g., *Paragioxenos*, *Nipponoxenos* and *Tachytixenos*), whereas others can even utilize hosts from three families (e.g., *Eupathocera*). The species diversity of a lineage depends on the ability to utilize new hosts which would also facilitate the dispersion and increase the range of distribution (Benda et al. 2019).

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Supplementary material 1

Table S1

Authors: Daniel Benda, Hans Pohl, Yuta Nakase, Rolf Beutel, Jakub Straka

Data type: occurences

Explanation note: Voucher names, hosts, and collection localities of the samples.

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Paper IV

Benda D., Pohl H., Beutel R., Straka J. (2022). Two new species of Xenos (Strepsiptera: Xenidae), parasites of social wasps of the genus Mischocyttarus (Hymenoptera: Vespidae) in the New World. *Acta Entomologica Musei Nationalis Pragae*, 62(1), 185-195.

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RESEARCH PAPER

Two new species of *Xenos* (Strepsiptera: Xenidae), parasites of social wasps of the genus *Mischocyttarus* (Hymenoptera: Vespidae) in the New World

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Abstract. Two new species of Strepsiptera of the genus *Xenos* Rossi, 1793 (Xenidae) from the New World are described. Both are endoparasites of social wasps of the genus *Mischocyttarus* Saussure, 1853 (Vespidae: Mischocyttarini). *Xenos bicolor* Benda & Straka, sp. nov., parasitizes *Mischocyttarus navajo* Bequaert, 1933, *Mischocyttarus flavitarsis* (Saussure, 1854), and *Mischocyttarus pallidipectus* (Smith, 1857), whereas *Xenos pallens* Benda & Straka, sp. nov., is a parasite of *Mischocyttarus costaricensis* Richards, 1945 (Vespidae: Polistinae: Mischocyttarini). Diagnoses and descriptions of female cephalothoraces are presented for all three species that parasitize species of *Mischocyttarus*. Diagnoses and descriptions of male cephalothecae are presented for *Xenos bicolor* sp. nov. and *Xenos pallens* sp. nov. Additionally, a key for *Xenos* species parasitic on *Mischocyttarus* is provided based on characters of the female cephalothorax and male cephalotheca. Identification of *Xenos* species based on external morphology is discussed.

Key words. Strepsiptera, *Xenos*, Hymenoptera, Vespidae, *Mischocyttarus*, cephalothorax, cephalotheca, morphology, taxonomy, wasp parasite, wasps, Neotropical Region

Zoobank: http://zoobank.org/urn:lsid:zoobank.org:pub:7EF65AA0-A787-4534-8514-2D29999D48F4

Introduction

Xenidae are insect endoparasites of wasps from four families, Crabronidae, Bembicidae, Sphecidae, and Vespidae (Benda et al. 2021). The family originated relatively late, approximately 50–60 million years ago (McMahon et al. 2011). Xenidae and its sister taxon Stylopidae are the groups with the highest degree of specialization in Strepsiptera. They belong to Stylopidia, a clade containing more than 97% of species of the order and parasitizing only neopteran pterygote insects (Pohl & Beutel 2008). Xenidae are mainly characterized by unique characters of the first instar larvae. These features enhance the attachment capacity to the smooth body surface of the wasp hosts. This includes enlarged and round adhesive tarsal pads and filamentous cuticular outgrowths of the labium, which strongly increase its wettability (Pohl & Beutel 2004, 2008).

Xenos Rossi, 1793 was previously classified as a genus using social species of Vespidae as hosts (Kinzelbach 1971), but Benda et al. (2019, 2021) revealed that the group is polyphyletic. It was subsequently subdivided into three monophyletic genera: *Nipponoxenos* Kifune & Maeta, 1975 (parasites of *Vespula* Thomson, 1869), *Brasixenos* Kogan & Oliveira, 1966 (parasites of Epiponini wasps), and *Xenos* (parasites of Vespini, Polistini, Mischocyttarini and Ropalidiini) (Benda et al. 2022). *Xenos* is deeply nested within Xenidae, representing the largest radiation with 32 described species (Benda et al. 2021). It occurs on all continents except for Australia and Antarctica. Its geographic origin is unclear, though the most likely options are the New World or Afrotropical Region (Benda et al. 2019). It is the sister group of *Deltoxenos* Benda, Pohl, Nakase, Beutel & Straka, 2022 (Benda et al. 2019; Straka & Benda, unpubl.).

In the New World, *Xenos* parasitizes only species of *Polistes* Latreille, 1802 and *Mischocyttarus* Saussure, 1853 (Vespidae: Polistinae). Seventeen species are known from *Polistes* in the New World, and only one species has been described from *Mischocyttarus* (Benda et al. 2022, Brèthes 1923). The first note of *Mischocyttarus* as a host

was presented by Pierce (1919), who recorded stylopised *Mischocyttarus flavitarsis* (Saussure, 1854) in Arizona, USA. This record was later cited in comprehensive lists of host records of Xenidae (Salt & Bequaert 1929) or Strepsiptera in general (Hofeneder & Fulmek 1943). Subsequently, Brèthes (1923) described *Clypoxenos americanus* parasitic on *Mischocyttarus flavicans* (Fabricius, 1804) from Bolivia. Until now, this was the only species described from *Mischocyttarus*. The genus *Clypoxenos* Brèthes, 1923 was established for the species parasitizing *Mischocyttarus* species but later was synonymized with *Xenos* by Bohart (1941). Günther (1949) recorded stylopised *Mischocyttarus surinamensis* (Saussure, 1854) from Trinidad without a species description due to poor preservation of males in puparia. Although the host-parasite association of *Xenos* with *Mischocyttarus* has been known for more than one hundred years, no other new species from this host genus has ever been described. Here we present two new species of *Xenos* associated with this host genus and compare the morphology of the female cephalothorax and male cephalotheca with described species parasitizing *Mischocyttarus* and *Polistes*.

Material and methods

Depository of examined specimens. For this study, specimens from the following institutions were analysed:

CNC Canadian National Collection of Insects, Arachnids, and Nematodes (Ottawa, Ontario, Canada);

KUNHM Natural History Museum, Division of Entomology, University of Kansas (Lawrence, Kansas, USA).

The newly described species were labelled in the following manner: "Holotypus ♀, name of taxon, Benda & Straka, sp. nov." on a red card; yellow cards were used for paratypes. Exact label data are cited only for the holotypes. Separate lines on the labels are indicated with a slash "/", and separate labels are indicated with a double slash "//".

Morphological studies. All host individuals were first relaxed in water vapour and then immediately dissected. The endoparasitic females and males were removed from the host's body. Female and male puparia used for morphological study were cleared using a mixture of lysis buffer ATL and proteinase K (Qiagen) heated to 56°C. The lysis procedure took several hours or overnight. Cleared specimens were cleaned in distilled water several times and then stored in vials with 96% ethanol. Whole female cephalothoraces and male puparia were air-dried using a micro-pad inserted into the cephalothorax to prevent the cuticle from collapsing during the process. The rest of the female body was usually extracted from the cephalothoracic cuticle before drying. After this step and the removal of the micro-pad, the dried specimens were glued onto card mounting points, which were pinned. The width and length of the female cephalothorax, the female head capsule, and the male cephalotheca were measured using a Leica S9D stereo microscope with a calibrated ocular micrometre. The length of the cephalothorax was measured from the apex of the clypeal lobe to the constriction of abdominal segment I; the cephalothorax width is the maximum distance between its lateral margins.

The general habitus of stylopised host specimens and the host's abdomen with protruding strepsipterans were documented. Multi-focus images were taken using Canon EOS 550D or 70D cameras equipped with EF 50mm and MP-E 65mm macro lenses. Lateral lights and a diffuser were used. For the documentation of the original colouration of the female cephalothorax and the male cephalotheca, air-dried specimens glued to the card mounting points were used. They were photographed with a Canon EOS 7D digital SLR equipped with a Canon MP-E 65mm macro lens (Canon, Krefeld, Germany) fitted with a StackShot macro rail (Cognisys, Traverse City, MI, USA). Each specimen was illuminated with two flashlights (Yongnuo Photographic Equipment, Shenzhen, China) fitted to a transparent cylinder for even and soft light. For the documentation of tiny structures on the head capsule, Canon EOS 70D camera attached to an Olympus BX40 Microscope was used. The microscope was equipped with lateral lights and a diffuser. Zerene Stacker (Zerene Systems LLC, Richland, USA) was used to process stacks of images with different focus. All images were processed and arranged into plates with Adobe Photoshop® CS5 (Adobe System Incorporated, San Jose, USA) software. CorelDraw® X8 (CorelDraw Corporation, Ottawa, ON, Canada) was used for the lettering of the plates.

Terminology and description style. The terminology used for the female cephalothorax and male cephalotheca is adopted from Benda et al. (2022), Richter et al. (2017), Löwe et al. (2016), and Kinzelbach (1971). Appropriate terminology was developed for morphological characters without specific names. Cephalothorax and cephalotheca are described in morphological orientation in figures although their functional orientation in the host's body is inverted.

Abbreviations: ♀ – female, MP – male puparium, EMP – empty male puparium.

Results

Xenos bicolor Benda & Straka, sp. nov.

(Figs 1–2, 5A)

Type locality. USA: Arizona, Cochise Country, Ash, Canyon Road 0.5 km W, Huachuca Mountains.

Type material. Holotype: ♀ (CNC), cephalothorax on mounting board (abdomen not preserved): "**USA: Arizona:** Ash, Cyn. / Rd 0.5 km W, Cochise Co. / Huachuca Mts., 14.ii.1994 / N. McFerland lgt. // **XFl9**, host: / *Mischocyttarus navajo* / Bequaert, 1933". Host: *Mischocyttarus navajo* Bequaert, 1933. Paratypes: **USA: Arizona:** 1 ♀ (CNC), with same data as for holotype; 3 EMP, same host, locality, and collector, 31.x.1993; 2 ♀♀, Ramsey Cyn., Siera Vista, Huachuca Mts., 26.viii.1967, R. Stenitzky lgt., host: *Mischocyttarus navajo*; 1 ♀ (KUNHM), Oak Criek Canyon, F. H. Snow lgt., host: *Mischocyttarus navajo*, J. Bequaert det.; 5 EMP (CNC), Miller Cyn., Huachuca Mts., Cochise Co., 19.viii.1993, M. Sharkey lgt., host: *Mischocyttarus navajo*; 1 EMP (CNC), same locality, 25.v.1969, R. Stenitzky lgt., host: *Mischocyttarus flavitarsis* (Saussure, 1854); 1 ♀ + 4 EMP (CNC), same host, locality, and collector, 5.xi.1969; 2 EMP (KUNHM), Santa Rica Mts., 19.vii.1938, L. W. Hepner lgt., host: *Mischocyttarus flavitarsis*; 1 ♀ + 1 EMP (KUNHM), South Arizona, locality and date unknown, F. H. Snow lgt., host: *Mischocyttarus flavitarsis*. **New Mexico**: 1 ♀ (KUNHM), Ponderosa env., 13.vii.1991, B. Alexander lgt., host: *Mischocyttarus navajo*; 2 ♀ (KUNHM), Jemez Springs env., 01.vii.1941, R. H. Beamer lgt., host: *Mischocyttarus flavitarsis*. **MEXICO: Nuevo León:** 1 ♀ (KUNHM), Linares env., 22.iii.1991,

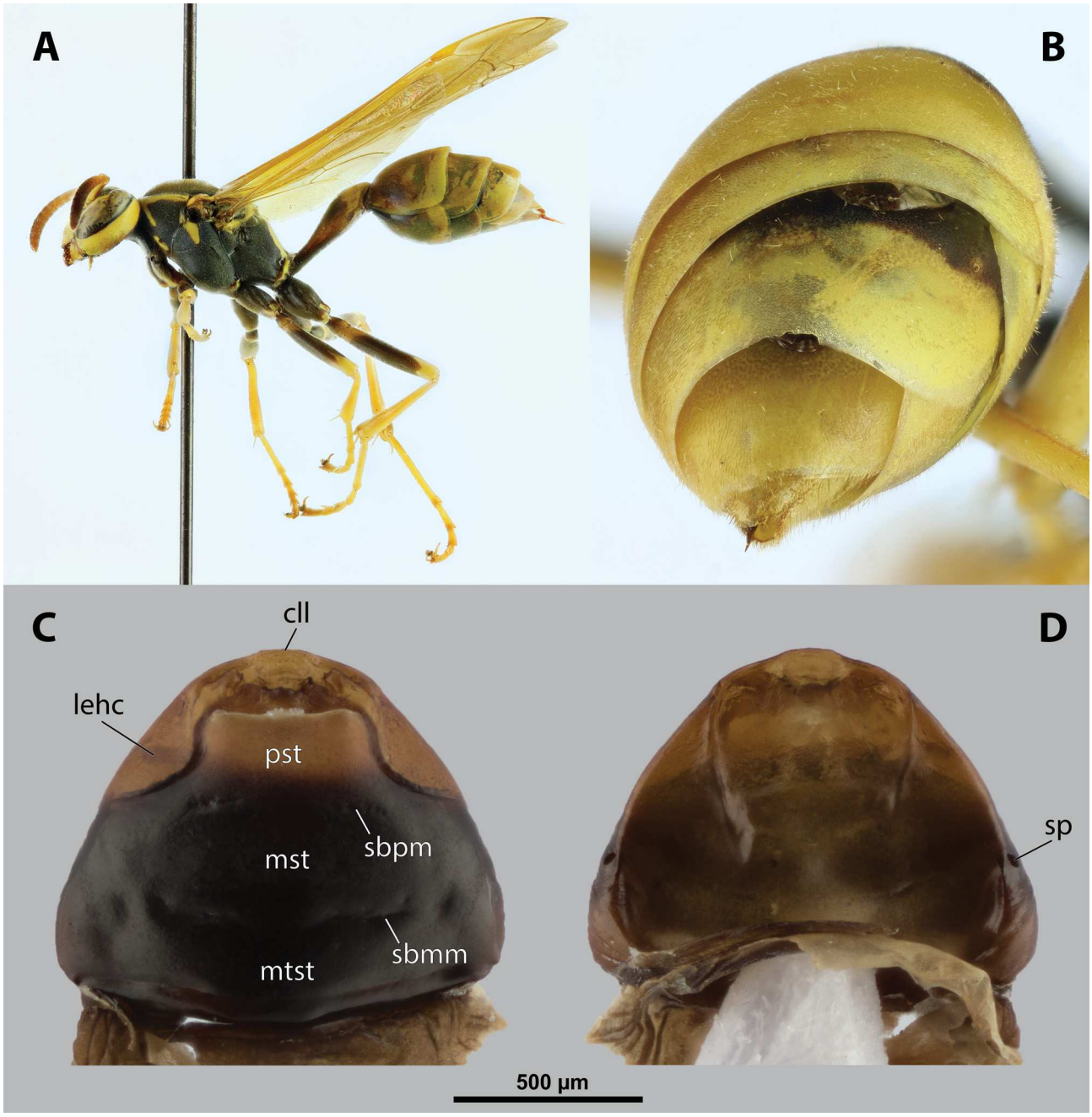

Fig. 1. *Xenos bicolor* Benda & Straka sp. nov., host, female, cephalothorax. A – *Mischocyttarus flavitarsis* (Saussure, 1854) stylopised by *X. bicolor* sp. nov., lateral view; B – detail of host abdomen of *M. navajo* Bequaert, 1933, with two adult females; C–D – holotype of *X. bicolor* sp. nov. from *M. navajo*, cephalothorax; C – ventral side; D – dorsal side. Abbreviations: cll – clypeal lobe, lehc – lateral extension of head capsule, mst – mesosternum, mtst – metasternum, pst – prosternum (prosternal extension), sbmm – segmental border between mesothorax and metathorax, sbpm – segmental border between prothorax and mesothorax, sp – spiracle.

R. Brooks & R. Leschen lgt., host: *Mischocyttarus pallidipectus* (Smith, 1857); 2 EMP (KUNHM). **Hidalgo:** 5 MP (KUNHM), Actopan env., 27.viii.1962, Ordway & Marston lgt., host: *Mischocyttarus pallidipectus*.

Diagnosis of female cephalothorax. *Xenos bicolor* sp. nov. differs from *X. pallens* sp. nov. and *X. americanus* (Brèthes, 1923) by colouration of the cephalothorax. Anterior third of cephalothorax pale, posterior two thirds dark in contrast to *X. pallens* sp. nov. and *X. americanus* with cephalothorax almost completely pale (Fig. 5). Differing from *X. pallens* sp. nov. by a larger size of cephalothorax: *X. bicolor* sp. nov. (length 0.90–1.14 mm, width 1.12–1.24 mm) versus *X. pallens* sp. nov. (length 0.80–0.86 mm, width 0.86–0.96 mm). Mesosternal and metasternal pigmented papillae not visible on dark background in *X. bicolor* sp. nov., but well visible in *X. pallens* sp. nov. *Xenos bicolor* sp. nov. differs from *X. americanus* by a smaller size of cephalothorax, *X. americanus*: length 1.43 mm, width 1.80 mm.

Xenos bicolor sp. nov. differs from superficially similar *Xenos pecki* Kirby, 1813 by following characters. Dark colouration of prosternum in *X. pecki* not reaching ventral border between head and cephalothorax; only prosternal extension pale brown in *X. bicolor* sp. nov. Maxilla elongated anteriorly in *X. pecki*; in *X. bicolor* sp. nov. maxilla shorter, rather wider than long. For visual impression compare Figure 1C with Figure 51 in Benda et al. (2022).

Description of female cephalothorax. ***Shape and colouration.*** Size of holotype cephalothorax: length 1.04 mm, width 1.22 mm. Cephalothorax variable but always wider than long, length 0.90–1.14 mm, width 1.12–1.24 mm. Meso-metathoracic segmental border very slightly constricted laterally, indistinct in some specimens. Anterior head margin slightly protruding, but distinctly in some individuals. Thorax distinctly widening posteriorly. Cephalothorax on ventral side with pale anterior part (head capsule and anterior part of prosternal extension) and dark posterior area (pst, Figs 1C, 5A).

Head capsule. Length of head less than half of cephalothorax. Length proportion of head/cephalothorax 0.40 mm (0.40–0.45 mm) including lateral cephalic extension. Head colouration predominantly pale, not forming specific pattern. Only lower edge of mouth opening and area along border between head and prothorax distinctly darker. Clypeal region well delimited from labral area. Apical margin of clypeal area forming slightly protruding clypeal lobe (cll, Fig. 1C), but distinctly protruding in some individuals (cll, Fig. 2A). Numerous distinct sensilla present on clypeal surface, more or less evenly scattered. Cuticle of frontal region slightly wrinkled, reticulated (fr, Fig. 2B). Segmental border between head and prothorax indicated by interrupted suture from dorsal side (sbhp, Fig. 2B). Head and prothorax distinctly separated by birth opening ventromedially, and laterally by suture.

Supraantennal sensillary field slightly wrinkled with dispersed sensilla. Not delimited or indistinctly delimited by furrow medially, but border usually still recognisable (ssf, Fig. 2B).

Antenna. Vestige of antennae present (details not investigated) (a, Fig. 2B).

Labrum. Ventral field elliptic, not protruding. Dorsal field elongated, slightly arcuate, protuberant, ~ 4–5× (4× in holotype) wider than long in midline (dlf, Fig. 2A). Dorsal field laterally as long as medially, with dispersed setae or sensilla inserted in small concavities.

Mandible anteromedially directed at angle of 40–50° (45° in holotype) and enclosed in mandibular capsule (md, Fig. 2A). Mandibular bulge more or less distinctly raised, with several sensilla. Cuticle almost completely smooth. Tooth narrow, pointed apically.

Maxilla. Maxillae only partially fused with labial area, well demarcated from it, slightly raised but not distinctly projecting anteriorly from head capsule (mx, Fig. 2A). Cuticle slightly wrinkled to reticulated, not distinctly sclerotized. Maxillary apex not projecting beyond mandible anteriorly, maxillary base not overlapping mandibular base, but at least in some individuals adjacent. Vestige of palp present, in some individuals very inconspicuous to almost invisible, located medially on ventral side of maxilla (mxp, Fig. 2A). Maxillary base indistinctly produced anterolaterally as submaxillary groove, which is not part of maxilla; adjacent to border between head and prothorax.

Labium. Labial area recognisable between maxillae, delimited anteriorly by mouth opening and posteriorly by birth opening (lba, Fig. 2A). Flat, as long as wide, in some individuals wider than longer. Cuticular surface very slightly reticulated. Anterior labial surface around mouth opening distinctly sclerotized and pigmented, posteriorly pale.

Mouth opening. Bisinuate in holotype. Very variable, widely arcuate, nearly straight or bisinuate, rarely nearly V-shaped, sclerotized along margin (os, Fig. 2A).

Thorax. Pro-mesothoracic and meso-metathoracic borders well demarcated ventrally by mesal furrows (sbpm, sbmm, Fig. 1C), indistinct dorsally. Border between metathorax and abdomen formed by ridge and indicated by change of cuticular sculpture. Thoracic segments constricted laterally between lateral cephalic extension and abdominal area around spiracles. Prosternal extension not indicated by specific cuticular sculpture or protuberance, evenly arched. Anterior part of prosternum pale, posterior part dark. Transition between colouration gradual, not sharp. Dark colouration reaches border between head and prosternum laterally. Cuticle of thoracic segments on ventral side with reticulate surface pattern. Prosternum with 7 to 37 (35 in holotype) conspicuous pigmented papillae in central pale area. Mesosternal and metasternal pigmented papillae not visible on a dark background. Colouration of meso- and metathorax dark ventrally and dorsally. Cuticle of dorsal side of thorax slightly reticulated, without papillae.

Abdominal segment I and spiracles. Lateral region of abdominal segment I below spiracles dark dorsally, similar as coloration of thorax. Spiracles located on posterior third of cephalothorax, slightly elevated, with anterodorsal (in holotype) or anterolateral orientation.

Diagnosis of male cephalotheca. *Xenos bicolor* sp. nov. differs from *Xenos pallens* sp. nov. by following characters. Cephalotheca with anterior protrusion, but apically blunt (Fig. 2D). Colouration predominantly dark with some slightly lighter areas forming specific pattern (cephalotheca of *Xenos pallens* sp. nov. paler). Gena between compound eye and mandible completely dark (gn, Fig. 2C); conspicuously pale in *Xenos pallens* sp. nov. Occipital bulge absent. Maxilla completely dark (mx, Fig. 2C).

Xenos bicolor sp. nov. differs from similar species *Xenos pecki* in several features. Cephalothorax shape of *X. bicolor* sp. nov. elliptic, occipital, and labial part not protruding; frontal impression (fi, Fig. 2C) more distinct; diameter of genae between maxillary base and compound eye ~ 2.5× larger than diameter of vestigial antenna (~ 3× larger in *X. pecki*). Compare Figure 2C with Figure 54 in Benda et al. (2022).

Description of male cephalotheca. ***Shape and colouration.*** In frontal view rounded and broadly elliptic, length 0.64–0.72 mm, width 0.84–0.92 mm; in lateral view slightly protruding anteriorly but with blunt apex. Colouration predominantly dark with some slightly lighter areas forming specific pattern.

Cephalothecal capsule. Entire compound eyes with light ground colour, well visible, with dark individual cornea lenses. Area around compound eye also slightly lighter than rest of cephalotheca. Genal region between compound eye and mandible completely dark (gn, Fig. 2C). Clypeus paler than frons and labrum. Clypeal lobe distinctly arcuate in frontal view, prominent in lateral

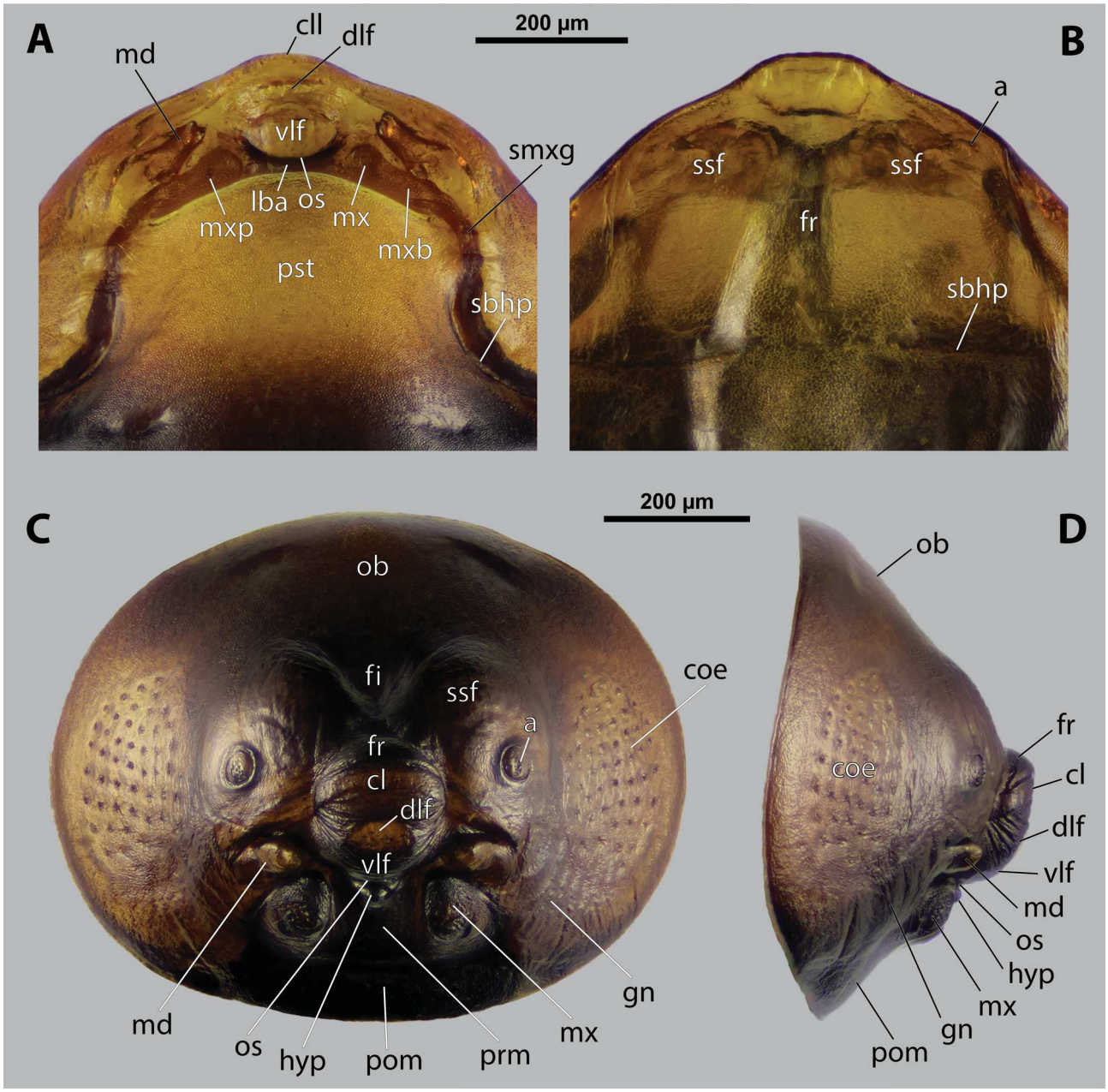

Fig. 2. *Xenos bicolor* Benda & Straka sp. nov., female, detail of cephalothorax, male, cephalotheca. A – detail of ventral side of cephalothorax from *Mischocyttarus navajo* Bequaert, 1933; B – detail of dorsal side of cephalothorax from *M. flavitarsis* (Saussure, 1854); C – frontal view of cephalotheca from *M. pallidipectus* (Smith, 1857); D – lateral view of cephalotheca from *M. pallidipectus*. Abbreviations: a – vestigial antenna, cl – clypeus, cll – clypeal lobe, coe – compound eye, dlf – dorsal labral field of labral area, fi – frontal impression, fr – frontal region, gn – gena, hyp – hypopharynx, lba – labial area, md – mandible, mx – vestige of maxilla, mxb – maxillary base, mxp – vestige of maxillary palp, ob – occipital bulge, os – mouth opening, pom – postmentum, prm – prementum, pst – prosternum (prosternal extension), sbhp – segmental border between head and prothorax, smxg – submaxillary groove, ssf – sensillum of supraantennal sensillary field, vlf – ventral labral field of labral area.

view but blunt anteriorly. Sensilla concentrated mainly on clypeal lobe. Frontal impression distinct (fi, Fig. 2C). Occipital bulge absent (ob, Figs 2C, D). Diameter of genal region between maxillary base and compound eye ~ 2.5× larger than diameter of vestigial antenna.

Supraantennal sensillary field. Dark, kidney-shaped and bulging, without furrows, delimited medially by distinct frontal impression.

Antenna of standard shape, dark, with small plates, and torulus usually complete, rarely incomplete (Fig. 2C). Periantennal area not clearly delimited from supraantennal sensillary field, dark.

Labrum. Labral area distinct, bulging. Dorsal field conspicuous, primarily dark with lighter central area, with dispersed setae well visible (dlf, Figs 2C, D). Ventral field inconspicuous, completely dark (vlf, Fig. 2C).

Mandible anteromedially to almost medially directed. Colouration lighter than that of maxilla, especially apically. Mandibular bulge with sensilla, separated from pointed tooth.

Maxilla distinct, prominent, completely dark (mx, Figs 2C, D). Vestige of palp present.

Labium and hypopharynx. Labium distinctly visible between and below maxillae. Prementum and postmentum

completely dark, separated by more or less distinct transverse furrow. Hypopharyngeal protuberance present but in some cases almost invisible (hyp, Figs 2C, D).

Mouth opening well visible, not covered by ventral labral field, slightly or distinctly arcuate.

Hosts. *Mischocyttarus navajo* Bequaert, 1933, *Mischocyttarus flavitarsis* (Saussure, 1854), and *Mischocyttarus pallidipectus* (Smith, 1857).

Phylogenetic relationships. Closely related species to *X. pallens*, part of a New World clade of *Xenos* containing a lineage parasitizing *Polistes* (Benda et al. 2021).

Etymology. From Latin, *bicolor* (= having two colours), referring to the colouration of the cephalothorax, with pale anterior region on both sides (head capsule and anterior part of prosternal extension) and dark posterior area; an adjective.

Distribution. USA: Arizona, New Mexico; Mexico: Hidalgo, Nuevo León.

Xenos pallens Benda & Straka, sp. nov.

(Figs 3–4, 5B)

Type locality. Costa Rica: Puntarenas, San Vito env., Las Alturas.

Type material. Holotype: ♀ (CNC), cephalothorax on mounting board (abdomen not preserved). "**COSTA RICA:** / **Puntarenas:** San Vito / env., Las Alturas, 1500 m / 16.viii.1995 / J. R. Vockeroth lgt. // **Brsp2** / host: *Mischocyttarus* / *costaricensis* Richards, / 1945" Host: *Mischocyttarus costaricensis* Richards, 1945. Paratypes: 2 ♀♀ + 1 MP (CNC), from the same host specimen as holotype.

Diagnosis of female cephalothorax. Differing from *X. bicolor* sp. nov. and *X. americanus* by following characters. Cephalothorax almost completely pale as in *X. americanus*, but in contrast to *X. bicolor* sp. nov. with posterior two thirds dark. Some parts of cephalothorax, especially maxillae and abdominal areas, dark and sclerotized. Smallest known species parasitizing *Mischocyttarus* wasps: cephalothorax length: 0.80–0.86 mm, width 0.86–0.96 mm. Other two species significantly larger: *X. bicolor* sp. nov. (length 0.90–1.14 mm, width 1.12–1.24 mm), *X. americanus* (length 1.43 mm, width 1.80 mm). Mesosternal and metasternal pigmented papillae well visible, in contrast to *X. bicolor* sp. nov. where papillae are unrecognizable on dark posterior part of cephalothorax.

Description of female cephalothorax. ***Shape and colouration.*** Size of cephalothorax of holotype: length 0.80 mm, width 0.86 mm. Shape of cephalothorax somewhat variable but always slightly wider than long, length 0.80–0.86 mm, width 0.86–0.96 mm. Meso-metathoracic segmental border region usually very slightly constricted laterally, but not in all individuals. Anterior head margin protruding in holotype, usually but not always slightly protruding. Thorax slightly widening posteriorly. Colouration mostly pale, with shades of light brown dominating. Some parts of cephalothorax, especially maxillae and abdominal regions, dark and sclerotized.

Head capsule. Length of head slightly less than half of cephalothorax, proportion head/cephalothorax 0.45 (0.44–0.47) including lateral cephalic extension. Head colouration predominantly pale, not forming specific pattern. Only maxillae, lower edge of mouth opening, and area along border between head and prothorax distinctly darkened. Clypeal region well delimited from labral area. Clypeal lobe on apical margin of clypeal area usually but not always protruding (compare cl on Figs 3C, 4A). Numerous sensilla present on clypeal surface, scattered through clypeal surface but mainly concentrated medially. Cuticle of frontal region slightly wrinkled, reticulated (fr, Fig. 4B). Segmental border between head and prothorax indicated by indistinct coloured stripes laterally and by transition of colouration on dorsomedian region (sbhp, Fig. 4B). On ventral side head and prothorax distinctly separated by birth opening medially, and by a suture laterally.

Supraantennal sensillary field slightly wrinkled, with dispersed sensilla (ssf, Fig. 4B). Medial paired furrows indistinct.

Antenna. Vestige not investigated.

Labrum. Ventral field elliptic to nearly circular, not protruding. Dorsal field elongated, slightly arcuate, distinctly protuberant, ~ 4–5× wider than long in midline (dlf, Fig. 4A). Dorsal field laterally as long as medially, with dispersed setae or sensilla inserted in small concavities.

Mandible anteromedially directed at angle of 45° (40–50°), enclosed in mandibular capsule (md, Fig. 4A). Mandibular bulge more or less distinctly raised, with several sensilla. Cuticle of mandible partially smooth and partially wrinkled. Tooth narrow, wider in some individuals, directing ventrally or apically.

Maxilla partially fused with labial area but distinguishable from it, slightly raised anterolaterally near mandible but not distinctly prominent (mx, Fig. 4A). Cuticle slightly wrinkled to reticulated, distinctly sclerotized. Maxillary apex not projecting beyond mandible anteriorly, maxillary base not overlapping with mandibular base but adjacent. Vestige of palp presented, very inconspicuous, located medially on ventral side of maxilla. Maxillary base distinctly produced anterolaterally as submaxillary groove, well visible as dark interrupted line parallel to border between head and prothorax (mdb, smxg, Fig. 4A).

Labium. Labial area recognisable between maxillae, delimited anteriorly by mouth opening and posteriorly by birth opening. Flat, wider than long, cuticular surface very slightly reticulated. Anterior labial surface around mouth opening distinctly sclerotized and darkened; posterior region pale (lba, Fig. 4A).

Mouth opening widely arcuate in holotype. Variable, slightly arcuate in some individuals, medially nearly straight, distinctly sclerotized at margin (os, Fig. 4A).

Thorax. Pro-mesothoracic and meso-metathoracic borders distinct ventrally, indicated by mesal furrows (sbpm, sbmm, Fig. 4A), borders on dorsal side indistinct. Border between metathorax and abdomen usually formed by ridge or indicated by change of cuticular sculpture. Thoracic segments constricted laterally between lateral cephalic extension and abdominal area around spiracles. Prosternal extension without different cuticular sculpture or protuberance, evenly arched. Whole prosternum pale. Cuticle of thoracic segments on ventral side reticulate to nearly smooth. Prosternum in the centre with conspicuous pigmented papillae. In three available cephalothoraces 29 (holotype), 32, or 51 prosternal papillae (pstp, Fig. 4A).

Fig. 3. *Xenos pallens* Benda & Straka sp. nov., host, female, cephalothorax. A – *Mischocyttarus costaricensis* Richards, 1945, stylopised by *X. pallens* sp. nov., lateral view; B – the same specimen, dorsal view; C – holotype of *X. pallens* sp. nov., ventral side of cephalothorax; D – holotype of *X. pallens* sp. nov., dorsal side of cephalothorax. Abbreviation: cl – clypeus.

Mesosternal papillae forming two groups each situated close to lateral margin, each group containing 7 (7–17) papillae. Metasternal group contains 5 (5–18) papillae. In contrast to prosternum, pigmented papillae absent medially on mesosternum and metasternum (mstp, mtstp, Fig. 4A). Colouration of meso- and metathorax pale on both sides. Cuticle of dorsal side of thorax slightly reticulated, without papillae.

Abdominal segment I and spiracles. Lateral region of abdominal segment I below spiracles darker on dorsal side, contrasting to pale thorax. Spiracles on posterior half of cephalothorax very slightly elevated, with anterolateral (holotype) or anterodorsal orientation.

Diagnosis of male cephalotheca. *Xenos pallens* sp. nov. differs from *X. bicolor* sp. nov. by a combination of characters. Cephalotheca protruding anteriorly, pointed apically (Fig. 4C). Colouration predominantly dark, but overall lighter than in *X. bicolor* sp. nov., with extensive bright areas forming specific pattern. Part of genal region bordering mandible, maxilla, and labium conspicuously pale (gn, Fig. 4C). Occipital bulge very indistinct but present (ob, Figs 4C, D); absent in *X. bicolor* sp. nov. Maxillary base pale, anterior part of maxilla and vestige of palp entirely dark (mx, Fig. 4C); maxilla completely dark in *X. bicolor* sp. nov.

Description of male cephalotheca. ***Shape and colouration.*** In frontal view rounded and broadly elliptic, length 0.74 mm, width 0.90 mm; in lateral view protruding anteriorly, pointed apically. Colouration predominantly dark with some extensive pale areas forming specific pattern.

Cephalothecal capsule. Entire compound eyes pale, well visible, with darker remnants of individual cornea lenses visible. Genal region around eyes pale laterally but darker medially. Areas of gena bordering with mandible, maxilla, and labium conspicuously pale (gn, Fig. 4C). Clypeus moderately pale. Clypeal lobe distinctly arcuate in frontal view, prominent in lateral view, pointed. Sensilla mainly concentrated on clypeal lobe. Frontal impression distinct (fi, Fig. 4C). Occipital bulge very indistinct (ob, Figs 4C, D). Diameter of gena between maxillary base and compound eye ~ 2.5 larger than diameter of vestigial antenna.

Supraantennal sensillary field dark, kidney-shaped, slightly bulging, without furrows, delimited medially by distinct frontal impression.

Antenna of standard shape, dark, with small plates and complete torulus (Fig. 4C). Periantennal area not clearly delimited from supraantennal sensillary field, dark coloured.

Labrum. Labral area distinct. Dorsal field conspicuous, mostly dark but lighter on central area, with dispersed setae well visible. Ventral field inconspicuous, entirely dark.

Mandible orientation almost straight towards midline. Colouration overall lighter than posterior part of maxilla. Mandibular bulge with sensilla, separated from pointed tooth.

Maxilla distinct, prominent. Vestige of palp present. Maxillary base bright, anterior part and vestige of palp entirely dark (mx, Fig. 4C).

Labium and hypopharynx. Labium distinctly visible between and below maxillae. Prementum and postmentum entirely dark, separated by more or less distinct transverse furrow. Hypopharyngeal protuberance absent.

Mouth opening. Well visible, not covered by ventral labral field, slightly arcuate.

Host. *Mischocyttarus costaricensis* Richards, 1945.

Phylogenetic relationships. Closely related species to *X. bicolor* sp. nov., part of a New World clade of *Xenos* containing a lineage parasitizing *Polistes* (Benda et al. 2021).

Etymology. From Latin *pallens* (= pale, yellowish), referring to the characteristic pale colouration of the female cephalothorax; an adjective.

Distribution. Costa Rica.

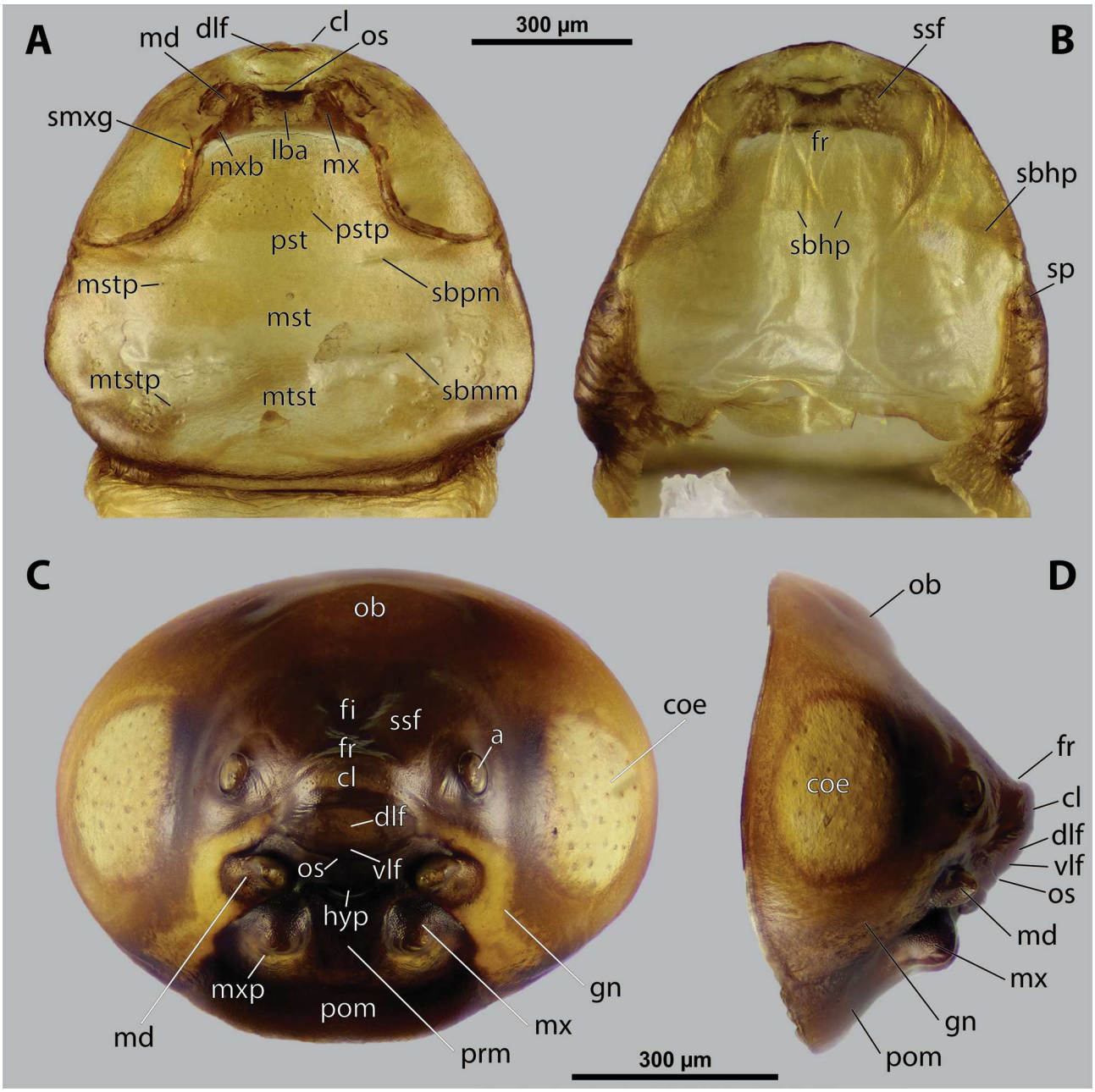

Fig. 4. *Xenos pallens* Benda & Straka sp. nov., female, cephalothorax, male, cephalotheca. A – ventral side of cephalothorax; B – dorsal side of cephalothorax; C – frontal view of cephalotheca; D – lateral view of cephalotheca. Abbreviations: a – vestigial antenna, cl – clypeus, coe – compound eye, dlf – dorsal labral field of labral area, fi – frontal impression, fr – frontal region, gn – gena, hyp – hypopharynx, lba – labial area, md – mandible, mst – mesosternum, mstp – mesosternal papilla, mtst – metasternum, mtstp – metasternal papilla, mx – vestige of maxilla, mxb – maxillary base (at mandible base), mxp – vestige of maxillary palp, ob – occipital bulge, os – mouth opening, pom – postmentum, prm – prementum, pst – prosternum (prosternal extension), pstp – prosternal papilla, sbhp – segmental border between head and prothorax, sbmm – segmental border between mesothorax and metathorax, sbpm – segmental border between prothorax and mesothorax, smxg – submaxillary groove, sp – spiracle, ssf – sensillum of supraantennal sensillary field, vlf – ventral labral field of labral area.

Xenos americanus (Brèthes, 1923)

(Fig. 5C)

Clypoxenos americanus Brèthes, 1923: 46 (original description, holotype not designated, location of syntypes unknown). Type locality: Bolivia.
Xenos americanus (Brèthes, 1923): Bohart (1941): 141 (new combination).

Diagnosis of female cephalothorax. Differing from *Xenos bicolor* sp. nov. and *X. pallens* sp. nov. by following characters. Cephalothorax almost entirely pale as in *X. pallens* sp. nov. but different from *X. bicolor* sp. nov. where posterior two thirds of cephalothorax are dark. Some parts of the cephalothorax, such as for instance the mouth opening distinctly sclerotized. Largest known species parasitizing wasps of *Mischocyttarus*: cephalothoracic length 1.43 mm, width 1.80 mm (Fig. 5C). Other two species distinctly smaller: *X. bicolor* sp. nov. (length 0.90–1.14 mm, width 1.12–1.24 mm), *X. pallens* sp. nov. (length: 0.80–0.86 mm, width 0.86–0.96 mm).

Description of female cephalothorax (modified from Brèthes 1923). ***Shape and colouration.*** Cephalothoracic length 1.43 mm, width 1.80 mm; width at spiracles 1.76 mm; distance between mandibles 0.26 mm; maximum length of head capsule 1.51 mm. Cephalothorax wider

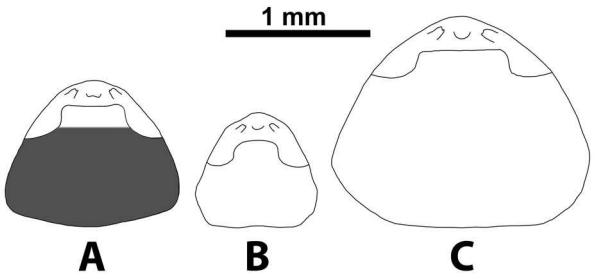

Fig. 5. Schematic drawing of cephalothorax of all known *Xenos* species parasitizing wasps of the genus *Mischocyttarus* Saussure, 1853. A – *X. bicolor* Benda & Straka sp. nov.; B – *X. pallens* Benda & Straka sp. nov., C – *X. americanus* (Brèthes, 1923).

than long, subtriangular, distinctly widening posteriorly, with corners rounded and spiracles not reaching the lateral border. Colouration light reddish-brown. Mouth opening distinctly sclerotized at margin. Mandibles sub-quadrate, with a small sharp tooth at inner corner.

Host. *Mischocyttarus flavicans* (Fabricius, 1804) (as *Clypeopolybia duckei* Brèthes, 1923) (Brèthes 1923).

Phylogenetic relationships. Unknown.

Distribution. Bolivia, precise locality not mentioned in original description.

Comments. The genus *Clypoxenos* was described by Brèthes (1923). He followed Pierce's concept that each genus of Xenidae is specialized to one host genus of wasps (Pierce 1908, 1909, 1911). Bohart (1941) treated *Clypoxenos* as a presumptive junior synonym of *Xenos* but remained somewhat ambivalent of this interpretation (Cook 2019). In contrast Kinzelbach (1971) clearly confirmed the synonymy. The phylogenetic placement of xenids parasitizing *Mischocyttarus* as a subordinate group of *Xenos* supports previous hypothesis that *Clypoxenos* is not a valid genus (Benda et al. 2021).

Key to *Xenos* species parasitizing wasps of the genus *Mischocyttarus* (cephalothorax of female)

1 Cephalothorax almost entirely pale, light reddish-brown. 2
– Anterior third of cephalothorax pale, posterior two thirds dark; dark colouration of prosternum reaches border between head and cephalothorax ventrally (Fig. 1C); maxilla short, wider than long (mx, Fig. 2A); hosts: *Mischocyttarus navajo* Bequaert, *M. flavitarsis* (Saussure), and *M. pallidipectus* (Smith). .... ***Xenos bicolor*** Benda & Straka **sp. nov.**
2 Cephalothorax very small (length 0.80–0.86 mm, width 0.86–0.96 mm), head capsule elongate, almost half as long as cephalothorax (Fig. 5B); host: *Mischocyttarus costaricensis* Richards. .... ***Xenos pallens*** Benda & Straka **sp. nov.**
– Cephalothorax large (length 1.43 mm, width 1.8 mm), head capsule shorter, about one third as long as cephalothorax (Fig. 5C); host: *Mischocyttarus flavicans* (Fabricius)..... ***Xenos americanus*** Brèthes, 1923.

Key to *Xenos* species parasitizing wasps of the genus *Mischocyttarus* (male puparium cephalotheca)

Note. Cephalotheca of *Xenos americanus* unknown.

1 Cephalotheca overall lighter with more extensive bright areas, region of gena bordering with mandible, maxilla, and labium conspicuously pale (gn, Fig. 4C); maxillary base pale, anterior part, and vestige of palp completely dark (mx, Fig. 4C); host: *Mischocyttarus costaricensis* Richards. .... ***Xenos pallens*** Benda & Straka **sp. nov.**
– Cephalotheca mostly dark, with some slightly lighter areas forming specific pattern; cephalotheca elliptic; frontal impression distinct (fi, Fig. 2C); diameter of genal region between maxillary base and compound eye ~ 2.5× larger than diameter of vestigial antenna; hosts: *Mischocyttarus navajo* Bequaert, *M. flavitarsis* (Saussure), and *M. pallidipectus* (Smith). .... ***Xenos bicolor*** Benda & Straka **sp. nov.**

Discussion

The *Xenos* species parasitizing species of *Mischocyttarus* are easily recognizable by a combination of cephalothoracic colouration, cephalothoracic shape, length proportion of head versus cephalothorax in females, and by the colouration and shape of the cephalotheca in males. Brèthes (1923) did not designate a type specimen of *Xenos americanus*, but he provided a detailed description and monochrome photographic documentation which facilitate the distinction from other species parasitizing *Mischocyttarus*. Unfortunately, the location of the original type specimen(s) of *Xenos americanus* is unknown and it may have been lost (Cook 2019). A lectotype (or neotype) should be designated in future studies, and stylopised specimens of *Mischocyttarus flavicans*, which were not at our disposition, should be examined.

Xenos bicolor sp. nov. is similar to *Xenos pecki* but can be distinguished from it by the dark colouration of the prosternum extending to the border between head and cephalothorax on the ventral side. Additionally, the maxilla is shorter in *Xenos bicolor* sp. nov. (compare with Figs 51 and 52 in Benda et al. 2022).

Distinctive colour patterns of the cephalothorax, with a pale anterior part and a dark posterior portion, are very common in the New World species of *Xenos* parasitizing *Polistes* (Hoffmann 1914, Brèthes 1923, Kifune 1979, Cook & Mathison 1997). However, almost entirely pale species of *Xenos* also occur in the New World (Brèthes 1923, this study). Although there are 20 known species of *Xenos* from the New World, some species using *Polistes* have identical host species or a similar distribution area indicating the need for revision (Table 1). Garza & Cook (2021) strongly recommended the taxonomic revision of New World species and the re-evaluation of species currently considered synonyms of *X. pecki*. In contrast, Günther (1949) suggested that some valid names should be synonymised.

Table 1. Overview of 20 currently valid species of *Xenos* Rossi, 1793 from the New World with general information on their distribution and hosts.

Species	Distribution	Hosts
X. americanus (Brèthes, 1923)	Bolivia	*Mischocyttarus flavicans* (Fabricius, 1804)
X. argentinus Brèthes, 1923	Argentina	*Polistes cavapyta* Saussure, 1853; *Polistes buyssoni* Brèthes, 1903
X. bicolor Benda & Straka **sp. nov.**	Mexico, USA (Arizona, New Mexico)	*Mischocyttarus navajo* Bequaert, 1933; *Mischocyttarus flavitarsis* (Saussure, 1854); *Mischocyttarus pallidipectus* (Smith, 1857)
X. boharti Hofmann, 1965	Chile	*Polistes peruvianus* Bequaert, 1934
X. bohlsi Hoffmann, 1914	Argentina, Brazil, Paraguay	*Polistes canadensis* (Linnaeus, 1758)
X. bonairensis Brèthes, 1923	Argentina, Brazil	*Polistes versicolor* (Olivier, 1792)
X. colombiensis Cook, Mayorga-Ch & Sarmiento, 2020	Colombia	*Polistes myersi* Bequaert, 1934
X. hamiltoni Kathirithamby & Hughes, 2006	Mexico	*Polistes carnifex* (Fabricius, 1775)
X. hospitus Oliveira & Kogan, 1962	Brazil, Ecuador	*Polistes versicolor* (Olivier, 1791)
X. hunteri (Pierce, 1909)	USA (Texas)	*Polistes* sp. near *P. minor* Palisot de Beauvois, 1818
X. indespectus Oliveira & Kogan, 1962	Brazil	*Polistes* sp.
X. iviei Kifune, 1983	Virgin Islands	*Polistes crinitus* (Felton, 1764)
X. kifunei Cook & Mathison, 1997	USA (Arizona)	*Polistes comanchus navajoe* Cresson, 1868
X. nigrescens Brues, 1903	USA (Texas)	*Polistes rubiginosus* Lepeletier, 1836
X. pallens Benda & Straka **sp. nov.**	Costa Rica	*Mischocyttarus costaricensis* Richards, 1945
X. pallidus Brues, 1903	USA (Florida, Texas), Mexico	*Polistes annularis* (Linnaeus, 1763); *Polistes crinitus* (Felton, 1764); *Polistes carnifex* (Fabricius, 1775); *Polistes bellicosus* Cresson, 1872
X. pecki Kirby, 1813	USA (California, Connecticut, Massachusetts, Michigan, Ohio, Texas)	*Polistes apachus* Saussure, 1857; *Polistes aurifer* Saussure, 1853; *Polistes carolina* (Linnaeus, 1767); *Polistes fuscatus* (Fabricius, 1793); *Polistes metricus* Say, 1831
X. peruensis Kifune, 1979	Peru	*Polistes lanio* (Fabricius, 1775)
X. rostratus Trois, 1984	Argentina, Brazil, Paraguay, Peru	*Polistes billardieri ruficornis* Saussure, 1853; *Polistes billardieri biglumoides* Ducke, 1904
X. rubiginosi (Pierce, 1909)	USA (Louisiana)	*Polistes rubiginosus* Lepeletier, 1836

Although the geographic origin of the New World *Xenos* is unclear, it is considered to be a monophyletic group (Benda et al. 2019, 2021). The latter phylogenetic study also suggested a New World *Xenos* clade comprising the lineage parasitizing species of *Polistes* and those using *Mischocyttarus* as their host. It is conceivable that ancestors of both host genera were initially infested by one species in the New World which subsequently diversified. The large genus *Mischocyttarus* is endemic to the New World. It comprises approximately 250 described species and is the only genus of Mischocyttarini (Silveira 2008). The great diversity of the host genus suggests that many species of the *Xenos* may still be undescribed.

Recommendations for future species descriptions

As was shown by Benda et al. (2022), the female cephalothorax and male cephalotheca provide important and convenient characters for distinguishing the genera of Xenidae. They are also very suitable for species differentiation when good quality colour photos are used. Especially in old dry museum specimens, cephalothoraces and cephalothecae are the only useful character systems, when internal unsclerotised parts are poorly preserved or males are in the pupal stage. For an introduction in characters suitable for the identifying genera of Xenidae we recommend the determination key in Benda et al. (2022). Although a scanning electron microscope (SEM) is required for certain characters, the host and distribution information are additional cues. Thus, in most cases identification to species level should be possible using only a light microscope.

Important diagnostic features of the female cephalothorax and male cephalotheca of *Xenos* species are the following:

– colouration of cephalothorax/cephalotheca; often with species-specific colour patterns;
– length ratio between head capsule (including lateral cephalic extensions) and cephalothorax;
– shape of clypeal lobe;
– shape of mandibular tooth (SEM micrographs are required) (Nakase & Kato 2013);
– shape of maxilla; similar shapes can occur in related species;
– shape of frontal impression (cephalotheca);
– diameter of genal region between maxillary base and compound eye (cephalotheca).

Some features are apparently unsuitable for identifying species of *Xenos*:

– shape of cephalothorax/cephalotheca; species may vary in proportions and series of individuals are required to assess intraspecific shape variability;
– mouth opening; the shape is usually very variable at intraspecific and interspecific levels;
– hypopharyngeal protuberance (cephalotheca); presence or absence usually very variable at intraspecific and interspecific levels;
– spiracles (cephalothorax);
– orientation and prominence of lateral projections of 1st abdominal segment can be variable.

In general, high-quality photos should be provided as important source of information. Additional schematic drawings can be helpful in some cases. Even decades-old cephalothoraces and cephalothacae can be used. The colour is usually clearly visible if specimens are cleaned with proteinase according to the protocol (see Methods). Reliable identification of other conspecific individuals can be possible without comparing them to the type specimens, but only when descriptions with a good documentation are provided (Pohl et al. 2012).

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Paper V

Benda D., Pohl H., Nakase Y., Beutel R., Straka J.: A new species of the genus *Paraxenos* Saunders, 1872 (Strepsiptera: Xenidae) from *Bembix* digger wasps (Hymenoptera: Bembicidae) and a redescription of *Paraxenos hungaricus* (Székessy, 1955) (accepted in *Europaean Journal of Taxonomy*)

A new species of the genus *Paraxenos* Saunders, 1872 (Strepsiptera: Xenidae) from *Bembix* digger wasps (Hymenoptera: Bembicidae) and a redescription of *Paraxenos hungaricus* (Székessy, 1955)

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Running title: Taxonomy of *Paraxenos* from *Bembix*.

Abstract. A new species of Strepsiptera of the genus *Paraxenos* Saunders, 1872 (Xenidae) from the United Arab Emirates is described. It was recorded from the host species *Bembix kohli* Morice, 1897 and represents the first occurrence of *Paraxenos* from *Bembix* Fabricius, 1775 in the Afrotropical region. A detailed redescription of the female cephalothorax of *Paraxenos hungaricus* (Székessy, 1955) is provided, together with the first description of the male cephalotheca. The holotype of *Paraxenos krombeini* Kifune & Hirashima, 1987 was redescribed. Additionally, a key for *Paraxenos* species parasitic on *Bembix* is provided based on characters of the female cephalothorax and male cephalotheca. Distribution and conservation status of *Paraxenos* spp., on *Bembix* are also discussed.

Key words. Wasp parasite, taxonomy, cephalothorax, cephalotheca, morphology

Introduction

Strepsiptera is a highly derived order of endoparasitic insects characterized by a unique and complex life cycle and extreme sexual dimorphism (Pohl & Beutel 2008). Whereas the adult males are free-living and have an excellent flying capacity, adult females of Stylopidia (ca. 95% of all strepsipteran species) are neotenic, lacking sensory organs and body appendages such as compound eyes, antennae, wings, and legs (Kinzelbach 1971; Pohl & Beutel 2005). Xenidae are a family of Strepsiptera with derived characters and are deeply nested within the clade Stylopiformia (Pohl & Beutel 2005; McMahon *et al.* 2011). Traditionally, the family was divided into four genera: *Paragioxenos* Ogloblin, 1923, *Paraxenos* Saunders, 1872, *Pseudoxenos* Saunders, 1872, and *Xenos* Rossi, 1794. Benda *et al.* (2022) provided a generic revision and a detailed checklist of Xenidae and delimited 13 genera based on previous molecular phylogenetic studies (Benda *et al.* 2019, 2021). The genus *Paraxenos* was identified as a lineage with Old World and Australasian distribution, with species parasitizing three genera of digger wasps: *Bembecinus* Costa, 1859, *Bembix* Fabricius, 1775, and *Stizus* Latreille, 1802 (Bembicidae: Bembicinae) (Benda *et al.* 2022). Its monophyly was supported in an analysis of molecular data, and represented a sister taxon to most other Xenidae (Benda *et al.* 2020: fig. 1). The genus diagnosis consists of a combination of characters because no morphological synapomorphies were found (Benda *et al.* 2022).

The species of the sand wasp genus *Bembix* are only little known as hosts of Strepsiptera. Stylopization of *Bembix* (i.e., infestation with strepsipterans) was recorded for the first time by Pierce (1909, 1919) based on correspondence with the British entomologist Robert Cyril Layton Perkins, who mentioned that many members of the family Bembicidae are stylopized in Australia (Perkins 1905). Pierce (1919) also presented a record of stylopized *Bembix texana* Cresson, 1873 from Louisiana (USA). Another finding was published by Ulrich (1930) who reported parasitized *Bembix rostrata* (Linnaeus, 1758) from Germany.

Although some other records of stylopized *Bembix* were mentioned in the literature (Hofeneder & Fulmek 1942a, 1942b), the first species of Strepsiptera from *Bembix* was described more than ten years later. Székessy (1955) described *Paraxenos hungaricus* (Székessy, 1955) based on material collected in Central Hungary and reported *Bembix oculata* Panzer, 1801 as host. In the same year, Beaumont (1955) published findings of stylopized *B. oculata* and *B. rostrata* from Spain. Székessy (1959) listed stylopized hosts from the Hungarian Natural History Museum with new data on *P. hungaricus* from Central Hungary. In his study he also reported *Bembix vespiformis* F. Smith, 1856 from Australia. Kinzelbach (1978) assigned all published

findings of stylopized *Bembix* from Europe to *Paraxenos hungaricus*. Previously, he recorded this species only from Hungary (Kinzelbach 1971). Kifune & Hirashima (1987) described three new species infesting *Bembix*, one from Sri Lanka and two from Australia. Until now, all *Paraxenos* species from *Bembix* were described mainly by female cephalothorax, first instar larvae are not known and adult male with puparium is only known in *P. hungaricus* (Székessy (1955).

In the molecular phylogeny of Benda *et al.* (2021), several strepsipteran samples from *Bembix* hosts were included, ranging from Italy to Mongolia. All of these samples formed a well-supported monophyletic lineage, and all applied species delimitation methods designated it as one putative species. Based on these findings and using a morphological approach, Benda *et al.* (2022) listed *P. hungaricus* as a widely distributed (transpalearctic) species, found in many countries but rarely collected. Here we describe a new species of *Paraxenos* associated with *Bembix* and compare the morphology of the female cephalothorax and male cephalotheca with characteristics observed in previously described species. We also describe the male cephalotheca of *P. hungaricus* for the first time.

Material and methods

Taxon sampling

A total of 41 females and 26 male puparia of *Paraxenos* were obtained from species of *Bembix* or investigated directly on the host. Material from the following public and private collections was examined:

HNHM = Hungarian Natural History Museum, Budapest, Hungary
JSPC = Jakub Straka personal collection, Prague, Czech Republic
KUMC = Kyushu University Museum Collection, Fukuoka, Japan
NMPC = National Museum, Prague, Czech Republic
OLML = Oberösterreichisches Landesmuseum, Linz, Austria

Fixation and preparation

All host individuals were first relaxed in water vapours and then immediately dissected. The endoparasitic females and males were removed from the host abdomina. Females and male puparia used for the morphological study were cleared using a mixture of lysis buffer ATL and proteinase K (Qiagen) heated to 56 °C. The lysis procedure took several hours or overnight. Cleared specimens were washed in distilled water several times and then stored in vials with

96% ethanol. Complete female cephalothoraces and male puparia were air-dried using a micro-pad inserted into the cephalothorax to prevent the cuticle from collapsing during the process. The female body was usually extracted from the cephalothorax before drying. After this step and removal of the micro-pad, the dried specimens were glued onto card mounting points, which were pinned.

Measurements

The width and length of the female cephalothorax, the female head capsule, and the male cephalotheca were measured using a Leica S9D Stereomicroscope with a calibrated ocular micrometer. The cephalothorax length was measured from the apex of the clypeal lobe to the constriction of abdominal segment I; the width of the cephalothorax is the maximum distance between its lateral margins.

Photomicrography

The general habitus of stylopized host specimens and the host abdomen with protruding strepsipterans were documented using microphotography. Multi-focus images were taken using Canon EOS 550D or 70D cameras equipped with EF 50 mm and MP-E 65 mm macro lenses. Lateral lights and a diffuser were used.

For the documentation of the original coloration of the cephalothoraces, cephalothecae, or puparia, we used air-dried specimens glued to card mounting points. The specimens were photographed with a Canon EOS 7D digital SLR equipped with a Canon MP-E 65 mm macro lens (Canon, Krefeld, Germany) fitted with a StackShot macro rail (Cognisys, Traverse City, MI, USA). Each specimen was illuminated with two flashlights (Yongnuo Photographic Equipment, Shenzhen, China) fitted to a transparent cylinder for even and soft light. For the documentation of tiny cuticular structures, a Canon EOS 70D camera attached to an Olympus BX40 Microscope was used. The microscope was equipped with lateral lights and a diffuser. Zerene Stacker (Zerene Systems LLC, Richland, USA) was used to process stacks of images with different focus.

Scanning electron microscopy (SEM)

Dried female cephalothoraces glued to card points were mounted on a rotatable specimen holder (Pohl 2010). Each specimen was sputter coated with gold with an Emitech K 500 (Sample preparation division, Quorum Technologies Ltd., Ashford, England). The SEM micrographs were taken with an ESEM XL30 (Philips, Amsterdam, Netherlands) equipped with Scandium FIVE (Olympus, Münster, Germany).

Image processing

All images were processed and arranged into plates with Adobe Photoshop® CS5 (Adobe System Incorporated, San Jose, USA) software. CorelDraw® X8 (CorelDraw Corporation, Ottawa, ON, Canada) was used for the lettering of the plates.

Morphological terminology and description style

The terminology used for the female cephalothorax and male cephalotheca is adopted from Benda *et al.* (2022), Richter *et al.* (2017), Löwe *et al.* (2016), and Kinzelbach (1971). Cephalothorax and cephalotheca are described in morphological orientation as in figures although their functional orientation in the host's body is inverted.

Taxonomy

Class Insecta Linnaeus, 1758

Order Strepsiptera Kirby, 1813

Suborder Stylopidia Kinzelbach, 1969

Family Xenidae Saunders, 1872

Paraxenos Saunders, 1872

Saunders, 1872: 45. Type species: *Paraxenos erberi* Saunders, 1872, subsequent designation by Pierce (1908). Type locality: Greece, Corfu.

= *Bembicixenos* Székessy, 1955: 280 (synonymized by Kinzelbach 1978: 82). Type species: *Pseudoxenos* (*Bembicixenos*) *hungaricus* Székessy, 1955, by original designation.

= *Paraxenos* (*Bembicixenos*); Kinzelbach 1971: 162.

Paraxenos arabicus Benda & Straka sp. nov.

Figs 1A-D, 2A-D, 4F

Diagnosis of female cephalothorax

Differing from *P. hungaricus* and *P. krombeini* by a maxilla shaped like an orthogonal triangle; maxillary base wide; maxilla narrowing anteromedially in contrast to anteriorly narrowed in *P. hungaricus* and *P. krombeini*. Maxillary base about 3× wider than anterior part of maxilla (mxb, mx, Fig. 2A), very slightly overlapping with mandible proximally. Mandible completely enclosed in mandibular capsule as in *P. hungaricus*, in contrast to *P. krombeini* where the mandible overtops the anterior edge of the head capsule. Mandibular base slightly bulging,

divided from genal area by furrow. Labial area between maxillae slightly wider than long (lba, Fig. 2A), but distinctly wider than long in *P. hungaricus* (lba, Fig. 6C). Dorsal labral field distinctly arcuate (dlf, Fig. 2A) as in *P. krombeini*, in contrast to slightly arcuate in *P. hungaricus*. Mouth opening very slightly arcuate to straight and not distinctly sclerotized around the margin (os, Fig. 2A), versus conspicuously sclerotized in most specimens of *P. hungaricus*. Lateral extensions of head capsule dull on ventral side (lehc, Fig. 2A), covered by conspicuous dense dark papillae, cuticle wrinkled between papillae, in contrast to lateral extensions pale, shiny to dull, without conspicuous densely arranged dark papillae in *P. hungaricus*. Lateral region of abdominal segment I below spiracles only slightly darker than dorsal side (asI, Fig. 1D), and not distinctly contrasting to pale thorax as in *P. hungaricus*. Anterior head margin distinctly protruding as in *P. krombeini*, versus only slightly protruding from head capsule in *P. hungaricus*.

Etymology

The specific epithet refers to Arabia, the region of origin of the new species. Adjective.

Material examined

Holotype

UNITES ARAB EMIRATES • ♀ (cephalothorax on mounting board); Umm al-Kuvajn; Biyatah env.; 50 m a.s.l.; 28 Feb. 2017; M. Halada lgt.; host: *Bembix kohli* Morice, 1897; NMPC.

Paratypes

UNITES ARAB EMIRATES • 2 ♀♀; 3 male puparia (MP); Umm al-Kuvajn; Biyatah env.; same collection data as the holotype; NMPC • 1 MP; Al Dhaid; Šardžá env.; 2 Mar. 2017; L. Bíca leg.; NMPC.

Description of holotype, female cephalothorax

SHAPE AND COLORATION. Size of holotype cephalothorax: length 1.32 mm, width 1.68 mm. Anterior head margin distinctly protruding. Thorax slightly widening posteriorly. Cephalothorax displaying multiple light brown shades, cuticle sclerotized and darker only around mandible. Cephalothorax less pigmented medially, darker laterally.

HEAD CAPSULE. Approximately ⅓ as long as entire cephalothorax including lateral extensions. Coloration pale to dark with specific pattern (Fig. 2A). Clypeal area well separated from labral area, protruding anteriorly and forming clypeal lobe. Surface slightly wrinkled on dorsal side. Lateral extensions of head capsule dull ventrally (lehc, Fig. 2A), covered by conspicuous dark

papillae, with cuticle wrinkled between. Clypeal sensilla mainly concentrated on clypeal lobe and extending to ventral side of clypeal area. Border between clypeal and frontal regions not clearly recognizable, but present. Frontal region distinctly wrinkled, not covered by dark papillae. Segmental border between head and prothorax indistinct on dorsal side, recognizable by change in cuticular surface structure.

SUPRA-ANTENNAL SENSILLARY FIELD. Slightly wrinkled, with dispersed sensilla, delimited by distinct furrow on medial side (fssf, Fig. 2B).

ANTENNA. Morphology of antennal vestige not observable.

LABRUM. Ventral field distinctly wider than long, elliptic. Dorsal field distinctly arcuate, > 4× wider than long in midline. Dorsal field with approximately 25 setae inserted in cavities (dlf, vlf, Fig. 2A).

MANDIBLE. Anteromedially directed at an angle of 30°, completely enclosed in mandibular capsule or very slightly protruding from it. Mandibular bulge not distinctly raised, with approximately 15 sensilla. Cuticle almost completely smooth, partially sculptured on articulatory area. Mandibular tooth narrow, pointed, sharply curved anteriorly. Mandibular base slightly bulging, divided by furrow from genal area (Fig. 2A).

MAXILLA. Well-developed and prominent, separated from labial area. Shaped like orthogonal triangle. Very wide basally, narrowing anteriorly, maxillary base about 3× wider than distal part (mxb, mx, Fig. 2A). Anteromedially directed, very slightly overlapping mandible. Cuticle smooth. Vestige of palp present, with more or less distinct plates, located anteriorly on ventral side of maxilla. Submaxillary groove distinctly produced posterolaterally (smxg, Fig. 2A).

LABIUM. Labial area between maxillae distinct, delimited anteriorly by mouth opening and posteriorly by birth opening. Flat and slightly wider than long in midline to almost square. Cuticular surface smooth to slightly wrinkled and reticulated.

MOUTH OPENING. Straight, not distinctly sclerotized along margin.

THORAX. Pro-mesothoracic and meso-metathoracic borders distinct on ventral side, separated by mesal furrows (sbpm, sbmm, Fig. 1C). On dorsal side only indistinctly indicated by differing cuticular sculpture. Border between metathorax and abdomen formed by ridge in combination with changed cuticular sculpture and coloration. Cuticle of thoracic segments reticulate on ventral side, with small, scattered pigmented papillae. Prosternal extension differentiated by cuticular sculpture and coloration (pst, Fig. 2A). Anterior part darker with conspicuous

pigmented papillae medially (pstp, Fig. 2A), posterior part pale and lacking distinct pigmented papillae. Meso- and metathorax unmodified in shape, transverse. Mesosternum with small paler area posteromedially, without pigmented papillae. Metasternum with large pale area medially without pigmented papillae and width reaching up to ⅔ of metathorax (Fig. 1C). Dorsal side of thorax smooth or slightly reticulated.

ABDOMINAL SEGMENT I AND SPIRACLES. Lateral region of abdominal segment I below spiracles only slightly darker on dorsal side, not distinctly contrasting to pale thorax (asI, Fig. 1D). Spiracles on posterior half of cephalothorax slightly elevated, with lateral or laterodorsal orientation.

VARIABILITY OF FEMALE CEPHALOTHORAX. Cephalothorax compact, nearly as long as wide or distinctly wider than long. Size slightly variable, length 1.18–1.32 mm, maximum width 1.32–1.68 mm. Dorsal labral field with about 21 to 25 setae inserted in cavities. Mandible anteromedially directed at an angle of 30–45°. Mandibular bulge not distinctly raised, with approximately 12–18 sensilla. Mouth opening very slightly arcuate to straight.

Diagnosis of male cephalotheca

Paraxenos arabicus sp. nov. differs from *P. hungaricus* by several characters. In lateral view, cephalotheca rounded anteriorly, versus protruding and acute anteriorly in *P. hungaricus*. Clypeus (clypeal lobe) not projecting in lateral view (cl, Fig. 2D), but prominent in *P. hungaricus*. Gena around compound eye nearly completely pale (gn, Fig. 2C), with dark area around mandibular base reduced; pale stripe between compound eye and mandibular base wide, about 2× wider than diameter of compound eye; in *P. hungaricus* about as long as diameter of compound eye. Maxilla not wide at base, about 1.5× wider than width of mandible (mx, Figs 2C, 4F), but about 2× wider than mandibular width in *P. hungaricus*. Length of clypeal lobe approximately equal to mandibular length (cll, Fig. 4F), versus clypeal lobe distinctly wider than length of mandible in *P. hungaricus*.

Description of male cephalotheca

SHAPE AND COLORATION. In frontal view rounded, slightly flattened, elliptic, length 0.66–0.82 mm, width 1.30–1.46 mm; in lateral view rounded anteriorly, not acute apically. Coloration predominantly pale with some dark areas forming specific pattern (Fig. 2C).

CEPHALOTHECAL CAPSULE. Compound eyes pale, with darker individual cornea lenses recognizable. Gena around compound eye almost completely pale, dark area around mandibular base missing; pale area between compound eye and mandibular base about 2× wider than

diameter of compound eye (gn, coe, Fig. 2C). Clypeus pale medially and darker laterally. Clypeal lobe straight in frontal view, blunt and not prominent in lateral view. Length of clypeal lobe nearly equal to mandibular length (cll, Fig. 4F). Sensilla mainly concentrated on clypeal lobe, visible. Frontal region with paired furrow of supra-antennal sensillary field, lacking impression or clearly recognizable occipital bulge (fssf, Fig. 2C). Occipital bulge indistinct (ob, Fig. 2C). Diameter of genae between maxillary base and compound eye more than 2× larger than diameter of vestigial antenna.

SUPRA-ANTENNAL SENSILLARY FIELD. Predominantly pale to partly dark (ssf, Fig. 2C, 4F), kidney-shaped and bulging, delimited medially by distinct furrow. Furrows wide, not connected anteriorly. Dark sensilla distinctly visible on pale surface.

ANTENNA. Of standard shape, pale or dark, small, with small plates, sensilla and complete torulus (a, Fig. 2C, 4F). Periantennal area not clearly delimited from supra-antennal sensillary field, pale or dark.

LABRUM. Labral area distinct. Dorsal field conspicuous, pale, with dispersed setae well visible. Ventral field darker (Fig. 2C).

MANDIBLE. Nearly medially directed. Tooth apically pointed, wide basally, but not reaching area of mandibular bulge. Coloration mostly pale, especially middle region, with darker parts apically and basally. Mandibular bulge with sensilla, separated from tooth. Length between mandibles nearly equal to mandibular length.

MAXILLA. Distinct, prominent, with darker and paler parts. Vestige of palp present, conspicuous (mxp, Figs 2C, 4F). Not very wide at base, approximately 1.5× wider than mandible (mx, Figs 2C, 4F).

LABIUM AND HYPOPHARYNX. Labium distinct, located between and below maxillae, darker. Praementum and postmentum more or less distinctly separated by furrow (Fig. 2C). Hypopharyngeal protuberance absent.

MOUTH OPENING. Visible, not covered by ventral labral field, slightly arcuate, sclerotized around margin.

Host

Bembix kohli Morice, 1897.

Distribution

United Arab Emirates.

Paraxenos hungaricus (Székessy, 1955)

Figs 3A-D, 4A-E, 5A-F, 6A-F

Pseudoxenos (*Bembicixenos*) *hungaricus* Székessy, 1955: 281 (type locality: Hungary, Bugac).
Paraxenos (*Bembicixenos*) *hungaricus*; Kinzelbach 1971: 162.

Diagnosis of female cephalothorax

Differing from *P. arabicus* sp. nov. and *P. krombeini* in several characters. Maxilla cone-shaped, narrowing anteriorly like in *P. krombeini*, but blunt apically (mx, Fig. 6E). Anteriorly directed, very slightly overlapping with mandible proximally. Maxillary base approximately 2–3× wider than anterior part of maxilla (mxb, mx, Fig. 6E, F). Mandible enclosed in mandibular capsule like in *P. arabicus* sp. nov., versus overtopping anterior edge of the head capsule in *P. krombeini*. Mandibular base flat, not divided by furrow from genal area. Labial area between maxillae distinctly wider than long in midline (lba, Fig. 6C), versus slightly wider than long in *P. arabicus* sp. nov. (lba, Fig. 2A). Dorsal labral field slightly arcuate (dlf, Fig. 6D), versus distinctly arcuate in *P. arabicus* sp. nov. and *P. krombeini*. Mouth opening usually conspicuously sclerotized, but only indistinctly in *P. arabicus* sp. nov. Lateral extensions of head capsule predominantly dull on ventral side, cuticle wrinkled, but shiny area near submaxillary groove without conspicuous dark papillae (lehc, Fig. 3C, lehc, smxg, Fig. 6A), versus lateral extensions completely dull and covered by dark papillae in *P. arabicus* sp. nov. Dark lateral region of abdominal segment I below spiracles distinctly contrasting to pale thorax from dorsal side (asI, Fig. 3D). Clypeal lobe slightly protruding from head capsule (cll, Fig. 3C), but distinctly protruding in *P. krombeini* and *P. arabicus* sp. nov.

Material examined

CZECH REPUBLIC • 1 MP; Bzenec env.; 12 Jun. 2015; M. Halada leg.; host: *Bembix rostrata* (Linnaeus, 1758); NMPC.

HUNGARY • Allotype ♀; Bugac env.; 30 Jul. 1941; Móczár leg.; host: *Bembix oculata* Panzer, 1801; HNHM • 2 ♀♀; Agasegyháza env.; 15 Jul. 1956; Bajári leg.; host: *Bembix oculata* Panzer, 1801; HNHM • 1 MP + 1 ♀; Agasegyháza env.; 16 Jul. 1956; Mihályi leg.; host: *Bembix oculata* Panzer, 1801; HNHM • 1 MP + 1 ♀; Fülophaza env.; 17 Jul. 2013; J. Straka leg.; host: *Bembix oculata* Panzer, 1801; NMPC • 1 ♀; Fülophaza env.; 12 Aug. 2011; P. Bogusch & J. Straka leg.; host: *Bembix oculata* Panzer, 1801; NMPC.

IRAN • 1 ♀; Kerman; 20 km E Ghobira; 5 Jun. 2010; Mi. Halada leg.; host: *Bembix* sp.; OLML.

ITALY • 1 MP; Sicilia; Mts. Süd. Etna, 21 Jun. 2012; J. Halada leg.; host: *Bembix rostrata* (Linnaeus, 1758); OLML • 3× 1 MP; 1 ♀; Sardinia; Sassari 30 km NW; 19 May 2013; J. Halada leg.; host: *Bembix rostrata* (Linnaeus, 1758); NMPC.

KAZAKHSTAN • 1 MP; Lepsi env.; 20 Jun. 1995; M. Múčka leg.; host: *Bembix* sp.; NMPC • 1 ♀; Lepsi env.; 20 Jun. 1995; M. Múčka leg.; hosts: *Bembix rostrata* (Linnaeus, 1758); OLML • 1 MP; 50 km S Balkhash; 28 Jun. 1992; K. Deneš leg.; host: *Bembix oculata* Panzer, 1801; OLML • 1 empty male puparium (EMP); 2 EMP; Matai desert; 25 Jun. 1995; J. Halada & M. Múčka leg.; host: *Bembix rostrata* (Linnaeus, 1758); OLML.

MONGOLIA • 3× 1 MP; Gobi; Khatansuudal 70 km SE; 11 Jul. 2005; P. Tyrner leg.; host: *Bembix* sp.; NMPC.

TURKEY • 1 MP; 40 km NE Muradiye; 5 Jul. 2000; M. Halada leg.; host: *Bembix rostrata* (Linnaeus, 1758); OLML • 24× 1 ♀; 2× 2 ♀♀; 1 ♀+ 1 EMP; 2 ♀♀ + 1 MP; 6× 1 MP; 20 km W Van, 5 Jul. 1997; M. Halada leg.; host: *Bembix rostrata* (Linnaeus, 1758); OLML.

Description of female cephalothorax

SHAPE AND COLORATION. Compact, widened, slightly or distinctly wider than long. Size variable, length 1.28–1.93 mm, maximum width 1.68–2.57 mm. Anterior head margin slightly or scarcely protruding from head capsule. Thorax widening posteriorly. Cephalothorax displaying multiple light brown shades, only around mandible and mouth opening cuticle more sclerotized and darker, but lighter in central region, darker laterally.

HEAD CAPSULE. Approximately ⅓ to ½ as long as entire cephalothorax including lateral extensions. Coloration pale to dark with specific pattern. Clypeal area distinctly or indistinctly separated from labral area, slightly protruding anteriorly or not protruding. Clypeal lobe blunt. Surface slightly wrinkled on dorsal side, reticulated. Lateral extensions of head capsule predominantly dull on ventral side, cuticle wrinkled, but shiny area near submaxillary groove lacking conspicuous dark papillae (lehc, Fig. 3C, lehc, smxg, Fig. 6A). Clypeal sensilla present on ventral side of clypeus, mainly concentrated on clypeal lobe (cls, Fig. 6D). Border between clypeal and frontal region indistinct, but still recognizable (Fig. 5F). Frontal region of head capsule distinctly wrinkled, not covered by dark papillae. Segmental border between head and prothorax indistinct on dorsal side, recognizable by change in cuticular surface structure.

SUPRA-ANTENNAL SENSILLARY FIELD. Predominantly smooth or slightly wrinkled, with dispersed sensilla (sssf, Fig. 5 C, D), delimited by distinct furrow medially (fssf, Fig. 6B).

ANTENNA. Preserved as cavity, rarely combined with rounded plates (a, Fig. 5C, D). Antennal sensilla or vestigial setae missing. Periantennal area smooth, reduced when supra-antennal sensillary field almost reaches vestige of antennae (paa, ssf, Fig. 5C, D).

LABRUM. Ventral field distinctly wider than long, elliptic or semicircular. Dorsal field slightly arcuate to nearly straight, > 4× wider than long in midline. Dorsal field with about 24 setae inserted in cavities (Fig. 6C, D).

MANDIBLE. Anteromedially directed at an angle of 30–35°, enclosed in mandibular capsule. Mandibular bulge not distinctly raised, with ca. 12–18 sensilla (mdbs, Fig. 6E, F). Cuticle completely smooth anteriorly, posteroventrally sculptured, reticulated. Mandibular tooth slightly widened, pointed apically, anteriorly directed, armed with distinct spines (mdt, mdts, Fig. 6E, F). Mandibular base flat, not divided by furrow from genal area.

MAXILLA. Well-developed and separated from labial area, prominent. Cuticle smooth. Maxilla cone-shaped, wide at base, but narrowing distally, maxillary base approximately 2–3× wider than distal part (mxb, mx, Fig. 6E, F). Anteriorly directed, very slightly overlapping with mandible proximally. Vestige of palp present, with more or less distinct plates, located anteriorly on ventral side of maxilla. Additional sensilla present on ventral maxillary surface (mxs, Fig. 6E, F). Submaxillary groove distinctly produced posterolaterally (smxg, Fig. 6A).

LABIUM. Labial area between maxillae distinct, delimited anteriorly by mouth opening and posteriorly by birth opening. Distinctly wider than long in midline, rectangular, flat. Cuticular surface very slightly wrinkled, reticulated.

MOUTH OPENING. Mouth opening straight, or bi-arcuate, sclerotized marginally.

THORAX. Pro-mesothoracic and meso-metathoracic borders distinct on ventral side, separated by mesal furrows (sbpm, sbmm, Fig. 3C). On dorsal side indistinct, indicated by different cuticular sculpture. Border between metathorax and abdomen formed by ridge in combination with changed cuticular sculpture and coloration. Cuticle of thoracic segments reticulate on ventral side, with small, scattered pigmented papillae. Prosternal extension variable, differentiated by cuticular sculpture and coloration (pst, Fig. 3C). Anterior part usually darker, with more or less distinct pigmented papillae medially. Posterior part usually pale and without conspicuous pigmented papillae. Meso- and metathorax unmodified in shape, transverse.

Posteromedial pale area on mesosternum and metasternum variable in shape, in some specimens indistinct (mst, mtst, Fig. 3C). Dorsal side of thorax smooth or slightly reticulated.

ABDOMINAL SEGMENT I AND SPIRACLES. Lateral region of abdominal segment I below spiracles conspicuously darkened on dorsal side, contrasting to pale thorax (asI, Fig. 3D). Spiracles on posterior half of cephalothorax slightly elevated, with lateral or laterodorsal orientation.

Diagnosis of male cephalotheca

See the Diagnosis section under *P. arabicus* sp. nov.

Description of male cephalotheca

SHAPE AND COLORATION. In frontal view rounded, slightly flattened, elliptic, length 0.63–0.78 mm, width 1.23–1.53 mm, in lateral view protruding anteriorly, pointed apically. Coloration forming pattern of pale and dark shades (Fig. 4A, B).

CEPHALOTHECAL CAPSULE. Compound eyes pale to dark, visible, with dark individual cornea lenses. Gena completely pale except dark area around mandibular base; pale area between compound eye and mandibular base narrowed (nearly as wide as diameter of compound eye) (gn, coe, Fig. 4A). Clypeus pale medially (on clypeal lobe) and darker laterally. Clypeus (clypeal lobe) straight in frontal view, prominent in lateral view, but blunt apically (cl, Fig. 4B). Clypeal lobe distinctly wider than mandibular length (cll, Fig. 4E). Clypeal sensilla mainly concentrated on clypeal lobe, visible or indistinct (Figs 4A, E). Frontal region with paired furrow of supra-antennal sensillary field, lacking impression or occipital bulge (fssf, Figs 4A, E). Diameter of genae between maxillary base and compound eye approximately 2× larger than diameter of vestigial antenna.

SUPRA-ANTENNAL SENSILLARY FIELD. Dark (ssf, Fig. 4A, E), kidney-shaped and bulging, delimited medially by distinct furrow. Furrows wide, not connected anteriorly. Dark sensilla visible (Fig. 4E).

ANTENNA. Of standard shape, dark, small, with small plates or sensilla, complete torulus (a, Fig. 4A, E). Periantennal area not clearly delimited from supra-antennal sensillary field, dark.

LABRUM. Labral area distinct. Dorsal field pale or dark, with dispersed setae visible. Ventral field conspicuously darkened (dlf, Fig. 4E).

MANDIBLE. Nearly medially directed. Tooth inconspicuous, apically pointed, wide basally, but not reaching area of mandibular bulge. Coloration pale with darker parts. Mandibular bulge

with sensilla, separated from pointed tooth. Distance between mandibles very distinctly exceeding mandibular length (Fig. 4E).

MAXILLA. Distinct, prominent, with darker and paler parts. Vestige of palp present, conspicuous (mxp, Fig. 4A, E). Wide at base, approximately 2× as wide as mandible (mx, Fig. 4A, E).

LABIUM AND HYPOPHARYNX. Labium distinct between and below maxillae, darker. Praementum and postmentum almost fused, indistinctly separated by furrow. Hypopharyngeal protuberance present or absent (hyp, Fig. 4E).

MOUTH OPENING. Visible, not covered by ventral labral field, slightly arcuate, sclerotized around margin.

Hosts

Bembix oculata Panzer, 1801; *Bembix rostrata* (Linnaeus, 1758); *Bembix sp*. (Kinzelbach 1978; Benda, Pohl, Nakase, *et al.* 2022).

Distribution

Palearctic: Czech Republic; Germany; Hungary; Italy; Mongolia; Spain (Székessy 1955; Kinzelbach 1978; Benda *et al.* 2021); Turkey (Benda *et al.* 2022); Iran; Kazakhstan (this tudy).

Paraxenos krombeini Kifune & Hirashima, 1987

Paraxenos krombeini Kifune & Hirashima, 1987: 155.

Diagnosis of female cephalothorax

Differing from *P. arabicus* sp. nov. and *P. hungaricus* in several characters. Mandible projects beyond anterior edge of the head capsule (md, Fig 7A), versus enclosed in mandibular capsule in *P. arabicus* sp. nov. and *P. hungaricus*. Maxilla triangular, similar to that of *P. hungaricus*, but pointed apically (mx, Fig. 7A). Maxilla not overlapping or touching mandible. Labial area between maxillae wide (lba, Fig. 7A). Dorsal labral field distinctly arcuate as in *P. arabicus* sp. nov. Clypeal lobe distinctly protruding from head capsule as in *P. arabicus* sp. nov., versus slightly protruding in *P. hungaricus*.

Material examined

SRI LANKA • Holotype ♀ on slide; Ratmalana airport, 19.-21.1.1975, K. V. Krombein, P. B. Karunaratne, P. Fernando, & N. V. T. A. Weragoda leg., host: *Bembix orientalis* (Handlirsch, 1893); KUMC.

Description of female cephalothorax (modified from Kifune & Hirashima 1987)

SHAPE AND COLORATION. Cephalothorax brown, wider than long, trapezoidal; anterior margin of oral portion (clypeal lobe) roundly protruding. Length and maximum width of cephalothorax 1.5–1.6 mm and 1.7–2.0 mm, respectively. Width of abdominal constriction 1.3–1.6 mm. Cephalothoraces of specimens from tristylopized hosts smaller than those from monostylopized hosts. Mandibles almost trapezoidal; each with sharp, but short, anteriorly directed tooth. Distal part of mandibles projects beyond anterior edge of head capsule. Maxillae triangular, apically pointed or very slightly rounded; not overlapping or touching mandibles. Spiracles dorsally oriented, placed roughly at basal third of cephalothorax.

Host

Bembix orientalis Handlirsch, 1893 (Kifune & Hirashima 1987).

Phylogenetic relationships

Unknown.

Distribution

Sri Lanka.

Key to *Paraxenos* species parasitizing digger wasps of the genus *Bembix* based on female cephalothorax

Modified and extended from Kifune & Hirashima (1987).

1. Old World distribution. 2
– Australian distribution. 4
2. Mandible completely enclosed in mandibular capsule (md, Figs 2A, 6C). 3
– Mandible projecting beyond anterior edge of head capsule (md, Fig. 7); host: *Bembix orientalis* (Handlirsch); Sri Lanka. ..... ***Paraxenos krombeini*** Kifune & Hirashima, 1987
3. Maxilla anteriorly directed, maxillary base ~ 2–3× wider than distal part of maxilla (mxb, mx, Fig. 6 E, F); labial area between maxillae distinctly wider than long in midline (lba, Fig. 6C); shiny area near submaxillary groove without conspicuous dark papillae on

lateral cephalic extensions (lehc, Fig. 3C, lehc, smxg, Fig. 6A); dark lateral region of abdominal segment I below spiracles distinctly contrasting to pale coloration of thorax on dorsal side (asI, Fig. 3D); hosts: *Bembix oculata* Panzer, *Bembix rostrata* (Linnaeus), *Bembix* sp.; distribution: widespread in the Palearctic Region. .... ***Paraxenos hungaricus*** (Székessy, 1955)

– Maxilla anteromedially directed, maxillary base ~ 3× wider than distal part (mxb, mx, Fig. 2A); labial area between maxillae slightly wider than long (lba, Fig. 2A); lateral cephalic extensions completely dull on ventral side (lehc, Fig. 2A), covered by conspicuous, densely arranged dark papillae; lateral region of abdominal segment I below spiracles only slightly darker on dorsal side (asI, Fig. 1D); host: *Bembix kohli* Morice; distribution: United Arab Emirates. ..... ***Paraxenos arabicus*** sp. nov.

4. Spiracles situated at about midlength of cephalothorax; maximum width of cephalothorax ca. 1.8 mm; host: *Bembix atrifrons* F. Smith, 1856; distribution: Western Australia. ..... ***Paraxenos occidentalis*** Kifune & Hirashima, 1987

– Spiracles situated at about basal third of cephalothorax; maximum width of cephalothorax ca. 1.6 mm; lateral margins of cephalothorax evenly curved; host: *Bembix musca* Handlirsch; distribution: Australia (Queensland). .... ***Paraxenos australiensis*** Kifune & Hirashima, 1987

Key to *Paraxenos* species parasitizing digger wasps of the genus *Bembix* based on male cephalotheca

Cephalotheca of *Paraxenos australiensis*, *P. krombeini*, and *P. occidentalis* unknown.

1. Clypeal lobe distinctly wider than length of mandible length (cll, Fig. 4E); distance between mandibles very distinctly exceeding mandibular length; maxilla at base ~ 2× wider than mandible (mx, Fig. 4A, E); clypeus (clypeal lobe) prominent in lateral view, but blunt anteriorly (cl, Fig. 4B); pale stripe between compound eye and mandibular base as wide as diameter of compound eye; hosts: *Bembix oculata* Panzer, *Bembix rostrata* (Linnaeus), *Bembix* sp., distribution: widespread in the Palearctic Region. ..... ***Paraxenos hungaricus*** (Székessy)

– Clypeal lobe ~ as wide as mandibular length (cll, Fig. 4F); maxilla at base ~ 1.5× wider than mandible (mx, Figs 2C, 4F); clypeus (clypeal lobe) not prominent in lateral view (cl,

Fig. 2D); pale stripe between compound eye and mandibular base ~ 2× wider than diameter of compound eye; host: *Bembix kohli* Morice, distribution: United Arab Emirates. ..... ***Paraxenos arabicus*** sp. nov.

Discussion

The *Paraxenos* species parasitizing *Bembix* hosts can be easily distinguished from other species of the genus by very wide cephalothecae and cephalothoraces. Important characters for species identification are the shape of the mandibles and maxillae, the sculpture and coloration of the cuticle, and the shape of the clypeus. These characters are confirmed with important diagnostic features of the female cephalothorax and male cephalotheca used for differentiation of *Xenos* species (Benda *et al.* 2022). Unfortunately, type material of the Australian species was not mentioned in the type catalogue of the National Museum of Natural History), and the specimens are probably lost. Although we examined the holotype of *Paraxenos krombeini*, the cuticular sculpture was not visible due to inadequate mounting on a slide (Fig. 7). For future research, we therefore recommend gluing specimens onto the tip of card mounting points for easy examination of the dorsal and ventral side of the cephalotheca or cephalothorax. This avoids or reduces artefacts caused by the preservation medium and facilitates documentation using scanning electron microscopy and other techniques (Benda *et al.* 2022).

The host genus *Bembix* is composed of more than 300 species of ground-nesting wasps inhabiting sandy substrates (Pulawski 2021; Frank 2022). They are distributed on all continents except for Antarctica, with the greatest diversity in the Afrotropical and Australian regions (Bohart & Menke 1976). Although these hosts are very large and attractive wasps easy to observe and collect in sandy habitats, relatively scarce data on *Paraxenos* have been available so far. For instance, only three Australian species were recorded and described in a single study (Kifune & Hirashima 1987). One reason may be their inconspicuousness on the host compared to other species of Xenidae. In addition to the generally flattened cephalothorax, *Paraxenos* species from *Bembix* also have flat male cephalothecae (Benda *et al.* 2022). Females and male puparia do not project from the host. Remaining concealed below the host abdominal tergites (see Figs. 1A, 3A), they can easily escape attention in contrast to species of other genera of Xenidae.

Unfortunately, there is no information on the phylogeography and evolutionary history of *Bembix*, which could explain the distribution of its *Paraxenos* parasites. Studies covering the

New World (Pierce 1908, 1909, 1919) tentatively suggested that *Paraxenos* did not disperse into New World biogeographic region or alternatively became extinct there. The absence of *Paraxenos* in the New World can also be explained by the rarity of potential hosts. In this context, the record of stylopized *Bembix texana* published by Pierce (1919) appears doubtful. This could be due to misidentification or a rare case of host switch by another genus of Xenidae (e.g., *Eupathocera* Pierce, 1918). *Paraxenos arabicus* sp. nov. is the first species from *Bembix* recorded in the Afrotropical region according to the presently known biogeographic distribution (Morrone 2002). Intensified screening of stylopized *Bembix* by researchers in this area would probably reveal more records. The great diversity of *Bembix* in the Afrotropics suggests that many more undescribed species of *Paraxenos* are likely in this region.

Although there are still insufficient data concerning the distribution of species of *Paraxenos* from *Bembix*, the first insight suggests possible differences in the size of their distributional areas (Fig. 8, Table 1). *Paraxenos hungaricus* has a transpalearctic distribution also including Mediterranean islands such as Sicily or Sardinia, which was also supported by a molecular phylogeny (Benda *et al.* 2021). In contrast, *P. krombeini* has only been found in Sri Lanka. Additional data of stylopized *Bembix* from the Oriental region are needed to confirm it as either endemic or a species with a wider distribution. In some parts of their distribution, species also may become endangered, especially if they occur only locally on a specific habitat of shifting sands with a high abundance of their hosts. In Central Europe, *P. hungaricus* is likely to be threatened due to habitat loss and decline of available nesting sites for *Bembix* species. The distribution area of its host *Bembix rostrata* is shrinking, and the number of populations has apparently declined in many parts of Europe, as well as in other *Bembix* species (Blösch 2000; Klein & Lefeber 2004).

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Figures and tables

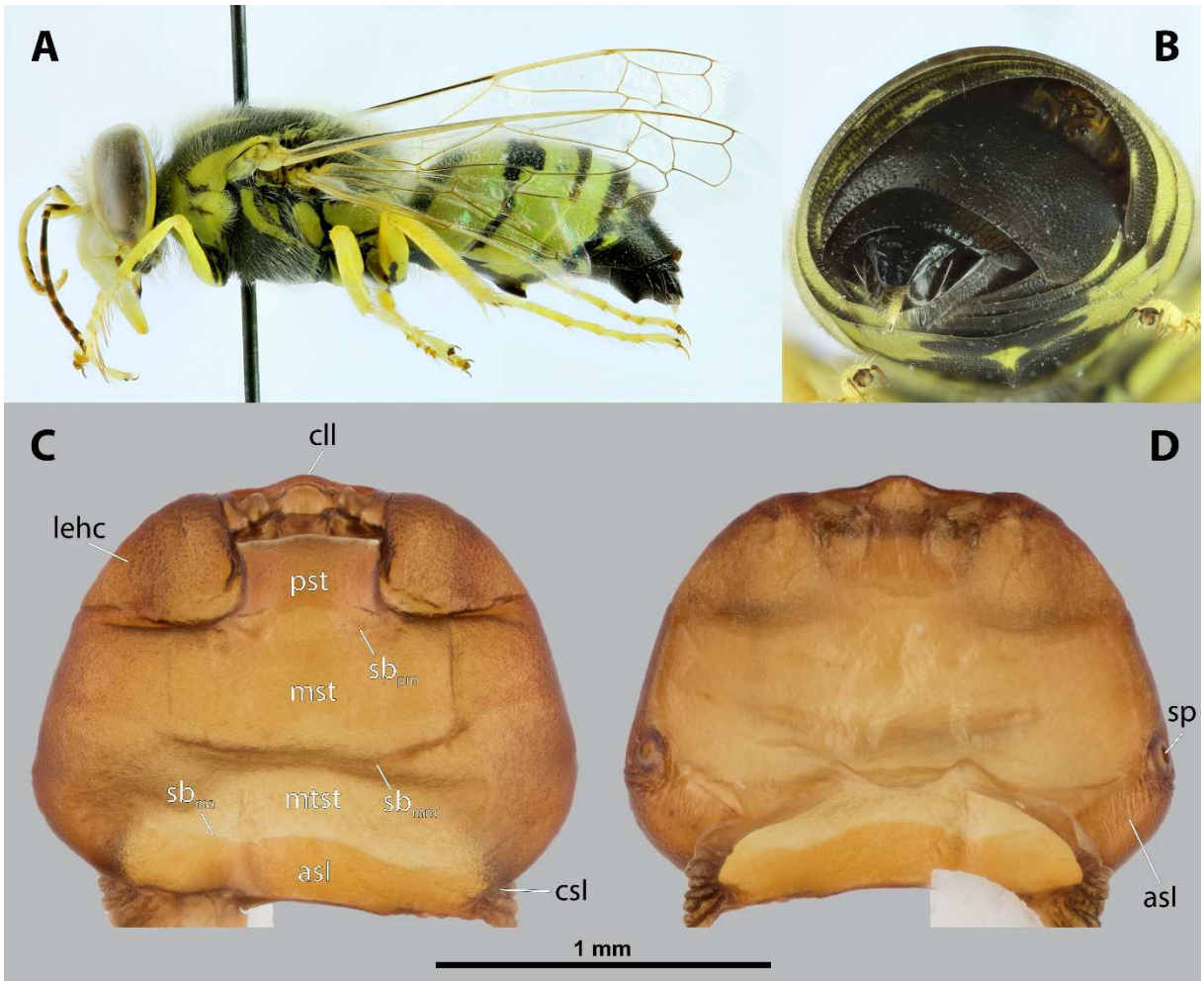

Fig. 1. *Paraxenos arabicus* Benda & Straka sp. nov., host, male puparium, female cephalothorax. **A**. *Bembix kohli* Morice, 1897 stylopized by *P*. *arabicus* sp. nov., lateral view. **B**. Detail of host abdomen of *B*. *kohli*, with male puparium. **C**. Holotype of *P*. *arabicus* sp. nov. from *B*. *kohli*, ventral side of cephalothorax. **D**. Holotype of *P*. *arabicus* sp. nov**.** from *B*. *kohli*, dorsal side of cephalothorax. Abbreviations: asI = abdominal segment I; cll = clypeal lobe; csI = constriction of abdominal segment I; lehc = lateral extension of head capsule; mst = mesosternum; mtst = metasternum; pst = prosternum (prosternal extension); sbma = segmental border between metathorax and abdomen; sbmm = segmental border between mesothorax and metathorax; sbpm = segmental border between prothorax and mesothorax; sp = spiracle.

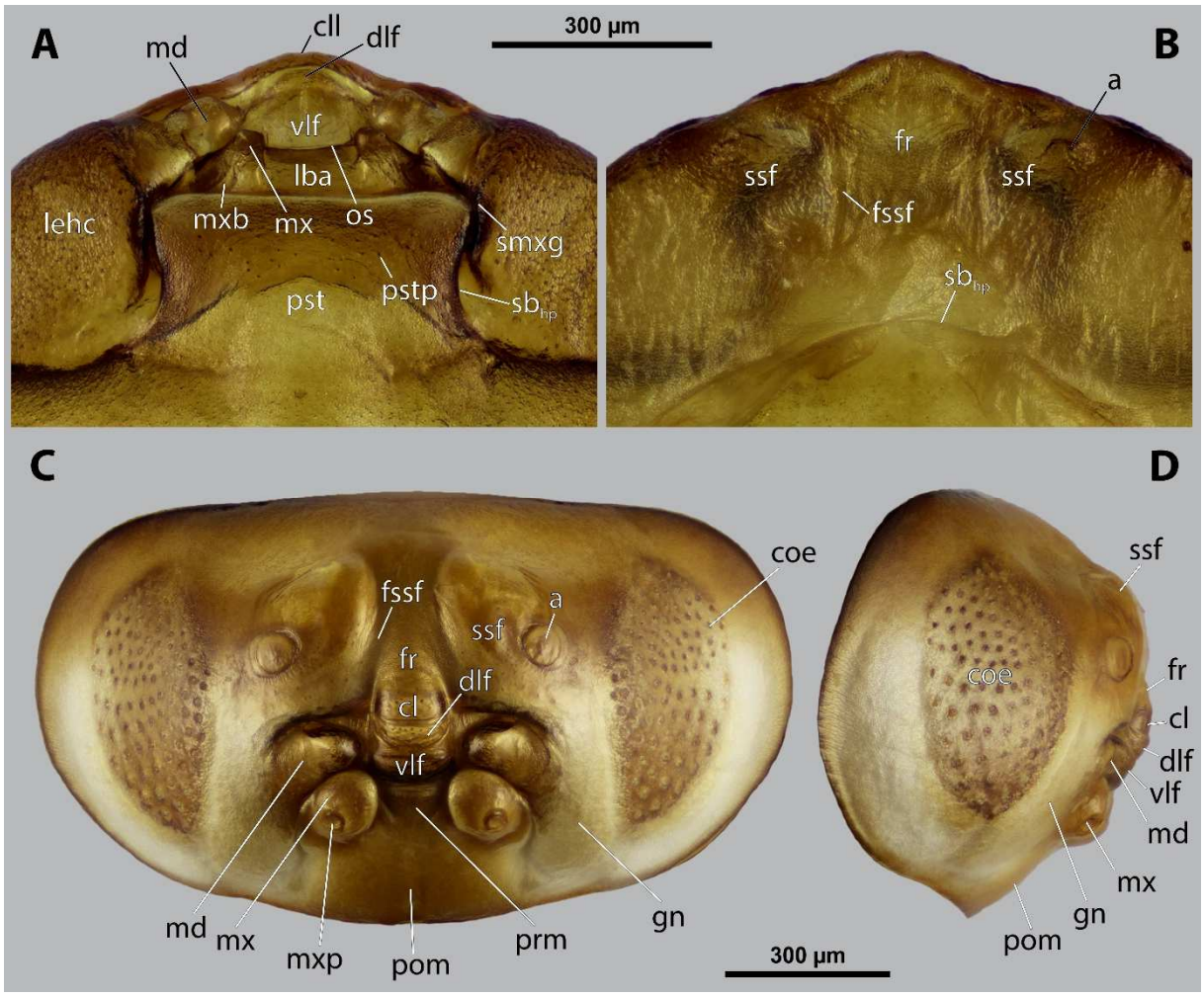

Fig. 2. *Paraxenos arabicus* Benda & Straka sp. nov., female, detail of cephalothorax, male, cephalotheca. **A**. Detail of ventral side of cephalothorax. **B**. Detail of dorsal side of cephalothorax. **C**. Frontal view of cephalotheca. **D**. Lateral view of cephalotheca. Abbreviations: a = vestigial antenna; cl = clypeus; cll = clypeal lobe; coe = compound eye; dlf = dorsal labral field of labral area; fr = frontal region; fssf = furrow of supra-antennal sensillary field; gn = gena; lba = labial area; lehc = lateral extension of head capsule (lateral cephalic extension); md = mandible; mx = vestige of maxilla; mxb = maxillary base; mxp = vestige of maxillary palp; os = mouth opening; pom = postmentum; prm = praementum; pst = prosternum (prosternal extension); pstp = prosternal papilla; sbhp = segmental border between head and prothorax; smxg = submaxillary groove; ssf = supra-antennal sensillary field; vlf = ventral labral field of labral area.

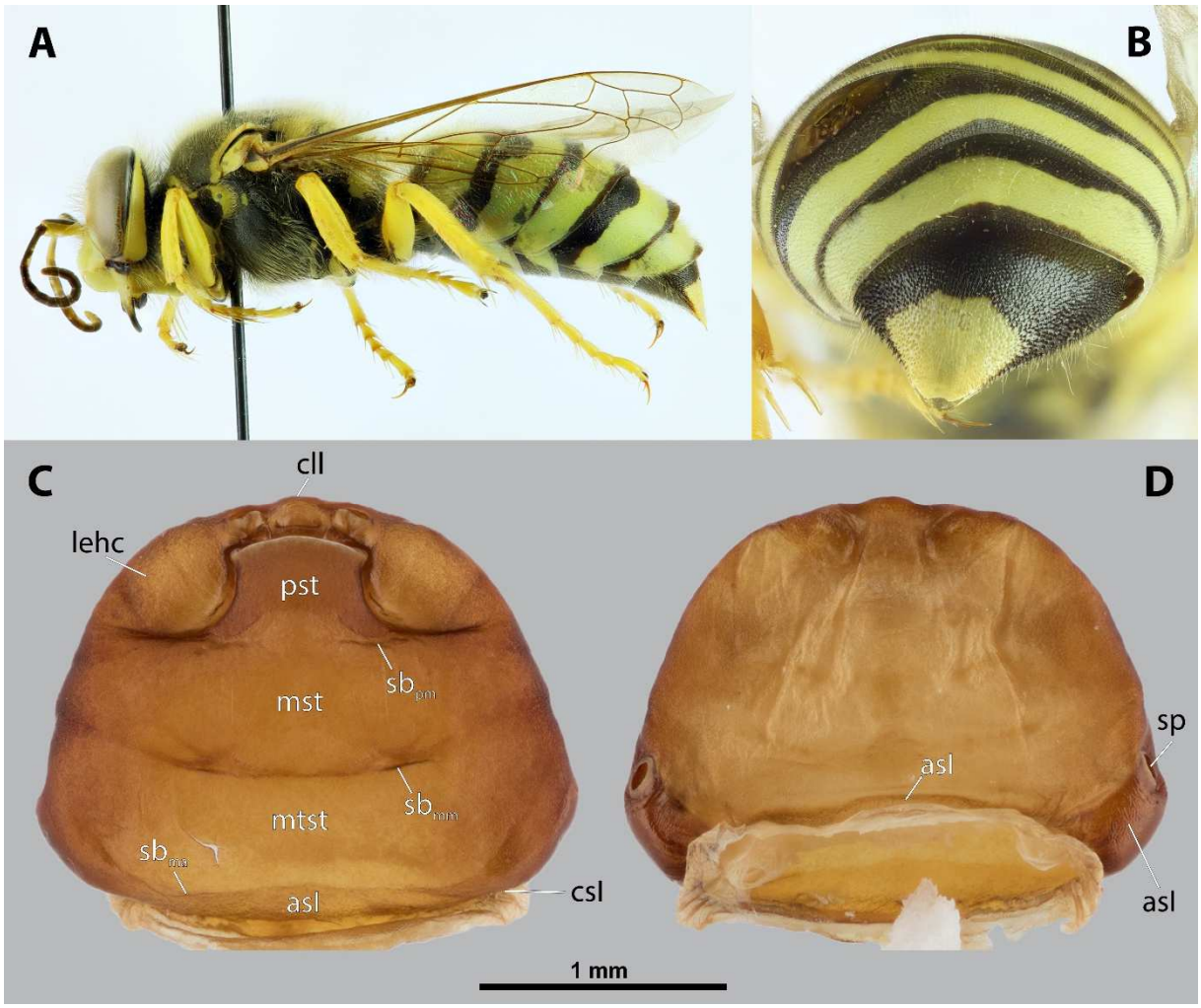

Fig. 3. *Paraxenos hungaricus* (Székessy, 1955), host, female cephalothorax. **A**. *Bembix rostrata* (Linnaeus, 1758) stylopized by *P*. *hungaricus*, lateral view. **B**. Detail of host abdomen of *B*. *rostrata*, with adult female. **C**. *P*. *hungaricus* from *B*. *rostrata*, ventral side of cephalothorax. **D**. *P*. *hungaricus* from *B*. *rostrata*, dorsal side of cephalothorax. Abbreviations: asI = abdominal segment I; cll = clypeal lobe; csI = constriction of abdominal segment I; lehc = lateral extension of head capsule; mst = mesosternum; mtst = metasternum; pst = prosternum (prosternal extension); sbma = segmental border between metathorax and abdomen; sbmm = segmental border between mesothorax and metathorax; sbpm = segmental border between prothorax and mesothorax; sp = spiracle.

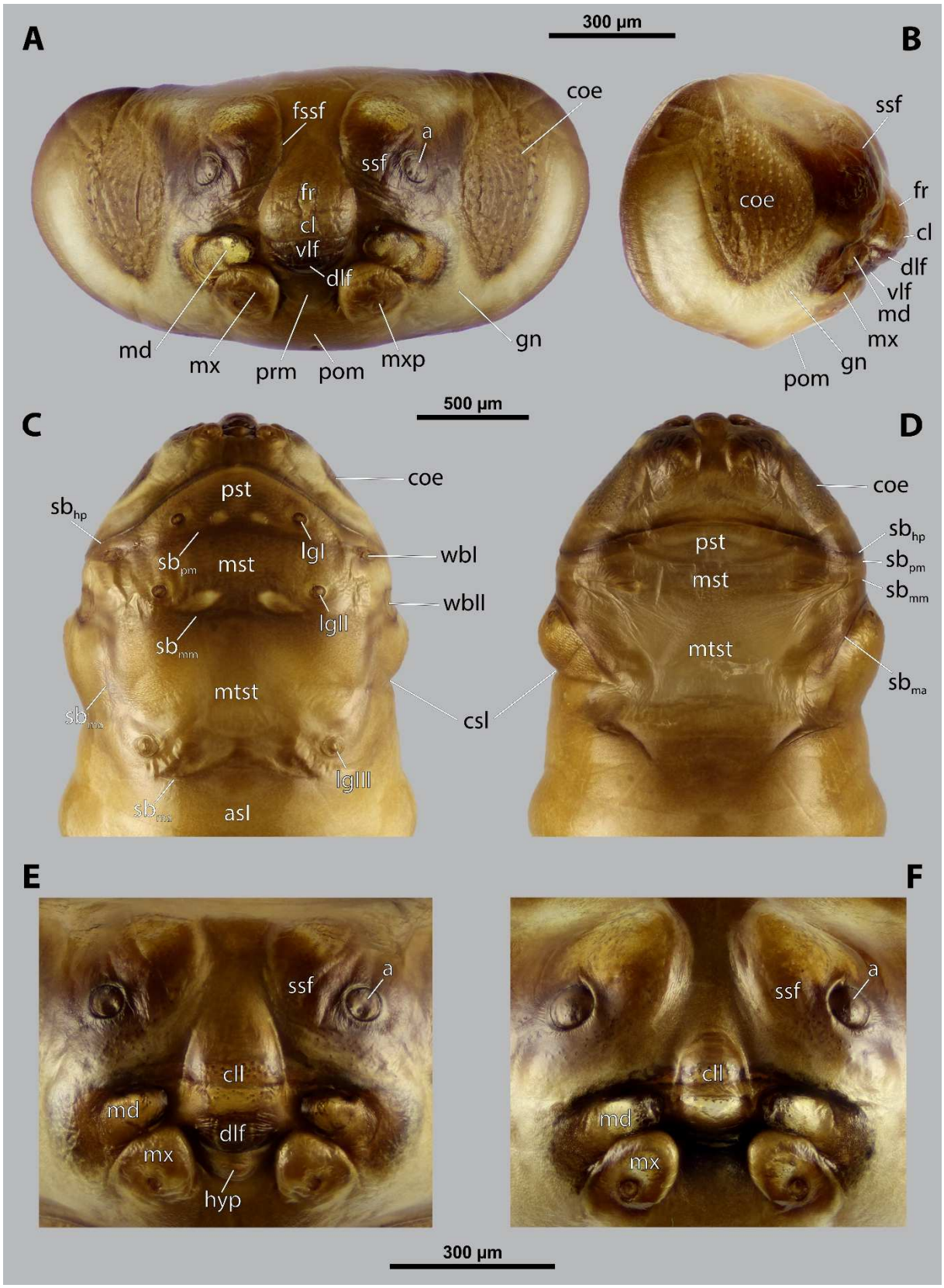

Fig. 4. *Paraxenos hungaricus* (Székessy, 1955), male, cephalotheca, anterior part of puparium, detail of cephalotheca; *Paraxenos arabicus* Benda & Straka sp. nov., detail of cephalotheca. **A**. Frontal view of cephalotheca (*P. hungaricus*). **B**. Lateral view of cephalotheca (*P. hungaricus*).

C. Ventral view of anterior part of the puparium (*P. hungaricus*). **D**. Dorsal view of anterior part of the puparium (*P. hungaricus*). **E**. Detail of cephalotheca, frontal view (*P. hungaricus*). **F**. Detail of cephalotheca, frontal view (*P. arabicus* sp. nov.). Abbreviations: a = vestigial antenna; asI = abdominal segment I; cl = clypeus; cll = clypeal lobe; coe = compound eye; csI = constriction of abdominal segment I; dlf = dorsal labral field of labral area; fr = frontal region (frons); fssf = furrow of supra-antennal sensillary field; gn = gena; hyp = hypopharynx; lgI = foreleg; lgII = middle leg; lgIII = hindleg; md = mandible; mst = mesosternum; mtst = metasternum; mx = maxilla; mxp = vestige of maxillary palp; pom = postmentum; prm = praementum; pst = prosternum; sbhp = segmental border between head and prothorax; sbma = segmental border between metathorax and abdomen; sbmm = segmental border between mesothorax and metathorax; sbpm = segmental border between prothorax and mesothorax; ssf = supra-antennal sensillary field; vlf = ventral labral field of labral area; wbI = wing buds I; wbII = wing buds II.

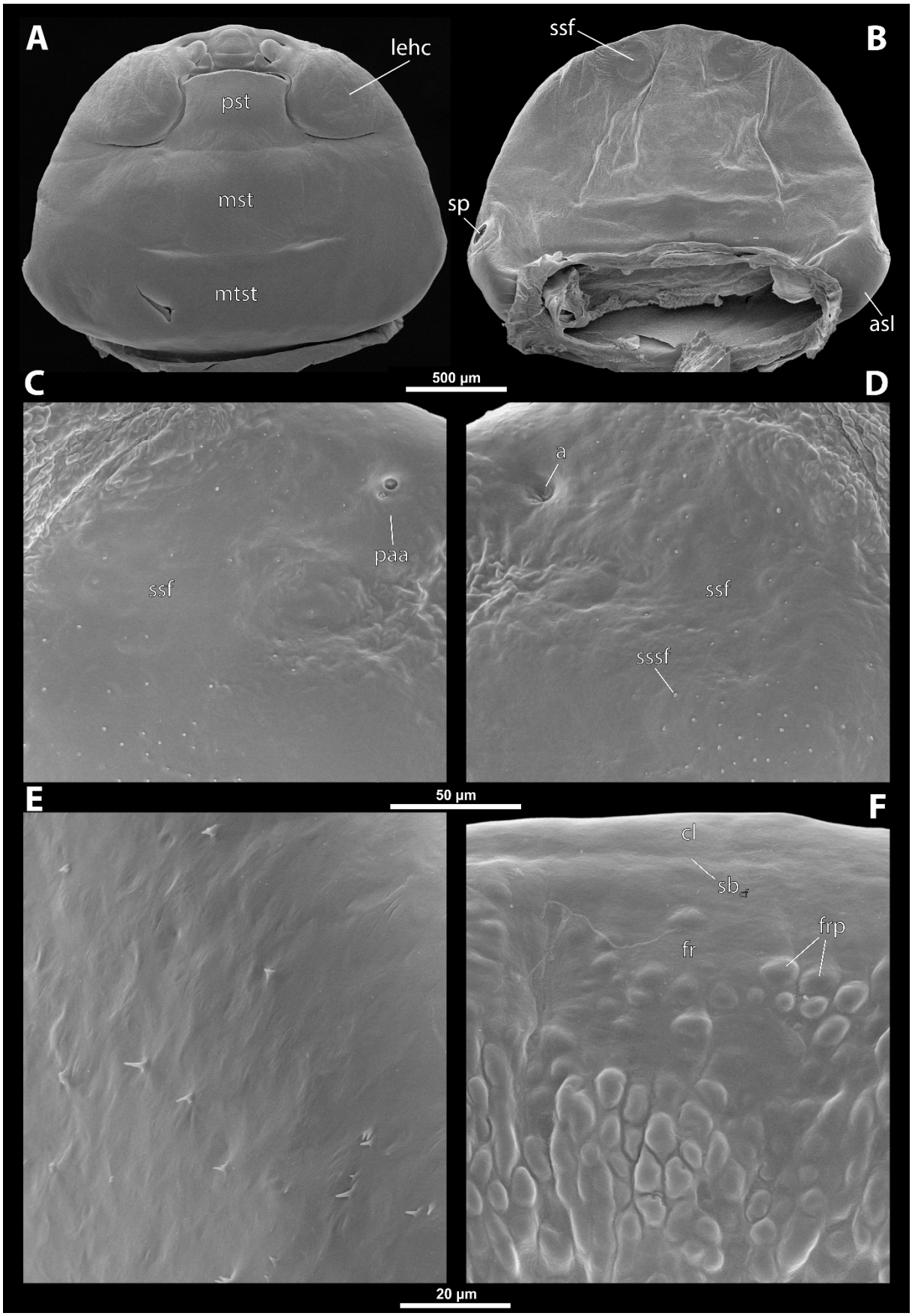

Fig. 5. *Paraxenos hungaricus* (Székessy, 1955), female, cephalothorax, SEM micrographs. **A**. Vental side. **B**. Dorsal side. **C**. Right vestigial antenna, dorsal side. **D**. Left vestigial antenna,

dorsal side. **E**. Left lateral border of abdominal segment I below spiracle, dorsal side. **F**. Detail of anterior border of cephalothorax, dorsal side. Abbreviations: a = vestigial antenna; asI = abdominal segment I; cl = clypeus; fr = frontal region; frons; frp = frontal papillae; lehc = lateral extension of head capsule; mst = mesosternum; mtst = metasternum; paa = periantennal area; pst = prosternum; sbcf = segmental border between clypeus and frons; sp = spiracle; ssf = supra-antennal sensillary field; sssf = sensillum of supra-antennal sensillary field.

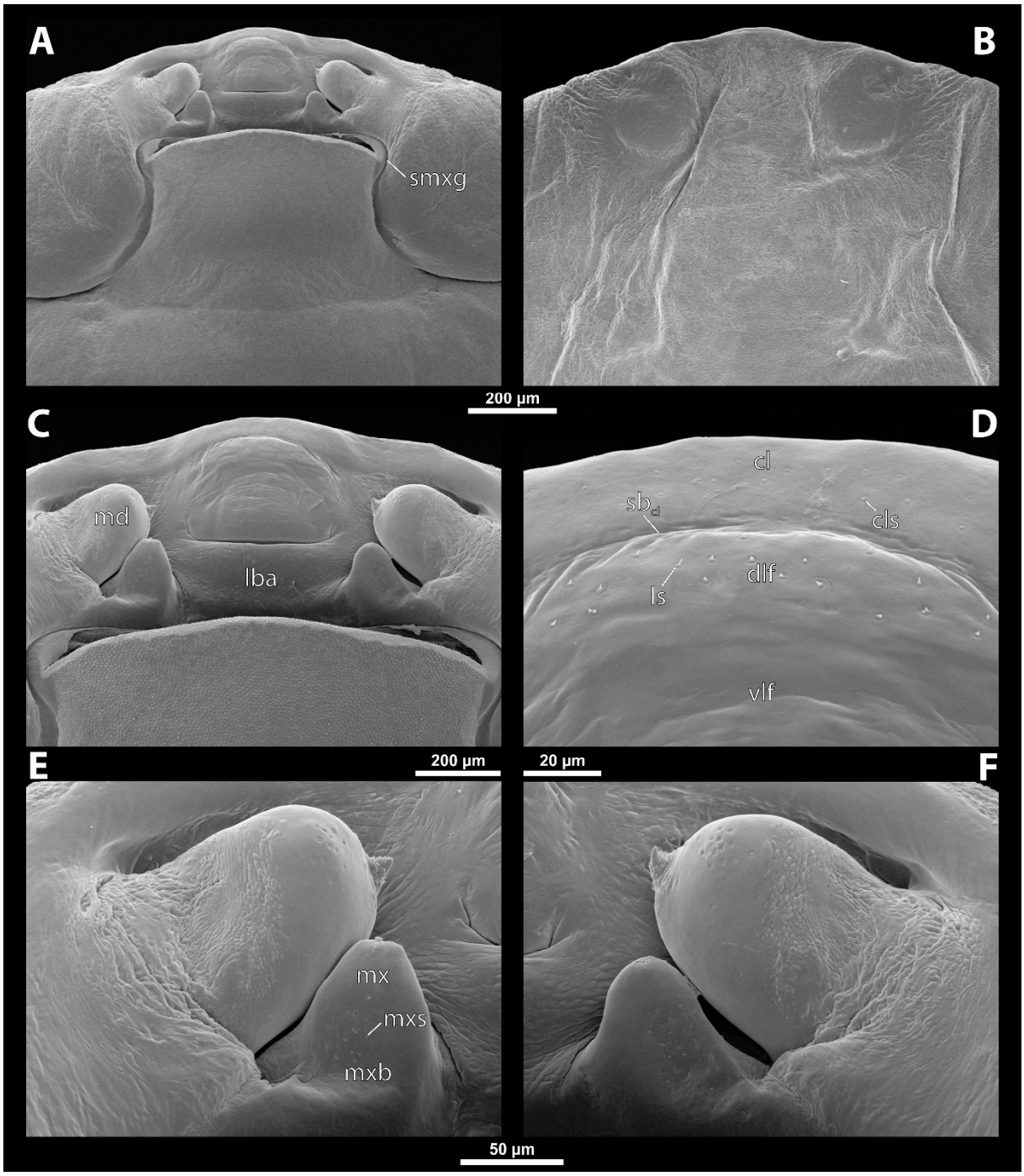

Fig. 6. *Paraxenos hungaricus* (Székessy, 1955), female, cephalothorax, SEM micrographs. **A**. Anterior part of cephalothorax, ventral side. **B**. Anterior part of cephalothorax, dorsal side. **C**. Mouthparts, ventral side. **D**. Detail of anterior border of cephalothorax, ventral side. **E**. Right mandible and maxilla, ventral side. **F**. Left mandible and maxilla, ventral side. Abbreviations: cl = clypeus; cls = clypeal sensillum; dlf = dorsal labral field of labral area; lba = labial area; ls = labral seta in cavity (spine-shaped sensillum); md = mandible; mx = vestige of maxilla; mxb = maxillary base; mxs = maxillary sensillum; sbcl = segmental border between clypeus and labrum; smxg = submaxillary groove; vlf = ventral labral field of labral area.

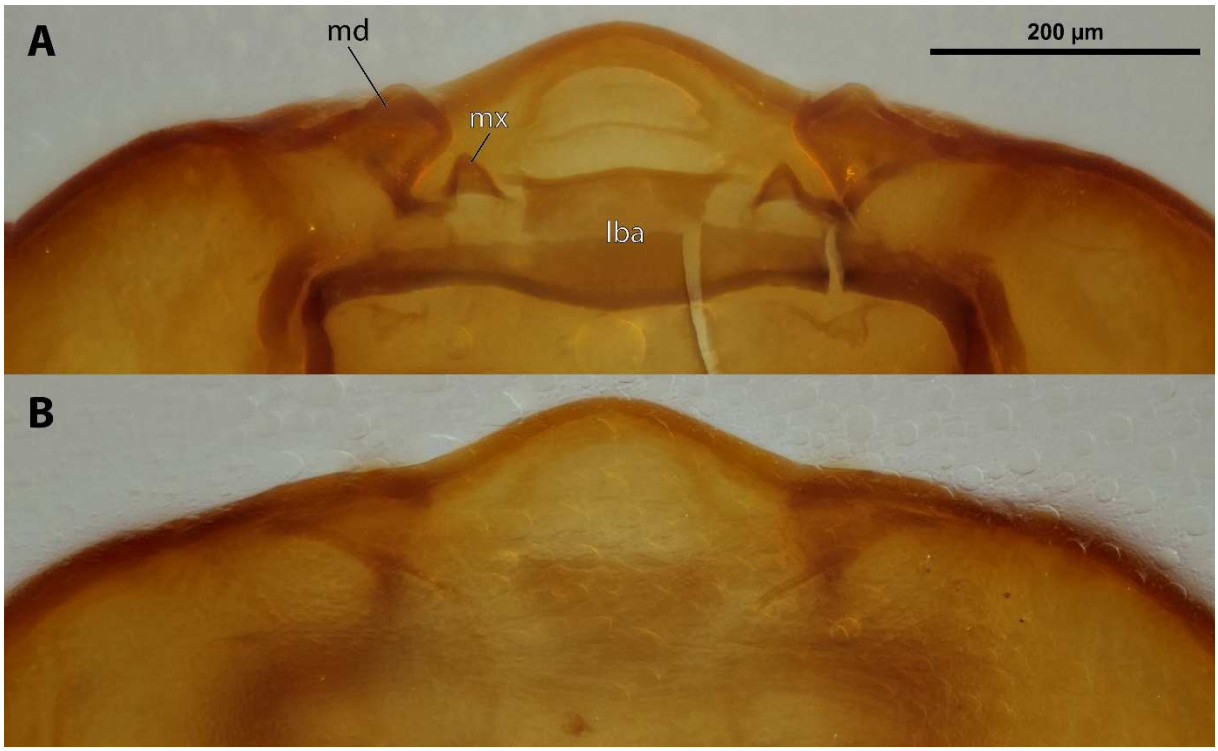

Fig. 7. *Paraxenos krombeini* Kifune & Hirashima, 1987, holotype, female, cephalothorax. **A**. Anterior part of cephalothorax, ventral side. **B**. Anterior part of cephalothorax, dorsal side. Abbreviations: lba = labial area; md = mandible; mx = vestige of maxilla.

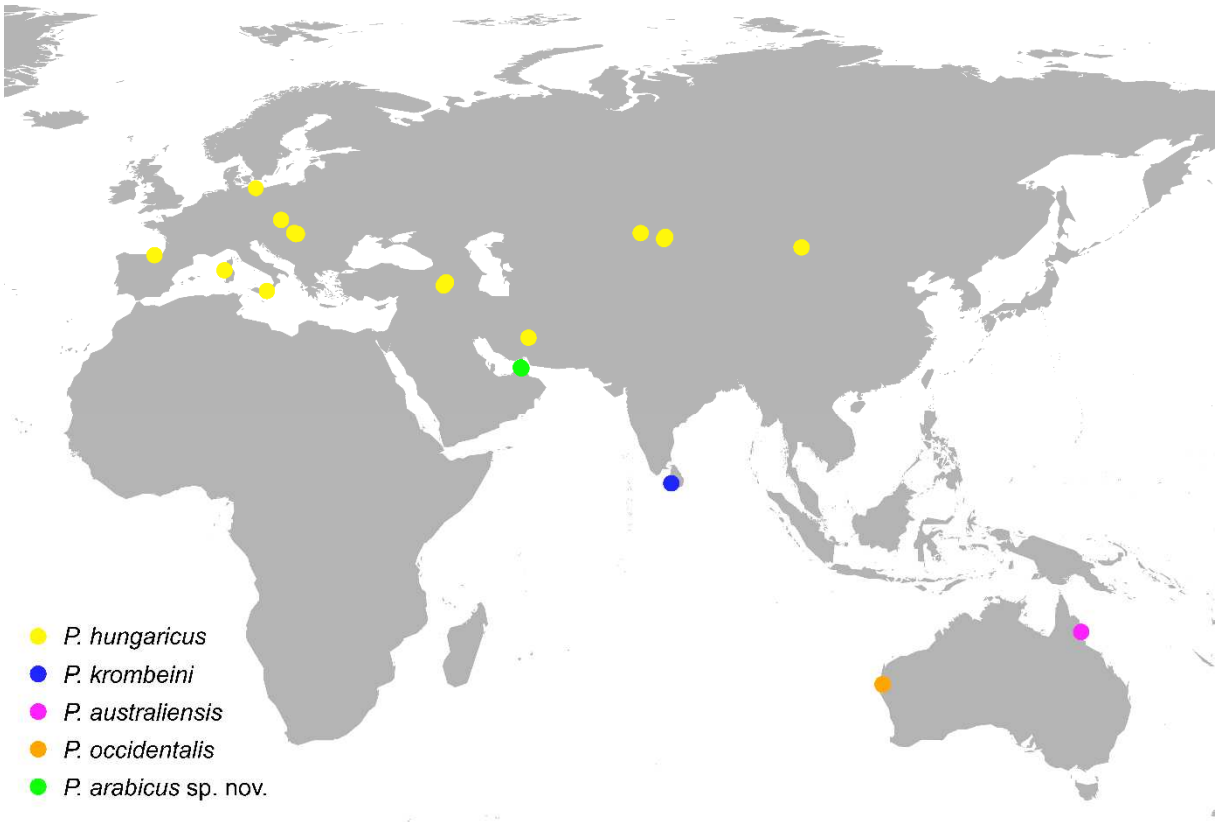

Fig. 8. Distribution of *Paraxenos* Saunders, 1872 species stylopized the host genus *Bembix* Fabricius, 1775. Distribution of each species is indicated by colored dots.

Table 1. Overview of 5 currently valid species of *Paraxenos* Saunders, 1872 stylopized the host genus *Bembix* Fabricius, 1775 with general information on their distribution and hosts.

Species	**Distribution**	**Hosts**
Paraxenos arabicus Benda & Straka sp. nov.	United Arab Emirates	*Bembix kohli* Morice, 1897
Paraxenos australiensis Kifune & Hirashima, 1987	Australia (Queensland)	*Bembix musca* Handlirsch, 1894
Paraxenos hungaricus (Székessy, 1955)	Palearctic	*Bembix oculata* Panzer, 1801; *Bembix rostrata* (Linnaeus, 1758); *Bembix sp*.
Paraxenos krombeini Kifune & Hirashima, 1987	Sri Lanka	*Bembix orientalis* Handlirsch, 1893
Paraxenos occidentalis Kifune & Hirashima, 1987	Australia (Western Australia)	*Bembix atrifrons* F. Smith, 1856