8. Prílohy

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A new species of *Chiroptella* Vercammen-Grandjean, 1960 (Acari: Trombiculidae) from diadem leaf-nosed bat *Hipposideros diadema* (Geoffroy) (Chiroptera: Hipposideridae) in Bali Island (Indonesia) with distribution records, hosts, and a key to the species of the genus.

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A new species of *Chiroptella* Vercammen-Grandjean, 1960 (Acari: Trombiculidae) from diadem leaf-nosed bat *Hipposideros diadema* (Geoffroy) (Chiroptera: Hipposideridae) in Bali Island (Indonesia) with distribution records, hosts, and a key to the species of the genus

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Abstract A new chigger mite *Chiroptella baliensis* n. sp. is described based on material from the diadem leaf-nosed bat *Hipposideros diadema* (Geoffroy) (Hipposideridae) in Bali Island (Lesser Sunda Islands, East Indonesia). With this newly described species, the genus *Chiroptella* includes 22 species, 13 in the subgenus *Chiroptella* Vercammen-Grandjean, 1960 (*sensu stricto*), and 9 in the subgenus *Neosomia* Vercammen-Grandjean, 1968. Species of *Chiroptella* are distributed throughout the Ethiopian region, the Oriental region, and the Australian region (and only marginally throughout the Palaearctic region) and are all associated with several families of bats. The paper also provides a key to all members of the genus and the new combinations for two species originally described as *Chiroptella*.

Introduction

The genus *Chiroptella* Vercammen-Grandjean, 1960 is one of the 19 genera of chigger mites known to parasitise bats (Yunker & Jones, 1961; Daniel & Stekol’nikov, 2003; Shatrov & Kudryashova, 2006; Kalúz & Ševčík, 2015). Vercammen-Grandjean (1960) erected the subgenus *Chiroptella* Vercammen-Grandjean, 1960 in the genus *Eltonella* Audy, 1956 with a type-species *Trombicula insolli* Philip & Traub, 1950. This taxonomic act was based on the following characteristics of the new subgenus *Chiroptella*: 6B or 5BS palpal tarsal formula; subtrapezoidal scutum; nude galeal seta; 3-pronged palpal claw; and 2 genualae on leg I. However, in his later work, Vercammen-Grandjean (1965a, b) proved that 6B or 5BS palpal tarsal formula was erroneous and corrected it for the subgenus *Chiroptella* to 7B and also transferred *Chiroptella* from *Eltonella* to *Leptotrombidium* Nagayo, Miyagawa, Mitamura & Imamura, 1916.

Based on his study of larval and nymphal characters, Nadchatram (1966) raised *Chiroptella* to full

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generic rank. He also transferred the subgenus *Neosomia* Vercammen-Grandjean & Nadchatram, 1965 from the genus *Riedlinia* Oudemans, 1914 to *Chiroptella* since *Neosomia* showed a greater affinity with the latter by sharing the following characters: palpal tarsus 7B or 7BS; 2 genualae I and III; femorala III; and ASB/PSB ratio 3.0. Vercammen-Grandjean & André (1966) transferred the subgenus *Lorillatum* Nadchatram, 1963 from the genus *Leptotrombidium* to *Chiroptella*. They also proposed a new subgenus *Oudemansidium* in the genus *Chiroptella* based on its palpal setal formula N/N/NNN/7BS, and the presence of only 1 genuala III. Vercammen-Grandjean & Langston (1971) raised *Lorillatum* to full generic rank, therefore, redefining *Chiroptella* as comprising of three subgenera: *Neosomia*, *Oudemansidium*, and the nominate subgenus. Subsequently, Vercammen-Grandjean & Langston (1976) proposed a fourth subgenus, *Willmannium*, characterised by nude galeala, and the absence of femorala III. Finally, Kudryashova (1991, 1992) revised two of these subgenera (*Oudemansidium* and *Willmannium* Vercammen-Grandjean & Langston, 1976) and raised them to full generic rank. Therefore, there are two subgenera (*Neosomia* and the nominate subgenus) currently recognised in the genus *Chiroptella* (see Brown, 1997, Brown et al., 2003).

In this paper, we present the description of a new species, *Chiroptella baliensis* from *Hipposideros diadema* (Geoffroy) in Bali Island, Indonesia. With this new species, the total number of *Chiroptella* species has risen to 22. We review all synonyms, hosts, and distribution records of all *Chiroptella* species. A key to all known species of the genus is also provided.

Materials and methods

All specimens of the new mite species (parasitic larvae) were collected from the diadem leaf-nosed bat *Hipposideros diadema* in Goa Peteng Cave (8°47’34.4”S, 115°08’14.6”E), in Jimbaran village, Bali Island, Indonesia. The bats were caught with a hand net during April 2018, February 2019, and March 2020. All body parts of live bats were inspected. The mites were collected using tweezers and were preserved in 90% ethanol. The bats were released unharmed after inspection.

The preparation of the new material was completed by M. Ševčík without any institutional support and by S. Kalúz from the Institute of Zoology, Slovak Academy of Sciences (Bratislava, Slovak Republic). The microscopic slides were prepared using SWAN’s embedding medium (Swan, 1936). All drawings were produced using standard light microscopy (Karl Zeiss Jena, Germany, Ergaval, equipped with a drawing tube) and enhanced with a computer program (GIMP2).

The standard terminology, abbreviations, and diagnostic formulae of the following authors that are generally accepted in trombiculid taxonomy were used: Vercammen-Grandjean & Langston (1976); Goff et al. (1982) and Kudryashova (1998). The taxonomy of bats follows Wilson & Mittermeier (2019).

All measurements (26 specimens measured unless otherwise specified) of chigger mites are provided as the range followed by the mean in parentheses. The holotype of the new species is deposited in the Slovak National Museum, Bratislava, Slovak Republic (SNM); the paratypes are deposited in SNM, in the private collection of the first author (CMŠ), and in the National Museum (Natural History), Prague, Czech Republic (NMP).

Family Trombiculidae Ewing, 1944

Subfamily Trombiculinae Ewing, 1944

Tribe Trombiculini Vercammen-Grandjean, 1960

Genus *Chiroptella* Vercammen-Grandjean, 1960 (*sensu lato*)

Remarks

Type-species: *Trombicula insolli* Philip & Traub, 1950

The subgenus *Chiroptella* Vercammen-Grandjean, 1960 (*sensu stricto*) is characterised by the following characters: SIF = 7BS.N.3.212(0)1(0). 1(0)1(0)2(0)1; fsp = 7.7.7; (ST, pST, PT’, PT”) = N; fPp = NN.NNN; fCx = 1.1.1; fSt = 2.2; fSc = PL > AM > AL; scutum subquadrate, punctate, with anterolateral shoulders and nearly straight posterior margin; SB situated anteriorly or in line with PL bases; sensilla filiform and sparsely barbed in distal half; mastisetae present or absent.

Chiroptella **(*****Chiroptella*****)** ***baliensis*** **n. sp.**

Type-host: *Hipposideros diadema* (Geoffroy) (Chiroptera: Hipposideridae), diadem leaf-nosed bat.

Type-locality: Goa Peteng cave (8°47'34.4"S, 115°08'14.6"E), Jimbaran village, Bali, Indonesia.

Type-material: Larval holotype (SNM SZ 10942) ex *Hipposideros diadema*, 5.iii.2020, collected by P. Šrámek; 25 larval paratypes (CMŠ, SNM SZ 10943, NMP P6A 7373–7378) ex 4 specimens of *H. diadema*, 26.iv.2018, 13.ii.2019 and 5.iii.2020, collected by P. Šrámek.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Chiroptella baliensis* n. sp. is urn:lsid:zoobank.org:act:A5C15C89-4CEB-41B7-B2EE-1420279D3CF7.

Etymology: The new species is named after the province (Bali, Indonesia) of the type-locality.

Description

Larva [Based on the holotype and 25 paratypes; measurements are given in Table 1; Figs. 1–4.] Dorsal aspect of idiosoma (Fig. 1A). Scutum nearly quadrate, posterior margin nearly straight with slight median concavity, bearing 1 AM, 1 pair of AL and 1 pair of PL setae, and 2 sensilla. Fine punctation covering most of the scutum. Paired oval AL windows present closed to bases of AL setae (see Fig. 3). Sensilla filiform with basal third nude, ciliated in second and apical thirds of sensilla; oval eyes (2 + 2) without ocular plates, anterior eyes laterally between AL and PL, posterior eyes at the level of PL (Fig. 3A). Subcuticular granulate structures present on the dorsal side laterally from scutum, more or less closed to scutum and eyes, visible in the majority of specimens studied (Fig. 3A).

Measurements of scutum given in Table 1. PW > AW = SD; ASB > SB > PSB. Two pairs (4H) of humeral setae 66–73 (69) long, dorsal setae (Fig. 3F) covered with thick barbs 53–72 (56) long, mostly arranged in regular rows.

Ventral aspect of idiosoma (Fig. 1B). Two pairs of sternal setae. Each coxa bearing 1 seta (Fig. 3D), ventral side of idiosoma with 38–42 setae (excluding

Table 1 *Chiroptella baliensis* n. sp. Measurements of holotype and paratypes

Variable	Sample size	Range	Mean ± SD	Holotype
AW	26	56–68	60 ± 3	58
PW	26	71–88	82 ± 4	71
SB	26	23–28	25 ± 1	23
ASB	26	39–49	44 ± 2	43
PSB	26	14–20	16 ± 1	15
SD	26	55–66	61 ± 3	58
AP	26	44–54	48 ± 3	45
AM	25	47–64	56 ± 4	63
AL	25	39–47	43 ± 3	45
PL	26	71–94	83 ± 4	88
S	4	75–83	80 ± 2	76
H	26	64–77	71 ± 3	75
DS	25	50–54	52 ± 2	50
VS	25	38–45	42 ± 2	40
NDV	25	84–99	92 ± 4	90
D_{min}	26	43–65	57 ± 5	55
D_{max}	26	60–75	67 ± 5	75
V_{min}	26	43–56	50 ± 3	53
V_{max}	26	49–66	59 ± 5	65
pa	26	355–445	417 ± 16	420
pm	26	337–375	354 ± 8	360
pp	26	375–425	403 ± 12	420
Ip	26	1,095–1,240	1,174 ± 30	1,200
TaIIIL	26	109–120	116 ± 3	115
TaIIIW	26	19–23	20 ± 1	19

Abbreviations: AW, distance between scutal anterolateral setae; PW, distance between scutal posterolateral setae; SB, distance between sensilla; ASB, distance from extreme anterior margin of scutum to the level of sensillary bases; PSB, distance from extreme posterior margin of scutum to the level of sensillary bases; SD, length of scutum (ASB + PSB); AP, distance from anterolateral to posterolateral scutal setae on one side; AM, length of scutal anteromedian seta; AL, length of anterolateral scutal setae; PL, length of posterolateral scutal setae; S, length of sensilla; H, length of humeral setae; DS, number of dorsal idiosomal and humeral setae; VS, number of ventral idiosomal setae (excluding coxal and sternal setae); NDV, number of idiosomal setae (DS + VS); Dmin, length of the shortest dorsal idiosomal seta; Dmax, length of the longest dorsal idiosomal seta; Vmin, length of the shortest ventral idiosomal seta; Vmax, length of the longest ventral idiosomal seta; pa, length of leg I (including coxa); pm, length of leg II (including coxa); pp, length of leg III (including coxa); Ip, total length of legs I-III; TaIIIL, length of leg tarsus III; TaIIIW, width of leg tarsus III; StD, standard deviation

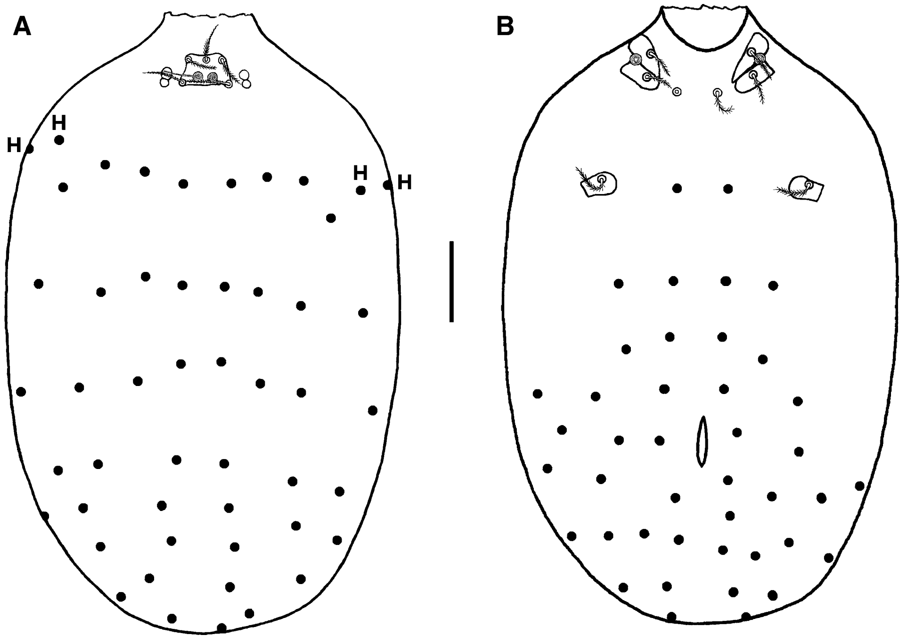

Fig. 1 *Chiroptella baliensis* n. sp., larva, holotype. A, Dorsal idiosoma; B, Ventral idiosoma. *Scale-bar*: 100 µm

sternal setae), setae covered with thick barbs (Fig. 3B, D, E), setae arranged in a mostly regular pattern. The length of ventral setae varies with proximal setae shorter, 47–53 (51) and caudal setae longer 56–64 (61).

Gnathosoma (Fig. 2). Galeala nude. Palps (including claw) 88–94 (92) long, palptibial claw slender, 28–32 (30) long, with 3 deeply indented prongs. Gnathobase (infracapitulum) with 1 pair of branched setae 34–39 (37) long, palpal femur, and genu each with 1 nude dorsal seta. Palpal tibia bears 2 nude dorsal setae and one nude ventro-lateral seta. Palpal tarsus bears 7 branched setae, nude subterminala, and a solenidion. Chelicerae missing in some specimens.

Legs (Fig. 4). Pretarsus, claws and normal empodia present. All setae on legs barbed, slender. Specialised setae on leg segments as follows. Leg I (pa): S_1 37–42 (39) f_1; (PT', ST, pST) = N; 2 tibialae (ta) 27–30 (29) and 29–31 (30), respectively, microtibiala (µta), 2 genualae (ga) 24–32 (29) and 24–28 (25); microgenuala (µga). Leg II (pm): S_2 22–28 (25) f_2; PT'' = N; 2 tibialae (tm) 29–30 (28) and 20–24 (23), respectively; genuala (gm) 22–27 (24). Leg III (pp): tibiala 23–29 (25); 2 genualae 37–41 (40) and 39–55 (48), respectively; femorala 37–55 (48). Number of normal barbed setae on leg segments as follows:

Leg I (pa): Cx (1) – Tr (1) – Bf (0) – Tf (5) – G (4) – Ti (8) – Ta (21);
Leg II (pm): Cx (1) – Tr (1) – Bf (0) – Tf (5) – G (3) – Ti (6) – Ta (16);
Leg III (pp): Cx (1) – Tr (1) – Bf (2) – Tf (3) – G (2) – Ti (6) – Ta (15);

Lengths of legs of holotype and paratypes (n = 26) are given in Table 1.

Diagnosis

SIF = 7BS.N.3.2121.0001; fsp = 7.7.7; fPp = NN.NNN; fCx = 1.1.1; fSt = 2.2; PL > AM > AL; fDS = 4H.8.8.6.6.4.4.2 = 50; VS = 38–42; NDV = 88–92; Ip = 1,121–1,240 (1,171).

Differential diagnosis

Chiroptella baliensis n. sp. is similar to *Chiroptella niehoffi* (Domrow, 1962) in having the same SIF = 7BS.N.3.2121.0001; fPp = NN.NNN; fCx = 1.1.1; fSt = 2.2 and numbers of dorsal and ventral setae but differs in the dorsal setal formula fDS = 4H.8.8.8.6.6.4.4.2 = 50 (*vs* fDS = 4H.10.10.8.+*c*.20). Other characters distinguishing these two species include in *Chiroptella baliensis* n. sp. the presence

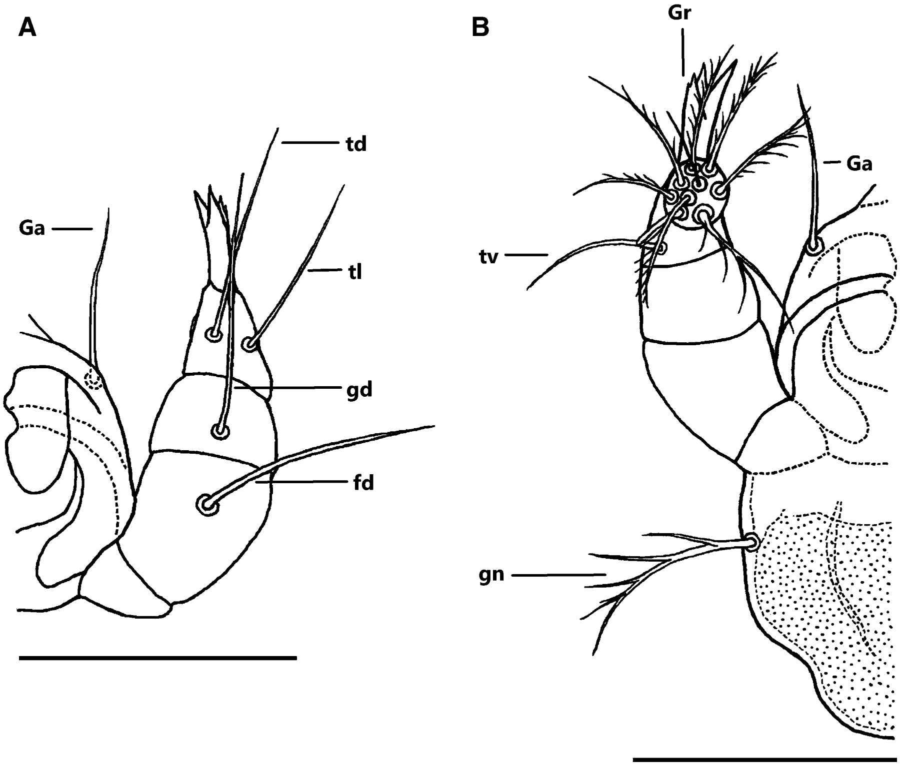

Fig. 2 *Chiroptella baliensis* n. sp., larva, paratype. A, Dorsal gnathosoma; B, Ventral gnathosoma. *Abbreviations*: Ga, galeal seta; fd, palpofemoral dorsal seta; gd, palpogenual dorsal seta; td, palpotibial dorsal seta; tl, palpotibial lateral seta;.gn, gnathobasal seta; tv, palpotibial ventral seta; Gr, palpotibial claw. *Scale-bar*s: 100 µm

of AL scutal windows; AM and AL setae bearing long barbs, PL with less long barbs, proximal third of sensillum nude, medial third with short ciliae, distal third with long ciliae, while in *Ch. niehoffi* AL scutal windows are absent; AM and PL setae bear very short barbs (AL with long barbs), proximal half of sensillum is nude, while distal half with long ciliae. Other *Chiroptella* spp. differ from *Chiroptella baliensis* n. sp. by different numbers of dorsal setae, different dorsal setal formula and by lengths of scutal and dorsal setae. More details are given in the key below.

Species previously included in the genus *Chiroptella*

Oudemansidium anhuiensis (Chen, Fan & Chen, 1980) n. comb.

Syns *Chiroptella* (*Oudemansidium*) *anhuiensis* Chen, Fan & Chen, 1980 of Lanza (1999); *Chiroptella anhuiensis* Chen, Fan & Chen, 1980 of Peng et al. (2015, 2016); Zajkowska et al. (2018)

Remark

Chen et al. (1980) state that *Chiroptella* (*Oudemansidium*) *anhuiensis* bears Ga = B, a typical taxonomic feature for the genus *Oudemansidium*. More comparative taxonomic details are provided by Kudryashova (1991; see below) and Kudryashova (1998).

Oudemansidium tanyei (Wen & Xiang, 1984) n. comb.

Syn. *Chiroptella* (*Oudemansidium*) *tanyei* Wen & Xiang, 1984

Remark

Wen & Xiang (1984) placed *Chiroptella* (*Oudemansidium*) *tanyei* within the subgenus *Oudemansidium* based on following diagnostic characters: telofemur III with 3B setae and 1N seta, Ga = B. The species is similar to *C.* (*O.*) *muscae* (syn. *Oudemansidium musca*), yet, it differs in following: (i) fDS = 36–40

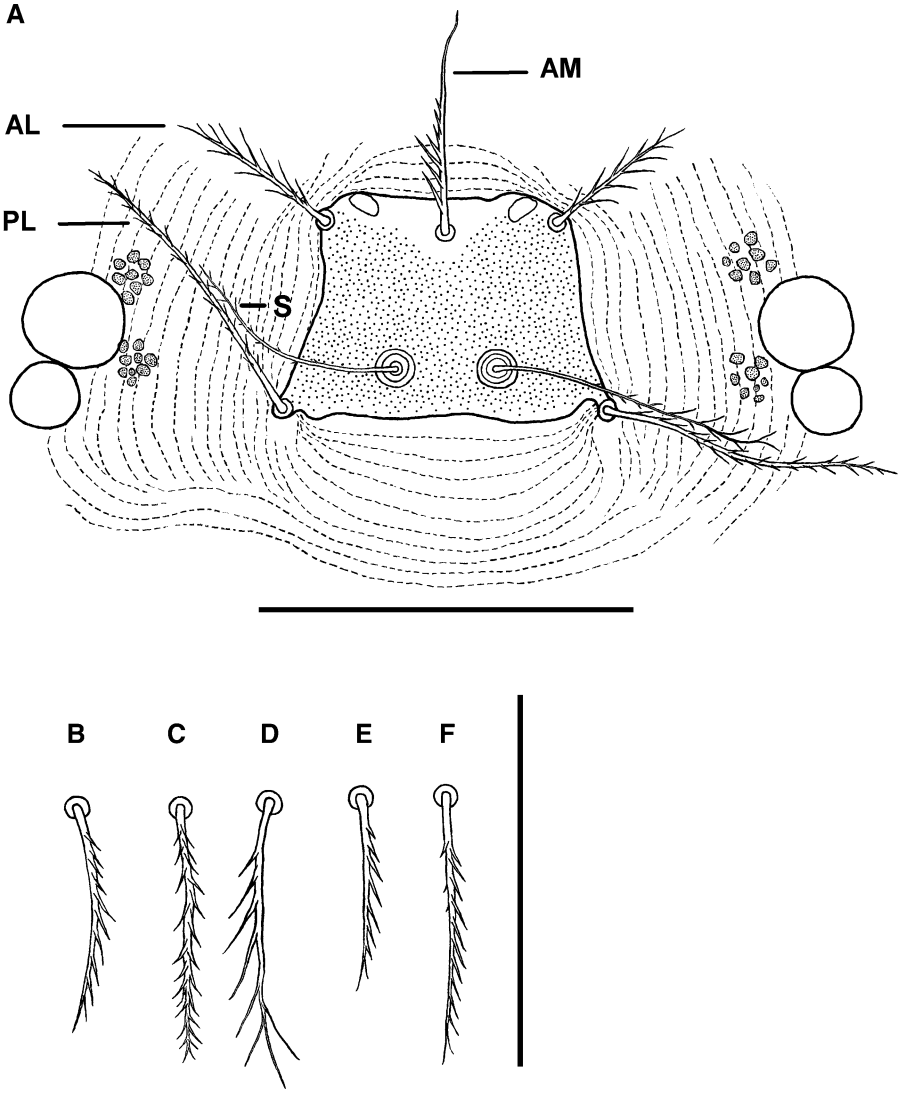

Fig. 3 *Chiroptella baliensis* n. sp., larva, holotype, and paratype. A, Scutum; B, Ventral seta; C, Humeral seta; D, Coxal seta; E, Sternal seta; F, Dorsal seta. *Abbreviations*: S, sensillum; AM, anteromedian scutal seta; AL, anterolateral scutal seta; PL, posterolateral scutal seta. *Scale bars*: 100 µm

(*vs* 40), fVS = 32–35 (*vs* 42), with arrangements being also different; (ii) AM ≥ PL > AL (*vs* PL > AM > AL); (iii) IP = 848 (*vs* 803); (iv) ScS, DS, PL slender, flat and broad-leafed shaped; (v) Chs hook-like in shape and curved upwards.

Kudryashova (1991) listed the following diagnostic characters of *Oudemansidium*: telofemur III with 3B setae and 1N femorala, Ga = B; while in *Chiroptella*: telofemur III with 3B setae and nude femorala, but Ga = N. Based on these characters both species belong to the valid genus *Oudemansidium* (Kudryashova, 1991).

Unidentified records of *Chiroptella*

Several records concerning the members of the genus *Chiroptella* from the Old World, without clearly mentioning the species names have been published: Congo (Adam & Vattier, 1965), Kirgizstan (Rybin et al. 1989), Ukraine (Bobkova, 2004) and Malaysia (Ahamad et al., 2013). These specimens were not physically available to us and require further research. The diagnostic characters of the genus *Chiroptella* were absent in these papers.

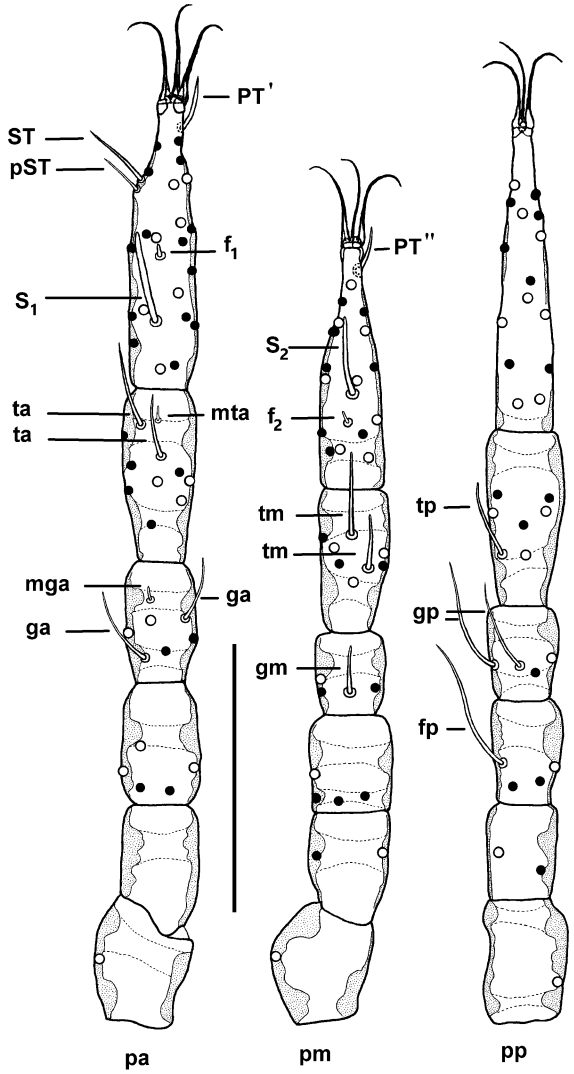

Fig. 4 *Chiroptella baliensis* n.sp., larva, paratype. Legs I-III. *Scale-bar*: 100 μm

Species inquirendae

Due to a different taxonomic delimitation of chigger genera in the past, several species were formerly included in the genus *Chiroptella*, e.g. *Ch. geikiensis* Goff, 1979. Goff (1979) included this species into the subgenus *Neosomia* based on the presence of 6BN.S on palpal tarsus. However, further detailed studies showed that species of *Neosomia* have tarsus with 7B, without tarsal subterminala (Chen et al., 1996, Brown, 1998, Brown et al., 2003). Another species, *Ch. heidemani* Brown, 1997, was known to have palpal tarsal formula 2N5BS and according to the author (Brown, 1997) this species was also included in the subgenus *Neosomia*. The taxonomic position of these two species need a more detailed study.

Chiroptella (*Ch.*) *daguana* Wen & Xiang, 1984 and *Ch.* (*Ch.*) *curvisetosa* Wang, 1995 appear to be nearly identical, differing only in two characters: pa = 439–489 and some idiosomal setae not curved in *Ch.* (*Ch.*) *daguana vs* pa = 359–411 and some idiosomal setae curved in *Ch.* (*Ch.*) *curvisetosa.* The type-material of these two species needs closer revision.

A key to larvae of the genus *Chiroptella*

1a Nude palpal subterminala absent (palpal tarsal formula: 7B or 6BN or 2N5B) (Subgenus *Neosomia* Vercammen-Grandjean, 1968)…13
1b Nude palpal subterminala always present (palpal formula: 7B.S) (Subgenus *Chiroptella* Vercammen-Grandjean, 1968)… 2
2a DS = 30–35; VS = 26, NDV = 56–61; fD = 2H.8.8.8.4.2.2 = 34; PW/SD = 1.91; PW/AP = 2.18…… *Ch.* (*Ch.*) *nocticola* Nadchatram, 1966
2b DS more than 39, NDV more than 69…........3
3a DS less than 49, NDV less then 85… 4
3b DS more than 49, NDV more than 86…..........7
4a SIF = 7BS.N.3.2100.1121; fD = 2H.8.8.9.6.2.4.4 = 43; AW = 45; PW = 78; AW/PW = 0.58…... *Ch.* (*Ch.*) *vavilovi* Kudryashova, 1975
4b SIF = 7BS.N.3.2121.0001 or SIF=7BS.N.2121.0000, AW/PW more than 0.7… 5
5a Nude mastifemorala on tarsus III absent; SIF = 7BS.N.2121.0000; fD = 2H.9.10.9.6.2.2 = 40; AW = 59; PW = 69; AW/PW = 0.86; SB = 24–25; Ip = 648….. ....*Ch.* (*Ch.*) *dianensis* (Wen & Xiang, 1984)
5b Nude mastifemorala on tarsus III present… ...6
6a SIF = 7BS.N.3.2121.0001; AW = 45, PW = 59, AW/PW = 0.76; SB = 19; Ip = 974….................. *Ch.* (*Ch.*) *hiregaudari* Fernandes & Kulkarni, 2003
6b SIF = 7BS.N.3.2121.0001; AW = 51, PW = 72, AW/PW = 0.71; SB = 29; Ip = 930….................. ....*Ch.* (*Ch.*) *bandupi* (Hiregaudar & Bal, 1956)
7a Two pairs of humerals (4H) ....…8
7b One pair of humerals (2H)...........................…9
8a fD = 4H.10.10.8+c.20, AL scutal windows absent; AM and PL setae with very short barbs (AL with long barbs), lateral para-scutal

subcuticular granulate structure absent……… ……………*Ch.* (*Ch.*) *niehoffi* (Domrow, 1962)

8b fD = 4H.8.8.8.6.6.4.4.2; AL scutal windows present; all scutal setae strongly barbed, lateral para-scutal subcuticular granulate structure mostly present……………………………… ………………*Chiroptella* (*Ch.*) *baliensis* n. sp.

9a AW < 70; scutal shoulders well developed…10

9b AW > 80, scutal shoulders very small or absent ………………………………………………11

10a fD = 2H.11.2.8.8.6.6.6.6.2; big shoulders (four times diameter of AL base), AW = 52; PW = 70; ASB/PSB = 3.67; PW/SD = 1.25; AL = 54… .. ……………………*Ch.* (*Ch.*) *adami* Taufflieb, 1972

10b fD = 2H.8.8.12.9.2.8.3.2; small shoulders (one diameter of AL base); AW = 64–69, PW = 78–102; ASB/PSB = 3.21; PW/SD = 1.51; AL = 43–52; AW/PW = 0.838, 0.854… ………………… …………*Ch.* (*Ch.*) *pipistrella* Chen & Hsu, 1963

11a pa < 420, pm < 360; fD = 2H.8.8.3.9.8.3.10.3.6.3 or 2H.9.10.9.3.10.4.7; pp = 426–458; Ip = 1198–1199; AW = 81–86; PW = 92–95; ASB = 41; PSB = 12–14; ASB/PSB = 3.0; AL = 46… ………………………………… ….*Ch.* (*Ch.*) *chrysentheum* Chen & Duan, 1996

11b pa > 430, pm > 370………………………………12

12a fD = 2H.(8.9).(10.4).8.2.8.6.3; pa = 439–489; pm = 374–415; pp = 425–465; Ip = 1234–1365; AW = 85–100; PW = 98–115; ASB = 38–40; PSB = 14–18; ASB/PSB = 2.6; AL = 53–55; dorsal and ventral setae not curved… ………….. …………*Ch.* (*Ch.*) *daguana* Wen & Xiang, 1984

12b fD = 2H.(8.9).11.11.10.7.2 = 60; pa = 359–411; pm = 363–406; pp = 411–449; Ip = 1182–1295; AW = 81–93; PW = 91–104; ASB = 39–43; PSB = 13–16; ASB/PSB = 2.5–3.0; AL = 51–64; some ventral and dorsal setae curved………… …………………*Ch.* (*Ch.*) *curvisetosa* Wang, 1995

13a Dorsal scutum with setae AM, AL and PL – normal scutum…………………………………………14

13b Dorsum with extrascutal PL setae – peniscutum ……………………………………………20

14a Palpal tarsus with all setae nude (7N); fD = 2H.8.8.8.6+(10–12); VS = 28–30; ASB = 40–42; PSB = 18–20; AW = 54–62; PW = 68–79………. *Ch.* (*N.*) *nudusetosa* Brown, 1998

14b Palpal tarsal formula with barbed setae ……15

15a Palpal tarsal formula 2N5BS; fD = 2H.8.8.8+(8–10); VS = 22–24; AL windows present; AW = 57–58; PW = 70–72; AP = 41–42; ASB = 36; PSB = 11–13………………… ……………………*Ch.* (*N.*) *heidemani* Brown, 1997

15b Palpal tarsal formula 7B ………………………16

16a fD = 2H.7.11.9.7.8.4.2; DS = 48; AW = 79; PW = 92; AM = 60; pa = 439; pm = 373; pp = 439; Ip = 1251…*Ch. (N.) megaseta* Chen & Duan, 1996

16b fD other… ……………………………………………17

17a fD = 2H.12.4.10.10.8+(8–14); very short scutum; VS = 46; NDV = 92; AW = 50; PW = 64; SD = 35; SB = 21; AP = 24; Ip = 644………… ………………………*Ch.* (*N.*) *revelae* (Audy, 1952)

17b fD = 2H.8.8.8.6.6 or 2H-8.8.8.8.6 …………18

18a fD = 2H.8.8.6.6.6=34; DS = 34; SD = 42–44; NDV = 68–70; PW = 64–65; AM = 28; PL = 55–56…*Ch.* (*N.*) *kanneliya* Brown, Udagama-Randeniya & Seneviratne, 2003

18b DS > 40; SD > 54; AM > 40; PW > 80……19

19a fD = 2H.8.8.8.6.6.4.4.2 or 2H.8.10.8.8.8.6.6.4.2; DS = 62; NDV = 98; PW = 69–70; SD = 59; AM = 42–45… ………………………………… ………… *Ch.* (*N.*) *insolli* (Philip & Traub, 1950)

19b fD = 2H.8.8.8.6.7.7.2; DS = 42; NDV = 80; PW = 82; SD = 62; AM = 55……………………………… ……………………… *Ch.* (*N.*) *piercei* (Ewing, 1931)

20a Palpal tarsus 7B; perirostral collar present; AM < AL…*Ch.* (*N.*) *audyi* (Vercammen-Grandjean & Nadchatram, 1965)

20b Palpal tarsus 6BN.S; perirostral collar absent; AM > AL………*Ch.* (*N.*) *geikiensis* Goff, 1979

Discussion

Twenty-two species of the genus *Chiroptella* are known today with 13 belonging to the nominate subgenus and 9 to the subgenus *Neosomia* (see the key and Table 2). These species can be found in the tropics of the Ethiopian (Senegal), Oriental (India, Sri Lanka, Vietnam, Thailand, Laos, Malaysia, Indonesia, Philippines) and Australian (Papua New Guinea, Solomon Islands, Australia) zoogeographic regions, only marginally in the Palaearctic (Iran, China) region. Except for 3 random records from other small mammals [*Ch. insolli* (Philip & Traub, 1950) from *Rattus rattus* (Linnaeus) (Brown, 1991); *Ch. nocticola* Nadchatram, 1966 and *Ch. revelae* (Audy, 1952) from *Mus pahari* Thomas (Gould et al., 1967; see Table 2)], all species

Table 2 Checklist of *Chiroptella* spp. with host and distribution data

Species	Synonyms	Hosts	Host family	Country (Continent)	Zoogeographical region	Source
Subgenus *Chiroptella* Vercammen-Grandjean, 1960						
Ch. adami Taufflieb, 1972		*Lissonycteris angolensis smithii* Thomas	Pteropodidae	Senegal (Africa)	Ethiopian	Taufflieb (1972)
Ch. baliensis n. sp.		*Hipposideros diadema* (Geoffroy)	Hipposideridae	Indonesia (Asia and Australia)	Oriental	This study
Ch. bandupi (Hiregaudar & Bal, 1956)	*Trombicula bandupi* Hiregaudar & Bal, 1956 of Mulyarskaya (1970); *Leptotrombidium* (*Chiroptella*) *bandupi* (Hiregaudar & Bal, 1956) of Vercammen-Grandjean (1965a)	*Rhinolophus rouxii* Temminck	Rhinolophidae	India (Asia)	Oriental	Hiregaudar & Bal (1956)
Ch. chrysentheum Chen & Duan, 1996		*Rhinolophus ferrumequinum* (Schreber)	Rhinolophidae	China (Asia)	Palaearctic	Chen et al. (1996)
Ch. curvisetosa Wang, 1995		*Myotis* sp.	Vespertilionidae	China (Asia)	Palaearctic	Wang (1995)
		Miniopterus sp.	Miniopteridae			
Ch. daguana (Wen & Xiang, 1984)		Chiroptera fam. gen. sp.		China (Asia)	Palaearctic	Wen & Xiang (1984)
Ch. dianensis (Wen & Xiang, 1984)		Chiroptera fam. gen. sp.		China (Asia)	Palaearctic	Wen & Xiang (1984)
Ch. giga (Schluger, Grokhovskaya, Dan & Nguyen, 1959)	*Trombicula giga* Schluger, Grokhovskaya, Dan & Nguyen, 1959 of Mulyarskaya (1970); *Leptotrombidium* (*Chiroptella*) *giga* (Schluger, Grokhovskaya, Dan & Nguyen, 1959) of Vercammen-Grandjean (1965a)	*Hipposideros armiger* (Hodgson)	Hipposideridae	Vietnam (Asia)	Oriental	Schluger et al. (1959)
Ch. hiregaudari Fernandes & Kulkarni, 2003		*Rhinopoma hardwickii* Gray	Rhinopomatidae	India (Asia)	Oriental	Fernandes & Kulkarni (2003)

Table 2 continued

Species	Synonyms	Hosts	Host family	Country (Continent)	Zoogeographical region	Source
Ch. niehoffi (Domrow, 1962)	*Trombicula niehoffi* Domrow, 1962; *Leptotrombidium* (*Chiroptella*) *niehoffi* (Domrow, 1962) of Vercammen-Grandjean (1965a)	*Hipposideros* spp.	Hipposideridae	Indonesia (Asia and Australia)	Oriental	Domrow (1962)
Ch. nocticola Nadchatram, 1966		*Hipposideros diadema* (Geoffroy)	Hipposideridae	Papua New Guinea (Australia)	Australian	Nadchatram (1966)
		Hipposideros spp. Gray	Hipposideridae	Solomon Islands (Australia); Thailand (Asia)	Australian	Nadchatram (1966); Gould et al. (1967)
		Emballonura raffrayana Dobson	Emballonuridae	Solomon Islands (Australia)	Australian	Nadchatram (1966)
		Emballonura sp. Temminck	Emballonuridae	Papua New Guinea (Australia)	Australian	Nadchatram (1966)
		Myotis adversus (Horsfield)	Vespertilionidae	Thailand (Asia)	Oriental	Gould et al. (1967)
		Chiroptera fam. gen. sp.		Solomon Islands (Australia)	Australian	Nadchatram (1966)
		Mus pahari Thomas	Muridae	Thailand (Asia)		Gould et al. (1967)
Ch. pipistrella (Chen & Hsu, 1963)	*Trombicula* (*Leptotrombidium*) *pipistrella* Chen & Hsu, 1963; *Chiroptella* (*Chiroptella*) *sandoshami* Nadchatram, 1966 of Nadchatram (1970); Gould et al. (1967); Anciaux de Faveaux (1985)	*Rhinolophus ferrumequinum* (Schreber)	Rhinolophidae	Thailand (Asia)	Oriental	Chen (1975)
		Hipposideros armiger (Hodgson)	Hipposideridae	China (Asia)	Oriental	Chen & Hsu (1963)
		Hipposideros diadema (Geoffroy)	Hipposideridae	Malaysia (Asia)	Oriental	Nadchatram (1966)
		Hipposideros larvatus (Horsfield)	Hipposideridae	Laos (Asia)	Oriental	Gould et al. (1967)
		Hipposideros pomona Andersen	Hipposideridae	Malaysia (Asia)	Oriental	Nadchatram (1966)
		Hipposideros sp.	Hipposideridae	Thailand (Asia)	Oriental	Nadchatram (1966)
Ch. vavilovi Kudryashova, 1975		*Rhinopoma hardwickii* Gray	Rhinopomatidae	Iran (Asia)	Palaearctic	Kudryashova (1975)
Subgenus *Neosomia* Vercammen-Grandjean & Nadchatram, 1965						

Table 2 continued

Species	Synonyms	Hosts	Host family	Country (Continent)	Zoogeographical region	Source
Ch. audyi (Vercammen-Grandjean & Nadchatram, 1965)	*Riedlinia* (*Neosomia*) *audyi* Vercammen-Grandjean & Nadchatram, 1965	*Hipposideros* sp.	Hipposideridae	Indonesia (Asia and Australia)	Oriental	Vercammen-Grandjean & Nadchatram (1965)
Ch. geikiensis Goff, 1979		*Rhinonicteris aurantia* (Gray)	Hipposideridae	Australia (Australia)	Australian	Goff (1979)
Ch. heidemani Brown, 1997		*Rhinolophus inops* Andersen	Rhinolophidae	Philippines (Asia)	Oriental	Brown (1997)
Ch. insolli (Philip & Traub, 1950)	*Trombicula insolli* Philip & Traub, 1950 of Audy (1952); Gunther (1952); Womersley (1952); *Trombicula* (*Trombicula*) *insolli* Philip & Traub, 1950 of Wharton & Fuller (1952); Audy (1954); Womersley & Audy (1957); *Eltonella* (*Chiroptella*) *insolli* (Philip & Traub, 1950) of Vercammen-Grandjean (1960) *Leptotrombidium* (*Chiroptella*) *insolli* (Philip & Traub, 1950) of Vercammen-Grandjean (1965a)	*Eonycteris spelaea* (Dobson)	Pteropodidae	Malaysia (Asia)	Oriental	Philip & Traub (1950)
		Hipposideros diadema (Geoffroy)	Hipposideridae	Philippines (Asia)	Oriental	Brown & Goff (1988)
		Taphozous melanopogon Temminck	Emballonuridae	Malaysia (Asia)	Oriental	Audy (1952)
		Rattus rattus (Linnaeus)	Muridae	Philippines (Asia)	Oriental	Brown & Goff (1988)
Ch. kanneliya Brown, Udagama-Randeniya & Seneviratne, 2003		*Hipposideros speoris* (Schneider)	Hipposideridae	Sri Lanka (Asia)	Oriental	Brown et al. (2003)
Ch. megaseta Chen & Duan, 1996		*Rhinolophus ferrumequinum* (Schreber)	Rhinolophidae	China (Asia)	Palaearctic	Chen & Duan (1996)
Ch. nudusetosa Brown, 1998		Chiroptera fam. gen. sp.		Philippines (Asia)	Oriental	Brown (1998)

Table 2 continued

Species	Synonyms	Hosts	Host family	Country (Continent)	Zoogeographical region	Source
Ch. piercei (Ewing, 1931)	*Trombicula piercei* Ewing, 1931 of Ewing (1937); Radford (1942); Michener (1946); Philip & Woodward (1946); Fuller (1948, 1952); Philip & Traub (1950); Womersley (1952); Gunther (1952); *Trombicula* (*Trombicula*) *piercei* Ewing, 1931 of Thor & Willmann (1947); Wharton & Fuller (1952); Audy (1954); Womersley & Audy (1957); *Leptotrombidium* (*Chiroptella*) *piercei* (Ewing, 1931) of Vercammen-Grandjean (1965a)	*Hipposideros diadema* (Geoffroy)	Hipposideridae	Philippines (Asia)	Oriental	Ewing (1931)
Ch. revelae (Audy, 1952)	*Trombicula revelae* Audy, 1952; *Leptotrombidium* (*Chiroptella*) *revelae* (Audy, 1952) of Vercammen-Grandjean (1965a)	*Hipposideros* sp.	Hipposideridae	Malaysia (Asia)	Oriental	Audy (1952); Gould et al. (1967)
		Mus pahari Thomas	Muridae	Thailand (Asia)	Oriental	Gould et al. (1967)

were found exclusively on bats and even in the past, this genus had been considered to be restricted to this group of vertebrates (Shatrov & Kudryashova, 2006). The new species only confirms this fact.

Despite the lack of significant data set, the majority of hosts recorded belong to genus *Hipposideros* Gray (Hipposideridae) and *Rhinolophus* Lacépède (Rhinolophidae) both belonging to superfamily Rhinolophoidea, suborder Yinpterochiroptera (Table 2). This fact supports the idea that some chigger mites genera might be tightly dependent on a specific family of bats (Kalúz & Ševčík, 2015).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national, and international guidelines for the care and use of animals were followed.

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8.2. Kalúz S., Ševčík M. 2014:

A new species of the genus *Grandjeana* (Koçak & Kemal, 2009) (Acari: Trombiculidae) from Mauritanian bat with a key to species of the genus.

International Journal of Acarology 40: 31–36.

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A new species of the genus *Grandjeana* (Koçak & Kemal, 2009) (Acari: Trombiculidae) from Mauritanian bat with a key to species of the genus

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The genus *Diplectria* (Vercammen-Grandjean 1967) nec Enderlain, 1939 in family Trombiculidae is a junior homonym of the genus *Diplectria* Enderlein 1939 in dipteran. On the basis of homonymy was proposed the replacement name *Grandjeana* Koçak & Kemal, 2009 and *Diplectria* was replaced by a new subgeneric name *Grandjeana* given by them. In the present article, the authors promote it to a full genus status. A new species *Grandjeana mauritanica* Kalúz & Ševčík from type host *Rhinopoma cystops* Thomas, 1903 (Mammalia: Chiroptera: Rhinopomatidae), from Mauritania (North-West Africa) is reported, and a key with a new combinations of names to the known species of this genus is given.

http://zoobank.org/urn:lsid:zoobank.org:pub:59F61AC1-C833-4DDE-9A6C-543C24202E76

Keywords: *Grandjeana*; taxonomy; replacement name; species novum; key; bat host

Introduction

The important feature characterizing the genus *Diplectria* (Vercammen-Grandjean 1967) in Trombiculidae is the presence of paired pretarsalae on tarsus I. These chigger mites parasitizing bats occur from Africa to South-East Asia, when the first chigger with a pair of pretarsalae I (*Trombicula taphozous*) was described by Womersley (1952) from Malaya (now Malaysia). Later, other representatives with this feature were described from Malaya and Thailand (Domrow 1962). After the description of fourth species occurring in South Africa (Vercammen-Grandjean and Nadchatram 1963) the new subgenus *Diplectria* within the genus *Trombicula* Berlese, 1905 was erected by Vercammen-Grandjean (1967). The full generic status *Diplectria* was promoted by Vercammen-Grandjean (1971) and the species *Trombicula (Trombicula) reticulata* (Vercammen-Grandjean & Nadchatram 1963) was designated as a type species. During the study of this genus the descriptions of another two species were added from South China by Zhao and Qiu (1979) and Wen and Xiang (1984). The last two papers bringing the descriptions of a new species of the genus *Diplectria* comes from Philippines. Wen and Corpuz-Raros (1997) described another new species (*D. asiatica*) and Brown (1997) brought the description of a new species (*D. manjuyodensis*). So far eight species were known from this genus until 2009. However, Koçak and Kemal (2009) found out, that the subgeneric name *Diplectria* of Vercammen-Grandjean was already used in dipteran since 1939 by Enderlein, and a nomen novum *Grandjeana* replaced for the junior synonym *Diplectria* Vercammen-Grandjean by them. There is no doubt on the reasons for generic status of *Grandjeana* status novus we proposed herewith. We also present the description of a new species *G. mauritanica* **sp. nov.**, together with the previous knowledge on this proposed genus, a key to the known species of this genus is also given.

Materials and methods

The newly described chigger mite was found on bats *Rhinopoma cystops* Thomas, 1903 (det. P. Benda, National Museum, Prague) captured in an abandoned house (details see below in definition of material examined). Hosts were caught using mist-nets and mites were collected from the ears by entomological tweezers and preserved in ethylalcohol. Specimens were mounted onto slides using Swann's medium, microscopically identified and described. All drawings were produced by using standard light microscopy and enhanced with computer software (GIMP 2). Diagnostic features and measurements were made with a standardized microscopy ocular micrometre, and in the text are stated in micrometres (μm). The measurements, other symbols and abbreviations follow Vercammen-Grandjean (1968), Vercammen-Grandjean and Langston (1976) and Kudryashova (1998). The scale bars for all figures are 100 μm. A key to the known species of the genus *Grandjeana* was also elaborated.

Results and discussion

Genus ***Grandjeana*** (Koçak & Kemal 2009)

Taxonomy

Diagnosis

Type species: *Trombicula (Trombicula) reticulata* Vercammen-Grandjean & Nadchatram, 1963

The genus *Grandjeana* is characterized by having: SIF = 5B-B(N)-3-3111-0(1)000 and flagelliform sensilla. Scutum trapezoidal, with coarse punctate prominent shoulders and with protruded earlike postero-lateral angles. AM, AL and PL setae on scutum. Two pairs of eyes rounded, with anterior lens much larger than posterior. All legs slender, seven segmented (fSP = 7.7.7), coxae narrow (fCx = 1.1.1). Tarsus I with S_1 and f_1, paired pretarsalae (PT′ = N) which are missing in related genera; ST, pST absent. Pretarsala on tarsus II present or absent, mastitarsala present or absent, the latter sometimes with proximal ciliation. Solenidia on legs long (20–44) and slender. Genualae III (23–81) and tibialae III (16–77), in African species longer and resembling mastisetae, IP = 795–1164. Distribution in Africa, South-East Asia, hosts bats.

Grandjeana mauritanica **Kalúz & Ševčík, sp. nov.**

(Figures 1–6).

Diagnosis

SIF = 5B-N-3-3111.0000; fPp = B-B-N.N.B; nude galeala, each coxa with a single barbed seta and two pairs of sternal setae; DS = 40(39); VS = 42; NDV = 82(81); fD = 2H-

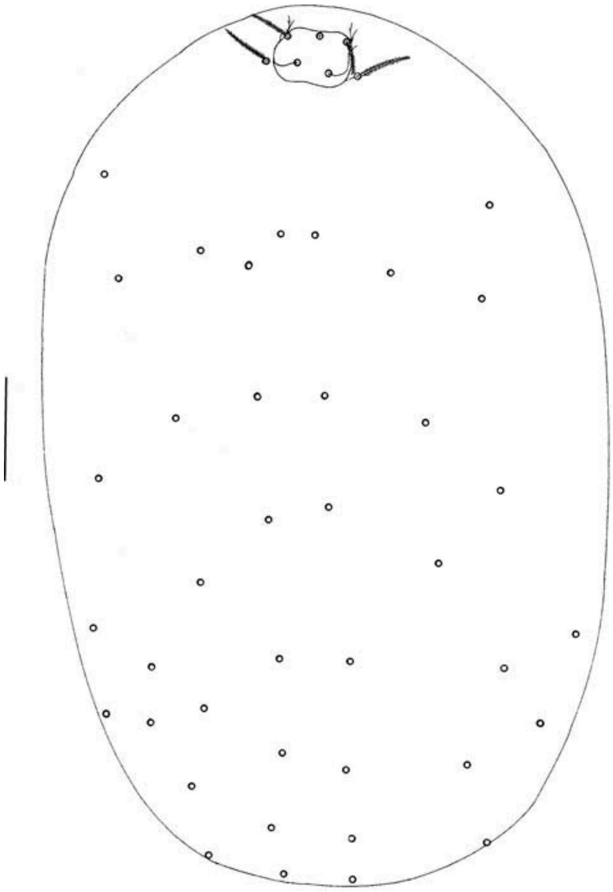

Figure 1. *Grandjeana mauritanica* **sp. nov.** (holotype) – idiosoma dorsal. Scale bar: 100 µm.

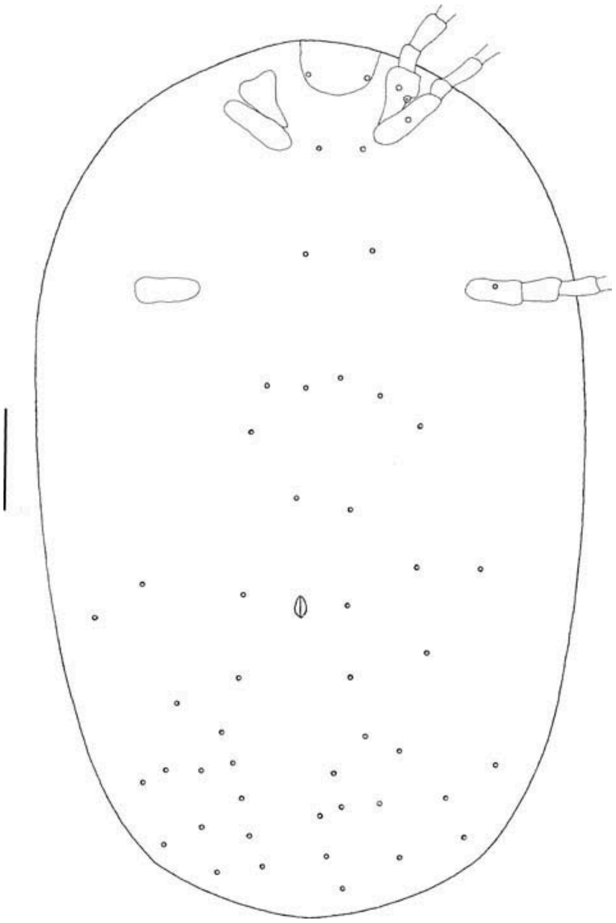

Figure 2. *Grandjeana mauritanica* **sp. nov.** (holotype) – idiosoma ventral. Scale bar: 100 µm.

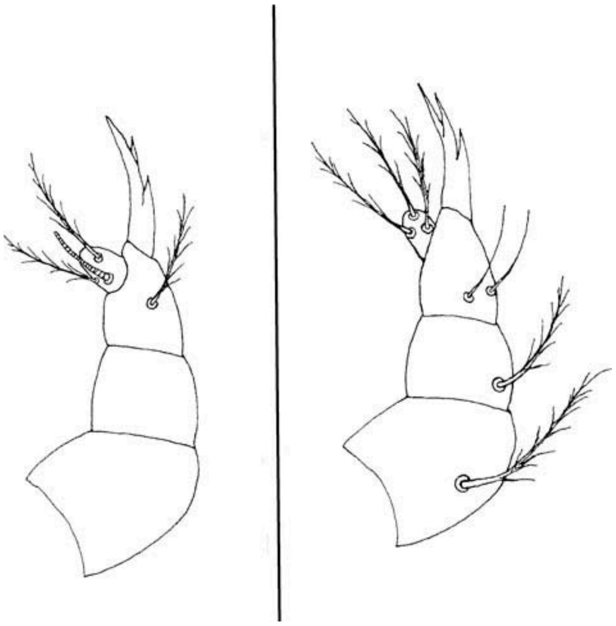

Figure 3. *Grandjeana mauritanica* **sp. nov.** (holotype) – gnathosoma (left – ventral, right – dorsal). Scale bar: 100 µm.

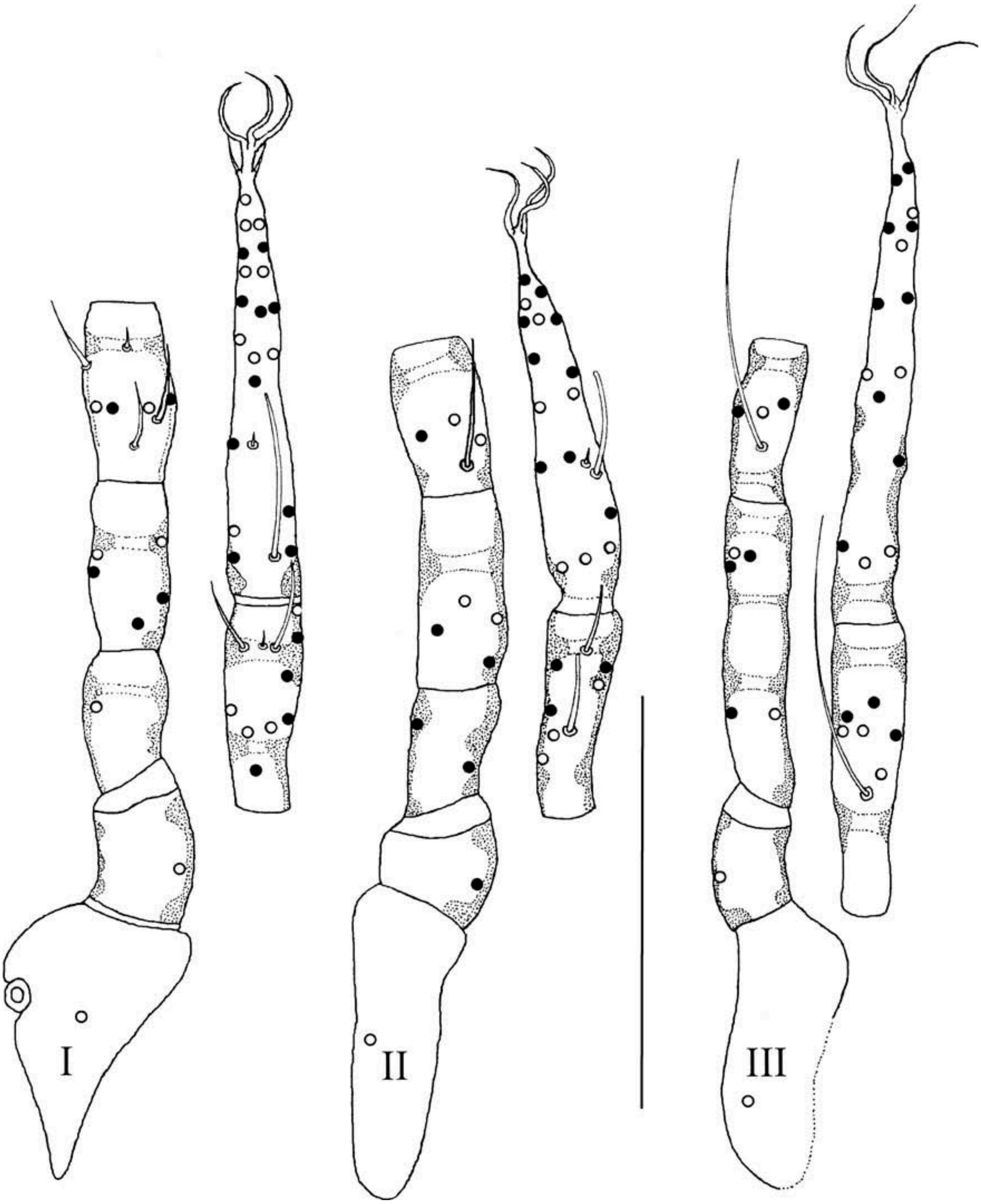

Figure 4. *Grandjeana mauritanica* **sp. nov.** (holotype) – legs I–III (dots – bases of setae; full dots – upper side; empty dots – bottom side. Numbers – lengths of solenidia and famuli. Scale bar: 100 μm.

6.6.6.6.4.4(3).4.2 = 40(39), very long genuala III (81) and tibiala III (77). This new species is the most similar to *Grandjeana koongi* (Domrow 1962). The main differences are: *G. mauritanica* **sp. nov.** has fPp = B-B-N.N.B, ASB = 39, wider distance between bases of sensilla (SB = 26–28) and longer scutal setae while *G. koongi* differs from the above-mentioned species by fPp = B-B-B.N.B, ASB = 25, shorter distance between bases of sensilla (SB = 16) and scutal setae (AM, AL, PL) are much more shorter. The differences between *G. mauritanica* **sp. nov.** and other known species of this genus are presented both in the Table 1 and a key below.

Description

Larva – measurements: mean (minimal–maximal); (holotype and paratypes; *n* = 4)

SIF = 5B-N-3-3111.0000; fsp = 7.7.7; fPp = B-B-N.N.B; Ga = N; fCx = 1.1.1; fSt = 2.2; DS = 40(39); VS = 42; NDV = 82(81); fD = 2H-6.6.6.6.4.4(3).4.2 = 40(39).

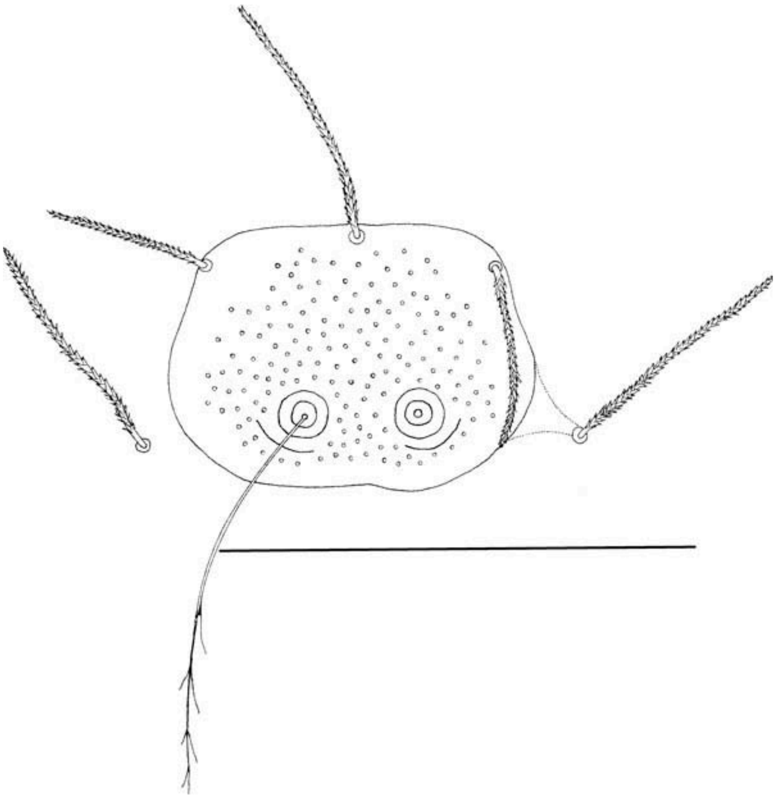

Figure 5. *Grandjeana mauritanica* **sp. nov.** (holotype) – scutum. Scale bar: 100 µm.

Idiosoma (Figures 1 and 2). Scutum trapezoidal, posterior margin semi-rounded with concave median depression, slightly wider than long, with AL, AM, PL setae and two sensilla. Small anterolateral scutal shoulders typical for the genus not developed laterally, position of AL setae laterally marginal. In some specimens scutum partially reduced (semi-peniscutum from left or right side). In that case the PL setae are situated on soft integument and

Table 1. Comparison of nine known species of the genus *Grandjeana*. Data from the paper of Wen and Corpuz-Raros (1997) used and adapted, two other species added.

	fPp	Ga	fD	DS	VS	NDV	IP
asiatica	B.B.BBB	N	2 + 9.8.8.6.6(4.5.4) =	54	52	106	795
calva	B.B.bbB	N	2 + 6.6.2.6.4.2 =	32	34	64	?
koongi	B.B.bNb	n/b	2 + 6.6.6.6.6.6.2 =	42	40	82	?
manjuyodensis	B.B.NBB, B.B.BNB	N	2 + 10.10.6.(28–30) =	54–56	44–47	102–110	637–698
mauritanica	B.B.NNB	N	2 + 6.6.6.6.4.4(3).4.2 =	39–40	42	81–82	1145
reticulata	B.B.BBB	b	2 + (2.6).(2.6).8.6.6.4.2 =	64	46	110	?
sinensis	B.B.BNB	N	2 + 12.12.10.8...(6–5) =	58	65	123	800
taphozous	N.N.NNN	N	2 + 10.10.10.10.8.6.4 =	50+	70	120+	846
wenquana	B.B.BBB	N	2 + 6.6.6.6.4.4 =	46	34	80	875

	AW	PW	SB	ASB	PSB	AP	AM	AL	PL	Sn	HS	DS	DS
asiatica	57	85	19	34	14	35	33	34	45	42+	37	32/25	23/31
calva	54	74	18	36	14	39	31	30	39	52	34	31/26	29
koongi	57	68	16	25	12	27	32	30	36	55	34	50/23	18–23
manjuyodensis	56	76	17	24	14	31	29	31	41	58	33–38	36/25	
mauritanica	58	86	27	39	17	40	49	36	49	85–88	44–46	51/43	31/34
reticulata	69	88	24	35	19	35	41	32	59	82	52	46/31	27/33
sinensis	63	85	21	31	14	34	35	35	45	59	34		19/27
taphozous	64	87	20	34	14	39	20+	40	50	56		30	28
wenquana	69	92	21	37	17	42	42	40	52	72	49	49/37	30/37

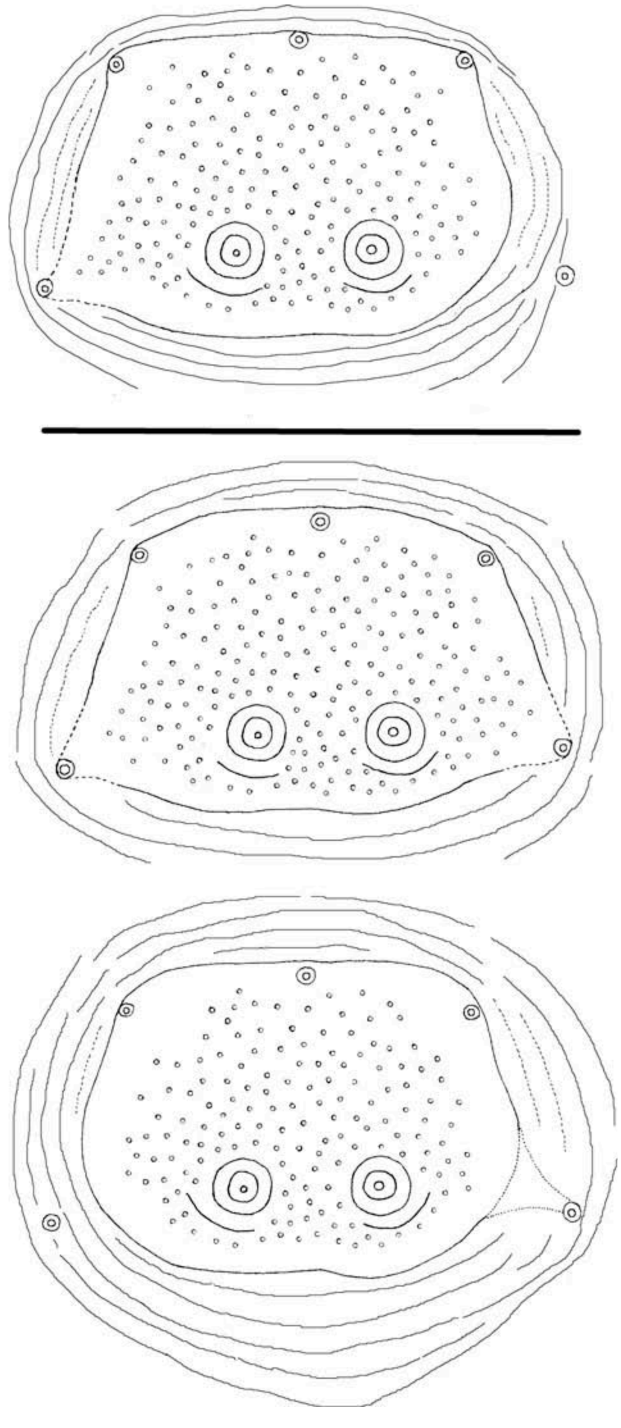

Figure 6. *Grandjeana mauritanica* **sp. nov.** (holotype and paratypes) – types of scutum. Scale bar: 100 μm.

cuticle striae cross PL angle and cover the posterior margin between scutum and PL setae (Figures 5 and 6). Scutal punctae spread and cover most of scutum. Sensilla ciliated 87(85–88 long), with 5–6 lateral cilia situated in apical half. Rounded eyes (2 + 2), anterior larger than posterior, ocular plates present. Humeral setae 45(44–46) long, dorsal anterior 47(43–51) long, central 38(34–42) and posterior setae 32(31–34) long, densely ciliated, dorsal setae arranged in regular rows, the rows slightly varying in position of setae. Scutal measurements: AW = 58(57–60), PW = 86(82–89), SB = 27(26–28), ASB = 39(39–40), PSB = 17(15–19), SD = 56(54–59), AP = 40(36–43), AM = 49(49–49), AL = 36(34–37), PL = 49(46–53), PL ≥ AM > AL, SD < AW < PW, PL situated slightly anteriorly from level of SB. Ventral side with two pairs of sternal setae (fSt = 2.2), fCx = 1.1.1. Ventral side with 42 irregularly arranged densely ciliated setae. Lengths of ventral setae vary, increase from anterior 25(25–26) to median 30(28–31) and posterior 33(31–36) setae.

Gnathosoma (Figure 3). Galeala nude (Ga = N). Palps 72(69–74) long, slender palpotibial claw 25(25–26) long, with three deeply indented prongs. Chelicera anteriorly with tricuspid cap.

Legs (Figure 4). With pretarsus, claws and normal empodia. All barbed setae on legs ciliated, slender. Specialized setae on leg segments – Leg I: Tarsus – S_1 44(42–46), f_1 2(2–2), paired nude pretarsalae 7(6–8) (PT′ = N), ST and pST absent, 2 tibialae 20(18–23), microtibiala 5(4–5), 3 genualae – anterior 20(18–21), lateral 23(23–23), posterior 18(17–20), microgenuala 5(5–6); Leg II: Tarsus – S_2 26(25–28), f_2 4(4–5), 2 tibialae – anterior 19(18–20), posterior 17(17–18), genuala 24(23–26); Leg III: very long tibiala 77(75–78) and genuala 81(77–84) resembling mastisetae. Number of barbed setae on leg segments (leg formula from coxa to tarsus): Leg I: 1-1-(1 + 5)-4-8-19; Leg II: 1-1-(2 + 4)-3-6-16; Leg III: 1-1-(2 + 3)-3-6-15. Length of tarsi I–III: I – 99(92–104), II – 92(89–94), III – 122(119–123). Leg length: pa 388(364–403), pm 350(341–357), pp 408(405–412), Ip 1145(1111–1164).

Material examined

Holotype: Mauritania, Ouadâne, old town, 20° 56′ N, 11° 37′ W, 440 m a. s. l., abandoned house, one larva from bat *Rhinopoma cystops*, 18 October 2010. **Paratypes:** The same locality, three larvae, same data as the holotype, collected by P. Benda, A. Reiter and M. Uhrin. Holotype and two paratypes are deposited in the Slovak National Museum, Bratislava, Slovak Republic, one paratype in the Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

Remarks

The most of species of the genus *Grandjeana* (7) are known from South-East Asia (Malaysia, Thailand, China, Philippines), one species *G. reticulata* comes from Africa. All known species have been occurring on bats. The new described species *Grandjeana mauritanica* **sp. nov.** comes from West Africa (Mauritania). The African representatives of the genus *Grandjeana* show longer solenidia on tarsi I (*G. reticulata* – 29, *G. mauritanica* **sp. nov.** – 44) and longer genualae and tibialae III (*G. reticulata* – 42 and 38, respectively; *G. mauritanica* **sp. nov.** – 81 and 77, respectively), while the species from Asia have shorter solenidia on tarsi I (20–25) and also shorter genualae III (23–26) and tibialae III (16–23).

Key to the known species of the genus *Grandjeana*

1(2) fPp = N-N-N.N.N, NDV ≥ 120, fD = 2H-10.10.10+ .... ***G. taphozous*** (Womersley 1952)
– fPp other **2**

2(1) fPp = B-B-B.B.B, or fPp = B-B-b.b.B **3**
– fPp = B-B-B.N.B or fPp = B-B-B.N.b or fPp = B-B-N.N.B **6**

3(2) NDV = 60–80 .... **4**
– NDV > 100 .... **5**

4(3) NDV = 64, Ip < 700, PW = 74 .... ***G. calva*** (Domrow 1962)
– NDV = 80, Ip > 800, PW = 92 .... ***G. wenquana*** (Wen & Xiang 1984)

5(3) Ga = B, PL > AM > AL .... ***G. reticulata*** (Vercammen-Grandjean & Nadchatram 1963)
– Ga = N, PL > AL > AM .... ***G. asiatica*** (Wen & Corpuz-Raros 1997)

6(2) fD = 2H-6.6.6.6.+ **7**
– fD = 2H-10.10. +... or fD = 2H-12.12.+ **8**

7(6) fPp = B-B-B.N.B, ASB = 25, AM = 32, SB = 16 .... ***G. koongi*** (Domrow 1962)
– fPp = B-B-N.N.B, ASB = 39, AM = 49, SB = 26 .... ***G. mauritanica* sp. nov.** Kalúz & Ševčík

8(7) fD = 2H-12.12.10. + , NDV = 123, Ip = 800, PW = 85 .... ***G. sinensis*** (Zhao & Qiu 1979)
– fD = 2H-10.10.6. + , NDV = 102-110, Ip < 700, PW = 76 ...... ***G. manjuyodensis*** (Brown 1997)

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We express our thanks to Petr Benda, Antonín Reiter and Marcel Uhrin for collecting the material of chiggers. We are very thankful to Prof. Ting-Huan Wen for valuable advice in taxonomy of chiggers and we also extend our thankfulness to anonymous reviewers for their review of the manuscript and critical comments.

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8.3. Kalúz S., Ševčík M. 2015:

A new species of *Grandjeana* (Acari: Trombiculidae) from heart-nosed bat (Chiroptera: Megadermatidae) in Ethiopia (Africa) with notes to biogeography of this genus.

Biologia 70: 380–385.

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Biologia **70**/3: 380—385, 2015
Section Zoology
DOI: 10.1515/biolog-2015-0043

A new species of *Grandjeana* (Acari: Trombiculidae) from heart-nosed bat (Chiroptera: Megadermatidae) in Ethiopia (Africa) with notes to biogeography of this genus

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Abstract: A new chigger mite *Grandjeana kanuchi* sp. n. collected from the bat *Cardioderma cor* (Peters, 1872) (Megadermatidae) in Ethiopia (North-East Africa) is described. Additional comments on hosts and distribution records of the chiggers of genus *Grandjeana* are provided. With the newly described species the genus *Grandjeana* includes 10 species, three of them are occurring in Africa and seven in South Asia. Present findings suggest that this genus has the geographic distribution in Ethiopian and Oriental regions with a strong association with selected families of Chiroptera.

Key words: *Grandjeana*; new taxon; parasite; distribution; Ethiopian region; Oriental region

Introduction

One of the most important features characterizing the genus *Grandjeana* Koçak et Kemal, 2009 is the presence of paired pretarsalae on tarsus I. Until recently, altogether nine species in this genus have been known (Kalúz & Ševčík 2014), all parasitizing bats. Species of this genus are widely distributed in Malaysia (Womersley 1952), Thailand (Domrow 1962), China (Zhao & Qiu 1979; Wen & Xiang 1984), Philippines (Brown 1997; Wen & Corpuz-Raros 1997), South Africa (Vercammen-Grandjean & Nadchatram 1963; Vercammen-Grandjean 1971, Vercammen-Grandjean et al. 1973) and Mauritania (Kalúz & Ševčík 2014).

In this paper we present the description of a new species, *Grandjeana kanuchi* sp. n. The comparison of species distribution within the genus and their host selectivity are also given.

Material and methods

The newly described chigger species was found on bats in Ethiopia (details are given below, in the definition of material studied). Hosts were caught using mist-nets and mites were collected from the ears by entomological tweezers and preserved in ethyl alcohol. Specimens were mounted onto slides using Swann's medium, microscopically identified and described. All drawings were produced by using standard light microscopy and enhanced with computer programme (GIMP2). Measurements were made with an ocular micrometer and dimensions presented in the text are shown in micrometers. Terminology follows Vercammen-Grandjean & Langston (1976) and Kudryashova (1998). The scale bar for all figures is 100 μm.

Genus ***Grandjeana*** Koçak et Kemal, 2009

Type species: *Trombicula* (*Trombicula*) *reticulata* Vercammen-Grandjean et Nadchatram, 1963, by original designation.

Species included

Known *Grandjeana* species, synonyms; host (family); locality; references

Grandjeana asiatica, *Diplectria asiatica* Wen et Corpuz-Raros 1997, *Trombicula* (*Grandjeana*) *asiatica* Koçak et Kemal 2009; *Pipistrellus* sp. (Vespertilionidae); Philippines, Luzon; Wen & Corpuz-Raros (1997)

Grandjeana calva, *Trombicula calva* Domrow 1962, Tenorio 1976, *Diplectria calva* Wen et Xiang 1984, Wen et Corpuz-Raros 1997; undetermined bat; Thailand; Domrow (1962)

Grandjeana kanuchi sp. n.; *Cardioderma cor* (Peters, 1872) (Megadermatidae); Ethiopia; this paper

Grandjeana koongi, *Trombicula koongi* Domrow 1962, Yang 1990, *Diplectria koongi* Wen et Xiang 1984, Wen et Corpuz-Raros 1997; *Megaderma spasma* (L., 1758) (Megadermatidae); Malaysia; Domrow (1962)

Grandjeana manjuyodensis, *Diplectria manjuyodensis* Brown 1997, *Trombicula* (*Grandjeana*) *manjuyodensis* Koçak et Kemal 2009; *Taphozous philippinensis*

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* Corresponding author

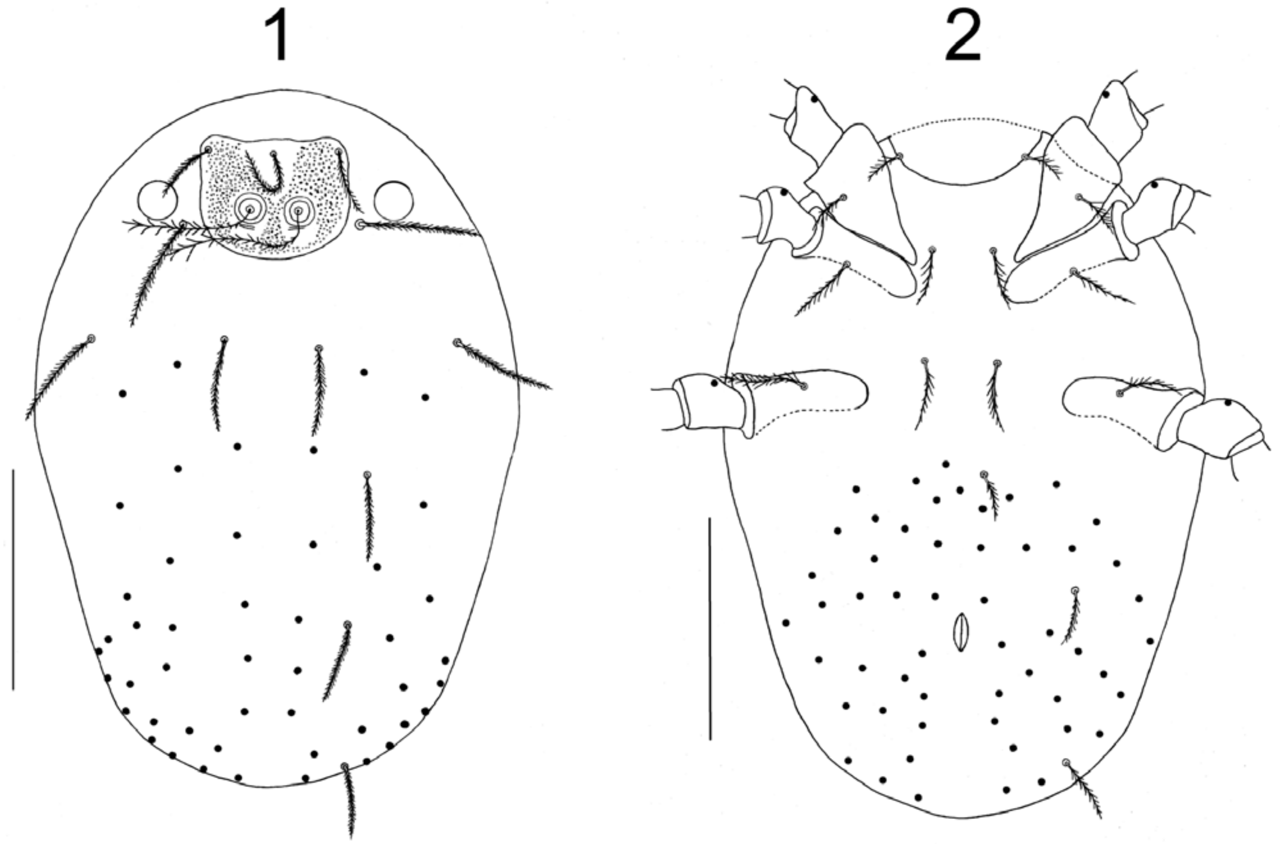

Fig. 1, 2. *Grandjeana kanuchi* sp. n. (holotype): 1 – idiosoma dorsal; 2 – idiosoma ventral. Scales 100 μm.

Waterhouse, 1845 (Emballonuridae); Philippines, Negros; Brown (1997)

Grandjeana mauritanica; *Rhinopoma cystops* Thomas, 1903 (Rhinopomatidae); Mauritania; Kalúz & Ševčík (2014)

Grandjeana reticulata, *Trombicula* (*Trombicula*) *reticulata* Vercammen-Grandjean et Nadchatram 1963, *Trombicula* (*Diplectria*) *reticulata* Vercammen-Grandjean 1967, 1968, 1971, 1973, Vercammen-Grandjean, Langston et Audy 1973, *Diplectria reticulata* Nadchatram et Dohany 1974, Wen et Xiang 1984, Wen et Corpuz-Raros 1997, *Trombicula* (*Grandjeana*) *reticulata* Koçak et Kemal 2009; *Rhinolophus geoffroyi zuluensis* Ortlepp, 1932 [= *Rhinolophus clivosus* complex] (Rhinolophidae); South Africa; Vercammen-Grandjean & Nadchatram (1963)

Grandjeana sinensis, *Diplectria sinensis* Zhao et Qiu 1979, Wen et Xiang 1984, Wen et Corpuz-Raros 1997, *Trombicula* (*Grandjeana*) *sinensis* Koçak et Kemal 2009; undetermined bat; China; Zhao & Qiu (1979)

Grandjeana taphozous, *Trombicula taphozous* Womersley 1952, *Diplectria taphozous* Wen et Xiang 1984, Wen et Corpuz-Raros 1997; *Taphozous melanophogon* Temminck, 1841 (Emballonuridae); Malaysia; Womersley (1952)

Grandjeana wenquana, *Diplectria wenquana* Wen et Xiang 1984, Wen et Corpuz-Raros 1997, *Trombicula* (*Grandjeana*) *wenquana* Koçak et Kemal 2009; undetermined bat; China; Wen & Xiang (1984)

Diagnosis

SIF = 5B-B(N)-3-3111-0(1)000; sensilla flagelliform. Scutum varies as scutum, peniscutum or semi-peniscutum. One or two pairs of eyes, with anterior lense much larger than posterior. All legs slender, seven segmented (fSP = 7.7.7), coxae narrow (fCx = 1.1.1). Tarsus I with S_1, f_1 and paired pretarsalae; ST and pST absent. Pretarsala on tarsus II present or absent, mastitarsala present or absent, sometimes with proximal ciliation. Solenidia on legs long and slender. The genus is occurring on bats in Africa and Asia.

***Grandjeana kanuchi* sp. n.** (Figs 1–5)

Description. Larva. SIF = 5B-N-3-3111-0000; fsp = 7-7-7; fPp = B-B-B.B.B; Ga = N; fCx = 1.1.1; fSt = 2.2; DS = 54, VS = 28+28 = 56; NDV = 110; fD = 2H+6.6.6.8.6.6.8/6 = 54.

Idiosoma (Figs 1, 2). Scutum reduced (peniscutum), lateral margins almost perpendicular and parallel, smooth protruded posterior margin. Scutum slightly wider than longer, with AL, AM setae and two sensilla. PL setae situated on soft cuticle, fine cuticular striae running between PL and latero-posterior scutal margin. Scutal reticulation covers most of scutum (Fig. 3). Sensilla with 16–24 branches, shorter branches situated in median and longer in apical thirds. Scutal measurements (means and ranges; sample size = 16): AW =

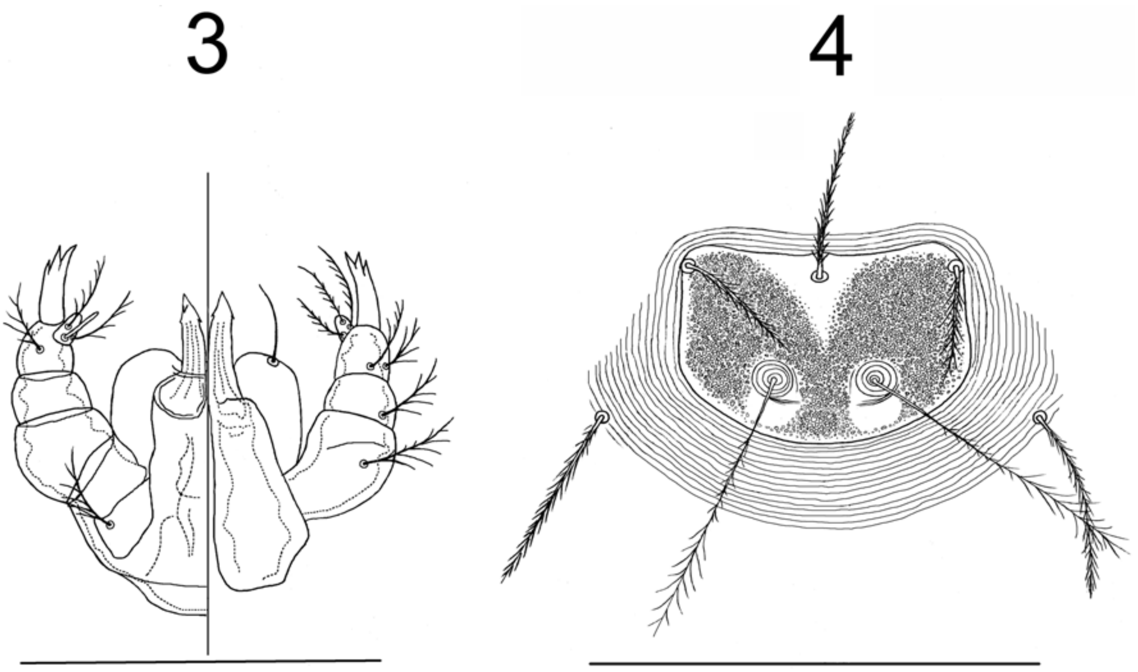

Fig. 3, 4. *Grandjeana kanuchi* sp. n. (paratype): 3 – scutum; 4 – gnathosoma (left – ventral, right – dorsal). Scales 100 μm.

60(58–63), PW = 98(78–105), SB = 21(20–23), ASB = 34(31–35), PSB = 16(15–18), SD = 50(48–51), AP = 44(40–46), AM = 46(40–49), AL = 30(26–31), PL = 49(46–51), S = 75(69–77). PL situated slightly posteriorly from level of SB, PL > AM > AL, SD < AW < PW. Large (18 in diameter) eyes (1+1), posterior pair of eyes reduced, not visible, ocular plates absent. Humeral setae 46(42–48) long, dorsal anterior 39(36–43) long, central 34(31–38) and posterior setae 29(28–35) long, densely barbed, dorsal setae arranged in regular rows, the rows slightly varying in position of setae. Sternal side with two pairs of sternal setae. Ventral side with irregularly arranged densely barbed setae, 28 setae anteriad and 28 posteriad from anus. Lengths of ventral setae vary, increase from anterior 23(18–26) to median 26(21–31) and posterior 29(25–34) setae.

Gnathosoma (Fig. 4). Galeala nude. Palps 72(69–74) long, slender palpotibial claw 24(23–25) long, with 3 deeply indented prongs. All palpal setae branched. Chelicera is positioned anteriorly with tricuspid cap.

Legs (Fig. 5). With pretarsus, claws and normal empodia. Setae on legs plumose, slender. Specialized setae on leg segments – Leg I: Tarsus – S_1, f_1, paired nude PT′, ST = B, pST absent, 2 tibialae, microtibiala, 3 genualae, microgenuala; Leg II: Tarsus – S_2, f_2, PT″ absent, 2 tibialae, genuala; Leg III: tibiala and genuala. Number of plumose setae on leg segments (leg formula fBP): Leg I: 1-1-5-3-7-21; Leg II: 1-2-4-3-6-16; Leg III: 1-2-3-3-6-15.

Leg length: pa 304(278–316), pm 284(251–290), pp 318(286–332), Ip 899(815–925).

Material studied. Holotype: Ethiopia, Mago National Park, E 36°15.67′, N 05°24.17′, 533 m a.s.l., one larva from the mixed collection of *Cardioderma cor* (2 males, 3 females; det. hosts and coll. mites by Peter Kaňuch & Josef Bryja), 14 November, 2012.

Paratypes: The same locality and host(s), 15 larvae. The holotype (SZ 7496) and four paratypes (SZ 7497) will be deposited in the Slovak National Museum, Bratislava, Slovak Republic; three paratypes (IG 32.960/001, IG 32.960/002, IG 32.960/003) will be deposited in the Royal Belgian Institute of Natural Sciences, Brussels, Belgium; remaining paratypes (4+4) are equally shared in the collections of the authors.

Etymology. Patronymic; named in honour of Slovakian zoologist Peter Kaňuch, researcher in bat biology in Slovakia.

Differential diagnosis. Morphologically the most similar species to *Grandjeana kanuchi* sp. n. is *G. reticulata* (Vercammen-Grandjean et Nadchatram, 1963), but *G. kanuchi* sp. n. differs from it by peniscutum with PL setae on soft tegument, fD = 2H-6-6-6-6-6… and single eyes (1+1) while *G. reticulata* has normal scutum with AL, AM, PL setae, fD = 2H-(2.6)-(2.6)-8-6-6… and paired eyes (2+2). Other similar species *G. asiatica* differs from *G. kanuchi* sp. n. by normal scutum with AL, AM, PL setae, fD = 2H-9-8-8-6… and paired eyes (2+2), while *G. kanuchi* sp. n. has peniscutum and dorsal setal formula fD = 2H-6-6-6-6-6…

Notes on the biogeography of *Grandjeana* species and their hosts

Species of *Grandjeana* are parasitic specifically on bats and all species of this genus were found in the Old World (Fig. 6). At the same time the order Chiroptera is also believed having its origin and distribution there. Inspecting closely the hosts group, all findings come from families Emballonuridae (distributed in the Old World), Rhinolophidae, Rhinopomatidae and Megadermatidae (Fig. 6, see Species included). The records of *Pipistrel-*

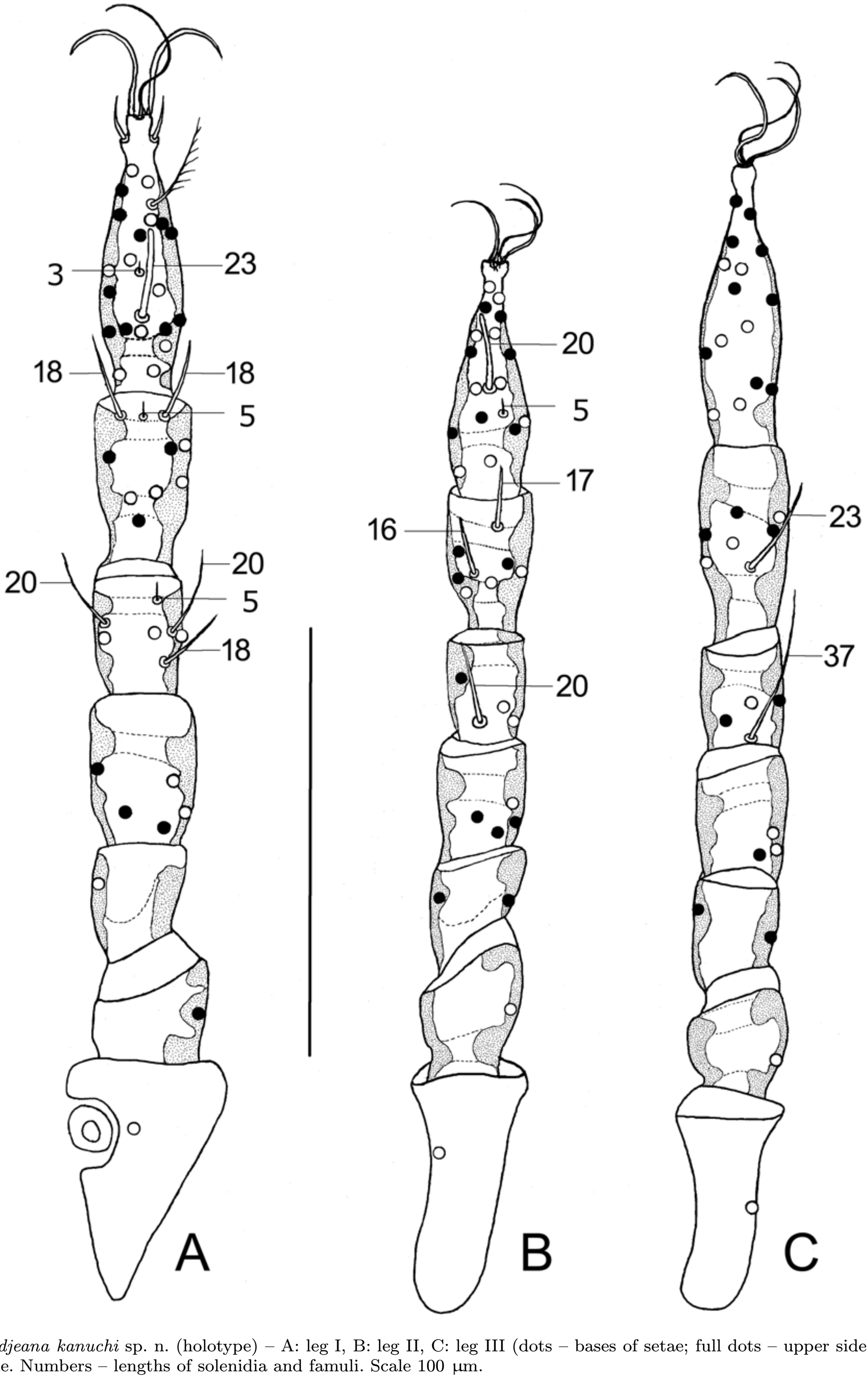

Fig. 5. *Grandjeana kanuchi* sp. n. (holotype) – A: leg I, B: leg II, C: leg III (dots – bases of setae; full dots – upper side; empty dots – bottom side. Numbers – lengths of solenidia and famuli. Scale 100 μm.

lus sp. (Vespertilionidae) (Wen & Corpus-Raros, 1997) and undetermined "bat host" (Domrow 1962; Zhao & Qiu 1979; Wen & Xiang 1984) are not included here. The records of *Grandjeana* on the genus *Pipistrellus* could be probably caused by their occurrence in the same habitat, "host switching" or absence of preferable host. The distribution of the above mentioned host families within the Old World comprises both African

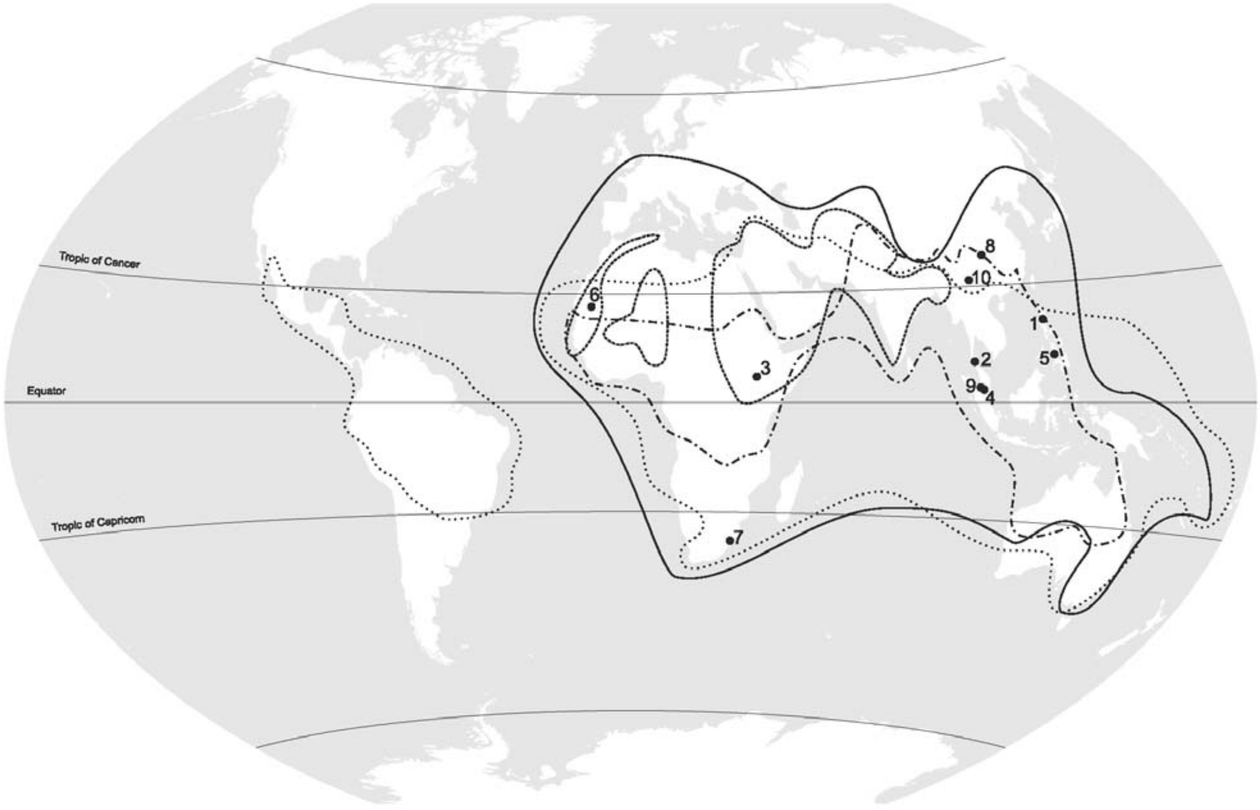

Fig. 6. Geographical distribution of host families and the species of *Grandjeana* in the World. Dotted line – Emballonuridae, dot dashed line – Megadermatidae, solid line – Rhinolophidae, dashed line – Rhinopomatidae. Dots: 1 – *G. asiatica*, 2 – *G. calva*, 3 – *G. kanuchi* sp. n., 4 – *G. koongi*, 5 – *G. manjuyodensis*, 6 – *G. mauritanica*, 7 – *G. reticulata*, 8 – *G. sinensis,* 9 –*G. taphozous,* 10 – *G. wenquana*.

and Oriental areas, where some species of the genus *Grandjeana* were also found; three species in Africa and seven in Asia. On the base of present findings we suppose the distribution of the species of *Grandjeana* within Ethiopian and Oriental zoogeographical regions between 24°55′ N and 29°33′ S latitudes. The records of *Grandjeana* are missing from the New World (Nearctic and Neotropical regions), despite the fact that bats of the family Emballonuridae are also distributed there. Moreover, the records are not known also from Australian region. However, taking into account the distribution of the family Emballonuridae and geographic proximity to Indo-Malaya, occurrence of *Grandjeana* in Australasia is likely in the future. Despite of minimum records, we suppose that this parasitic genus seems strictly linked to the above-mentioned families of Chiroptera in the Old World (cf. Teeling et al. 2005).

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8.4. Kalúz S., Šrámek P., Ševčík M. 2021:

Rudnicula goffi **n. sp. (Acariformes: Trombiculidae) from the diadem leaf-nosed bat** ***Hipposideros diadema*** **(Geoffroy) (Chiroptera: Hipposideridae) on Bali, Indonesia.**

Systematic Parasitology 98: 17–24.

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Rudnicula goffi n. sp. (Acariformes: Trombiculidae) from the diadem leaf-nosed bat *Hipposideros diadema* (Geoffroy) (Chiroptera: Hipposideridae) on Bali, Indonesia

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Abstract A new species of chigger mite, *Rudnicula goffi* n. sp., is described from the diadem leaf-nosed bat *Hipposideros diadema* (Geoffroy) (Hipposideridae) caught in the Goa Peteng Cave on Bali (Indonesia). The new species differs from the closely related species by the presence of scutum with AL windows, lateral scutal margins with cuticular striations and fSt = 2 + 2 + 2. A list of all available records of *Rudnicula* species is provided. The genus is restricted to the tropics of Southeast Asia, Australia, Melanesia, and the subtropics of eastern Asia. A key to the known species of *Rudnicula* based on the morphology of the larvae is also provided.

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Introduction

All species of the chigger mite genus *Rudnicula* Vercammen-Grandjean, 1964 are strictly confined to association with bats (Vercammen-Grandjean 1964). The first scientific information related to this taxon was published by Vercammen-Grandjean (1964), who proposed *Rudnicula* as a new subgenus of *Trombicula* Berlese, 1905 for *T. tibbettsi* Vercammen-Grandjean, 1961, a species of bat-infesting chigger found in South Korea. In the following year, this author elevated *Rudnicula* to full generic status and in the same paper (Vercammen-Grandjean 1965) replaced the name *T. tibbettsi* with *T. tibbi* since his studies showed that *T. tibbettsi* was a primary homonym of *Trombicula tibbettsi* Brennan & White, 1960. In the past, many more species currently placed elsewhere, were included in the genus *Rudnicula.* The last revision of the genus was published by Brown et al. (1988). Yet, further descriptions of new *Rudnicula* spp. have been published since then (Goff & Easton 1989; Brown 1997). After the recent transfer of four species, namely *Rudnicula aethiopica* (Hirst, 1926), *R. melvini* Traub & Evans, 1950, *R. knighty* (Radford, 1954) and *R. balcanica* Kolebinova, 1966 to the genus *Trisetica* Traub & Evans, 1950 by Stekolnikov & Quetglas (2019), the genus contains nine species of *Rudnicula.*

Herein, we describe a new species, *Rudnicula goffi* n. sp. collected from the bat *Hipposideros diadema* (Geoffroy) (Hipposideridae) in the Goa Peteng Cave on Bali (Indonesia), and summarise the data for the species currently placed in *Rudnicula* and provide a key to the species based on the morphology of the larvae.

Materials and methods

All individuals of the new species (holotype and paratypes) were collected from diadem leaf-nosed bat in Goa Peteng cave, 8°47’34”S, 115°08’14”E, in Jimbaran village, Bali, Indonesia. The bats were caught by a hand net in February 2019. All body parts (the face, ears, chiro-, plagio-, uro- paragia and fur on the back and abdominal part) of the live host were inspected with a naked eye. The bats were released within the cave unharmed after inspection. Mites were collected using tweezers and preserved in 90% ethanol.

Mite specimens were mounted onto slides using Swan’s embedding medium (Swan, 1936), then microscopically identified, using the standard microscopy (Karl Zeiss Jena, Germany, Ergaval, equipped with a drawing tube), then measured and described.

The drawings were produced from light microscopy using the drawing tube, then enhanced with computer software (Adobe Photoshop). All measurements presented in the paper were taken using a standardised ocular micrometer. The symbols and chigger mite terminology used in the text and figures follow Vercammen-Grandjean (1968), Vercammen-Grandjean & Langston (1976) and Goff et al. (1982), with later adaptations by Kudryashova (1998) and Stekolnikov et al. (2014). All measurements are presented in micrometres as the range followed by the mean in parentheses.

Bat taxonomy follows Wilson & Mittermeier (2019).

The holotype and paratypes of the new species are deposited in the Slovak National Museum, Bratislava, Slovak Republic (SNM) and the National Museum (Natural History), Prague, Czech Republic (NMP).

Family Trombiculidae Ewing, 1944
Subfamily Trombiculinae Ewing, 1944
Tribe Trombiculini Vercammen-Grandjean, 1960

Genus *Rudnicula* Vercammen-Grandjean, 1964

Type-species: *Trombicula* (*Rudnicula*) *tibbi* Vercammen-Grandjean, 1964 [= *Trombicula tibbettsi* Vercammen-Grandjean (1961) (necessity: preoccupied) *Trombicula tibbettsi* Brennan & White, 1960].

References: Vercammen-Grandjean (1964: p. 309–311, figures 1–4; 1965: p. 295, 296); Nadchatram (1968: p. 467).

Species included: Species of *Rudnicula* are listed below with the relevant data as follows: synonym and reference; country of record; host; family of the host; source of record.

(i) *Rudnicula agurensis* Goff & Easton, 1989; Papua New Guinea; *Hipposideros calcaratus* (Dobson); Hipposideridae; Goff & Easton (1989).

(ii) *Rudnicula barbarae* (Domrow, 1964); syns *Trombicula barbarae* Domrow, 1964 of Domrow & Lester (1985), *Sasatrombicula* (*Rudnicula*) *barbarae* of Anciaux de Faveaux (1976a, b), *Trisetica* (*Rudnicula*) *barbarae* of Anciaux de Faveaux (1985); Australia; *Taphozous georgianus* Thomas; Emballonuridae; Domrow (1964).

(iii) *Rudnicula becki* Nadchatram, 1968; syns *Sasatrombicula* (*Rudnicula*) *becki* of Anciaux de Faveaux (1976a, b), *Trisetica* (*Rudnicula*) *barbarae* of Anciaux de Faveaux (1985); Malaysia; *Rhinolophus sedulus* Andersen; Rhinolophidae; Nadchatram (1968).

(iv) *Rudnicula dimolinae* (Audy, 1952); syns *Trombicula* (*?Neotrombicula*) *dimolinae* Audy, 1952, *Trombicula* (*Trombicula*) *dimolinae* of Chen & Hsu (1963), *Riedlinia* (*Trombigastia*) *dimolinae* of Vercammen-Grandjean (1964), *Trombicula dimolinae* of Yang (1990); Malaysia; China; *Rhinolophus* sp., Chiroptera fam. gen. sp.; Rhinolophidae; Audy (1952); Chen & Hsu (1963).

(v) *Rudnicula hexasternalaea* Brown, 1997; Philippines; *Emballonura alecto* (Eydoux & Gervais); Emballonuridae; Brown (1991, 1997).

(vi) *Rudnicula leytensis* Brown, Goff & Nadchatram 1988; Philippines; *Ptenochirus jagori* (Peters), *Macroglossus minimus* (Geoffroy), *Macronus striaticeps* Sharpe; Pteropodidae, Timaliidae; Brown et al. (1988).

(vii) *Rudnicula meilingensis* Zhao, 1984; syn. *Sasatrombicula* (*Rudnicula*) *meilingensis* of Lanza (1999); China; *Rhinolophus* sp.; Rhinolophidae; Zhao (1984).

(viii) *Rudnicula templei* Nadchatram & Wilson, 1965; syns *Sasatrombicula* (*Rudnicula*) *templei* of Anciaux de Faveaux (1976a, b), *Trisetica* (*Rudnicula*) *templei* of Anciaux de Faveaux (1985); New Guinea; *Hipposideros semoni* Matschie; Hipposideridae; Nadchatram & Wilson (1965).

(ix) *Rudnicula tibbi* (Vercammen-Grandjean, 1964); syns *Trombicula* (*Rudnicula*) *tibbettsi* Vercammen-Grandjean, 1964, *Trombicula* (*Rudnicula*) *tibbi* of Vercammen-Grandjean (1965), *Sasatrombicula* (*Rudnicula*) *tibbi* of Anciaux de Faveaux (1976a), Lanza (1999), *Rudnicula tibbettsi* of Zhao (1984); South Korea; *Myotis mystacinus* (Kuhl); Vespertilionidae; Vercammen-Grandjean (1964).

(x) *Rudnicula* spp.; Malaysia; *Hipposideros bicolor* (Temminck) (also as *Rhinolophus bicolor*), *H. cervinus* (Gould), *H. diadema* (Geoffroy), *H. larvatus* (Horsfield), *Rhinolophus affinis* Horsfield, *R. luctus* Temminck, *R. pussilus* Temminck, *Tylonycteris pachypus* (Temminck); Hipposideridae, Rhinolophidae, Vespertilionidae; Ahamad et al. (2013).

(xi) *Rudnicula* sp.; Sri Lanka; *Hipposideros speoris* (Schneider); Hipposideridae; Brown et al. (2003).

Rudnicula (*Rudnicula*) *goffi* n. sp.

Type-host: *Hipposideros diadema* (Geoffroy) (Chiroptera: Hipposideridae), diadem leaf-nosed bat.

Type-locality: Goa Peteng cave, (8°47’34”S, 115°08’14”E) in Jimbaran village, Bali, Indonesia.

Type-material: Larval holotype (unengorged larva) (SNM SZ 10 944), ex *Hipposideros diadema*, 13.ii.2019, collected by P. Šrámek; 8 larval paratypes (unengorged and engorged) (SNM SZ 10 945, NMP P6A 7379–7380), same host, locality and date, collected by P. Šrámek.

ZooBank registration: *ZooBank registration*: To comply with the regulations set out in Article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature* (ICZN 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Rudnicula goffi* n. sp. is urn:lsid:zoobank.org:act:87F09D6E-A2CB-4C11-A551-4997EC2810AF.

Etymology: The species is named in honour of the entomologist M. Lee Goff, in recognition of his significant contribution to the knowledge of chigger mites of the Southeast Asia.

Description

Larva [Based on the holotype and 8 paratypes; measurements are given in Table 1; Figs. 1, 2, 3, 4, 5, 6] Dorsal aspect of idiosoma (Figs. 1, 3). Scutum somewhat subquadrate, PL corners extended, anterior margin biconcave, anterior lateral AL shoulders absent, lateral margins of scutum with cuticular striations, posterior margin medially slightly convex; scutum bearing 1 AM, 1 pair of AL and PL setae, and 2 sensilla (sensilla lacking in all studied specimens). Punctuation covering most of scutum. Oval eyes (2 + 2) in ocular plates present, laterally close to scutum (not visible in some studied specimens), anterior eyes laterally between AL and PL, posterior eyes at level of PL (Fig. 1). Scutal windows 7–15 (9) long, 6–12 (8) wide, mediolaterally close to AL setae. Measurements of scutum given in Table 1. Dorsum with pair (2H) of humeral setae 34–44 (39) long, dorsal setae (Fig. 5) covered with thick barbs, 26–40 (34) long, mostly arranged in regular rows (anterior rows, e.g. fDS = 2H.13.2.11….; 2H.13.10.2.9….; 2H.13.13.9….; 2H.13.13.8….; 2H.13.13.10….; 2H.12.12.2….; 2H.12.2.12….; 2H.13.13.12….); PW > AW > SD; ASB > SB > PSB).

Ventral aspect of idiosoma (Fig. 2). Three pairs of sternal setae (fSt = 2 + 2 + 2). Each coxa bearing 1 seta, ventral side generally with 52–67 (58) (depending on projection of engorged or unengorged specimens) setae (excluding coxal and sternal setae), setae covered with thick barbs (Fig. 5), mostly regularly arranged in rows. Length of ventral setae vary, proximal and medial setae shorter and caudal longer; sternal and coxal setae longer than ventral (Fig. 5).

Gnathosoma (Fig. 4). Galeala nude. Palps (including claw) 68–76 (73) long, 3-pronged palptibial claw slender, 19–22 (21) long, with deeply indented prongs. Gnathobase (infracapitulum) bearing pair of branched setae 22–27 (24) long, palpal femur and palpal genu each with 1 heavily barbed dorsal seta. Palpal tibia with 1 nude dorsal seta, 1 sparsely barbed dorsolateral seta, and 1 barbed ventro-lateral seta. Palpal tarsus bears 4 branched setae and solenidion. Cheliceral blade short, terminating with tricuspid cup.

Legs (Fig. 6). With pretarsus, claws and lanceolate empodia. All setae on legs barbed, slender. Specialised

Table 1 Measurements of the holotype and paratypes of *Rudnicula goffi* n. sp. (n = 9)

Variable	Paratypes		Holotype
	Range	Mean ± StD	
AW	49–59	53 ± 1	57
PW	68–78	73 ± 4	75
SB	19–23	21 ± 1	21
ASB	27–33	29 ± 2	28
PSB	15–21	18 ± 1	18
SD	44–51	47 ± 3	46
AP	38–46	41 ± 3	41
AM	41–50	44±3	44
AL	32–39	35 ± 2	34
PL	51–61	55 ± 3	58
H	34–44	39 ± 3	39
DS	43–58	51 ± 2	54
VS	52–67	58 ± 6	55
NDV	97–120	109 ± 8	109
D_{min}	26–36	31 ± 3	28
D_{max}	36–40	38 ± 1	38
V_{min}	21–26	23 ± 2	24
V_{max}	28–34	31 ± 2	29
pa	294–345	326 ± 16	304
pm	237–276	262 ± 13	237
pp	270–320	304 ± 15	270
Ip	811–940	892 ± 39	811
TaIIIL	85–95	92 ± 3	93
TaIIIW	14–19	15 ± 1	15

Abbreviations: AW, distance between scutal anterolateral setae; PW, distance between scutal posterolateral setae; SB, distance between sensilla; ASB, distance from extreme anterior margin of scutum to the level of sensillary bases; PSB, distance from extreme posterior margin of scutum to the level of sensillary bases; SD, length of scutum (ASB + PSB); AP, distance from anterolateral to posterolateral scutal setae on one side; AM, length of scutal anteromedian seta; AL, length of anterolateral scutal setae; PL, length of posterolateral scutal setae; H, length of humeral setae; DS, number of dorsal idiosomal and humeral setae; VS, number of ventral idiosomal setae (excluding coxal and sternal setae); NDV, number of idiosomal setae (DS + VS); D_{min}, length of the shortest dorsal idiosomal seta; D_{max}, length of the longest dorsal idiosomal seta; V_{min}, length of the shortest ventral idiosomal seta; V_{max}, length of the longest ventral idiosomal seta; pa, length of leg I (including coxa); pm, length of leg II (including coxa); pp, length of leg III (including coxa); Ip, total length of legs I-III; TaIIIL, length of leg tarsus III; TaIIIW, width of leg tarsus III; StD, standard deviation

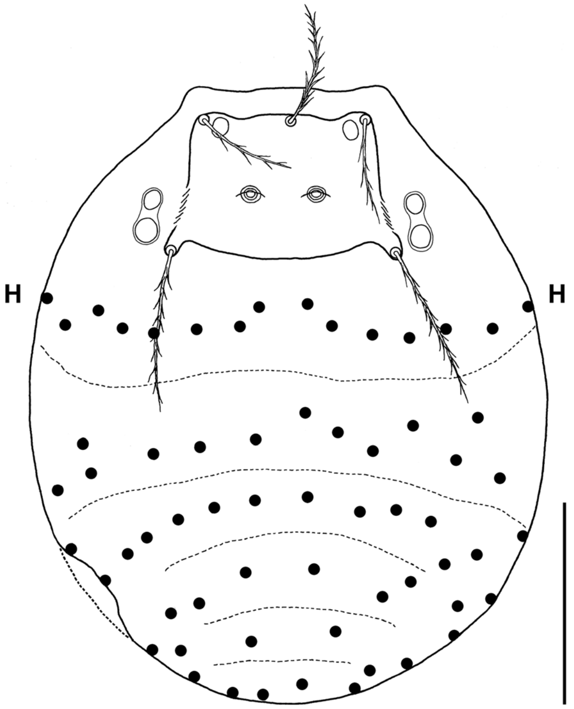

Fig. 1 *Rudnicula goffi* n. sp., larva, holotype. Idiosoma, dorsal view. *Scale-bar*: 100 μm

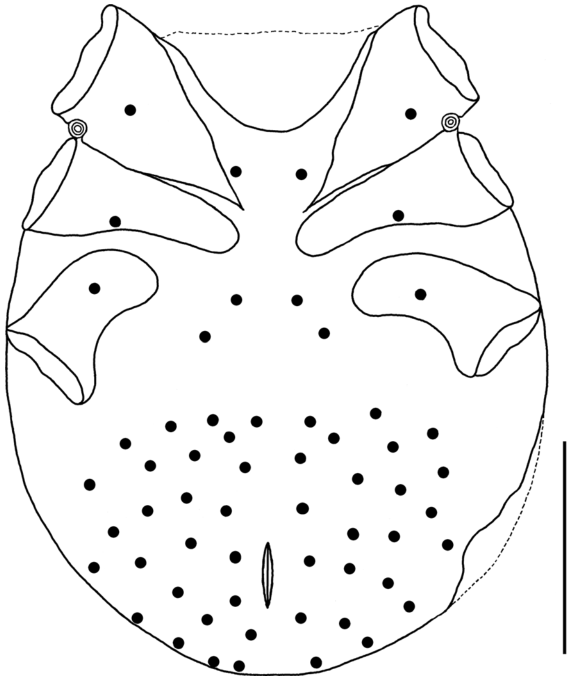

Fig. 2 *Rudnicula goffi* n. sp., larva, holotype. Idiosoma, ventral view. *Scale-bar*: 100 μm

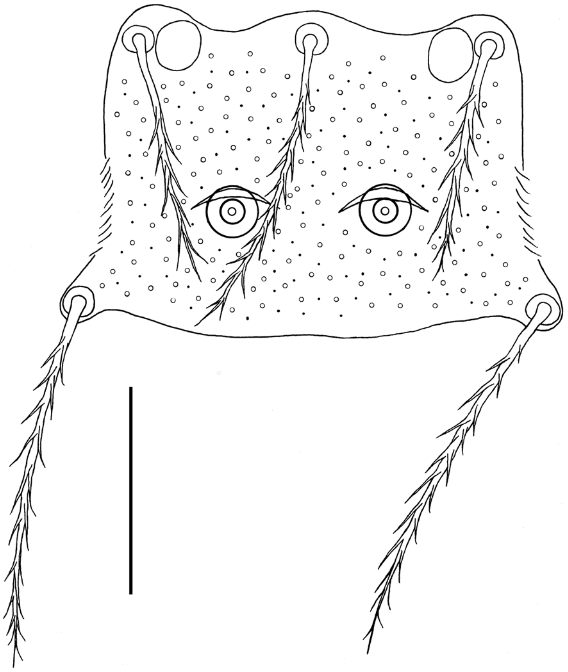

Fig. 3 *Rudnicula goffi* n. sp., larva, paratype. Scutum. *Scale-bar*: 50 µm

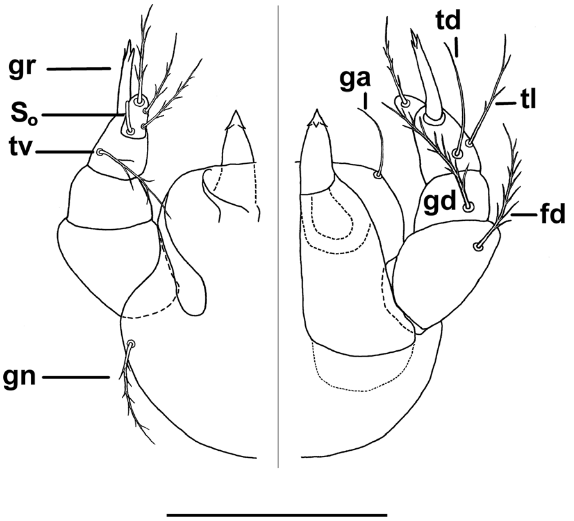

Fig. 4 *Rudnicula goffi* n. sp., larva, paratype. Gnathosoma, ventral view (left); dorsal view (right). *Abbreviations*: Ga, galeal seta; fd, palpofemoral dorsal seta; gd, palpogenual dorsal seta; td, palpotibial dorsal seta; tl, palpotibial lateral seta;. gn, gnathobasal seta; tv, palpotibial ventral seta; S_o, palpotarsal externobasal solenidion; Gr, palpotibial claw. *Scale-bar*: 50 µm

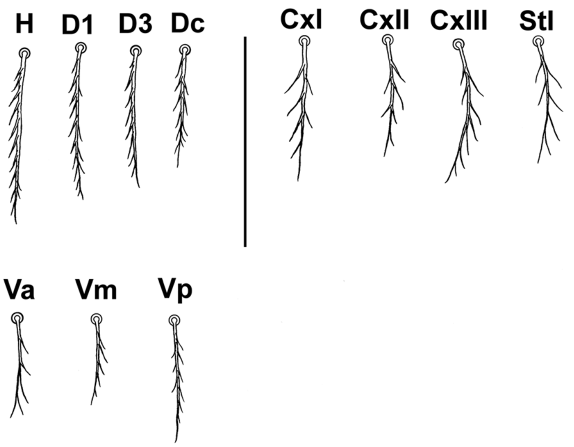

Fig. 5 *Rudnicula goffi* n. sp., larva, paratype. Body setae. *Abbreviations*: H, humeral seta; D, dorsal setae: D1, first row; D3, third row; Dc, caudal seta; V, ventral setae; Va, anterior seta; Vm, medial seta; Vp, posterior seta; C, coxal setae: CxI, first coxal seta; CxII, seta on coxa II; CxIII, seta on coxa III; StI, anterior sternal seta. *Scale-bar*: 50 µm

setae on leg segments: Leg I (pa) – S_1 27(25–29) f_1, (PT′, ST, pST) = N, 2 tibialae 21–27 (23) long and 17–22 (20) long, microtibiala, 3 genualae each 21–26 (24) long, microgenuala; Leg II (pm) – S_2 23 (22–25) f_2, PT″ = N, 2 tibialae 18–24 (20) long and 18–22 (20) long, genuala 16–20 (18) long; Leg III (pp) – tibiala 23–36 (31) long and 1 genuala 29–44 (34) long. Numbers of barbed setae on leg segments (leg formula):

Leg I (pa): Cx (1) – Tr (1) – Bf (1) – Tf (5) – G (4) – Ti (8) – Ta (21)
Leg II (pm): Cx (1) – Tr (1) – Bf (2) – Tf (4) – G (3) – Ti (7) – Ta (16)
Leg III (pp): Cx (1) – Tr (1) – Bf (2) – Tf (3) – G (4) – Ti (6) – Ta (15)

Diagnostic formulae

SIF = 4B.N.3.3111.000; fsp = 7.7.7; fCx = 1.1.1; fPp = BB.NbB; fSt = 2 + 2 + 2; PL > AM > AL; DS = 43–58; VS = 52–67; NDV = 97–120; Ip = 811–940.

Diagnosis

The new species is similar to *Rudnicula tibbi* Vercammen-Grandjean, 1965, *R. dimolinae* (Audy, 1952), *R. becki* Nadchatram, 1968 and *R. hexasternalaea* Brown, 1997 in having fSt = 2 + 2 + 2 but differs from these species by the presence of scutal AL windows as well as by other characters (see the key to the species below). *Rudnicula goffi* n. sp. is similar to *R. barbarae* (Domrow, 1964) in the presence of

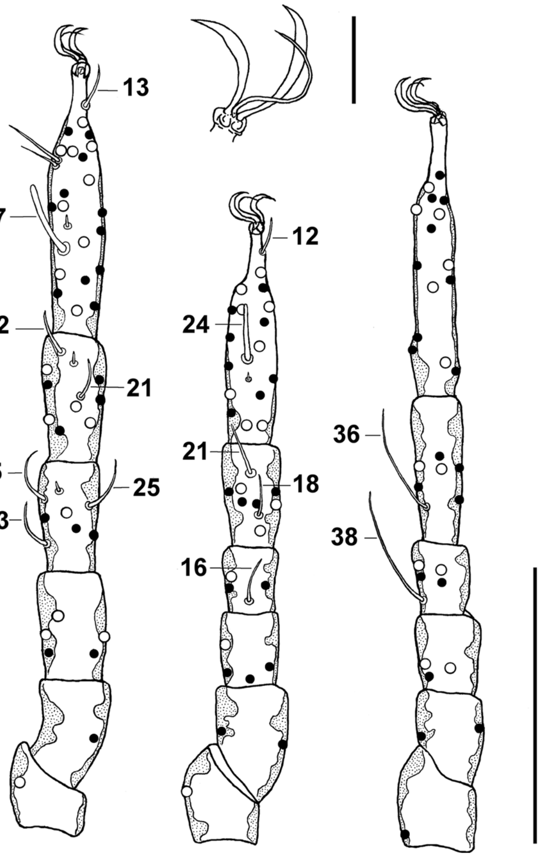

Fig. 6 *Rudnicula goffi* n. sp., larva, paratype. Free parts (trochanter-tarsus) of legs I, II and III (from left to right). *Scale-bar*: 100 μm; Enlarged pretarsus I with claws and empodium (above) *Scale-bar*: 30 μm

scutum with AL windows and in lateral margins with cuticular striations. However, *R. barbarae* differs in having fSt = 2 + 4 + 2 (*vs* fSt = 2 + 2 + 2 in *R. goffi* n. sp.) Other species of *Rudnicula*, namely *R. templei* Nadchatram & Wilson, 1965, *R. meilingensis* (Zhao, 1984), *R. agurensis* Goff & Easton, 1989 and *R. leytensis* Brown, Goff & Nadchatram, 1988 differ from *R. goffi* n. sp. in having fSt = 2 + 2. More details on other differential features are stated in the key below.

Key to the larvae of *Rudnicula* spp.

1a fSt = 2 + 4 + 2; Ga = B; scutum with AL windows; lateral margins with cuticular striations; posterior margin convex; SB = 20–22 μm; DS = 50; VS = 54 ……………………………………………………*Rudnicula barbarae*

1b fSt different ………………………………… 2

2a fSt = 2 + 2 ………………………………… 3

2b fSt = 2 + 2 + 2 ……………………………… 6

3a Scutal AL windows present; Ga = N; fPp = BB.NBB; pST on tarsus I absent; empodia lancetous; lateral scutal margins with cuticular striations ………………… *Rudnicula templei*

3b Scutal AL windows absent ………………….. 4

4a DS = 49–55; VS = 36; NDV < 100 ……………………… *Rudnicula meilingensis*

4b DS = 70–72; VS = 42–56; NDV > 110 ………………………………………………… 5

5a Ga = B; AM = 35–40 μm; PL = 43–47 μm; anterolateral shoulders large; lateral scutal margins without cuticular striations; pm = 246–254 μm …………… *Rudnicula agurensis*

5b Ga = N; AM = 46–53 μm; PL = 56–63 μm; anterolateral shoulders weakly developed; lateral scutal margins with cuticular striations; pm = 221–226 μm …………… *Rudnicula leytensis*

6a Scutal AL windows present; SB = 19–23 μm; Ga = N; length of AL setae < 40 μm; lateral scutal margins with cuticular striations …… ………………………….*Rudnicula goffi* n. sp.

6b Scutal AL windows absent ………………… 7

7a NDV = 116; AL windows absent; other scutal windows present posterior to SB–SB; SB = 31 μm; scutum trapezoidal; Ga = B; length of AL setae > 60 μm ……………………………… ……………………………...*Rudnicula tibbi*

7b 7b NDV < 100; scutum without any scutal windows …………………………………… 8

8a Basal third of scutal setae thickened; length of AL setae 40–50 μm; SB = 17–19 μm; Ga = N; pa = 230 μm; pm = 185 μm; pp = 210 μm; Ip = 625 μm; empodia clawlike …………………….. …………………………..*Rudnicula dimolinae*

8b Basal third of scutal setae not thickened; length of AL setae < 40 μm; legs longer like in previous species ……………………….…. 9

9a SB = 18–19 μm; empodia lanceolate; Ga = N; posterior scutal margin convex; lateral and posterior scutal margins with cuticular striations; pa = 288–292 μm; pm = 234–236 μm; pp = 272–276 μm; Ip = 769–803 μm ……………………………… *Rudnicula becki*

9b SB = 24–26 μm; empodia clawlike; Ga = bifurcate; posterior scutal margin nearly straight, only lateral scutal margins with cuticular striations; pa = 253–278 µm; pm = 214–226 µm; pp = 237–253 µm; Ip = 708–743 µm .... *Rudnicula hexasternalaea*

Discussion

Zhao (1984) mentioned in his original description of *Rudnicula meilingensis* its similarity with *R. tsaochiensis* (Chen & Hsu, 1963), i.e. the synonym of *Trombicula* (*T.*) *tsaochiensis* (Chen & Hsu, 1963). His comparison of the two species is based on different characters, most notably fT = 4B in *R. meilingensis* versus 5B in *R. tsaochiensis*. These differences show that *R. tsaochiensis* was incorrectly placed within the genus *Rudnicula*, which is characterised by palpal tarsal formula 4B. Further revision of the type-material of *T. tsaochiensis* is required to verify its generic status.

Thus, there are ten species of *Rudnicula* currently known, with the new species *R. goffi* included. All species have been found in a relatively geographically restricted area of Asia-Pacific, namely in Sri Lanka, China, South Korea, Malaysia, Philippines, Indonesia, Papua New Guinea and Australia. Almost all the records are from bats (Chiroptera) of the following families: Hipposideridae (7 host species recorded within the family), Rhinolophidae (4 species, +2 undetermined species of the genus *Rhinolophus*), Emballonuridae (2 species), Pteropodidae (2 species), Vespertilionidae (2 species) and Chiroptera fam. gen. sp. (1 species); except for one record of *R. leytensis* from *Macronus striaticeps*, a species of the family Timaliidae (Passeriformes) in the Old World, which is considered accidental. The vast majority of *Rudnicula* spp. can be found in the tropics with only one exception, *Rudnicula tibbi* from the subtropics (Palaearctic zoogeographical region; host *Myotis mystacinus* (Vespertilionidae)).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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8.5. Kalúz S., Šrámek P., Ševčík M. 2023:

A new species of chigger (Acari: Trombiculidae) from Indonesia and current differenciation of *Trombicula* Berlese, 1905 sensu stricto group.

Manuskript zaslaný do časopisu Acta Parasitologica.

A new species of chigger (Acari: Trombiculidae) from Indonesia and current differentiation of *Trombicula* Berlese, 1905 sensu stricto group

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Background Two new species of chiggers in the two genera (*Chiroptella* Vercammen-Grandjean, 1960 and *Rudniculla* Vercammen-Grandjean, 1964) have been described in Indonesia since 2020. The checklist of all known bat-infesting chiggers has also been compiled for Indonesia. It summarizes 16 species in 9 genera of chiggers parasitizing 12 bat species belonging to 5 families collected on 8 Indonesian islands. Nine specimens collected in 2020 on Nusa Penida Island were not included in the checklist because of their uncertain taxonomy. In the current paper, we resolve the taxonomy of these specimens belonging to *Trombicula* sensu stricto group. We also revise the existing characters of this group and provide new characters helping with the clear definition of this taxa.

Methods Hosts and chiggers were captured in February 2020 in Nusa Penida Islands in Indonesia using standard bat-capturing and parasite-collecting methods. For the specimens examined in this study were provided drawings, measurements, and microscopy images. This type material is deposited in the Slovak and Czech National museums.

Result In the paper, we describe a new species parasitizing diadem leaf-nosed bat in the Goa Peteng cave on Nusa Penida Island (Lesser Sunda Islands, East Indonesia). This new species differs from all related species by the presence of a very long tarsala I (S_1). A key to the larvae of all seven known *Trombicula* s. s. species is provided. The differentiation of *Trombicula* s. s. group is based on leg segmentation fsp = 6.6.6, rugose scutum with complete AM, AL and PL setation, and fT = 5B, 5BN or 4B2N.

Conclusion A new species within the genus *Trombicula*, group *minor* has been described. The new species also increases the number of known chiggers in Indonesia and the number of species parasitizing the host species *Hipposideros diadema. Trombicula* s. s. has also been revised and more clearly defined.

Keywords: *Trombicula*, *minor* group, new species, morphology description, South Asia

Introduction

A genus *Trombicula* Berlese, 1905 has been formed by the description of *Trombicula minor* Berlese 1905. The species characters were based on a post-larval specimen collected from bat guano in a cave near Tjiampea (Bogor Regency) in West Java, Indonesia [1].

Characters used to separate/diagnose genera of chiggers were only incomplete at that time. The main reason was that some descriptions of new species lacked diagnostic characters needed for unambiguous generic placement. It led to a higher taxonomy uncertainty of newly described species. Therefore, a relatively widely defined genus *Trombicula* was considered a good option for placing these problematic new species. This caused a significant increase of species within the genus currently classified as *Trombicula* sensu lato. Despite that, a group of species within the genus, now regarded as *Trombicula* sensu stricto (next s.s.), has been formed. The first species belonging to this group can be found in Womersley and Heaslip [2]. They described five *Trombicula* species from South Australia and for two of them mentioned specific differences: *T. chiroptera* Womersley and Heaslip 1943 [...] „Can be separated from other species as in the key and by the strongly rugose scutum", and *T. quadriense* Womersley and Heaslip, 1943 [...] „A very characteristic species in the shape of the dorsal scutum, and the arrangement of the DS".

Vercammen-Grandjean [3] newly characterizes *Trombicula* s. s. by a leg segmentation fsp = 6.6.6. Later authors describing other new species within *Trombicula* s. s. formalized also other diagnostic features of the taxon, like fT = 5B, 5BN or 4B2N [4, 5].

Twenty species of *Trombicula* s. s. have been described so far. They have been found in four (Oriental, Australasian, Neotropical and Afrotropical) zoogeographic region: *T. chiroptera* Womersley and Heaslip 1943; *T. dasyphloea* Domrow, 1958; *T. dewae* Domrow, 1964; *T. geckobia* (Womersley, 1952); *T. lukoschusi*

(Goff, 1983); *T. mitchellensis* (Goff, 1983); *T. quadriense* Womersley and Heaslip, 1943; *T. rugosa* (Goff, 1979); *T. southcotti* Womersley, 1952; *T. thomsoni* Womersley, 1954 (Australia, Australia); *T. hampii* Hiregaudal and Bal, 1956; *T. schmitzi* Oudemans, 1914; *T. hypodermata* Nadchatram and Traub, 1966 (India, South Asia); *T. macclurei* Vercammen-Grandjean and Nadchatram 1963; *T. reticulata* Vercammen-Grandjean and Nadchatram 1963 (Malaysia, South Asia); *T. stoliczkai* Daniel and Stekolnikov 2005 (Pakistan, South Asia); *Trombicula walkerae* Stekolnikov and Matthee, 2019 (South Africa, Africa); *T. minor* Berlese, 1905 (type species) (Indonesia, Malaysia, South Asia); and *T. thresca* Brennan 1967 (Carribean, America) [1, 2, 4–17].

Two new species of the genus *Chiroptella* Vercammen-Grandjean, 1960 and *Rudnicula* Vercammen-Grandjean, 1964 have been recently described from bat hosts in Indonesia (both from host species *Hipposideros diadema* Geoffroy, 1813, Hipposideridae Lydekker, 1891). The authors have also recorded *Whartonia diosi* and *Grandjeana sinensis* for the first time in Indonesia and have provided the checklist of all bat-infesting chigger species known to date [18–20]. All these new records are from a small collection of bat parasites originating from Bali and Nusa Penida islands (Lesser Sunda Islands, East Indonesia). One of these newly collected species could not be included in the above-mentioned papers since it required a more detailed taxonomical study. In this paper, we describe a new species belonging to *Trombicula* s. s. The new species is closely related to *T. minor* Berlese, 1905, a type species of the genus, as well as the entire family.

We have also compiled the key of all known chigger species of the genus *Trombicula* s. s.

Materials and methods

Collection and Preparation of Material

The description of new species is based on material collected from diadem leaf-nosed bat *Hipposideros diadema* (Geoffroy). The host(s) was caught by a hand net on 27 February 2020 in Nusa Penida Island, Ped – Goa Kentung (cave), East Indonesia. All body parts (the face, ears, chiro-, plagio-, uro- patagia, and fur on the back and abdominal parts) of the live hosts were inspected. Mites were collected using tweezers and preserved in 90% ethyl alcohol. After inspection, the hosts were released unharmed within the cave. Swan's embedding medium [21] was used for making permanent microscopic slides. Specimens were placed on slides with ventral and dorsal side continuously.

Terminology

The terminology, abbreviations, and diagnostic formulae of the following authors that are generally accepted in trombiculid taxonomy were used: Vercammen-Grandjean and Langston [22], Goff *et al.* [23], Kudryashova [24] and Stekolnikov [25] with the following abbreviations: Dmin and Dmax, minimal and maximal dorsal setal length; Vmin and Vmax, minimal and maximal length of ventral setae, i.e., setae on the ventral surface of idiosoma excluding coxal and sternal setae; DS, number of dorsal idiosomal and humeral setae; VS, number of ventral idiosomal setae; TaIII, length of tarsus III; m–t, the ratio between the distance from mastitarsala to the base of tarsus III and length of tarsus III. The scale bar in all figures is 50 µm. The taxonomy of hosts follows Wilson and Mittermeier [26].

Images

Drawings were made using a trinocular microscopy Omax 40x2500 with a digital camera and phase contrast optics employed. The figures were prepared with Inkscape v.1.0.1.

The images were taken by an Olympus XC30 digital camera, mounted on the Nikon E600 light microscope. As techniques, the bright-feld and interference contrasts (Nomarsky DIC) were applied. For processing the photos, analySIS Docu v. 5.1 and Corel Photopaint X5 were employed. All measurements are in micrometers.

Depositories

The holotype and paratypes of the new species are deposited in the Slovak National Museum, Bratislava, Slovak Republic [SNM SZ – 10979 (holotype), SZ – 10980 (four paratypes)]; and the National Museum, Prague, Czech Republic [NMP P6A 7392, P6A 7393, P6A 7394, P6A 7495 (four paratypes)].

ZooBank registration: To comply with the regulations set out in Article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature [27].

RESULTS

Systematics

Family Trombiculidae Ewing, 1944

Subfamily Trombiculinae Ewing, 1944

Tribe Trombiculini Vercammen-Grandjean, 1960

Genus *Trombicula* Berlese, 1905

Trombicula Berlese, 1905 [1], p. 155; 1912, pp. 83, 88. Type-species: *Trombicula minor* Berlese, 1905 [1], p. 155; [28], p. 94, by monotypy and subsequent designation.

Trombicula danieli **sp. n.**

(Figs. 1–6)

Description

Diagnosis

SIF = 5BN–B.3.2111.0000; fsp = 6.6.6; fPp = BB.NNB; fCx = 1.1.1; fSt = 2.2; PL> AL > AM; fD = 2H.8(9).6.6.6.2 = 30(31); fV = 4.6.4.3.2.2 = 21 or fV = 4.4.5.5.2.2 = 22 or fV = 4.4.5.5.2.2 = 22 or fV = 4.4.4.4.4.2 = 22 or fV = 4.4.6.4.2 = 20; VS = 20–22; NDV = 50–52; Ip = 790 (765–807).

Larva (Based on the holotype and 8 paratypes; measurements are given in Table 1). Dorsal aspect of idiosoma (Fig. 1 a): Scutum two times wider than long, posterior margin rounded, bearing one AM, one pair of AL and PL setae, and two sensilla. Scutum heavily punctated, posteriorly rugose, puncta cover most of the scutum (Fig. 3a). Sensilla were missing nearly in all specimens, but one filiform sensillum (64 long) was present (Fig. 3b); oval eyes (1 + 1) without ocular plates, anterior eyes 14 (13–14) long, and 12 (12–13) wide, positioned laterally between AL and PL, posterior eye not visible. Measurements of scutum are given in Table 1. PW>AW>SD; ASB>PSB >SB. Dorsum with 1 pair (2H) of humeral setae (Fig. 5a) 46 (43–49) long, dorsal setae (Fig. 5b, c) covered by thick barbs, with minimal length 37 (33–39) and maximal length 42 (38–45), arranged in regular rows. Ventral aspect of idiosoma (Fig. 1b): Two pairs of sternal setae. Each coxa bearing 1 seta (Fig. 6a, b, c), ventral side with 20–22 setae covered by thin barbs (Fig. 5e), regularly arranged (see diagnosis). Ventral setae vary in length, proximal setae shorter 28 (24–30) and caudal longer 40 (36–48). Gnathosoma (Figs. 2a, b): Galeala branched (2–3 barbs). Palps (excluding claw) 48 (43–51) long, 3-pronged palptibial claw slender, 15 (13–17) long, with deeply indented prongs. Ventral side of gnathosoma with one pair of branched setae, hypognathum distinctly punctate, both palpal femur and genu with one branched dorsal seta. Palpal tibia bears 2 branched dorsal setae and one nude (or with 1 branch) ventro-lateral seta. Palpal tarsus bears 5 branched setae, 1 nude (or with 1 branch) subterminala and a solenidion. Cheliceral blade with tricuspid cap. Legs (Fig. 4): Pretarsus and claws present, empodia normal. All setae on legs barbed, slender, relatively short. Specialized setae on leg segments: Leg I (pa) – S1 39 (38–43), f1 4 (4–5) long, (PT′, ST, pST) = N, 2 tibialae (ta) – anterior 21 (18–22), posterior 22 (20–25) long, microtibiala (µta) 6 (5–7), 2 genualae (ga) – anterior 23 (21–27), posterior 22 (20–25), microgenuala (µga) 4 (3–6) long; Leg II (pm) – S2 19 (17–19), f2 2 (2–2), PT′′ = N, 2 tibialae (tm) – anterior 18 (17–20), posterior 18 (16–20), genuala (gm) 18 (18–20); Leg III (pp) – tibiala (tp) 24 (22–25), genuala (gp) 23 (22–25), no more specialized setae. Number of barbed setae on leg segments (leg formula): Leg I (pa): Cx (1) – Tr (1) – F (6) – G (3) – Ti (8) – Ta (20); Leg II (pm): Cx (1) – Tr (1) – F (6) – G (3) – Ti (6) – Ta (15); Leg III (pp): Cx (1) – Tr (1) – F (4) – G (3) – Ti (6) – Ta (13). Legs lengths of holotype and paratypes (n = 8) are given in Table 1.

Type host: *Hipposideros diadema* (Geoffroy), diadem leaf-nosed bat or diadem roundleaf bat (Chiroptera: Hipposideridae).

Type locality and date: Ped – Goa Kentung (cave) (Lat. -8.69104, Long. 115.49926), Nusa Penida Island, East Indonesia, 27 February 2020, collected by P. Šrámek.
Deposited specimens: Holotype SNM SZ–10979 (unengorged larva), four paratypes SNM SZ–10980, four paratypes NMP P6A 7392, P6A 7393, P6A 7394.
Etymology: Patronymic; named in honor of Dr. Milan Daniel (Czech Republic) for his valuable contribution to the taxonomy and ecology of chiggers.

Differential diagnosis

The new species differs from all related species by the presence of a very long tarsala I (S_1). *Trombicula danieli* sp. is in general similar to *T. chiroptera* and *T. thresca*, but differs from both species by fD = 2H.8.6.6.4., while in *T. chiroptera* and *T. thresca* dorsal formulas start with fD = 2H.6.6.4.4.; moreover *T. danieli* sp. n. has fT = 5BN, while other related species *T. minor*, *T. chiroptera* and *T. hypodermata* have fT = 5B. Other differences from similar species are in palpal setal formula, where for *T. danieli* n. sp. is fPp = BB.NNB, while in *T. thresca* is fPp = BN.NNB and in all remaining species (*T. quadriense*, *T. walkerae*, *T. minor*, *T. chiroptera* and *T. hypodermata*) is fPp = BB.BBB. More detailed differential features are stated in the key.

A key to larvae of the genus *Trombicula* sensu stricto with leg segmentation fsp = 6.6.6;
rugose scutum with complete AM, AL and PL setation; fT = 5B, 5BN or 4B2N

1a Tarsala I (S1) very long (38–43), as long as half of tarsus including pretarsus, Indonesia;
.... *T. danieli* sp. n. Kalúz, Šrámek and Ševčík
1b Tarsala I (S1) short (14–22), as long as one-third of tarsus including pretarsus ....
2a Dorsal formula starting fD = 2H.6.6.6, Ga = B, fT = 5B, fV = 4.4.4.4.2, Australia;
.... *T. chiroptera* Womersley and Heaslip 1943
2b Dorsal formula starting fD = 2H.9.6.6 or fD = 2H.8.6.6 ....
33a fSt = 2+2+2, dorsal eyes single (1+1), Pakistan;
.... *T. hypodermata* Nadchatram and Traub, 1966
3b fSt = 2+2, dorsal eyes doubled (2+2) ....
4a VS = 32, NDV = 64, Indonesia; .... *T. minor* Berlese, 1905
4b VS = less than 25, NDV = 44–60 5
5a VS = 22, NDV = 60, Australia; .... *T. southcotti* Womersley, 1952
5b VS = 16–21, NDV = 44–50 6
6a fV = 2.6.2.4.2, AW = 56–62, PW = 81–92, AP = 18–20, SB = 22–24, PL = 40–44, Australia;
.... *T. quadriense* Womersley and Heaslip, 1943
6b fV = 4(5).4.2.4.4.2, AW = 48–53, PW = 68–75, AP = 23–27, SB = 14–18, PL = 32–36, Africa;
.... *T. walkerae* Stekolnikov and Matthee, 2019

Discussion

Current status of *Trombicula* sensu stricto

The taxonomy of *Trombicula* s. s. is complicated. Some of the above-mentioned species [see Introduction, 1, 5, 13] have already been moved into other genera, while the status of remaining remains is uncertain. Among species formerly placed within the genus *Trombicula* s. s. the taxonomic positions have been changed for the following taxa with leg segmentation fsp = 7.7.7, rugose scutum and very long S1: *T. dasyphloea* Domrow, 1958 = *T. (C.) dasyphloea* [29]; *T. lukoschusi* (Goff, 1983) = *T.* (*C.*) *lukoschusi* [30]; *T. macclurei* Vercammen-Grandjean and Nadchatram 1963 = *T.* (*C.*) *macclurei* [31]; *T. mitchellensis* (Goff, 1983) = *T.* (*C.) mitchellensis* [30]; *T. rugosa* (Goff 1979) = *T. (C.) rugosa* [30].
The status of the other species with fsp = 7.7.7 and rugose scutum is also uncertain: *T. dewae* Domrow, 1964; *T. reticulata* Vercammen-Grandjean and Nadchatram 1963; *T. schmitzi* Oudemans, 1914; *T. thomsoni* Womersley, 1954 and *T. stoliczkai* Daniel and Stekolnikov 2005. The species with fsp = 6.6.6 – *Traghardula geckobia* (Womersley, 1952) = *Womersleyna* (*Womersleyna*) *geckobia* [32], but Domrow and Lester [33] placed this species in *Trombicula* s. s. – taxon including species with galeal setae enormous and plumose, with not rugose pentagonal scutum. Therefore, we doubt that this species belongs to *Trombicula* s. str. The taxonomic position of *T. southcotti* Womersley, 1952 from Australia [33] has not changed [30]. We also

regard it as correctly placed. We obtained the information about the characters of this species from the original paper. Despite missing fsp formula and incomplete figures the majority of characters clearly places this species within *Trombicula s. s.* Therefore, we have decided to include *T. southcotti* in the key. The description of *T. thresca* Brennan, 1967 is also incomplete, fsp is missing. But sparsely punctate subpentagonal scutum is figured as well as palpal tarsus with 5B.S. Since we lack all characters needed for an unambiguous taxonomical decision we, tentatively, don´t include the species in the *Trombicula* s. str. and, therefore, don´t mention it in the key.

Species *T. hampii* is considered a *nomen nudum* its precise taxonomic status remains uncertain. It is tentatively retained in the genus *Trombicula* [34]. The description of two other *Trombicula* species, namely *T. caparti* Vercammen-Grandjean, 1962 and *T. cooremani* Vercammen-Grandjean, 1962 has been based on nymphal specimens only and, such as, they cannot be included in the key.

Habitat (?) of *Trombicula* sensu stricto species

All specimens of *Trombicula danieli* sp. n. come from just one host species *Hipposideros diadema*. It was caught by a hand net in Ped – Goa Kentung (cave). This bat species roosts during the day in small groups in caves, old mines, sheds, hollow trees, tree branches and buildings [26, 35].

Records of other *Trombicula* s. s. species from different bat hosts don´t shed light on a typical habitat of this parasitic taxon. The host species either utilize a wide range of habitats or their habitat is unknown: *T. chiroptera* of Chiroptera indet., unknown habitat; *T. minor* of both *Taphozous affinis* and *T. melanopogon* guano deposits inhabits caves, hollow trees and rock crevices; *T. southcotti* of bandicoot wide variety of habitats, from rainforests to wet and dry woodlands to heath. Bandicoots are nocturnal and spend a day in shallow holes in the ground; *T. quadriense* of *Chalinolobus gouldii* inhabits tree hollows, foliage and man-made structures [1, 2, 13, 36].

Two species – *T. hypodermata* from Pakistan, India; and *T. walkerae* from South Africa; Nadchatram and Traub [4], Stekolnikov and Matthee [5] have also noted records from Eulipotyphla and Rodentia (*Bandicota* Gray, *Mastomys* Thomas, *Mus* Clerck, *Nesokia* Gray, *Rattus* Fischer de Waldheim, *Suncus* Ehrenberg and *Tatera* Lataste). These hosts utilize a wide range of habitats with a preference for human settlement and its surroundings.

Data Availability Statement

Data is available upon request from the corresponding author.

Acknowledgements

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Declarations

Conflict of interest

The authors declare there are no conflicts of interest.

Ethical approval

All applicable institutional, national and international guidelines for the care and use of animals were followed.

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Figures

Fig. 1. *Trombicula danieli* sp. n. from *Hipposideros diadema* Geoffroy; Photomicrograph parts of larva specimen; holotype (SNM SZ–10979). **a** Dorsal aspect of idiosoma; **b** Ventral aspect of idiosoma; Scale: 100 µm

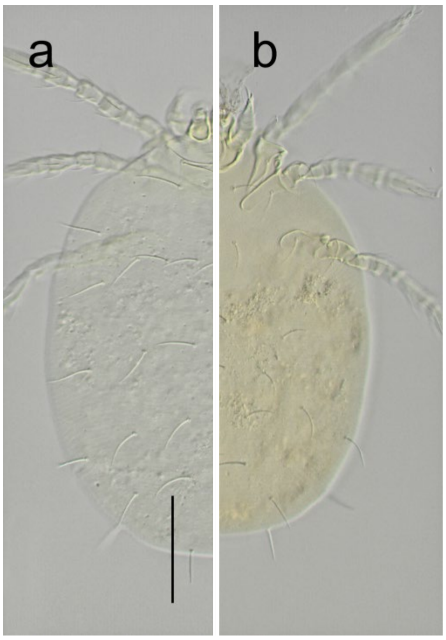


Fig. 2. *Trombicula danieli* sp. n. from *Hipposideros diadema* Geoffroy; Photomicrograph parts of larva specimen; paratype (SNM SZ–10980). **a** Ventral aspect of gnathosoma; **b** Dorsal aspect of gnathosoma; Scale: 50 µm
Abbreviations: Cb – cheliceral base; Cs – cheliceral blade; Ga – galeal (deutorostral) seta; gns – gnathocoxal (tritorostral) seta; ic – infracapitulum (gnathobase, gnathocoxa); PC – palpal claw (odontus); PF – palpal femur; PG – palpal genu; PTa – palpal tarsus; PTi – palpal tibia

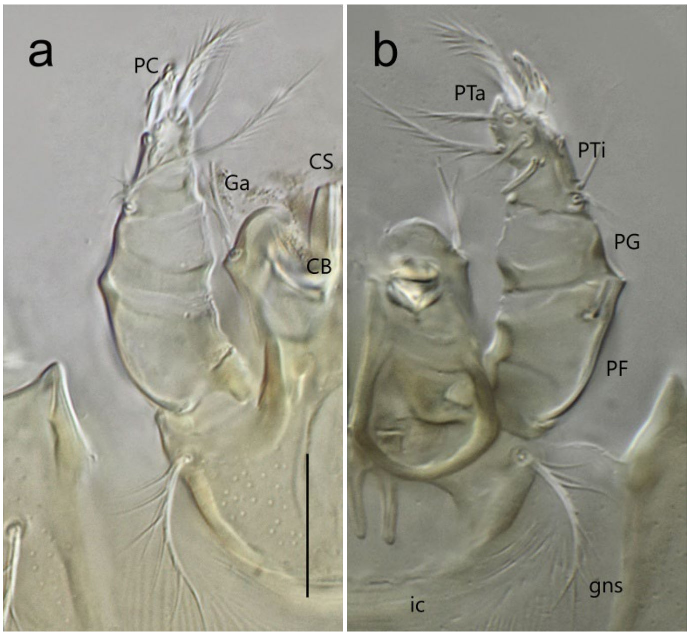


Fig. 3 *Trombicula danieli* sp. n. from *Hipposideros diadema* Geoffroy; Photomicrograph parts and line drawing of larvae specimens; paratype (SNM SZ–10980). **a** scutum; **b** enlarged sensillum. Scale: 50 µm
Abbreviations: AL – anterolateral scutal seta; AM – anteromedian scutal seta; PL – posterolateral scutal seta; S – sensillum (trichobothrium); Scale: 50 µm

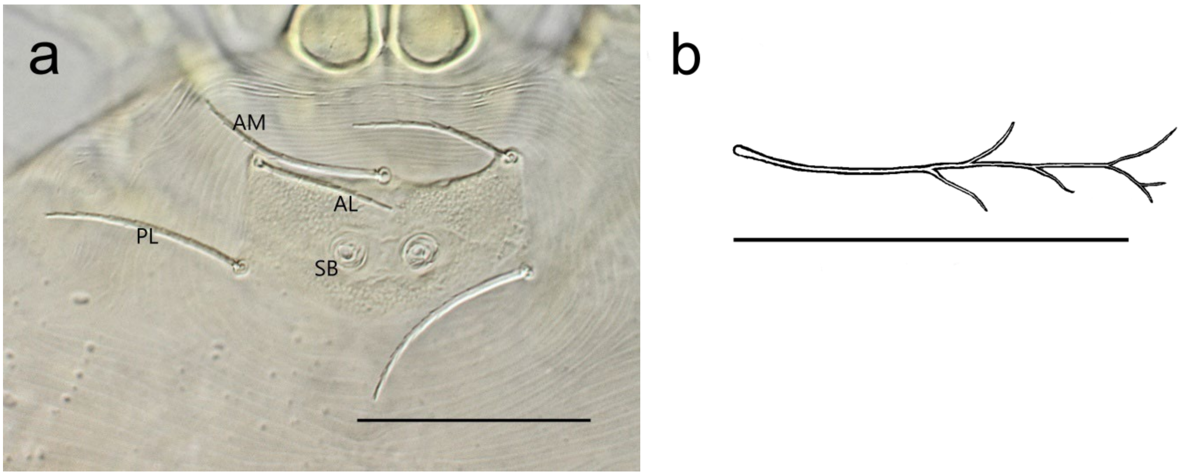


Fig. 4 *Trombicula danieli* sp. n. from *Hipposideros diadema* Geoffroy; Line drawing of larva specimen; holotype (SNM SZ–10979). From left to the right – Leg I; Leg II; Leg III. Scale: 100 µm
Abbreviations: f1 – microtarsala I (ε); f2 – microtarsala II (ε); ga – genuala I (σ); gm – genuala II (σ); gp – genuala III (σ); µga – microgenuala I (κ); µta – microtibiala I (κ); pST – parasubterminala (z); PT' – pretarsala I (ζ); PT'' – pretarsala II (ζ); S1 – leg tarsala I (ω); S2 – leg tarsala II (ω); ST – subterminala (ζ); ta – tibiala I (φ); tm – tibiala II (φ); tp – tibiala III (φ); Scale: 100 µm

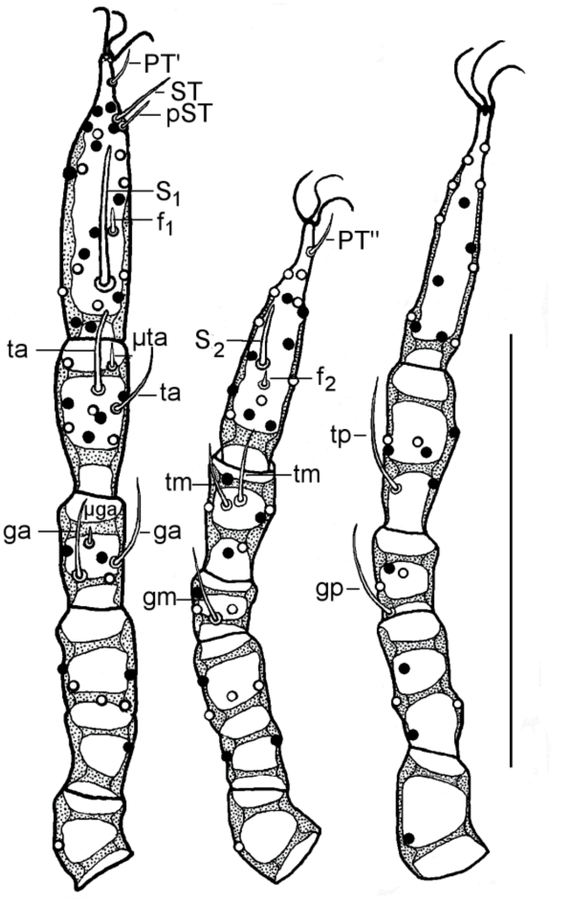
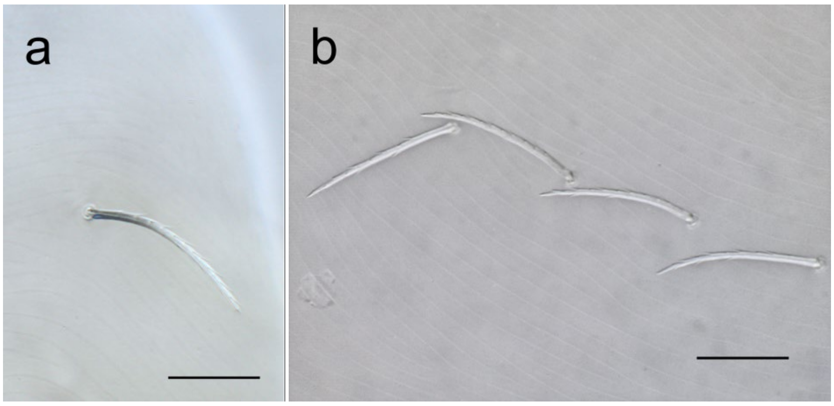


Fig. 5 *Trombicula danieli* sp. n. from *Hipposideros diadema* Geoffroy; Photomicrograph parts of larva specimen; holotype (SNM SZ–10979). **a** humeral seta; **b** dorsal seta (1-st row); **c** dorsal seta (2-nd row); **d** caudal and terminal setae; **e** ventral seta. Scale: 50 μm



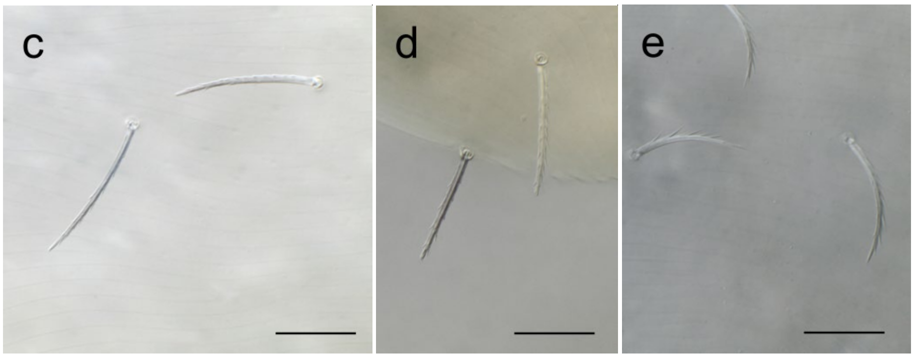


Fig. 6 *Trombicula danieli* sp. n. from *Hipposideros diadema* Geoffroy; Photomicrograph parts of larva specimen; holotype (SNM SZ–10979). **a** coxal seta I; **b** coxal seta II. Scale: 50 µm

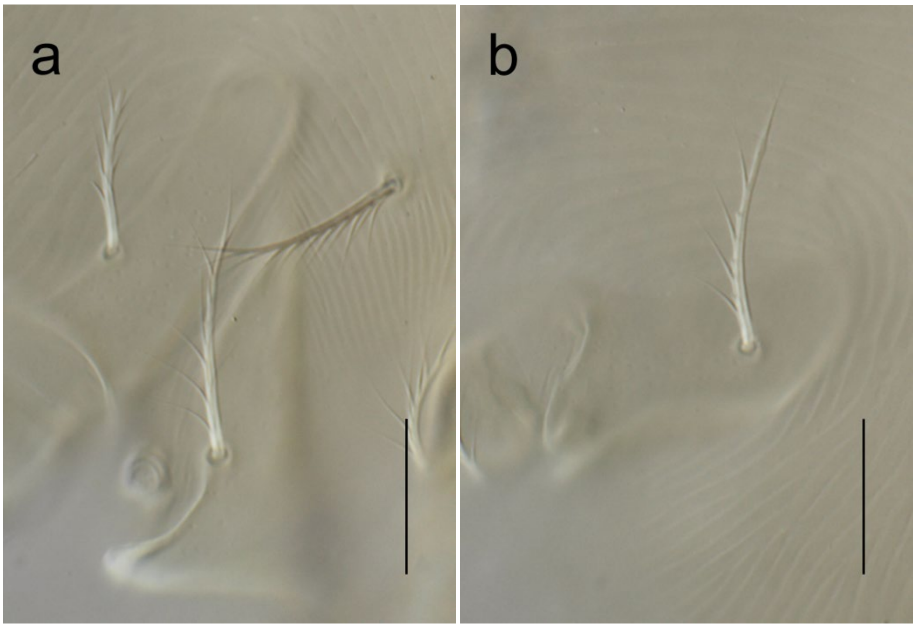


Table 1 Measurements of holotype and paratypes of *Trombicula danieli* sp. n.

Variable	Sample size	Range	Mean ± StD	Holotype
AW	9	57–61	59± 1	57
PW	9	65–77	71±4	70
SB	9	17–19	18±1	17
ASB	9	20–27	23±2	22
PSB	9	16–24	19±2	24
SD	9	38–48	42±3	46
AP	9	24–28	27±1	24
AM	9	43–49	45±2	46
AL	9	36–41	38±2	38
PL	9	42–49	47±2	46
S	1	64	64	–
H	9	43–49	46±2	45
DS	9	30–31	30±0	30
VS	9	20–22	22±1	22
NDV	9	50–52	52±1	52
D_{min}	9	33–39	37±2	38
D_{max}	9	38–45	42±2	43
V_{min}	9	24–30	28±2	26
V_{max}	9	36–48	40±3	36
pa	9	265–295	283±10	270
pm	9	231–248	240±6	232
pp	9	261–275	267±5	263
Ip	9	765–807	790±15	765
TaIIIL	9	70–77	73±2	71
TaIIIW	9	13–15	14±1	13

Abbreviations: AW – distance between scutal anterolateral setae; PW – distance between scutal posterolateral setae; SB – distance between sensilla; ASB – distance from extreme anterior margin of scutum to the level of sensillary bases; PSB – distance from extreme posterior margin of scutum to the level of sensillary bases; SD – length of scutum (ASB + PSB); AP – distance from anterolateral to posterolateral scutal setae on one side; AM – length of scutal anteromedian seta; AL – length of anterolateral scutal setae; PL – length of posterolateral scutal setae; S – length of sensilla; H – length of humeral setae; DS – number of dorsal idiosomal and humeral setae; VS – number of ventral idiosomal setae (excluding coxal and sternal setae); NDV – number of idiosomal setae (DS + VS); D_{min} – length of the shortest dorsal idiosomal seta; D_{max} – length of the longest dorsal idiosomal seta; V_{min} – length of the shortest ventral idiosomal seta; V_{max} – length of the longest ventral idiosomal seta; pa – length of leg I (including coxa); pm – length of leg II (including coxa); pp – length of leg III (including coxa); Ip – total length of legs I–III; TaIIIL – length of leg tarsus III; TaIIIW – width of leg tarsus III; StD – standard deviation.

8.6. Ševčík M., Kalúz S., Šrámek P. 2022:

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ORIGINAL PAPER

Bat-Infesting Chiggers (Trombiculidae) in Indonesia: Current Review, Distribution, and Hosts with Three New Records and their Morphometric Data

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Abstract

Purpose Even though the taxonomy of bat-infesting chiggers has been studied extensively, information about the distribution and morphological variability of many species is still lacking. In fact, the only available distribution records for several species are their type localities. The purpose of this paper is to broaden the knowledge of the Indonesian group.

Methods Hosts and chiggers were captured from April 2018 to March 2020 in Bali and Nusa Penida Islands in Indonesia by using standard bat-capturing and parasite-collecting methods. A list of bat-infesting chiggers in Indonesia was compiled by searching the public databases using specific keywords.

Results When compared with already published data on type specimens, the physical measurements and morphology of *Whartonia diosi*, *W. maai* and *Grandjeana sinensis* revealed only minor differences that fell within intraspecific variability of the mentioned species. Two species (*W. diosi* and *G. sinensis*) are recorded here for the first time outside of their type localities (Panay Island in the Philippines and China) and, in both cases, represent the first records for Indonesia. The checklist summarizes all 16 species in 9 genera of bat-infesting chiggers collected from 12 bat species belonging to 5 families all collected on 8 Indonesian islands.

Conclusion Indonesia comprises more than 17,000 islands, is home to over 200 bat species, and harbors hundreds of still undiscovered caves. With such a huge potential for chigger diversity and endemism, it is likely that our results document only a fraction of the actual diversity.

Keywords Chiggers · Chiroptera · Indonesian Islands · Taxonomy · Distribution · Southeast Asia

Introduction

“Chiggers” (larvae of the mite family Trombiculidae) can infest a wide range of hosts from insects to humans [1, 2]. The most commonly infested taxa are rodents, followed by insectivores, birds, and primates, which are rarely infested [1, 3]. Among these hosts, a relatively low host fidelity can be observed. However, a different situation is known in bats, which also serve as hosts. Chiggers infesting bats appear to be significantly more host-specific and can be only very rarely found on any other animal group. The specific life history (ability to actively fly) and the unique habitat that bats occupy (e.g., cave walls, rock crevices, and tree hollows) are the main reasons for their isolation, which leads to very limited physical contact with other mammals [4].

Twenty trombiculid genera considered to be bat-specialized parasites are known to date in both the Old and New World [3, 5–8]. The number of species in this group reaches its peak in the tropics [cf. 9]. Even though the taxonomy of bat-infesting chiggers has been studied extensively and results published in many papers, information about the distribution and morphological variability of many species is still lacking. Indeed, the only available distribution records for several species are their type localities. Even though the summary of South-East Asian chiggers has been published recently [10, 11], it does not mention two new

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recently described species of chiggers that we discovered on the Indonesian island of Bali: *Chiroptella baliensis* Ševčík, Kalúz and Šrámek, 2020 and *Rudnicula goffi* Kalúz, Šrámek and Ševčík, 2020 [12, 13].

In this article, we include an updated summary of all published records of chigger species infesting Indonesian bats. We also provide records for several species that we newly collected in the field. Three of them are recorded from Indonesia for the first time, with two of them being firstly recorded outside their type localities. For these three new Indonesian species, we provide new morphometric data and compare them with already published type specimens data.

Materials and Methods

Study Area

The study area includes the Indonesian Archipelago from a geopolitical point of view. It comprises more than 17,000 islands with the following major groups: Greater Sunda Islands (Borneo, which is divided among Brunei, Malaysia, and Indonesia; Sumatra; Java), Lesser Sunda Islands (Bali, Lombok, Sumbawa, Flores, Sumba, and Timor, which is divided between East Timor and Indonesia), Maluku Islands and New Guinea, which is divided between Papua New Guinea and Western New Guinea.

Literature Sources

The Indonesian Archipelago belongs geographically within the "Tsutsugamushi triangle" (South Asia together with Myanmar, Malaysia, Thailand, and Vietnam). The local chigger fauna has been intensively studied during the last 60 years since scrub typhus, which is caused by *Orientia tsutsugamushi* and transmitted by chigger mites, is endemic there. The result is many papers that deal with bat-infesting chiggers in South-East Asia either as their main subject or just marginally, thus providing (first) records of genera or species in Indonesia: Womersley [14, 15], Domrow [16, 17], Nadchatram and Wilson [18], Nadchatram [19, 20], Vercammen-Grandjean [21] and Goff [22].

The completeness of the checklist, together with the revision of the synonyms, was attained by searching the following databases: Web of Science, Biodiversity Heritage Library, and Google Scholar.

Collection of Material

In April 2018, February 2019, February 2020, and March 2020, all bat host specimens for the study were collected by using hand nets in three localities on Bali and Nusa Penida Islands (Bali province, Lesser Sunda Islands). All chiggers were removed from the plagiopatagium, uropatagium, and dactylopatagium of the hosts by forceps and placed in 96% ethanol. The hosts were released unharmed after photographic evidence was taken and morphological identification established according to the keys in Csorba *et al.* [23] and Francis and Barrett [24].

Chigger specimens were then prepared in a laboratory. All were mounted onto slides using Swan's embedding medium [25]. They were identified and measured by employing standard microscopy (Karl Zeiss Jena, Germany). All collected specimens were compared to published descriptions and measurements of type materials in the articles of Nadchatram and Wilson [18], Zhao and Qiu [26], Brown [27], and Fain [28].

Terminology and Construction of the Checklist

The terminology used in Tables 1, 2 and 3 follows Vercammen-Grandjean and Langston [1], Goff *et al.* [29], and Kudryashova [30] with the following abbreviations: D_{min} and D_{max}, minimal and maximal dorsal setal length; V_{min} and V_{max}, minimal and maximal length of ventral setae, i.e., setae on the ventral surface of idiosoma excluding coxal and sternal setae; DS, number of dorsal idiosomal and humeral setae; VS, number of ventral idiosomal setae; TaIII, length of tarsus III; m–t, the ratio between the distance from mastitarsala to the base of tarsus III and length of tarsus III. The scale bar in all figures is 50 µm.

The checklist is arranged in taxonomical, alphabetical, and chronological orders and includes information for each taxon in the following order: family, subfamily, tribe, genus, species, synonyms review, new material examined, known material already published, added remarks (only for the three species new to Indonesia), type specimens data (only holotype) and deposition, published records from Indonesia as well as extralimital records (if published).

The taxonomy of bat species follows Burgin *et al.* [31]. The family-level systematics of chiggers is based on the classification by Kudryashova [30] and Shatrov and Kudryashova [32], who both regard Trombiculinae, Leeuwenhoekiinae, Apoloniinae, and Gahrliepiinae as subfamilies within the Trombiculidae. For the generic-level systematics, we follow Stekolnikov [33, 34] and within the species-level we follow Berlese [35], Schluger *et al.* [36], Womersley [15], Nadchatram and Wilson [18], Vercammen-Grandjean [21], Nadchatram and Lakshumy [37], Nadchatram [20], Goff [22], Fain [28], Kalúz and Ševčík [38], Kalúz *et al.* [12] and Ševčík *et al.* [13]. Species are ordered alphabetically regardless of whether the findings were already published or not in the refereed scientific literature.

Table 1 Measurements and counts for *Whartonia diosi*

Variable	Range (of 19)*	Mean ± StD*	Range (of 3)**	Mean ± StD**	Holotype***	Paratype***
AW	91–97	95 ± 2	90–98	93 ± 4	87	90
PW	95–107	101 ± 3	98–100	99 ± 1	99	99
SB	35–40	38 ± 3	38–41	39 ± 2	39	–
ASB	35–40	38 ± 2	33–38	36 ± 6	42	39
PSB	15–17	16 ± 1	15–17	16 ± 1	15	15
SD	52–57	53 ± 2	48–54	52 ± 3	57	53
AA	9–13	11 ± 1	10–13	12 ± 2	–	–
PP	22–27	24 ± 2	20–25	23 ± 3	–	–
AP	22–27	24 ± 1	22–24	23 ± 1	21	18
AM	60–70	64 ± 3	60–62	62 ± 2	60	63
AL	45–60	52 ± 4	43–55	50 ± 6	53	51
PL	75–85	81 ± 3	80–85	82 ± 3	82	80
H	71–80	75 ± 3	73–77	75 ± 2	66	73
DS	44–56	51 ± 3	44–55	48 ± 6	51	–
VS	44–56	46 ± 3	43–48	46 ± 3	59	–
NDV	91–104	96 ± 4	91–98	94 ± 4	110	–
$\mathrm{D}_{\mathrm{min}}$	62–72	67 ± 3	62–72	67 ± 5	63	75
$\mathrm{D}_{\mathrm{max}}$	70–85	78 ± 4	70–85	78 ± 8	84	85
$\mathrm{V}_{\mathrm{min}}$	40–60	47 ± 6	46–50	48 ± 2	45	45
$\mathrm{V}_{\mathrm{max}}$	65–75	71 ± 4	65–80	71 ± 8	80	75
pa	438–490	458 ± 17	420–440	430 ± 10	486	–
pm	375–420	403 ± 20	310–390	340 ± 44	402	–
pp	423–481	448 ± 17	440–490	460 ± 27	450	–
Ip	1225–1380	1304 ± 43	1180–1280	1230 ± 50	1322	1338
TaIIIL	116–124	121 ± 2	114–122	119 ± 4	–	–
TaIIIW	20–25	21 ± 2	20–25	23 ± 3	–	–

* and **Specimens discovered by examining new material; *** after Fain (2002) [28]

AW distance between scutal anterolateral setae, *PW* distance between scutal posterolateral setae, *SB* distance between sensilla, *ASB* distance from extreme anterior margin of scutum to the level of sensillary bases, *PSB* distance from extreme posterior margin of scutum to the level of sensillary bases, *SD* length of scutum (ASB + PSB), *AA* distance between bases AM–AM, *PP* distance between bases of PL and caudal scutal margin, *AP* distance from anterolateral to posterolateral scutal setae on one side, *AM* length of scutal anteromedian seta, *AL* length of anterolateral scutal setae, *PL* length of posterolateral scutal setae, *H* length of humeral setae, *DS* number of dorsal idiosomal and humeral setae, *VS* number of ventral idiosomal setae (excluding coxal and sternal setae), *NDV* number of idiosomal setae (DS + VS), D_{min} length of the shortest dorsal idiosomal seta, D_{max} length of the longest dorsal idiosomal seta, V_{min} length of the shortest ventral idiosomal seta, V_{max} length of the longest ventral idiosomal seta, *pa* length of leg I (including coxa), *pm* length of leg II (including coxa), *pp* length of leg III (including coxa), *Ip* total length of legs I–III, *TaIIIL* length of leg tarsus III, *TaIIIW* width of leg tarsus III, *StD* standard deviation

Images

The images were taken by an Olympus XC30 digital camera, mounted on the Nikon E600 light microscope. As techniques, the bright-field and interference contrasts (Nomarsky DIC) were applied. For processing the photos, analySIS Docu v. 5.1 and Corel Photopaint X5 were employed.

Voucher specimens and photo vouchers of hosts are deposited in the National Museum (Natural History), Prague, Czech Republic.

Depositories

The list of depositories containing type series of Indonesian chigger species is included. The following abbreviations are used:

BPBM Bernice Pauahi Bishop Museum, Honolulu, Hawaii, US.

BMNH The Natural History Museum (formerly, British Museum of Natural History), London, UK.

IMR Institute for Medical Research, Kuala Lumpur, Malaysia.

Table 2 Measurements and counts for *Whartonia maai*

Variable	Range (of 5)*	Mean	Holotype	Mean (of 8)**	Range**	Range (of 7)***	Mean***
AW	135–148	142 ± 4	146	146	140–148	137–153	143
PW	139– 149	141 ± 4	154	152	148–155	137–148	143
SB	46–50	48 ± 2	53	50	49–53	45–48	47
ASB	48–57	52 ± 4	51	50	48–52	45–50	46
PSB	18–20	19 ± 1	18	19	18–20	14–16	15
SD	68–75	71 ± 4	69	69	68–71	–	–
AA	16–20	18 ± 2	–	–	–	–	–
AP	34–39	37 ± 2	35	35	32–36	27–36	31
AM	78–85	81 ± 4	86	82	80–86	68–81	75
AL	59–73	67 ± 5	60	66	60–68	58–68	63
PL	93–98	93 ± 3	93	86	82–93	75–91	85
H	93–107	101 ± 5	–	–	100	–	–
DS	57–68	61 ± 4		–	51–64	58–62	
VS	55–60	58 ± 2		–	88–100	90–96	
NDV	112–122	118 ± 4	–	–	138–164	138–148	–
D_{min}	59–71	65 ± 6	–	75	–	–	–
D_{max}	78–90	86 ± 7	–	88	–	–	–
V_{min}	39–50	47 ± 5	–	50	–	–	–
V_{max}	69–84	78 ± 6	–	75	–	–	–
pa	580–634	603 ± 21	–	–	–	468–536	–
pm	514–536	525 ± 8	–	–	–	427–428	–
pp	570–601	587 ± 11	–	–	–	463–531	–
Ip	1687–1749	1715 ± 31	–	–	–	–	–
TaIIIL	158–170	162 ± 5	–	–	–	–	123
TaIIIW	20–23	21 ± 1	–	–	–	–	21

Abbreviations see under Table 1

*Specimens discovered by examining new material; ** after Nadchatram and Wilson [18]; *** after Brown [27]

MHNG Natural History Museum of Geneva, Geneva, Switzerland.

NMP National Museum (Natural History), Prague, Czech Republic.

PNMM Philippine National Museum of Manila, Philippines.

SAM South Australian Museum, Adelaide, South Australia, Australia.

SNM Slovak National Museum, Bratislava, Slovak Republic.

USNM National Museum of Natural History (formerly, United States National Museum), Washington D.C., US.

Other abbreviations: P Preparate.

Results

Family Trombiculidae Ewing, 1944

Subfamily Leeuwenhoekiinae Womersley, 1944

Tribe Leeuwenhoekiini Vercammen-Grandjean, 1968

Genus *Whartonia* Ewing, 1944

Whartonia caobangensis Schluger, Grochovskaja, Ngu, Hoe and Tung, 1959

Whartonia caobangensis Schluger, Grochovskaja, Ngu, Hoe and Tung, 1959 [36], p. 422, Fig. 3: Domrow [17], p. 2; Audy *et al.* [39], p. 14; Chau *et al.* [40], p. 188; Stekolnikov [10], p. 51.

Type data and deposition.

Whartonia caobangensis: Vietnam: North Vietnam, *Hipposideros armiger* (H.) (Hipposideridae), ZMMU Tdt 3175 (syntypes) [36]; *Whartonia recurvata*: China: Kukong, Mapa, Kwangtung (Guangdong), *Pipistrellus pipistrellus* (S.) (Vespertilionidae), *H. larvatus* (H.) (as *Hipposideres poutensis*), ZSM [41].

Table 3 Measurements and counts for *Grandjeana sinensis*

Variable	Range (of 13)*	Mean ± StD*	Min**	Max**	Mean**
AW	51–87	61 ± 9	62	63	62,5
PW	70–92	85 ± 6	85	84	84,5
SB	13–24	19 ± 3	20	22	21
ASB	24–35	30 ± 3	29	33	31
PSB	12–21	17 ± 3	14	14	14
SD	36–53	46 ± 4	43	47	45
AP	28–43	36 ± 4	34	34	34
AM	34–41	39 ± 2	36	34	35
AL	30–40	36 ± 4	34	36	35
PL	40–57	47 ± 5	43	47	45
H	30–44	39 ± 5	–	–	–
DS	46–64	55 ± 5	–	–	60
VS	44–65	55 ± 6	–	–	50
NDV	90–128	110 ± 10	–	–	110
D_{min}	22–36	27 ± 4	–	–	–
D_{max}	28–43	34 ± 4	–	–	–
V_{min}	15–25	22 ± 3	–	–	–
V_{max}	24–36	30 ± 3	–	–	–
pa	250–341	281 ± 33	250	278	–
pm	221–267	240 ± 15	222	236	–
pp	237–340	268 ± 28	250	264	–
Ip	705–948	788 ± 72	–	–	–
TaIIIL	68–97	83 ± 8	–	–	–
TaIIIW	13–18	15 ± 2	–	–	–

Abbreviations see under Table 1

*Specimens discovered by examining new material; **after Zhao and Qiu [26]

Indonesian Record.

Tjiampea caves, near Bogor, Java; *Hipposideros* spp. [possibly *H. larvatus* and/or *H. diadema* (G.)] [17].

Other records.

China: Yunnan; *Rhinolophus ferrumequinum* (S.) (Rhinolophidae) [42].

Malaysia: Batu Caves, Selangor; *Hipposideros* sp. (Hipposideridae); Kepong, Selangor; *Hipposideros* sp. (Hipposideridae) [17];

Maliau Basin; *H. bicolor* (T.), *H. cervinus* (G.) (Hipposideridae);

Royal Belum; *H. bicolor* (Hipposideridae);

Gunung Mandi Angin; *H. larvatus* (Hipposideridae);

Gunung Stong; *H. larvatus* (Hipposideridae), *Kerivoula hardwickii* (H.) (Vespertilionidae);

Ulu Muda; *R. affinis* H. (Rhinolophidae) [43].

Gunung Stong Forest Reserve, Kelantan; *H. bicolor*, *H. larvatus* (Hipposideridae) [44].

Ulu Muda Forest Reserve, Kedah, *R. affinis* [45].

Whartonia diosi Fain, 2002

New material examined.

3 specimens (NMP [P]) ex *Hipposideros diadema*, 26 April 2018 and 16 specimens (NMP [P]), ex *H. diadema*, 05 March 2020, Goa Peteng, cave, Jimbaran Bali Island, Lat. - 8.79288, Long. 115.13740*; 3 specimens (NMP [P]), ex *Rousettus amplexicaudatus* (G.), 25 April 2018, Crystal Bay, pasture, Nusa Penida Island, Lat. - 8.71661, Long. 115.46119**, coll. P. Šrámek.

Additional material.

13 specimens ex *H. diadema*, 13 February 2019, Goa Peteng, cave, Jimbaran Bali Island, Lat. - 8.79288, Long. 115.13740; 41 specimens ex *H. diadema*, 05 March 2020, Goa Peteng, cave, Jimbaran Bali Island, Lat. - 8.79288, Long. 115.13740.

Remarks Differences in two characters were found between *W. diosi* collected in Bali Island and the data published by Fain [28]. Firstly, the anterior margin of scutum above AL–AL in *W. diosi* from the Philippines is straight [28] while in specimens from Bali the anterior margin of the middle part of the scutum is slightly convex (Fig. 1). The second difference is the relative position of PL setae to the bases of sensilla (SB). In *W. diosi* from the Philippines, the bases of PL setae are situated more anteriorly (the distance AL–PL is equal to longitudinal distance PL–SB) [28]. However, in Indonesian specimens, AL–PL distance is longer than the longitudinal distance PL–SB, and also the PL–PL line is situated slightly above the anterior edge of SB–SB (Fig. 1). We interpret these differences as falling within the range of the intraspecific variability of the species.

Type data and deposition.

Panay Island, *Megaderma spasma* (L.), (Megadermatidae), PNMM [28].

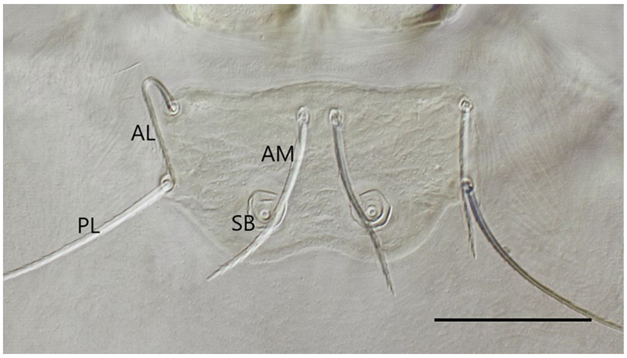

Fig. 1 Morphological details of scutum in larval specimen of *Whartonia diosi* Fain, 2002 (NMP). *AL* anterolateral scutal setae, *AM* anteromedian scutal setae, *PL* posterolateral scutal setae, *SB* sensilary base; scale: 50 µm

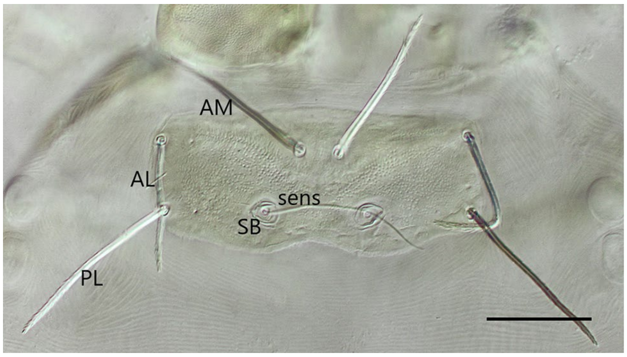

Fig. 2 Morphological details of scutum in larval specimen of *Whartonia maai* Nadchatram and Wilson, 1965 (NMP). *AL* anterolateral scutal setae, *AM* anteromedian scutal setae, *PL* posterolateral scutal setae, *SB* sensilary base, *sens* sensilla; scale: 50 µm

Whartonia kulumadouensis Goff, 1980

Whartonia kulumadouensis Goff, 1980 [46], p. 494, 495, Fig. 1(A–F): Takahashi *et al.* [47], p. 131; da Silveira *et al.* [48], p. 4.

Type data and deposition.

Papua New Guinea: Milne Bay Distr[ict], Woodlark I, Kulumadou, *Dobsonia pannietensis remota* C. (Pteropodidae), (A.F. #14796); Indonesia: NW New Guinea, Yapen Island (as Japen I), Samberbaba (as Sumberbaba), *D. moluccensis* (Q. and G.) (Pteropodidae) [46].

Whartonia maai Nadchatram and Wilson, 1965

Whartonia maai Nadchatram and Wilson, 1965 [18], p. 222–224, figs. 26–33: Kaneko and Kano [49], p. 171; Bhat [50], p. 585; Tenorio [51], p. 28; Brown [27], p. 20; Fernandes and Kulkarni [52], p. 64; Zajkowska *et al.* [9], p. 148; Stekolnikov [10], p. 51.

Whartonia (*Fascutonia*) *maai* Nadchatram and Wilson, 1965: Takahashi *et al.* [47], p. 131.

New material examined.

5 larvae (NMP [P]) (Fig. 2) ex *Rousettus amplexicaudatus* or *leschenaultii* (D.), date 25 April 2018, Nusa Penida, Crystal Bay, pasture, Lat. - 8.71661, Long. 115.46119*, coll. P. Šrámek.

Remarks Eight mites from the type series of *W. maai* from Vogelkop, Kebar Valley, West of Manokwari, from eastern Indonesian part of New Guinea [18] and twelve specimens from Leyte Island of Philippines [27] were measured. Nadchatram and Wilson [18] published morphological characteristics of the species (idiosoma, scutum, gnathosoma, legs, and setae). By comparing all these features with new material from Bali, different results were obtained for AW, PW, and SB, with the mean values being smaller for mites from Bali, while PL setae were a little longer in the Balinese animals (Table 2). Other characters were within the corresponding range and mean values.

Type data and deposition.

Indonesia: NW New Guinea, Vogelkop, Kebar Valley, W of Manokwari, *R. amplexicaudatus brachyotis* (D.) (Pteropodidae); BPBM 4028 [18, 51].

Other records.

Philippines: Leyte Island, Mahaplag, Mt. Kabalanti, “small bat”; Sta Cruz, *R. amplexicaudatus* (as *Rousettus amplexicaudatis*) (Pteropodidae) [53]; Mindanao Island, Davao Prov., Mt Mayo, *Hipposideros diadema* (Hipposideridae) [27].

Thailand: Chanthaburi (Khao Sa Bap, Namtok Phliu), Prachin Burl (Aranyaprathet), *R. leschenaultii* (as *Rouseltus leschenaultia*) (Pteropodidae) [54].

Whartonia penthetor Womersley, 1957

Whartonia penthetor Womersley, 1957 [15], p. 103, Fig. 1(A–E): Domrow [17], p. 4; Audy *et al.* [39], p. 14; Zajkowska *et al.* [9], p. 149; Stekolnikov [10], p. 51.

Whartonia (*W*) *penthetor* Womersley, 1957: Takahashi *et al.* [47], p. 132.

Type data and deposition.

Malaysia: Ulu Langat, Selangor, *Penthetor lucasi* (D.) (Pteropodidae); no data [15, 17, 39]; Bukit Lagong, Kepong, Selangor, *P. lucasi* (Pteropodidae); Gunong Brinchang, *Macroglossus lagochilus* M. (Pteropodidae) [17, 46].

Indonesian record.

Tjiampea caves, near Bogor, Java; *Eonycteris spelaea* (D.) (Pteropodidae) [17].

Whartonia teongwahi Nadchatram, 1980

Whartonia teongwahi Nadchatram,1980 [20], p. 324, 326–327, Fig. 8–15; da Silveira *et al.* [48], p. 4; Zajkowska *et al.* [9], p. 150; Stekolnikov [10], p. 52.

Whartonia (*W*) *teongwahi* Nadchatram, 1980: Takahashi *et al.* [47], p. 133.

Type data and deposition.

Indonesia: Timbang, Serdang, Sumatra Island; *Rhinolophus acuminatus sumatranus* A. (Rhinolophidae); IMR MZ75958; Nias Island, Nigeia River, Sumatra

Island; *Hipposideros larvatus neglectus* S. (Hipposideridae) [20].

Subfamily Trombiculinae Ewing, 1929

Tribe Schoengastiini Vercammen-Grandjean, 1960

Genus *Bishoplinia* Vercammen-Grandjean and Nadchatram, 1965

Bishoplinia wilsoni Vercammen-Grandjean and Nadchatram, 1965

Bishoplinia wilsoni Vercammen-Grandjean and Nadchatram, 1965 [55], p. 321, figs. on p. 322: Zajkowska *et al.* [9], p. 101; Stekolnikov [10], p. 77.

Riedlinia (*Bishoplinia*) *wilsoni* Vercammen-Grandjean, 1965b [21], p. 130.

Type data and deposition.

Indonesia: New Guinea, Dawai River, Yapen Island, Samberbaba; *Hipposideros* sp.; BPBM (# 11162/T/1) [55].

Tribe Trombiculini Vercammen-Grandjean, 1960

Genus *Chiroptella* Vercammen-Grandjean, 1960

Chiroptella audyi (Vercammen-Grandjean and Nadchatram, 1965)

Riedlinia (*Neosomia*) *audyi* Vercammen-Grandjean and Nadchatram, 1965 [55], p. 319–321, Figs. 1–7.

Chiroptella audyi (Vercammen-Grandjean and Nadchatram, 1965): Stekolnikov [56], p. 9; Zajkowska *et al.* [9], p. 101; Ševčík *et al.* [13], p. 11; Stekolnikov [10], p. 104.

Type data and deposition.

Indonesia: Dawai River, New Guinea; *Hipposideros* spp.; BPBM (declared) [55]; MHNG L: 11162/R/1 [56].

Chiroptella baliensis Ševčík, Kalúz and Šrámek, 2020

Chiroptella baliensis Ševčík, Kalúz and Šrámek, 2020 [13], p. 3–9, Figs. 1–4.

Type data and deposition.

Indonesia: Jimbaran vill.[age], Bali; *Hipposideros diadema* (Hipposideridae); SNM SZ 10942 [13].

Chiroptella niehoffi (Domrow, 1962)

Trombicula niehoffi Domrow, 1962a [16], p. 43, Figs. 26–33.

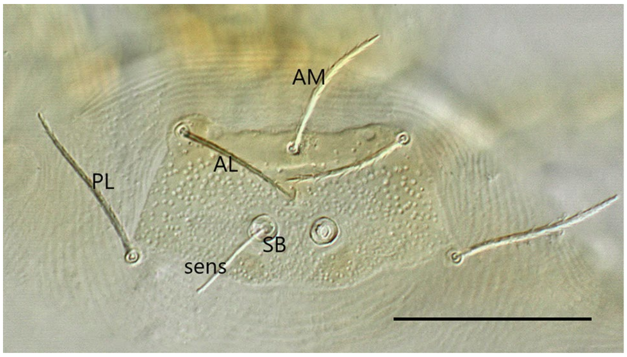

Fig. 3 Morphological details of scutum in larval specimen of *Grandjeana sinensis* (Zhao and Qiu, 1979) (NMP). *AL* anterolateral scutal setae, *AM* anteromedian scutal setae, *PL* posterolateral scutal setae, *SB* sensilary base, *sens* sensilla; scale: 50 μm

Leptotrombidium (*Chiroptella*) *niehoffi* Domrow, 1962: Vercammen-Grandjean [57], p. 46.

Chiroptella (*Chiroptella*) *niehoffi* Domrow, 1962: Nadchatram [19], p. 21; Vercammen-Grandjean and Langston [1], p. 898, 899.

Chiroptella niehoffi (Domrow, 1962) Zajkowska *et al.* [9], p. 103; Ševčík *et al.* [13], p. 10; Stekolnikov [10], p. 104.

Type data and deposition.

Indonesia: Tjiampea caves, Bogor, Java; *Hipposideros* spp. (probably *H. diadema* and *larvatus*); BMNH 1964.8.13.1. [16, 19].

Other records.

Malaysia: Kuala Lumpur, *H. diadema* (Hipposideridae) [1].

Genus *Grandjeana* Koçak and Kemal, 2009

Grandjeana sinensis (Zhao and Qiu, 1979)

New material examined.

9 larvae (NMP [P]) ex *Megaderma spasma*, 24 April 2018, Nusa Penida, Tembeling forest, forest, Lat. - 8.76401, Long. 115.50235*; 4 larvae (NMP [P]) ex *Hipposideros diadema*, 27 February 2020, Nusa Penida, Ped Goa Kentung cave, cave, Lat. - 8.79288, Long. 115.13740*; coll. P. Šrámek.

Remarks Individuals of *G. sinensis* from Indonesia are nearly identical to those from China. The majority of characters are identical and within the range of the character values described by Zhao and Qiu [26]. The only notable differences are in the shapes and positions of several scutal

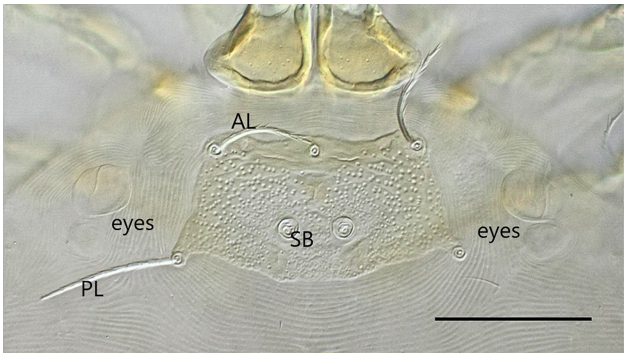

Fig. 4 Morphological details of scutum in larval specimen of *Grandjeana sinensis* (Zhao and Qiu, 1979) (NMP). *AL* anterolateral scutal setae, *AM* anteromedian scutal setae, *PL* posterolateral scutal setae, *SB* sensilary base, *sens* sensilla; scale: 50 µm

features. In *G. sinensis* from China the base of AM is situated anteriorly to the bases of AL–AL, while in individuals from Indonesia the base of AM is situated slightly posteriorly to the bases of AL–AL (Figs. 3 and 4). The shape of some scutal features in Indonesian individuals also varies: e.g., AP to PW or the distance between the bases of sensilla (Figs. 3 and 4). Specimens from both countries also differ in the length of several scutal setae: the setae AM and AL are slightly longer in the Indonesian specimens and exceed the nearest base of the sensillum. Corresponding setae in the Chinese individuals are shorter and do not go beyond the nearest base of the sensillum. This variability may be interesting, but we still consider it as intraspecific variability of *G. sinensis*. Figure 4 shows eye detail since the original description in Zhao and Qiu [26] depicts only eye outlines.

Type data and deposition.

China: Guangxi Zhuang Autonomous, Linshan Country; Chiroptera fam. gen. sp. (orig. "bat"); no data [26].

Genus *Microtrombicula* Ewing, 1950

Microtrombicula spelaea Goff, 1990

Microtrombicula spelaea Goff, 1990 [22], p. 175, 176, Fig. 1(A–G): Zajkowska *et al.* [9], p. 120; Stekolnikov [10], p. 135.

Type data and distribution.

Indonesia: Halmahera (as Halmabera), Kampung Pasir Putih; *Eonycteris spelaea* (Pteropodidae); USNM [22].

Genus *Myotrombicula* Womersley and Heaslip, 1943

Myotrombicula philipi (Womersley, 1952)

Trombicula philipi Womersley, 1952 [14], p. 135–136, Plate 25, fig. F–J: Radford [58], p. 258; Audy [59], p. 148; [60], p. 238; Womersley and Audy [61], p. 261.

Myotrombicula philipi (Womersley, 1952): Nadchatram and Lakshumy [37], p. 283; Zajkowska *et al.* [9], p. 122; Stekolnikov [10], p. 137.

Myotrombicula (*Myotrombicula*) *philipi* (Womersley, 1952): Vercammen-Grandjean [21], p. 62; [62], p. 75; Anciaux de Faveaux [63], p. 128.

Type data and deposition.

Indonesia: Hollandia, Dutch New Guinea; *H. cervinus* (Hipposideridae); SAM (Womersley 1952) [14].

Genus *Rudnicula* Vercammen-Grandjean, 1964

Rudnicula goffi Kalúz, Šrámek and Ševčík, 2020

Rudnicula goffi Kalúz, Šrámek and Ševčík, 2020 [12], p. 19–23, Figs. 1–6.

Type data and deposition.

Indonesia: Jimbaran vill.[age], Bali; *Hipposideros diadema* (Hipposideridae); SNM SZ 10 944 [12].

Genus *Trombicula* Berlese, 1905

Subgenus *Cotrombicula* Vercammen-Grandjean, 1960

Trombicula gressitti Nadchatram and Wilson, 1965

Trombicula (*Cotrombicula*) *gressitti* Nadchatram and Wilson, 1965 [18], p. 220, figs. 10–17: Tenorio 1976 [51], p. 26; Zajkowska *et al.* [9], p. 137; Stekolnikov [10], p. 142.

Type data and deposition.

Indonesia: Yapen Island (as Japen I.), Sumberbaba, NW New Guinea; *Aselliscus tricuspidatus* (Temminck, 1835) (Hipposideridae); SNM SZ 10 944 [18].

Subgenus *Trombicula* Berlese, 1905

Trombicula minor Berlese, 1905

Trombicula minor Berlese, 1905 [35], p. 155, Fig. 4 (adult): Ewing [64], p. 384; Ewing [65], p. 837; Thor and Willmann [66], p. 347, figs. 412, 413 (nymph); Philip and Traub [67], p. 29; Gunther [68], p. 477; [69], p. 66; [70], p. 5; Willmann [71], p. 131; Fuller [72], p. 15; Womersley [14], p. 4; Audy [73], p. 51; Womersley and Audy [61], p. 258; Cooreman [74], p. 10; Audy and Vercammen-Grandjean [75], p. 125;

Vercammen-Grandjean [76], p. 369; Audy *et al.* [39], p. 1, pls. I–III; Vercammen-Grandjean *et al.* [77], p. 275, Fig. 1 (fragments of nymph); Goff [78], p. 118;

Trombicula minor Berlese, 1904 [= 1905]: Womersley and Heaslip [79], p. 92, pl. V (2);

Trombicula minor Berlese, [1905]: Loomis [80], p. 1253;

Trombicula minor Berlese, 1910 [= 1905]: Leclerc *et al.* [81], p. 1814;

Trombicula (*Trombicula*) *minor* Berlese, 1905: Wharton and Fuller [82], p. 61; Stekolnikov [10], p. 142.

Type data and deposition.

Indonesia: Tjiampea near Bogor, Java, (formerly Buitenzorg); bat guano [35]; originally in Zoologisches Museum Hamburg (adult), destroyed in 1943 [69].

Malaysia: Bukit Lagong, Kepong, Selangor, USNM (neotype), *Saccolaimus saccolaimus affinis* (Dobson, 1875) (as *Taphozous affinis* et *T. melanopogon* T.) (Pteropodidae) [39, 59, 83 (as Trombicula "NOR"), 84].

Trombiculidae indet.

Indonesian Record.

Kampung Pasir Putih, Jailolo District, Halmahera; *Dobsonia crenulata* Andersen, 1909 (Pteropodidae), *Eonycteris spelaea* (Pteropodidae), *Rousettus amplexicaudatus* (Pteropodidae) [85].

Discussion

The chaetotaxy of the new Indonesian species *Whartonia maai*, *W. diosi,* and *Grandjeana sinensis* from Bali Island has been reported above in comments on the relevant species. All noted differences in characters between Indonesian specimens and published data of type specimens are considered as intraspecific variabilities of the aforementioned species.

Two of the species new to Indonesia (*W. diosi* and *G. sinensis*) are also recorded for the first time outside of their terra typica. The first of them, *W. diosi,* had previously been known only from its type locality in the Philippines with the host *Megaderma spasma* (family Megadermatidae) [28]. Our specimens were collected from a new host species *Hipposideros diadema* (Hipposideridae). Both host species live sympatrically and utilize similar roosts: caves and hollow trees [86].

The second species, *G. sinensis,* had been previously recorded only from China from an unidentified bat host [26]. Our specimens come from hosts *M. spasma* and *Rhinolophus acuminatus* P. Since none of these host species lives in China, we consider them as new hosts of *G. sinensis.* Both host species share suitable habitats like caves and hollow trees. There have also been South-East Asian records of mixed-species day roosts of *M. spasma* and *Rhinolophus* species in hollow trees [87].

All Indonesian trombiculid records from bats come from a mere eight islands [Geographically arranged from west to east, they are Sumatra, Nias Island (Sumatra), Java, Bali, Nusa Penida (Bali), Halmahera, New Guinea, Yapen Island (Papua)]. Eight is a surprisingly low number when compared to Indonesia's more than 17,000 islands. *Leptotrombidium deliense* (Walch, 1922) has not been included in the checklist since it is a typical chigger species infesting Rodentia [10], and we are considering its two records from bats—Sulawesi Island from host *Rousettus celebensis* (Pteropodidae) [88] and Java Island from host *Pipistrellus* sp. (Vespertilionidae) [89]—to be only accidental.

More factors imply that the true diversity of trombiculids in Indonesia is vastly under-recorded. Just including factors like the diversity of host species and the habitats they inhabit leads to interesting estimates. The checklist in this paper includes all published Indonesian chiggers and shows that they have been recorded from 12 species of bats belonging to 5 families: Pteropodidae (5 species), Hipposideridae (4), Rhinolophidae (1), Megadermatidae (1), and Vespertilionidae (1). Taking into consideration the full diversity of the 239 Indonesian bat species—Pteropodidae (81 species), Rhinopomatidae (1), Emballonuridae (11), Nycteridae (2), Megadermatidae (1), Rhinolophidae (22), Hipposideridae (32), Vespertilionidae (68), Miniopteridae (9) and Mollosidae (12) [90]—it is clear that more species are waiting to be discovered.

Another factor to consider is the extent of suitable host habitat. Caves are just one example of it. There are hundreds, perhaps thousands, of very large and complex caves and cave systems recorded in Indonesia with many more waiting to be discovered and mapped [91]. According to Stekolnikov [92], several trombiculid-infested, cave-dwelling bats have very limited distribution (e.g., monotypic genera *Alexfainia* Yunker and Jones, 1961; *Vergrandia* Yunker and Jones, 1961; or *Tectumpilosum* Feider, 1983). It is believed that each of these genera originated and subsequently evolved in a single isolated cave.

All of these facts imply that Indonesia hosts many more species of trombiculids restricted to cave-dwelling bat species than we currently know about.

By gathering published data and combining them with our new findings, we hereby confirm 9 genera and 16 species of bat-infesting chiggers in Indonesia. Four of these genera (*Whartonia, Chiroptella, Grandjeana,* and *Rudnicula*) are strictly confined to bats.

Since bats not only use caves but also often occupy another type of habitat, i.e., hollow trees, they host cave-dwelling species of chiggers along with species commonly inhabiting hollow trees, e.g., *Ascoschoengastia* Ewing, 1946 [93]. Nadchatram [4] estimates that 60% of bat-infesting chiggers are specific to hollow-tree habitats. This fact reveals our insufficient knowledge of the diversity of Indonesian bat-infesting chiggers. Our research is still largely incomplete and further study is needed to understand these trombiculids better.

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Declarations

Conflict of interest The authors declare there are no conflicts of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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8.7. Rešerš a základ výsledkov zakladajúcich prípravu publikácie o vzťahoch zamatovcov a netopierov.

Manuskript.

Rešerš a základ výsledkov zakladajúcich prípravu publikácie o vzťahoch zamatovcov a netopierov

Štúdium fylogenetických vzťahov a cesty evolúcie u zamatovcov (Trombiculidae) je v súčasnosti ťažko uchopiteľné. Neexistuje žiadny paleontologický materiál o tejto skupine, aj keď zástupcovia blízko príbuzných recentných čeľadí Erythraeidae Oudemans, 1902 a Trombidiidae Leach, 1815 sensu lato sa našli v baltskom jantáre (oligocén) (Dubinin 1962). Okrem toho spektrum potenciálnych hostiteľov zamatovcov je mimoriadne široké a ich hostiteľská špecifickosť sa zdá byť extrémne nízka. Preto použitie asociácie zamatovec–hostiteľ pri konštrukcii fylogenézy tohto taxónu sa zdá byť nereálne.

Väčšina zamatovcov prejavuje preferencie voči určitému mikrobiotopu v rámci ktorého napádajú a parazitujú všetky alebo väčšinu druhov stavovcov, ktoré tento biotop zaberajú. Inak povedané sú úzko spojené s partikulárnymi biotopmi v ktorých sa dokážu živiť na takmer každom druhu stavovca. To znamená že spojenie zamatovec–hostiteľ môže byť založené kedykoľvek priestorové možnosti roztoča a hostiteľa sa zhodujú (Kudrâšova 1998). Aj napriek tomu však v danom priestore môže preferovať určitý druh zamatovca špecifického hostiteľa medzi stavovcami. Ide však o ekologické súvislosti, ktoré môžu súvisieť so spoločným výskytom druhu zamatovca a hostiteľského druhu v biotope, hustoty hostiteľskej populácie, charakteristické správanie hostiteľa, ako aj ekologické vlastnosti konkrétneho druhu roztoča.

Pri pohľade na ekologickú jednotku akou je habitat, vieme že netopiere sú skupinou ktorých „stanovište“ je od ostatných stavovcov prevažne izolované. Ak si zoberieme túto skupinu do porovnania s ostatnými stavovcami, predstavujú jediný subjekt väzby s taxónmi zamatovcov. Respektíve inak povedané, jedine netopiere sú hostiteľskou skupinou u ktorej sa zdajú byť zamatovce najviac špecifické (cf. Shatrov and Kudryashova 2008).

V súčasnosti viac ako 260 druhov zamatovcov patriacich k 19 rodov je považovaných za špecializovaných na netopiere (Yunker a Jones 1961, Shatrov a Kudryashova 2008, Stekolnikov 2014, Kalúz a Ševčík 2015): *Alexfainia* Yunker et Jones, 1961, *Audytrombicula* Vercammen-Grandjean, 1963, *Chiroptella* Vercammen-Grandjean, 1960, *Grandjeana* (Koçak & Kemal, 2009), *Myotrombicula* Womersley et Heaslip, 1943, *Oudemansidium* Vercammen-Grandjean et André, 1966, *Pentagonaspis* Vercammen-Grandjean et André, 1966, *Perissopalla* Brennan et White, 1960, *Riedlinia* Oudemans, 1914, *Rudnicula* Vercammen-Grandjean, 1964, *Sasatrombicula* Vercammen-Grandjean, 1960, *Speleocola* Lipovsky, 1952, *Tectumpilosum* Feider, 1983, *Tecomatlana* Hoffmann, 1947, *Trisetica* Traub et Evans, 1950, *Trombigastia* Vercammen-Grandjean et Brennan, 1957, *Vergrandia* Yunker et Jones, 1961, *Whartonia* Ewing, 1944 a *Willmannium* Vercammen-Grandjean et Langston, 1976.

Asociácia zamatovce–netopiere bola uvedená viacerými autormi: Anciaux de Faveaux (1971a, b), Baker a Craven (2003), Lanza (1999), Marinkelle a Grose (1981), Reed a Brennan (1975) a Walters a kol. (2011). Jedná sa však len o súhrny nálezov. Ďalšie údaje súvisiace s uvedenými asociáciami sú rozptýlené v rade publikácií a boli sumarizované v recentnej práci Zajkowska a kol. (2018). Ide o prvý ucelený zoznam druhov zamatovcov v kombinácii s uvedením hostiteľa a geografického územia (vymedzeného politicky).

Uvedený zoznam Zajkowska et al. (2018) a ďalšie publikované nálezy a opisy druhov zamatovcov pochádzajúcich z netopierov do obdobia roku 2023 nám slúžili ako podklad pre hľadanie vzťahov zamatovce–netopiere. Cieľom bolo zistiť, či sú určité taxóny zamatovcov spojené s konkrétnymi taxónmi netopierov častejšie, ako len náhodne. To by mohlo naznačiť evolučné prepojenie alebo špecializáciu parazitov na ich netopierích hostiteľov.

Vstupné údaje tvorili matice reprezentujúce zoogeografické oblasti Starého a Nového sveta. Riadky v týchto matriciach zodpovedali rodom zamatovcov, stĺpce predstavovali čeľade netopierov. Tie boli tvorené zo všetkých doteraz známych nálezov v kritériu druh zamatovca–druh alebo rod netopiera.

Hodnoty buniek v matriciach sa pohybovali od 0 (indikovala absenciu záznamu taxónu parazita na taxóne netopierov) do „n“ (predstavovala počet zaznamenaných výskytov taxónu parazita na taxóne netopierov).

Na identifikáciu nenáhodných asociácií boli použité Pearsonove chí-kvadrát testy. Každý test porovnával pozorované frekvencie asociácií parazit-netopier v údajoch s očakávanými frekvenciami podľa nulovej hypotézy o žiadnej asociácii (náhodný výskyt). Pre každý pár parazitov a taxónov netopierov bola vypočítaná štatistika chí-kvadrát testu a zodpovedajúca p-hodnota. Hodnota p označovala pravdepodobnosť pozorovania asociácie tak silnej, ako je tá, ktorá bola zistená podľa nulovej hypotézy. Nízka p-hodnota naznačila odmietnutie nulovej hypotézy, čo bolo uvedené ako nenáhodné, potenciálne evolučne významné spojenie. Výsledky boli uvedené do tabuľky ukazujúce testovacie kritérium chí-kvadrát a hodnoty p pre každý pár rod zamatovca–čeľaď netopiera. Tabuľka bola doplnená grafickou vizualizáciou kde čierne bodky v matrici naznačujú prítomnosť asociácie medzi parazitom a taxónom netopierov. Čierne bodky uzatvorené v červenom kruhu predstavujú asociácie štatisticky významné, čo naznačuje nenáhodný vzťah a potenciálnu špecializáciu parazita na konkrétny taxón netopierov.

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Tabuľky

Tabuľka 1 Analýza nenáhodných asociácií medzi taxónmi zamatovcov a netopierov v Starom svete

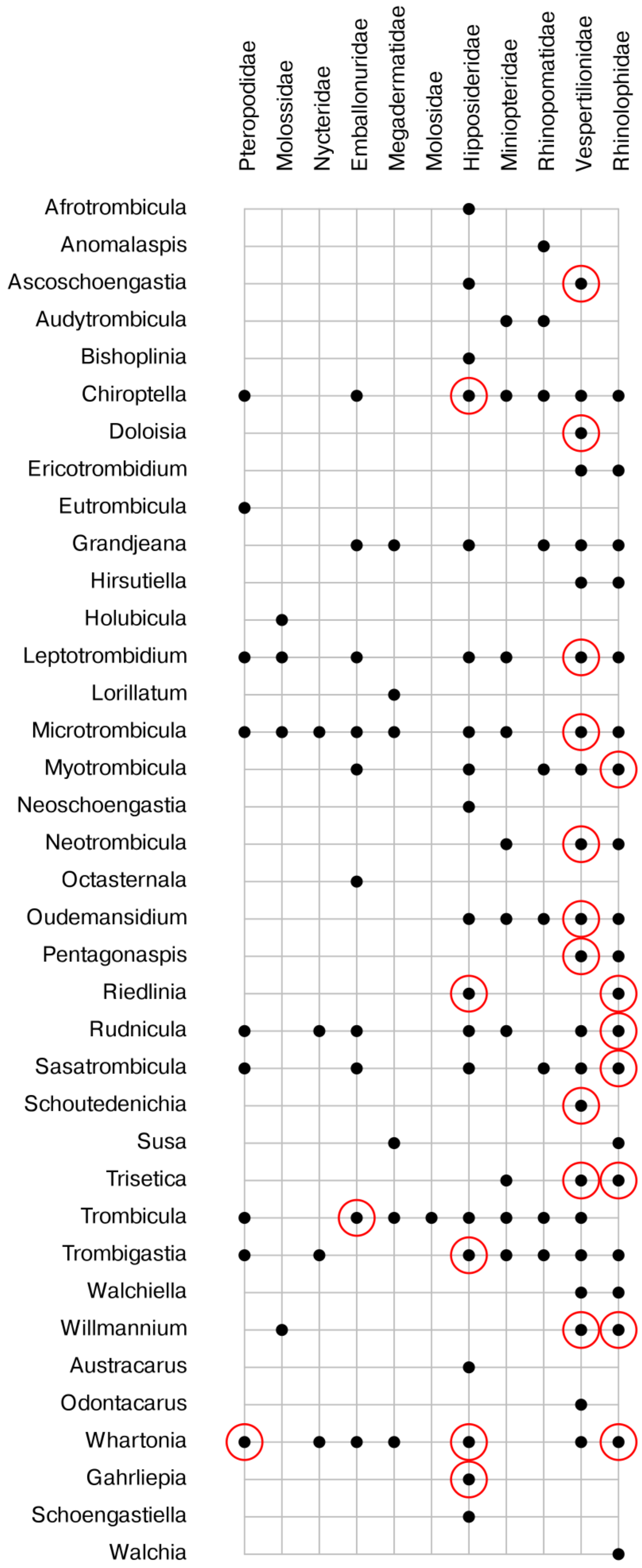
Tabuľka 2 Analýza nenáhodných asociácií medzi taxónmi zamatovcov a netopierov v Novom svete

taxa	parasite	nx	Pteropodidae	Molossidae	Nycteridae	Emballonuridae	Megadermatidae	Molosidae	Hipposideridae	Miniopteridae	Rhinopomatidae	Vespertilionidae	Rhinolophidae	Pteropodidae	Molossidae	Nycteridae	Emballonuridae	Megadermatidae	Molosidae	Hipposideridae	Miniopteridae	Rhinopomatidae	Vespertilionidae	Rhinolophidae	χ^2	**df**	**p-value**	
Trombiculinae	*Afrotrombicula*	1	0	0	0	0	0	0	1	0	0	0	0	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	3,162	-0,316	-0,316	-0,316	-0,316	**10**	**10**	**0,441**	
Trombiculinae	*Anomalaspis*	1	0	0	0	0	0	0	0	0	1	0	0	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	3,162	-0,316	-0,316	**10**	**10**	**0,441**	
Trombiculinae	*Ascoschoengastia*	4	0	0	0	0	0	0	1	0	0	5	0	-0,775	-0,775	-0,775	-0,775	-0,775	-0,775	0,645	-0,775	-0,775	**6,326**	-0,775	**41,67**	**10**	**<0.001**	***
Trombiculinae	*Audytrombicula*	1	0	0	0	0	0	0	0	1	2	0	0	-0,548	-0,548	-0,548	-0,548	-0,548	-0,548	-0,548	1,461	3,469	-0,548	-0,548	**15,33**	**10**	**0,12**	
Trombiculinae	*Bishoplinia*	1	0	0	0	0	0	0	1	0	0	0	0	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	3,162	-0,316	-0,316	-0,316	-0,316	**10**	**10**	**0,441**	
Trombiculinae	*Chiroptella*	18	2	0	0	3	0	0	15	2	2	1	6	-0,511	-1,761	-1,761	0,114	-1,761	-1,761	**7,611**	-0,511	-0,511	-1,136	1,988	**69,42**	**10**	**<0.001**	***
Trombiculinae	*Doloisia*	1	0	0	0	0	0	0	0	0	0	3	0	-0,548	-0,548	-0,548	-0,548	-0,548	-0,548	-0,548	-0,548	-0,548	**5,477**	-0,548	**30**	**10**	**<0.001**	***
Trombiculinae	*Ericotrombidium*	2	0	0	0	0	0	0	0	0	0	1	1	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	2,012	2,012	**9**	**10**	**0,532**	
Trombiculinae	*Eutrombicula*	1	1	0	0	0	0	0	0	0	0	0	0	3,162	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	**10**	**10**	**0,441**	
Trombiculinae	*Grandjeana*	10	0	0	0	3	2	0	2	0	1	1	1	-1	-1	-1	2,3	1,2	-1	1,2	-1	0,1	0,1	0,1	**12**	**10**	**0,285**	
Trombiculinae	*Hirsutiella*	2	0	0	0	0	0	0	0	0	0	1	1	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	2,012	2,012	**9**	**10**	**0,532**	
Trombiculinae	*Holubicula*	1	0	1	0	0	0	0	0	0	0	0	0	-0,316	3,162	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	**10**	**10**	**0,441**	
Trombiculinae	*Leptotrombidium*	22	2	2	0	1	0	0	2	4	0	39	7	-1,466	-1,466	-2,387	-1,927	-2,387	-2,387	-1,466	-0,545	-2,387	**15,581**	0,838	**251,58**	**10**	**<0.001**	***
Trombiculinae	*Lorillatum*	1	0	0	0	0	1	0	0	0	0	0	0	-0,316	-0,316	-0,316	-0,316	3,162	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	**10**	**10**	**0,441**	
Trombiculinae	*Microtrombicula*	28	5	4	4	1	1	0	4	5	0	9	2	1,069	0,481	0,481	-1,283	-1,283	-1,871	0,481	1,069	-1,871	**3,421**	-0,695	**23,14**	**10**	**0,01**	**
Trombiculinae	*Myotrombicula*	16	0	0	0	1	0	0	4	0	1	1	9	-1,265	-1,265	-1,265	-0,395	-1,265	-1,265	2,214	-1,265	-0,395	-0,395	**6,562**	**52,75**	**10**	**<0.001**	***
Trombiculinae	*Neoschoengastia*	1	0	0	0	0	0	0	1	0	0	0	0	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	3,162	-0,316	-0,316	-0,316	-0,316	**10**	**10**	**0,441**	
Trombiculinae	*Neotrombicula*	10	0	0	0	0	0	0	0	1	0	10	4	-1,225	-1,225	-1,225	-1,225	-1,225	-1,225	-1,225	-0,327	-1,225	**7,757**	2,368	**70,8**	**10**	**<0.001**	***
Trombiculinae	*Octasternala*	1	0	0	0	1	0	0	0	0	0	0	0	-0,316	-0,316	-0,316	3,162	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	**10**	**10**	**0,441**	
Trombiculinae	*Oudemansidium*	5	0	0	0	0	0	0	1	1	1	25	3	-1,761	-1,761	-1,761	-1,761	-1,761	-1,761	-1,136	-1,136	-1,136	**13,858**	0,114	**195,03**	**10**	**<0.001**	***
Trombiculinae	*Pentagonaspis*	2	0	0	0	0	0	0	0	0	0	4	1	-0,707	-0,707	-0,707	-0,707	-0,707	-0,707	-0,707	-0,707	-0,707	**5,515**	0,849	**32,4**	**10**	**<0.001**	***
Trombiculinae	*Riedlinia*	8	0	0	0	0	0	0	5	0	0	0	4	-0,949	-0,949	-0,949	-0,949	-0,949	-0,949	**4,849**	-0,949	-0,949	-0,949	**3,689**	**41,11**	**10**	**<0.001**	***
Trombiculinae	*Rudnicula*	9	1	0	1	2	0	0	1	1	0	1	5	-0,091	-1,095	-0,091	0,913	-1,095	-1,095	-0,091	-0,091	-1,095	-0,091	**3,925**	**19,17**	**10**	**0,038**	*
Trombiculinae	*Sasatrombicula*	15	1	0	0	4	0	0	2	0	2	2	17	-1,016	-1,673	-1,673	0,956	-1,673	-1,673	-0,359	-1,673	-0,359	-0,359	**9,502**	**96,93**	**10**	**<0.001**	***
Trombiculinae	*Schoutedenichia*	3	0	0	0	0	0	0	0	0	0	2	0	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	**4,472**	-0,447	**20**	**10**	**0,029**	*
Trombiculinae	*Susa*	1	0	0	0	0	1	0	0	0	0	0	1	-0,447	-0,447	-0,447	-0,447	2,012	-0,447	-0,447	-0,447	-0,447	-0,447	2,012	**9**	**10**	**0,532**	
Trombiculinae	*Trisetica*	4	0	0	0	0	0	0	0	1	0	3	3	-0,837	-0,837	-0,837	-0,837	-0,837	-0,837	-0,837	0,478	-0,837	**3,108**	**3,108**	**22,86**	**10**	**0,011**	*
Trombiculinae	*Trombicula*	17	1	0	0	7	3	1	2	1	1	2	0	-0,522	-1,342	-1,342	**4,398**	1,118	-0,522	0,298	-0,522	-0,522	0,298	-1,342	**24,78**	**10**	**0,006**	**
Trombiculinae	*Trombigastia*	24	4	0	2	0	0	0	13	4	1	1	2	1,035	-1,643	-0,304	-1,643	-1,643	-1,643	**7,06**	1,035	-0,974	-0,974	-0,304	**58,96**	**10**	**<0.001**	***
Trombiculinae	*Walchiella*	2	0	0	0	0	0	0	0	0	0	1	1	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	-0,447	2,012	2,012	**9**	**10**	**0,532**	
Trombiculinae	*Willmannium*	11	0	2	0	0	0	0	0	0	0	18	7	-1,643	-0,304	-1,643	-1,643	-1,643	-1,643	-1,643	-1,643	-1,643	**10,407**	**3,043**	**126,59**	**10**	**<0.001**	***
Leeuwenhoekiinae	*Austracarus*	1	0	0	0	0	0	0	1	0	0	0	0	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	3,162	-0,316	-0,316	-0,316	-0,316	**10**	**10**	**0,441**	
Leeuwenhoekiinae	*Odontacarus*	1	0	0	0	0	0	\	0	0	0	1	0	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	3,162	-0,316	**10**	**10**	**0,441**	
Leeuwenhoekiinae	*Whartonia*	25	12	0	1	1	1	0	22	0	0	3	11	**3,587**	-2,258	-1,771	-1,771	-1,771	-2,258	**8,458**	-2,258	-2,258	-0,797	**3,1**	**113,14**	**10**	**<0.001**	***
Leeuwenhoekiinae	*Gahrliepia*	3	0	0	0	0	0	0	3	0	0	0	0	-0,548	-0,548	-0,548	-0,548	-0,548	-0,548	**5,477**	-0,548	-0,548	-0,548	-0,548	**30**	**10**	**<0.001**	***
Leeuwenhoekiinae	*Schoengastiella*	2	0	0	0	0	0	0	1	0	0	0	0	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	3,162	-0,316	-0,316	-0,316	-0,316	**10**	**10**	**0,441**	
Gahrliepinae	*Walchia*	1	0	0	0	0	0	0	0	0	0	0	1	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	-0,316	3,162	**10**	**10**	**0,441**	

taxa	parasite	nx	Phyllostomidae	Mormoopidae	Molossidae	Emballonuridae	Vespertilionidae	Noctilionidae	Natalidae	Hipposideridae	Phyllostomidae	Mormoopidae	Molossidae	Emballonuridae	Vespertilionidae	Noctilionidae	Natalidae	Hipposideridae	χ^2	**df**	**p-value**	
Trombiculidae	*Alexfainia*	1	2	2	0	0	0	0	0	0	2,268	2,268	-0,756	-0,756	-0,756	-0,756	-0,756	-0,756	**12**	**7**	**0,101**	
Trombiculidae	*Batmanacarus*	1	0	1	0	0	0	0	0	0	-0,378	2,646	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	**7**	**7**	**0,428**	
Trombiculidae	*Beamerella*	3	17	4	4	3	1	0	0	0	**7,51**	0,211	0,211	-0,351	-1,474	-2,035	-2,035	-2,035	**62,31**	**7**	**<0.001**	***
Trombiculidae	*Blankaartia*	1	1	0	1	0	0	0	0	0	1,604	-0,535	1,604	-0,535	-0,535	-0,535	-0,535	-0,535	**6**	**7**	**0,539**	
Trombiculidae	*Bramkeria*	1	0	1	0	0	0	0	0	0	-0,378	2,646	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	**7**	**7**	**0,429**	
Trombiculidae	*Brennanacarus*	1	1	0	2	0	0	2	0	0	0,507	-0,845	1,859	-0,845	-0,845	1,859	-0,845	-0,845	**9,4**	**7**	**0,225**	
Trombiculidae	*Chiroptella*	2	0	0	4	0	2	0	0	0	-0,926	-0,926	**4,012**	-0,926	1,543	-0,926	-0,926	-0,926	**20,67**	**7**	**0,004**	**
Trombiculidae	*Colicus*	5	14	0	0	2	2	0	0	0	**8,374**	-1,604	-1,604	-0,178	-0,178	-1,604	-1,604	-1,604	**72,67**	**7**	**<0.001**	***
Trombiculidae	*Eltonella*	2	2	1	0	0	0	0	0	0	2,837	1,091	-0,655	-0,655	-0,655	-0,655	-0,655	-0,655	**10,33**	**7**	**0,171**	
Trombiculidae	*Euschoengastia*	6	1	1	0	0	17	0	0	0	-0,954	-0,954	-1,648	-1,648	**10,145**	-1,648	-1,648	-1,648	**103,53**	**7**	**<0.001**	***
Trombiculidae	*Euschoengastoides*	3	0	0	0	0	4	0	0	0	-0,756	-0,756	-0,756	-0,756	**5,292**	-0,756	-0,756	-0,756	**28**	**7**	**<0.001**	***
Trombiculidae	*Eutrombicula*	10	17	4	1	3	5	0	1	0	**7,128**	0,068	-1,561	-0,475	0,611	-2,104	-1,561	-2,104	**57**	**7**	**<0.001**	***
Trombiculidae	*Farrellioide*	1	1	0	0	0	0	0	0	0	2,646	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	**7**	**7**	**0,429**	
Trombiculidae	*Hoffmanniella*	4	9	1	1	3	2	0	0	0	**5,292**	-0,756	-0,756	0,756	0	-1,512	-1,512	-1,512	**32**	**7**	**<0.001**	***
Trombiculidae	*Hooperella*	5	30	1	3	8	1	0	0	0	**11,355**	-2,017	-1,095	1,21	-2,017	-2,478	-2,478	-2,478	**138,4**	**7**	**<0.001**	***
Trombiculidae	*Hyponeocula*	2	0	1	1	0	0	0	1	0	-0,655	1,091	1,091	-0,655	-0,655	-0,655	1,091	-0,655	**5**	**7**	**0,66**	
Trombiculidae	*Leptotrombidium*	2	0	0	1	0	28	0	0	0	-2,035	-2,035	-1,474	-2,035	**13,686**	-2,035	-2,035	-2,035	**187,55**	**7**	**<0.001**	***
Trombiculidae	*Loomisia*	8	26	0	1	10	3	0	0	0	**10,04**	-2,39	-1,912	2,39	-0,956	-2,39	-2,39	-2,39	**117,2**	**7**	**<0.001**	***
Trombiculidae	*Microtrombicula*	8	26	15	3	0	3	1	4	0	**8,177**	**3,564**	-1,468	-2,726	-1,468	-2,306	-1,048	-2,726	**92**	**7**	**<0.001**	***
Trombiculidae	*Miyatrombicula*	2	0	0	0	0	2	0	0	0	-0,535	-0,535	-0,535	-0,535	3,742	-0,535	-0,535	-0,535	**14**	**7**	**0,051**	
Trombiculidae	*Myotrombicula*	4	0	5	0	0	0	0	0	0	-0,845	**5,916**	-0,845	-0,845	-0,845	-0,845	-0,845	-0,845	**35**	**7**	**<0.001**	***
Trombiculidae	*Nahuacarus*	2	2	0	0	0	0	0	0	0	3,742	-0,535	-0,535	-0,535	-0,535	-0,535	-0,535	-0,535	**14**	**7**	**0,051**	
Trombiculidae	*Neotrombicula*	2	0	0	0	0	2	0	0	0	-0,535	-0,535	-0,535	-0,535	3,742	-0,535	-0,535	-0,535	**14**	**7**	**0,051**	
Trombiculidae	*Nycterinastes*	2	9	1	0	0	0	0	0	0	**7,41**	-0,239	-1,195	-1,195	-1,195	-1,195	-1,195	-1,195	**55,6**	**7**	**<0.001**	***
Trombiculidae	*Parascoschoengastia*	3	6	0	0	0	0	0	0	0	**6,481**	-0,926	-0,926	-0,926	-0,926	-0,926	-0,926	-0,926	**42**	**7**	**<0.001**	***
Trombiculidae	*Parasecia*	4	10	1	0	1	4	0	0	0	**6,047**	-0,756	-1,512	-0,756	1,512	-1,512	-1,512	-1,512	**43**	**7**	**<0.001**	***
Trombiculidae	*Perates*	5	5	15	0	0	1	0	0	0	1,567	**8,165**	-1,732	-1,732	-1,072	-1,732	-1,732	-1,732	**74,62**	**7**	**<0.001**	***
Trombiculidae	*Perissopalla*	9	25	0	0	6	2	0	0	0	**10,988**	-2,171	-2,171	0,987	-1,119	-2,171	-2,171	-2,171	**128,2**	**7**	**<0.001**	***
Trombiculidae	*Phalcophila*	2	0	2	0	0	0	0	0	0	-0,535	3,742	-0,535	-0,535	-0,535	-0,535	-0,535	-0,535	**14**	**7**	**0,051**	
Trombiculidae	*Pseudoschoengastia*	1	1	0	0	0	0	0	0	0	2,646	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	**7**	**7**	**0,429**	
Trombiculidae	*Quadraseta*	1	0	0	0	1	0	0	0	0	-0,378	-0,378	-0,378	2,646	-0,378	-0,378	-0,378	-0,378	**7**	**7**	**0,429**	
Trombiculidae	*Rudnicula*	1	0	0	0	0	0	0	0	1	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	2,646	**7**	**7**	**0,429**	
Trombiculidae	*Sasatrombicula*	1	0	0	0	0	0	0	0	2	-0,535	-0,535	-0,535	-0,535	-0,535	-0,535	-0,535	3,742	**14**	**7**	**0,051**	
Trombiculidae	*Speleocola*	5	11	0	6	3	1	1	0	0	**5,318**	-1,773	2,095	0,161	-1,128	-1,128	-1,773	-1,773	**39,09**	**7**	**<0.001**	***
Trombiculidae	*Tecomatlana*	3	5	1	1	4	1	0	0	0	**3,055**	-0,436	-0,436	2,182	-0,436	-1,309	-1,309	-1,309	**17,333**	**7**	**0,015**	*
Trombiculidae	*Trombicula*	9	4	0	3	2	0	0	0	1	2,63	-1,195	1,673	0,717	-1,195	-1,195	-1,195	-0,239	**14**	**7**	**0,051**	
Trombiculidae	*Vergrandia*	1	0	1	0	0	0	0	0	0	-0,378	2,646	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	**7**	**7**	**0,429**	
Trombiculidae	*Whartonacarus*	1	0	0	0	0	1	0	0	0	-0,378	-0,378	-0,378	-0,378	2,646	-0,378	-0,378	-0,378	**7**	**7**	**0,429**	
Trombiculidae	*Willmannium*	2	1	0	1	1	1	0	0	0	0,756	-0,756	0,756	0,756	0,756	-0,756	-0,756	-0,756	**4**	**7**	**0,779**	
Leeuwenhoekiinae	*Albeckia*	1	0	0	0	0	4	0	0	0	-0,756	-0,756	-0,756	-0,756	**5,292**	-0,756	-0,756	-0,756	**28**	**7**	**<0.001**	***
Leeuwenhoekiinae	*Antrohoekia*	1	0	1	0	0	0	0	0	0	-0,378	2,646	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	**7**	**7**	**0,429**	
Leeuwenhoekiinae	*Odontacarus*	3	8	0	0	1	1	0	0	0	**6,454**	-1,195	-1,195	-0,239	-0,239	-1,195	-1,195	-1,195	**42,8**	**7**	**<0.001**	***
Leeuwenhoekiinae	*Wagenaaria*	1	2	4	0	0	0	0	0	0	1,543	**4,012**	-0,926	-0,926	-0,926	-0,926	-0,926	-0,926	**22,67**	**7**	**0,004**	**
Leeuwenhoekiinae	*Whartonia*	13	42	5	3	7	19	1	0	0	**11,156**	-1,594	-2,283	-0,905	**3,23**	-2,972	-3,317	-3,317	**152,51**	**7**	**<0.001**	***
Leeuwenhoekiinae	*Xenodontacarus*	1	1	0	0	0	0	0	0	0	2,646	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	**7**	**7**	**0,428**	
Leeuwenhoekiinae	*Apolonia*	1	1	0	0	0	0	0	0	0	2,646	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	-0,378	**7**	**7**	**0,428**	

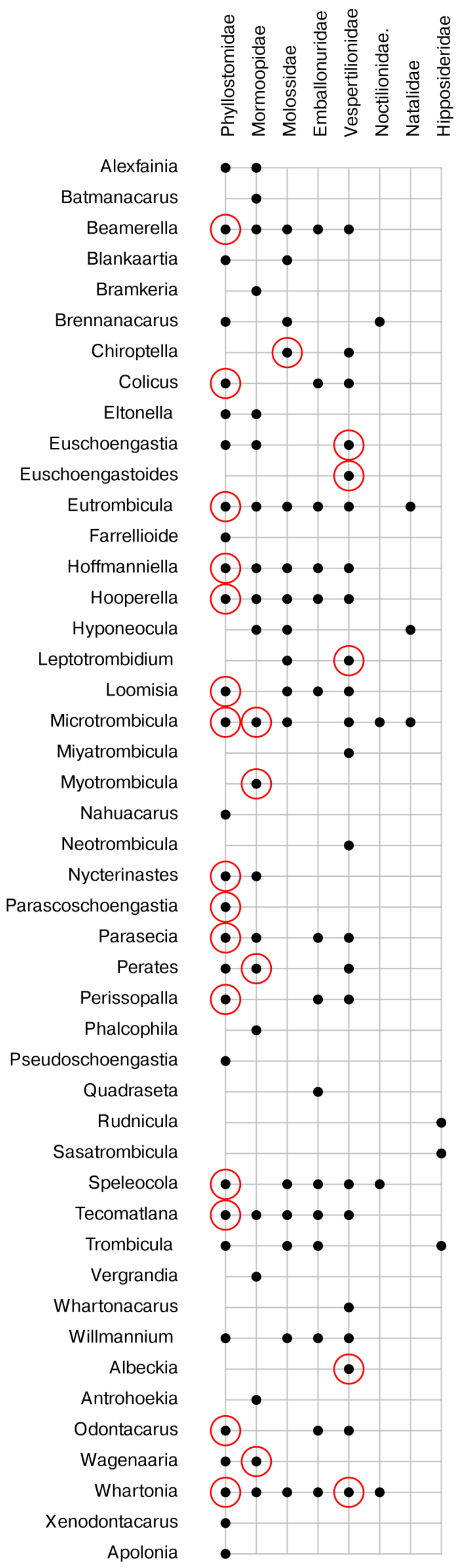
Obrázky

Obrázok 1 Matrica vzťahov taxónov zamatovcov a netopierov v Starom svete



	Pteropodidae	Molossidae	Nycteridae	Emballonuridae	Megadermatidae	Molosidae	Hipposideridae	Miniopteridae	Rhinopomatidae	Vespertilionidae	Rhinolophidae
Afrotrombicula							●				
Anomalaspis									●		
Ascoschoengastia							●			◉	
Audytrombicula								●	●		
Bishoplinia							●				
Chiroptella	●			●			◉	●	●	●	●
Doloisia										◉	
Ericotrombidium										●	●
Eutrombicula	●										
Grandjeana				●	●		●		●	●	●
Hirsutiella										●	●
Holubicula		●									
Leptotrombidium	●	●		●			●	●		◉	●
Lorillatum					●						
Microtrombicula	●	●	●	●	●		●	●		◉	●
Myotrombicula				●			●		●	●	◉
Neoschoengastia							●				
Neotrombicula								●		◉	●
Octasternala				●							
Oudemansidium							●	●	●	◉	●
Pentagonaspis										◉	●
Riedlinia							◉				◉
Rudnicula	●		●	●			●	●		●	◉
Sasatrombicula	●			●			●		●	●	◉
Schoutedenichia										◉	
Susa					●						●
Trisetica								●		◉	◉
Trombicula	●			◉	●	●	●	●	●	●	
Trombigastia	●		●				◉	●	●	●	●
Walchiella										●	●
Willmannium		●								◉	◉
Austracarus							●				
Odontacarus										●	
Whartonia	◉		●	●	●		◉			●	◉
Gahrliepia							◉				
Schoengastiella							●				
Walchia											●

Obrázok 2 Matrica vzťahov taxónov zamatovcov a netopierov v Novom svete



	Phyllostomidae	Mormoopidae	Molossidae	Emballonuridae	Vespertilionidae	Noctilionidae.	Natalidae	Hipposideridae
Alexfainia	●	●						
Batmanacarus		●						
Beamerella	◉	●	●	●	●			
Blankaartia	●		●					
Bramkeria		●						
Brennanacarus	●		●			●		
Chiroptella			◉		●			
Colicus	◉			●	●			
Eltonella	●	●						
Euschoengastia	●	●			◉			
Euschoengastoides					◉			
Eutrombicula	◉	●	●	●	●		●	
Farrellioide	●							
Hoffmanniella	◉	●	●	●	●			
Hooperella	◉	●	●	●	●			
Hyponeocula		●	●				●	
Leptotrombidium			●		◉			
Loomisia	◉		●	●	●			
Microtrombicula	◉	◉	●		●	●	●	
Miyatrombicula					●			
Myotrombicula		◉						
Nahuacarus	●							
Neotrombicula					●			
Nycterinastes	◉	●						
Parascoschoengastia	◉							
Parasecia	◉	●		●	●			
Perates	●	◉			●			
Perissopalla	◉			●	●			
Phalcophila		●						
Pseudoschoengastia	●							
Quadraseta				●				
Rudnicula								●
Sasatrombicula								●
Speleocola	◉		●	●	●	●		
Tecomatlana	◉	●	●	●	●			
Trombicula	●		●	●				●
Vergrandia		●						
Whartonacarus					●			
Willmannium	●		●	●	●			
Albeckia					◉			
Antrohoekia		●						
Odontacarus	◉			●	●			
Wagenaaria	●	◉						
Whartonia	◉	●	●	●	◉	●		
Xenodontacarus	●							
Apolonia	●							

8.8. Pocora I., Ševčík M., Uhrin M., Bashta A-T., Pocora V. 2013:

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Morphometric notes and nymphal stages description of mite species from the *Spinturnix myoti* group (Mesostigmata: Spinturnicidae) from Romania and Ukraine

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The morphometric comparison of two similar species from *Spinturnix myoti* group, *S. myoti* and *Spinturnix andegavina* based on the records from Romania and Ukraine was undertaken. Discriminant characteristics for nymph stadium are provided. Morphometric features of adult stages of these two species are discussed with respect to their applicability for species determination. Description and key features of nymphal stages of *S. andegavina* were compiled using metric and non-metric body characteristics. Records of *S. andegavina* from Romania and Ukraine are reported for the first time and previous records of spinturnicid mites from *Myotis daubentonii* (Kuhl, 1817) in northern Europe and Asia are also discussed.

Keywords: differences; *Spinturnix myoti*; *Spinturnix andegavina*; protonymphs; deutonymphs; description; identification key; records

Introduction

According to current knowledge, the *Spinturnix myoti* group includes six species: *Spinturnix andegavina* (Kolenati, 1857); *Spinturnix bechsteinii* Deunff, Walter, Bellido & Volleth, 2004; *Spinturnix dasycnemi* Kolenati, 1856; *Spinturnix emarginata* (Kolenati, 1856); *S. myoti* (Kolenati, 1856) and *Spinturnix mystacina* (Kolenati, 1857). Two species from this group show a very close resemblance, *S. andegavina* parasitizing on main host *Myotis daubentonii* (Kuhl, 1817) and *S. myoti* parasitizing on main hosts *Myotis myotis* (Borkhausen, 1797) and *Myotis blythii* (Tomes, 1857). *S. andegavina* was distinguished by Deunff (1977) based on the smaller body size compared to *S. myoti* in combination with ecological and biological characteristics of the host species and some additional differences of adult stages were described. The length and width body of both sexes in *S. andegavina* are significantly shorter; the space between coxae I is reduced in males; however, the distance separating the base of the shield gnathosoma jugular is larger than in *S. myoti*. In males and females, intercoxaux I–II and III–IV spaces are still narrowed or absent. Description of this species on body size and characteristics of body (body length and width, width and length of the sternal plate) was also mentioned by Haitlinger (1978) and the species was referred to as *Spinturnix daubentoni*. Peribáñez-López et al. (1989) stated differences in the number of dorsal opisthosomal setae for *S. andegavina* females with the existence of only 70–78 as compared to 80–100 indicated by Deunff (1977) and 90–130 reported by Rudnick (1960) for species *S. myoti*. The numbers of ventral opisthosomal setae observed in *S. andegavina* are only 46–49, while *S. myoti* has 57–88 setae. In males, differences were found only in morphology of the cuticle surrounding the dorsal shield of these two species.

Up to now, the differences between immature stages of the nymphs (protonymph and deutonymph) of both species have not been described. Evans (1968) described the nymphal stages of *S. myoti*, but without comparisons to other species from the group. Differences for both species in the intercoxal space (coxae I and II) were found by Estrada-Peña (1990). This space is larger or smaller but still visible in *S. andegavina* in contrast to *S. myoti*, where the intercoxal space is missing.

Morphometric features of adult stages (body length and width; dorsal shield length and width; ventral shield length and width, number of pairs of setae in sternal shield and in outside sternal shield, number of opisthoventral and opisthodorsal setae, surface of dorsal shield, dorsal pattern, peritreme length and width) of *S. andegavina* and *S. myoti* are presented and their applicability to determination is discussed. A description and key to the deutonymphal and protonymphal stages of *S. andegavina* are also presented, supplemented by characteristics useable to distinguish the species from *S. myoti*. Our records of *S. andegavina* individuals from *M. daubentonii* from south and east Europe (Romania and Ukraine) are discussed with reference to records of *Spinturnix* species previously published from

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northern Europe and Asia (e.g. Dusbábek 1966; Jaunbauere et al. 2008; Orlova and Pervushina 2010; Orlova 2011; Orlova et al. 2011).

Material and methods

Mites were collected from bats by inspection of fur and patagia using entomological tweezers and were stored in 75% alcohol. Specimens were dipped into 5% KOH and washed in distilled water. Slides were mounted in “Liquido de Swan”. Diagnostic features and measurements of adult specimens were observed using a binocular stereo-microscope and its micrometer facility (Krüss MSZ54, A.KRÜSS Optronic, Germany), measurements of the peritreme of nymphal specimens were observed with a microscope (Konus Campus BM-100-FM, Konus, Italia). Drawings were made by the first author using a Reichart drawing tube. The samples from which the drawings were made are deposited in the Slovak National Museum, Bratislava. Remaining samples are deposited in the second author’s collection.

Material examined

Spinturnix andegavina

Romania: Constanţa County, northern Dobrogea: **Liliecilor Cave** (44° 46′ N, 28° 48′ E, 45 m a. s. l.), 1 August 2006 and 28 August 2006, four females and ten males collected from mist-netted *M. daubentonii* in front of the cave (leg. I. Pocora, V. Pocora); **Limanu Cave** (43° 48′ N, 28° 31′ E, 26 m a. s. l.), 26 June 2006, 30 July 2006 and 27 August 2006, and 14 June 2007, ten females and eight males, three deutonymph females and two deutonymph males and seven protonymphs collected from mist-netted *M. daubentonii* in front of the cave (leg. I. Pocora, V. Pocora); Tulcea County, Danube Delta: **Letea Forest** (45° 30′ N, 29° 50′ E, 0–10 m a. s. l.), 17 June 2006, 26 June 2007, 1 July 2007, 3 July 2007, 6 July 2007, 7 July 2007, 8 July 2007, sixteen females and twelve males, six deutonymph females, twelve deutonymph males and ten protonymphs collected from mist-netted *M. daubentonii* above water and in the forest (leg. I. Pocora, V. Pocora).

Ukraine: Yavoriv District, Lviv region: **Ivano-Frankove village** (49° 54′ N, 23° 44′ E, 286 m a. s. l.), 18 August 2011, five females, six males, one deutonymph male and three protonymphs collected from four specimens of bat hosts *M. daubentonii* (leg. A.-T. Bashta). Bats were caught by mist-net set up under a bridge near the roost occupied by the migrating colony in the bridge crevices (between concrete blocks).

Spinturnix myoti

Romania: Constanţa County, northern Dobrogea: **Liliecilor Cave,** 27 June and 31 July 2006, eight females and seven males, five deutonymph females, five deutonymph males and four protonymphs collected from mist-netted *M. blythii* in front of the cave (leg. I. Pocora, V. Pocora); Harghita County, Varghis Gorge: **Meresti Cave** (46° 22′ N, 25° 54′ E, 640 m a. s. l.), 16 August 2004, 22 August 2006, seven females and ten males, five deutonymph females, seven deutonymph males and ten protonymphs collected from *M. blythii* and *M. myotis* in front of the cave and **Calului Cave** (46° 22′ N, 25° 54′ E, 618 m a. s. l.), 17 August 2004, two males, one deutonymph female and one protonymph collected from mist-netted *M. myotis* in front of the cave (leg. I. Pocora, V. Pocora); Suceava County, Rarau Mountains: **Liliecilor Cave** (47° 27′ N, 25° 33′ E, 1492 m a. s. l.) 6 August 2005, 26 August 2005 and 27 August 2005, 4 October 2007 and 9 September 2008, fifteen females and eleven males, four deutonymph females, three deutonymph males and five protonymphs collected from mist-netted *M. myotis* in front of the cave (leg. I. Pocora, V. Pocora).

Results and discussion

Adult stage measurements

As main diagnostic features to determine between *S. andegavina* and *S. myoti* body length and width and length

Table 1. Comparison of basic metric data of body parts of adult males of *Spinturnix andegavina* from Romania and Ukraine compared to *Spinturnix myoti* from Romania and literature data.

	BL	BW	DL	DW	VL	VW
S. andegavina						
Romania ($n = 30$)	729−843	586−729	571−643	429−529	336−379	217−250
Ukraine ($n = 5$)	779−857	714−736	586−643	464−500	336−357	243−257
Haitlinger (1978)	830−950	670−780			376−404	246−292
Peribáñez-López et al. (1989)	952−1095	750−869				274−280
Estrada-Peña and Sanchez (1989)	800−830	640−700	630−660	540−560	280−299	195−220
S. myoti						
Romania ($n = 30$)	829−1000	636−786	643−771	450−600	364−443	243−286
Dusbábek (1962)	972−1000	702−743			351−374	266−285
Pinchuk (1971)	902−1064	792				
Haitlinger (1978)	960−1130	760−870			403−468	272−320
Peribáñez-López et al. (1989)	1000−1119	738−952	720−821	500−607	411−446	250−399
Estrada-Peña and Sanchez (1989)	1029−1162	840−910	743−810	527−607	354−371	266−285

Notes: Measurements are in μm. Numbers of measurements individuals and locality for published data are not provided; see the respective source. BL – body length, BW – body width, DL – dorsal shield length, DW – dorsal shield width, VL – sternal shield length and VW – sternal shield width.

Table 2. Comparison of basic metric data of body parts of adult females of *Spinturnix andegavina* from Romania and Ukraine compared to *Spinturnix myoti* from Romania and literature data.

	BL	BW	DL	DW	VL	VW
S. andegavina						
Romania ($n = 30$)	907−1329	714−1057	714−843	514−643	186−229	143−200
Ukraine ($n = 5$)	1000−1286	786−1071	714−771	521−586	186−214	151−171
Haitlinger (1978)	1000−1360	750−970			218−246	182−224
Peribáñez-López et al. (1989)	1029−1559	739−1178			220	217
Estrada-Peña and Sanchez (1989)	908−960	790−900	600−670	490−520	160−180	130−152
S. myoti						
Romania ($n = 30$)	1100−1829	929−1214	800−900	571−657	189−229	186−229
Dusbábek (1962)	1453−1607	1013−1175			228−232	217−239
Pinchuk (1971)	1250−1466	902−1135				
Haitlinger (1978)	1210−1650	880−1200			212−254	228−256
Peribáñez-López et al. (1989)	1083−1827	809−1458	821−1107	512−1023	202−262	202−256
Estrada-Peña and Sanchez (1989)	1190−1722	938−1330	850−922	620−669	213−270	217−239

Notes: Measurements are in μm. For abbreviation explanations see Table 1. Numbers of measurements, individuals and locality for published data are not provided; see the respective source.

Table 3. Comparison of additional metric data of body parts of adult females of *Spinturnix andegavina* from Romania and Ukraine compared *Spinturnix myoti* from Romania and literature data.

	*n*S	*n*O	*n*V	*n*D	SD	P	PL	PW
S. andegavina								
Romania ($n = 30$)	3−4	1	65−85	80−110	fovea	ds	18−59	8−13
Ukraine ($n = 5$)	3	1	65−80	85−95	fovea	ds	41−87	8−13
Peribáñez-López et al. (1989)			46−49	70−78				
Estrada-Peña and Sanchez (1989)			70−80	80−100				
S. myoti								
Romania ($n = 30$)	3	1	70−105	95−130	fovea	ds	29−100	11−13
Peribáñez-López et al. (1989)			57−88	71−136				
Estrada-Peña and Sanchez (1989)			65−100	90−130				

Note: Measurements are in μm. Numbers of measurements, individuals and locality for published data are not provided; see the respective source. Numbers of measurements, individuals and locality for published data are not provided; see the respective source. *n*S – number of pairs of setae in sternal shield, *n*O – number of pairs outside sternal shield, *n*V – number of opisthoventral setae, *n*D – number of opisthodorsal setae, SD – surface of dorsal shield, P – dorsal pattern, PL – peritreme length, PW – peritreme width, and ds – denticulate scales.

Table 4. Comparison of additional metric data of body parts of adult males of *Spinturnix andegavina* from Romania and Ukraine compared to *Spinturnix myoti* from Romania and literature data.

	*n*S	*n*O	*n*V	*n*D	SD	P	PL	PW
S. andegavina								
Romania ($n = 30$)	4	1	20−30	16−44	pitted	cs	40−75	8−13
Ukraine ($n = 5$)	3−4	1−2	24−28	26−42	pitted	cs	66−74	8−11
Peribáñez-López et al. (1989)				28−34				
S. myoti								
Romania ($n = 30$)	4	1	18−28	32−46	pitted	cs	34−92	11−13
Peribáñez-López et al. (1989)			17−28	32−55				

Notes: Measurements are in μm. Numbers of measurements, individuals and locality for published data are not provided; see the respective source. For abbreviation explanations see Table 3. cs – conical scales.

and width of sternal shield were confirmed (Deunff 1977; Haitlinger 1978). Our values supported this determination picture (see Tables 1 and 2). Deunff (1977) proposed additional patterns to determine these two species: space between coxae I is reduced in males; however, the distance separating the base of the shield gnathosoma jugular is larger than in *S. myoti*. In males and females, the intercoxaux I–II and III–IV spaces are narrowed or absent. This features had not been reported by other authors (cf. Haitlinger 1978; Estrada-Peña and Sanchez 1989; Peribáñez-López et al. 1989). Peribáñez-López et al. (1989) mentioned in females *S. andegavina* 70–78 opisthodorsal setae, and consider this number a differentiator with comparison to the number in Deunff (1977, 80–100 setae) and Rudnick (1960, 90–130 setae) for *S. myoti*. Our measured values (80–110) were different and correspond with data provided by Estrada-Peña and Sanchez (1989). They found in *S. andegavina* 80–100 setae. Thus, the number of setae

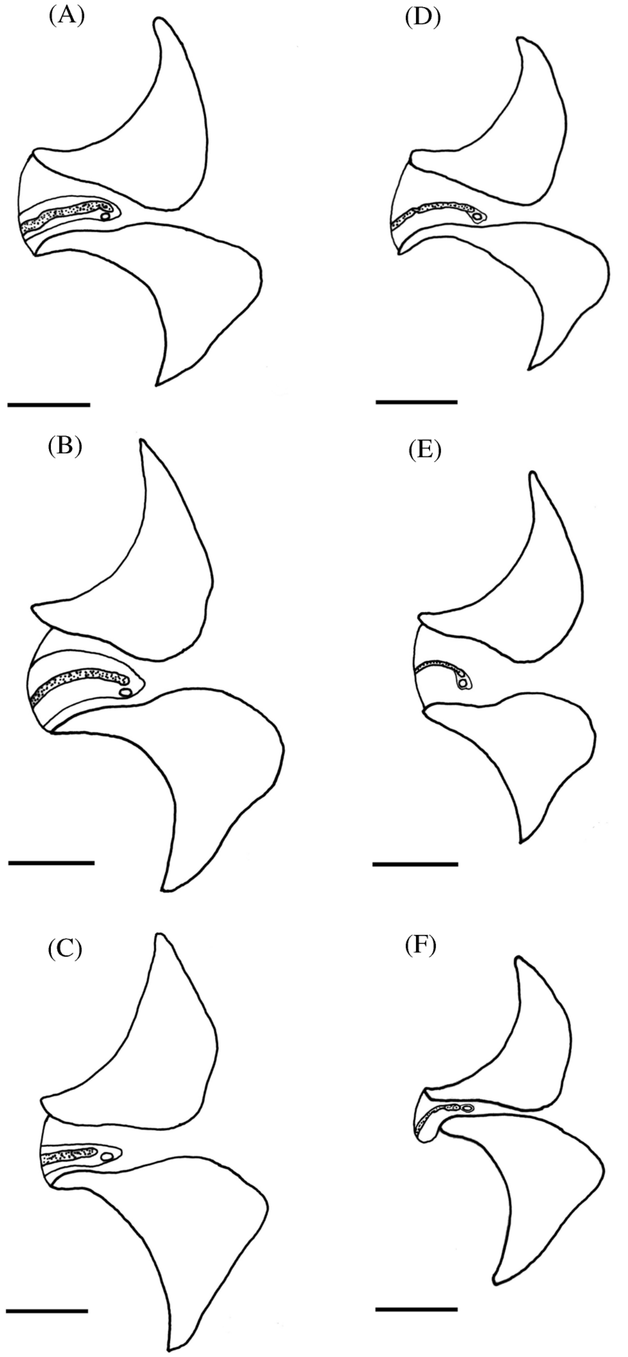

Figure 1. (A–F) Scheme of the peritremal ventral area in the vicinity of coxae II and III. Deutonymphs of *Spinturnix myoti* from Romania: A – male, B – female, C – male; deutonymphs of *Spinturnix andegavina* (from Romania): D – male, E – female; (from Ukraine): F – male. Scale-bars 100 µm.

overlap with the number of setae of *S. myoti* (see Table 3) and we cannot take it as a relevant feature for species determination. It could be also considered, that measurements provided by Peribáñez-López et al. (1989) are based only on four specimens. The author also points to the number of ventral opistosomales: for *S. andegavina* 46–49 compared with *S. myoti* 57–88, but measurements from *S. andegavina* were also provided from minimum (three) specimens. In further features, number of pairs of setae in sternal shield and outside sternal shield, surface of dorsal shield, dorsal pattern, peritreme length and peritreme width, we have not found any differences appropriate to distinguish between these species (see Tables 3 and 4) and they are reported here for an overview and comparison in further studies.

Nymphal stages description

Deutonymphs (Figures 1A–F, 2A–D)

According Estrada-Peña (1990), *S. andegavina* has larger or smaller but still visible intercoxal space (coxae II and III), while *S. myoti* has coxae II and III closely placed against each other without intercoxal gap.

The intercoxal spaces in both species measured on specimens from our collection are nearly the same size (Figure 1A–F) and we cannot take them as discriminant characteristics. On the other hand, we consider the peritrema as a suitable feature for identification. Although, the lengths of the peritrema in male deutonymph and female deutonymph of *S. andegavina* and *S. myoti* overlap, the width of the peritrema is different (Table 5, identification key). The peritrema in *S. myoti* is thicker than in *S. andegavina* (Figure 1A–F). Further, features suitable for identification in both sexes are the number of opisthodorsal setae (Table 5). Females of *S. andegavina* had 45–55 setae in contrast to 23–38 in *S. myoti,* and males of *S. andegavina* had 26–32 setae compared to 18–26 in *S. myoti*.

Protonymphs (Figures 1A–F, 2E–F)

For both species *S. andegavina* and *S. myoti*, some parts of the body are the same: six opisthodorsal setae, eight opisthoventral setae, ventral peritremal shield and pore absent, three pairs of setae and two pairs of pores on sternal shield. Our specimens of *S. andegavina* and *S. myoti* differed in their dorsal shields. *S. andegavina* has both length and width of dorsal shields about 10% smaller than *S. myoti* (see measurements in Table 6) and the shape of the pygidial shield are triangular. However, there were differences in the pygidial shield microsetae between specimens of *S. andegavina* from Romania and Ukraine. Specimens from Ukraine had one pair of microsetae in the pygidial shield compared to two pairs of microsetae and one pair of pores in specimens from Romania. However, these may merely be regional differences in these characteristics. Ventral peritreme width is also a different characteristic in these two species. The peritrema in *S. andegavina* is thinner compared with *S. myoti*, the same as in deutonymphs (see Table 6).

Identification key for protonymphs and deutonymphs of *Spinturnix andegavina* and *Spinturnix myoti*

Deutonymphs

1. Dorsal shield without ornamentation ...... **female, 2**
– Dorsal shield pitted .... **male, 3**

Table 5. Comparison of metric data of body part of deutonymphal stages of *Spinturnix andegavina* and *Spinturnix myoti* from Romania and Ukraine with literature data.

	BL	BW	DL	DW	VL	VW	PL	PW	nD
S. andegavina									
Romania, F (*n* = 9)	771–851	638–745	585–638	426–479	229–266	170–186	66–118	5–9	45–55
Romania, M (*n* = 14)	745–830	606–723	532–612	404–479	223–255	170–213	24–111	6–11	32–46
Ukraine, M (*n* = 1)	782	638	559	404	245	165	84	9	32
Peribáñez-López et al. (1989)	910–1047	750–857	714–738	482–547					38–46
S. myoti									
Romania, F (*n* = 15)	840–1011	660–872	606–723	426–521	197–319	170–213	61–126	11–13	23–38
Romania, M (*n* = 15)	798–958	638–819	585–681	479–540	245–309	160–213	61–116	12–13	36–44
Peribáñez-López et al. (1989)	845–1119	714–857	595–839	428–607	256–381	179–226			22–42

Notes: Measurements are in μm; average of metric parameters are in parentheses. Numbers of measurements, individuals and locality for published data are not provided; see the respective source. For abbreviations explanations see Tables 1 and 3. The author does not distinguish gender of deutonymphal stage.

Table 6. Comparison of metric data of body part of protonymphal stages of *Spinturnix andegavina* and *Spinturnix myoti* from Romania and Ukraine with literature data.

	BL	BW	DL	DW	VL	VW	PL	PW
S. andegavina								
Romania (*n* = 17)	692–766	564–692	479–559	367–404	192–234	122–170	40*	7*
Ukraine (*n* = 3)	692–745	585–617	489–553	372–372	192–197	133–154		
Peribáñez-López et al. (1989)	797–975	637–809	630–648	404–440				
S. myoti								
Romania (*n* = 20)	713–851	585–702	564–665	399–447	186–245	160–192	13–66	10–13
Peribáñez-López et al. (1989)	768–940	548–762	381–726	393–470				

Notes: Measurements are in μm, average of metric parameters are in parentheses. Numbers of measurements, individuals and locality for published data are not provided; see the respective source. For abbreviation explanations see Tables 1 and 3.

*Only from one specimen.

2. Number of opisthodorsal setae 45–55; length of proteronotal setae is one-fourth of the body length; anterior end of sternal shield is more elongated; width of ventral peritreme 6 μm; peritremal shield is reduced at the free end of peritreme and pore (Figure 1E) .... ***S. andegavina***

– Number of opisthodorsal setae 23–38; length of proteronotal setae is one-fifth of the body length; width of ventral peritreme 12.4 μm; peritremal shield is wider and more visible (Figure 1B) .... ***S. myoti***

3. Opisthoventral setae 26–32; anterior end of sternal shield is more elongated; width of ventral peritreme 7.9 μm; peritremal shield is reduced at the free end of peritreme and pore (Figure 1D, F) .... ***S. andegavina***

– Opisthoventral setae 18–26; width of ventral peritreme 13 μm; peritremal shield is wider and more visible (Figure 1A, C) .... ***S. myoti***

Protonymphs

1. The pygidial shield is triangular in shape with 1–2 pairs of microsetae (Fig 2E); width of ventral peritreme 7.8 μm................... ***S. andegavina***
2. The pygidial shield is rounded in shape at the posterior end reaching near opisthosomal setae, with two pairs of microsetae; width of ventral peritreme 11 μm .... ***S. myoti***

Notes on distribution

The previous findings of *S. andegavina* from Portugal, Spain and France (Deunff 1977) were later complemented by records from western and central Europe (Haitlinger 1978; Peribáñez-López et al. 1989; Estrada-Peña 1990; Haitlinger and Ruprecht 1992; Dietz and Walter 1995; Walter 1996; Haitlinger and Walter 1997; Ferenc et al. 2003; Giorgi et al. 2004; Rupp et al. 2004; Lučan 2006; Bruyndonckx et al. 2009).

Our records from Romania are the first findings from southern Europe and from Romania itself, where we recorded them from *M. daubentonii* as their host at three localities.

From the territory of Ukraine, our findings come from a single site, and they could also be considered as the first findings from this area. In Ukraine, the spinturnicid mites from *M. daubentonii* were already recorded (Bobkova 2002, 2005; Naglov and Tkatch 2002); however, these authors indicated these as other species from the *myoti* group, i.e. as *S. myoti* and *S. mystacinus* (Kolenati, 1857). We did not have any opportunity to revise these samples, and their re-identification was therefore not possible.

The range of *S. andegavina* could most probably cover the same extent as its main host, *M. daubentonii*; i.e. Scandinavia and Eastern Europe from northern Russia to the Caucasus (Mitchell-Jones et al. 1999). With respect to exceptional records of *S. andegavina* from further bat species [e.g. *Myotis dasycneme* (Boie, 1825) or *Myotis*

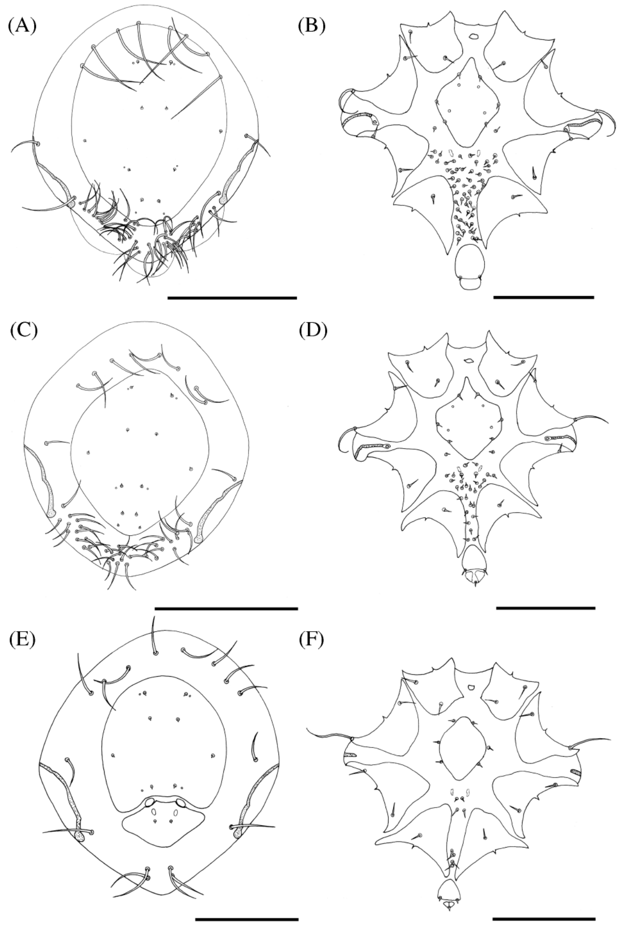

Figure 2. (A–F) View of nymphal stages of *Spinturnix andegavina*. A – dorsum of idiosoma of the deutonymphs (female); B – venter of idiosoma of deutonymph (female); C – dorsum of idiosoma of the deutonymph (male); D – venter of idiosoma of deutonymph (male) (both specimens from Romania); E – dorsum of idiosoma of protonymph; F – venter of idiosoma of protonymph (from Ukraine). Scale-bars 300 μm.

nattereri (Kuhl, 1817); Haitlinger and Walter 1997], its range could be probably wider. However, regarding relationship to main host species, mite records from *M. daubentonii* from Finland (Mrciak and Nyholm 1967), Latvia (Jaunbauere et al. 2008), Russia (Orlova and Pervushina 2010; Orlova 2011; Orlova et al. 2011) and Mongolia (Dusbábek 1966) indicated as *S. myoti,* almost certainly represent *S. andegavina*. Parasitic individuals were reported in great abundance in these references, and *S. andegavina* occurrence was not considered. *S. andegavina* was not considered even in the key of spinturnicid mites of Russia and adjacent countries (Stanyukovich 1997).

On the other hand, records of mites incorporated in the "*Spinturnix myoti* species group" from Asia (e.g. Dusbábek 1966; Uchikawa et al. 1994) also need to be verified as a consequence of changes in the species affiliation of their host. Mouse-eared bats from this region, which used to be considered as a subspecies of Daubenton's bat (*M. daubentonii*), were more recently revalidated as a separate species, *Myotis petax* Hollister, 1912 (Matveev et al. 2005; Kruskop et al. 2012).

According to Rudnick (1960), hosts of *S. myoti* are bat species *M. myotis*, *M. blythii* (Tomes, 1857), *M. capaccinii* (Bonaparte, 1837) and *M. nattereri* (Kuhl, 1817). Uchikawa et al. (1994) argued that verification of these *myoti* type species from *M. blythii*, *M. capaccinii* and *M. nattereri* is needed. So we expect extension of this parasite range based on distribution of *M. myotis* as its main host species [western, central and southern Europe, with individual records from southern England and southern Sweden, and in Asia Minor and in the Levant (Mitchell-Jones et al. 1999)]. All records outside this area and of other hosts should be verified.

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RESEARCH

Reticulinasus salahi (Acarina: Argasidae), a tick of bats and man in the Palaearctic and Afrotropics: review of records with the first pathogens detected

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Abstract

The soft ticks of the genus *Reticulinasus* Schulze, 1941 (family Argasidae Koch, 1844) are ectoparasites of the fruit bats of the Old World (Pteropodidae). *Reticulinasus salahi* (Hoogstraal, 1953) is the only representative of this genus that occurs in the western part of the Palaearctic. This unusual distribution reflects the distributon range of its primary host, *Rousettus aegyptiacus* (Geoffroy, 1810). In this contribution, we present a revised review of records of this tick that were made in two periods, 1951–1966 (records from Egypt, Israel, Jordan, Spain) and 2005–2019 (Cyprus, Iran, Oman), and additionally, we present notes, re-determinations, new records, and summary of hosts of this tick. Besides the primary host, the revised list of hosts comprises two bats (*Taphozous perforatus* Geoffroy, 1818, *Otonycteris hemprichii* Peters, 1859) and the human (*Homo sapiens* Linnaeus, 1758). We also tried to identify pathogens in specimens of this tick collected from *R. aegyptiacus* in Oman. The DNA of the Mouse herpesvirus strain 68 (MHV-68), of two bacteria, *Borellia burgdorferii* sensu lato, and *Ehrlichia* sp. almost identical (98%) with Candidatus *Ehrlichia shimanensis* was detected in several larvae specimens.

Keywords *Reticulinasus* · Summary · New records · Mediterranean · Middle East · Pathogens

Introduction

In accordance with a recent revision of the Argasidae ticks based on molecular genetics, the subgenus *Reticulinasus* Schulze, 1941 has to be raised to the genus level (Mans et al. 2021). This genus represents soft ticks parasitizing on the fruit bats (Pteropodidae) of the Old World, and only one species of this genus extends by its distribution to the Palaearctic, the Salah's Egyptian fruit-bat tampan, *Reticulinasus salahi* (Hoogstraal 1953). This range pattern is a direct consequence of the range extent of its primary host, the Egyptian fruit bat, *Rousettus aegyptiacus* (Geoffroy, 1810); the Palaearctic populations of this bat represent the only geographical offshoot of the fruit bat family (Pteropodidae) out of the tropics.

Records of this tick are rather scarce and most of them were made in the period of 1951–1966 with an additional finding in 2009 (Estrada-Peña et al. 1989; Hoogstraal 1953; Theodor and Costa 1960; Saliba et al. 1990; Benda et al. 2010). The evidence was reviewed by Sándor et al. (2021), who mapped a distribution range stretching from Spain to the Levant and Egypt. The latter authors also suggested a possible distribution extent of the tick based on the known range of its primary host

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and added a full list of the recorded hosts besides *R. aegyptiacus*, i.e. *Eptesicus serotinus* (Schreber, 1774), *Taphozopus perforatus* Geoffroy, 1818, and *Homo sapiens* Linnaeus, 1758.

Besides the primary parasitation of the Egyptian fruit bat, *R. salahi* has been evidenced to be a secondary parasite of humans (cf. Hoogstraal 1953; Lavoipierre and Riek 1955); despite this, only marginal attention has been paid to the distribution and ecology of this thick as well as its potential as a vector of pathogens. In the 1950s, only few attempts were made to find spirochaetes and/or salmonellas; however, these surveys failed in finding any of these pathogens (Hoogstraal 1953; Floyd and Hoogstraal 1956). In other bat ticks of the western Palaearctic and Afrotropics, of the genera *Carios* Latreille, 1796 and *Secretargas* Hoogstraal, 1957, the presence of pathogens is enormous. More than twenty species of bacteria and piroplasmids (namely, of the genera *Rickettsia* Da Rocha-Lima, 1916, *Coxiella* Philip, 1948, *Anaplasma* Theiler, 1910 /*Ehrlichia* Moshkovski, 1945, *Bartonella* Strong, Tyzzer, Brues et Sellards, 1915, *Borrelia* Swellengrebel, 1907, and *Babesia* Starcovici, 1893) were reported to be found in two argasid tick species parasitizing bats, which distribution range overlap with the range of *R. salahi* (Sándor et al. 2021).

Taking the aim of a revision of the current status of *R. salahi*, we complemented the review by Sándor et al. (2021) with new and/or revised data from the Middle East collected in 2005–2019. A small part of the newly obtained materials was subjected to a survey of bacteria and piroplasmids of the abovementioned genera. Additionally, a possibility of presence of the Mouse herpesvirus strain 68 (MHV-68), recently confirmed in bats of Europe and Central America (Briestenská et al. 2018; Janíková et al. 2020), was tested.

Materials and methods

Study area and materials

The study area covers the distribution range of the Egyptian fruit bat, *Rousettus aegyptiacus*, in the Middle East and north-eastern Africa, at the transition area of two zoogeographic regions, the Palaearctic and Afrotropics. Politically, it comprises wholes or parts of the following countries: Turkey, Cyprus, Syria, Lebanon, Jordan, Egypt, Sudan, Yemen, Oman, United Arab Emirates, and Iran. In these countries, the fruit bats were caught by standard methods, using mist or hand nets. All the body parts of the captured fruit bats (pelage, face, ears, wing membranes) were examined for the parasite presence, and all the ticks found were removed directly in the field by using tweezers and preserved in 96% ethanol. Some additional tick specimens were obtained secondarily, by examinations of the host specimens deposited in the collection of the National Museum, Prague, Czech Republic (NMP). In addition, one record from the Cave at the Sâsân Springat Bishapur, Iran, was realized by a random collection from the cave bottom under the fruit bat colony. Five specimens of *Reticulinasus salahi* were selected for a detailed examination and mounted onto slides using the Swan's embedding medium (Swan 1936).

Morphology determination

The adult and nymph ticks specimens stored in alcohol (Table 1) were examined employing standard microscopy (Karl Zeiss Jena, Germany) and compared to published morphology description of the type materials by Hoogstraal (1953: 256–258, Figs. 1–5). Additionally, all larva stages collected in Oman (Table 1) were prepared sufficiently to be identified according to their morphology, and the crucial characters like chelicera, hypostome, palp, Haller's organ, and dorsal plate (Dumbleton 1959: 307, Text–Fig. 18, Theodor and Costa 1960: 380–381, Text–Fig. 25 a, b, and Sonenshine et al. 1966: 118–120, Figs. 49–50).

Literature sources

All published records of *Reticulinasus salahi* were summarized, starting in 1953 when the species was described (Hoogstraal 1953), till mid-2022 (Table 2). Taxonomy and nomenclature of *Reticulinasus salahi* follow the revision of the family Argasidae by Mans et al. (2021).

Images

The images of the larvae specimens of *Reticulinasus salahi* from Oman (Fig. 1a, b) were taken by an Olympus XC30 digital camera installed on the Nikon E600 light microscope, where the bright-field and interference contrasts (Nomarsky-DIC) were applied. For processing the photos, analysis Docu v. 5.1 and Corel Photopaint X5 were employed. The photos of an adult female of *R. salahi* from Iran (Fig. 2a, b) were made using a Canon EOS 30D digital camera and Canon MP-E 65/2.8 Macro lens in multiple layers, stacked using Helicon Focus and edited in Corel Photopaint X3.

Depositories

The list of depositories containing specimens of *Reticulinasus salahi* species is included. The following abbreviations are used: BMNH, Natural History Museum (formerly, British Museum of Natural History), London, UK; FNHM, Field Museum of Natural History (formerly, Chicago Natural History Museum), Chicago, USA; CMŠ, private collection of Martin Ševčík, Nitra, Slovak Republic; CRBH, collection of Dr. R. B. Heisch, Nairobi, Kenya; DVSO, Division of Veterinary Services, Onderstepoort,

Table 1 New and corrected published records of *Reticulinasus salahi* arranged by country and date of collection

Country	Locality	Coordinates	Date	Host		Collector/s; host depository†	Number and stage	Detail of collection
Cyprus**	Afendrika	35° 39′ N, 34° 26′ E	17 October 2005	*Rousettus aegyptiacus*	1 ma	leg. R. Lučan	1 f [P]	
Iran*	Bishapur, cave at the Sâsân spring	29° 47′ N, 51° 35′ E	6 October 2011	under colony		leg. A. Reiter	1 n [A]	Benda et al. (2012)
Oman**	Al Hoota cave	23° 06′ N, 57° 22′ E	8 April 2011	*Rousettus aegyptiacus*	1 mj	leg. P. Benda, A. Reiter, M. Uhrin; NMP 93781	5 l [A][1]	
	Bidbid	23° 25′ N, 58° 08′ E	26 March 2011	*Rousettus aegyptiacus*	1 ma	leg. P. Benda, A. Reiter, M. Uhrin; NMP 93713	2 l [A]	
	Ain Sahnawt	17° 09′ N, 54° 11′ E	27 March 2012	*Rousettus aegyptiacus*	5 ma, 1 mj	leg. P. Benda, A. Reiter, M. Uhrin; NMP 94027–94029	23 l [A, P][2]	
	Shihayt, Wadi Darbat	17° 09′ N, 54° 28′ E	28 March 2012	*Rousettus aegyptiacus*	2 mj	leg. P. Benda, A. Reiter, M. Uhrin	9 l [A]	
	Wadi Hannah	17° 03′ N, 54° 37′ E	30 March 2012	*Rousettus aegyptiacus*	3 ma, 2 mj, 1 fa, 2 fj	leg. P. Benda, A. Reiter, M. Uhrin; NMP 94064	29 l [A, P][3]	
	dtto		22 October 2019	*Rousettus aegyptiacus*	1 ma	leg. P. Benda, J. Hájek, A. Reiter; NMP 97051	1 l [A]	

Explanations: *redetermination specimen, first record from country; **new and first record from the country; † the released bats are not mentioned; [1] 3 specimens from this collection used in pathogens study; [2] 2 specimens from this collection used in pathogens study; [3] 1 specimen from this collection used in pathogens study

Pretoria, South Africa; FIES, Fouad I Entomological Society, Cairo, Egypt; MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, USA; NMP, National Museum (Natural History), Prague, Czech Republic; RML, Rocky Mountain Laboratory, Hamilton, MT, USA; USNM, National Museum of Natural History (formerly,United States National Museum), Washington D.C., USA; ZIN, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia; others, A, alcoholic preparation; a, adult; f, female; j, juvenil; m, male; l, larva; n, nymph; P, mounted (tick) preparation.

Pathogen screening and phylogenetic analyses

Selected tick specimens (Table 1) were washed with fresh 70% ethanol, then with sterile water, dried, transferred individually to tubes, and crushed with a sterile Carbon Steel Surgical Scalpel Blade (Surgeon, JAI Surgicals Ltd., India). The DNA from the samples was isolated using QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The concentration and purity of the DNA were measured by NanoPhotometer Pearl (Implen, Germany). The DNA samples were stored at −20 °C and later used as templates for the PCR amplifications. Tick samples were screened by PCR-based methods for the presence of the MHV-68 virus, bacteria *Rickettsia* spp., *Anaplasma/Ehrlichia* spp., *Borrelia burgdorferi* sensu lato, *Bartonella* spp., and piroplasms *Babesia* spp. (Table 3). The PCR amplicons were purified and analyzed by sequencing in both directions in Macrogen Inc. (Amsterdam, The Netherlands). The DNA sequences were compared with those available in GenBank using the Basic Local Alignment Search Tool (Blast) (http://blast.ncbi.nlm.nih.gov). The new sequence generated in this study was submitted to GenBank under accession number OQ466707.

Results

Comments on records

Hoogstraal (1953: 256) reported the first record of *Reticulinasus salahi* from Israel; he mentioned the specimens collected in Jerusalem by O. Theodor. An additional locality of this tick from Israel was mentioned by Theodor and Costa (1960: 381), who found it on *Rousettus aegyptiacus* "in a cave in Herzliah together with specimens of *Ornithodorus tholozani* in 1951." However, Theodor and Costa (1960: 381) also commented Hoogstraal's (1953) report as follows:

Table 2 Published records of *Reticulinasus salahi* arranged by country and date of collection

Country	Locality	Coordinates	Date	Host	Sex and age host	Collector and deposited	Detail of collection	References
Spain	Prat de Llobregat (Barcelona)	41° 32′ N, 2° 09′ E	23 June 1955	*Eptesicus serotinus*	ma	leg. F. Lukoschus; not listed	1 l	Estrada-Peña et al. (1989)
Egypt	Hall under Mohammed Ali Mosque, Citadel area, (Cairo)	30° 03′ N, 31° 15′ E	9 May 1951	on the walls		leg. H. Hoogstraal; USNM, No. 2008	m (holotype), f (allotype)	Hoogstraal (1953)
	dtto		various time in 1951–52	on the walls and floors		leg. H. Hoogstraal, A. A. Salah, S. Mitwally, I. S. Khetr, S. Gaber; USNM, FIES, RML, MCZ, FNHM, BMNH, CRBH, DVSO, H. Hoogstraal private collection and other person private collections	400 m, 400 f, 400 n, 80 l (paratypes), 300 laboratory reared larvae from paratype parents	
	Nearby Sultan Hassan Mosque (Cairo)	30° 03′ N, 31° 15′ E		dtto; *Taphozous perforatus*			–; 1 l	
	Fom el Khalig aqueduct in Old Cairo (Cairo)	30° 01′ N, 31° 14′ E		dtto; man [= *Homo sapiens*]			–; “very frequently” records of these ticks “that many engorge”	
	Aquarium grotto, (Cairo)	30° 03′ N, 31° 13′ E		roosts of *Rousettus aegyptiacus*			unknown number l	
	Wadi Natroum, Western Desert	30° 22′ N, 30° 21′ E		dtto			unknown number l	
	Heliopolis (a suburb of Cairo)	30° 06′ N, 31°20′ E		street			1 n	
	The same localities as paratypes					leg. H. Hoogstraal; ZIN AL A853	1 f, 3 m, 1n (paratypes)	Filippova (2008)
	Citadel (Cairo)	30° 03′ N, 31° 15′ E		*Rousettus a. aegypticus* [= *Rousettus aegyptiacus*]			6 l	Sonenshine et al. (1966)
	Gezira Island (Cairo)	30° 03′ N, 31° 13′ E		dtto		leg. H. Hoogstraal; RML 25458	4 l	
Israel	Cave in Herzliah	32° 16′ N, 34° 81′ E	1951	*Rousettus aegyptiacus*		not listed	details of larva were added	Theodor and Costa (1960)
Jordan	Azraq-Shishan	31° 50′ N, 36° 49′ E	2 May 1966	*Myotis* sp. [= *Otonycteris hemprichii*]		leg. S. Atallah; not listed	8 l	Saliba et al. (1990)
Jordan	Iraq Al Amir	31° 55′ N, 35° 45′ E	10 May 2009	under the colony of *R. aegyptiacus*		leg. A. Reiter; CMŠ	4 m, 3 n [A]	Benda et al. (2010)

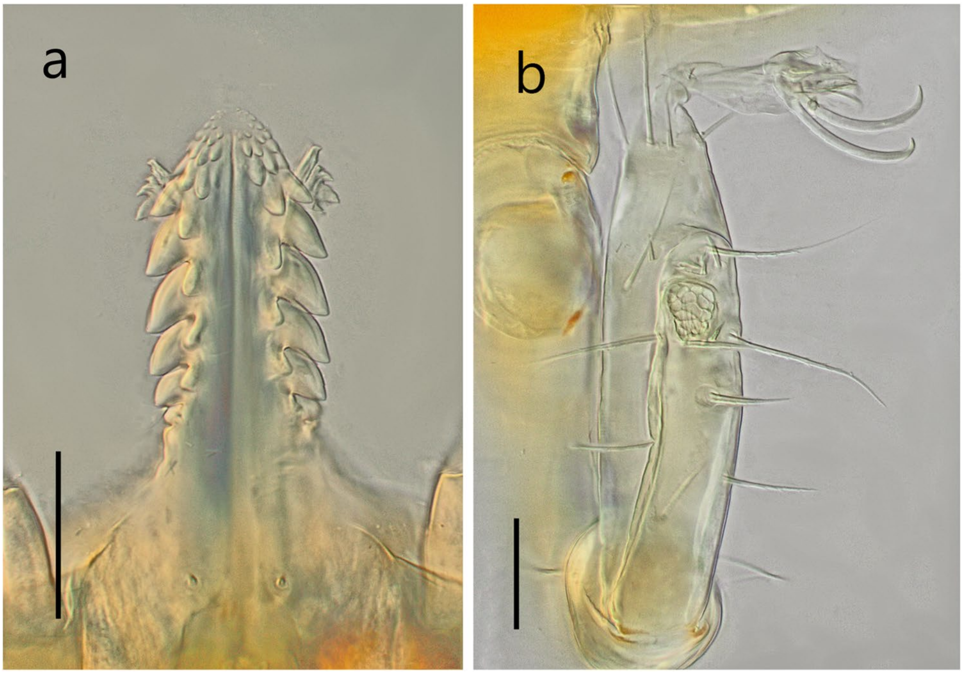

Fig. 1 Photomicrograph parts of capitulum of *Reticulinasus salahi* larvae from locality Ain Sahnawt, Oman. **a** Hypostome and partially protruding chelicerae. **b** Terminal segment of the first leg with the Haller's organ. Scale 50 µm

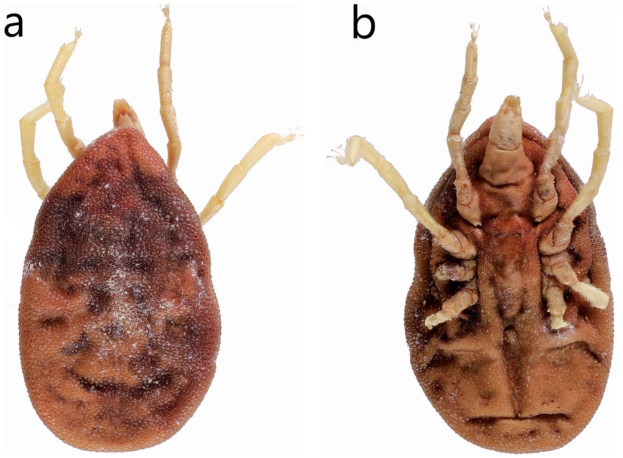

Fig. 2 Slightly engorged nymph of *Reticulinasus salahi* from locality Bishapur, cave at the Sâsân spring, Iran, originally identified as *Ornithodoros* sp. in Benda et al. (2012: 530). **a** Dorsal aspect. **b** Ventral aspect

"Hoogstraal, in a footnote in his paper on *O. salahi* [=*Reticulinasus salahi*] mentions that it has been found in Jerusalem. This is not correct, the only locality in which it has been found so far is Herzliah in the coastal plain. This mistake has been taken over by Leeson (1955 [=1956]) in his second paper on the distribution of species of *Ornithodorus*."

A similar situation appeared, when Hoogstraal (1962: 185) discussed the distribution of the genus *Reticulinasus* in Lebanon as follows: "Members of this subgenus are […] [*Ornithodoros* (*Reticulinasus*)] *salahi* Hoogstraal of Egypt, Lebanon, and Palestine." Nevertheless, we did not find any record of this tick from Lebanon (for a review, see Benda et al. 2016). Thus, we are unsure whether this note refers to an unpublished record or represents just an assumption based on the range of its primary host, *R. aegyptiacus*, in the Middle East.

Benda et al. (2012: 530) reported a single record of *Ornithodoros* sp. from Iran originally published with the following note: "An adult female of the tick *Ornithodoros* sp. was sampled from the bottom of the cave at the Sasan spring at Bishapur (Fars) where colonies of *Rousettus aegyptiacus*, *Rhinopoma microphyllum*, *R. muscatellum*, *Myotis blythii*, and *Miniopterus pallidus* were found." We examined the specimen in detail and clearly identified it as a nymph of *Reticulinasus salahi* (Fig. 2a, b).

On the other hand, we regard the reported finding of *R. salahi* from *Eptesicus serotinus* in Prat de Llobregat (Barcelona), Spain (Estrada-Peña et al. 1989, also in Cordero del Campillo et al. 1994), as doubtful, requiring a revision. The authors mentioned the deposition of the specimen at the Parasitology Unit of the Faculty of Veterinary Medicine of Zaragoza; however, we were not able to obtain it for a revision. Regarding the geographic distance to other record sites and the reported host species, we consider the species identification of the tick as erroneous (at least temporarily), until other records supporting such geographically and ecologically extraordinary findings are available. Estrada-Peña et al. (1989) considered this record unusual and accidental. In the western part of the Mediterranean, the bat species of the genus *Eptesicus* are primary hosts of other tick species, *Secretargas*

Table 3 Primers used for the detection and/or identification of different vector-borne bacteria in the examined ticks collected from bats

Assay (virus and bacteria)	Primer name	Primer sequence (5′- 3′)	Target gene	A. g. (bp)	A. t. (°C)	Reference
PCR	ORF50 F1	CCACCTGATCAAATATGCCA	ORF50 gene of MHV-68	969	57	Kabát et al. (2021)
MHV–68	ORF50 R1	TGTGGGTTTCTTGTTTGGAC	ORF50 gene of MHV-68			
	ORF50 F2	TGGCATATCCAGAGAAGTTGAG	ORF50 gene of MHV-68	581	57	
	ORF50 R2	TGGGAGTAGGTATGTAGCTCTG	ORF50 gene of MHV-68			
PCR						
Rickettsia spp.	SFGF	GAM AAA TGA ATT ATA TAC GCC GCA AA	hypothetical protein (RC0338 gene)	109	60	Socolovschi et al. (2010)
	SFGR	ATT ATT KCC AAA TAT TCG TCC TGT AC				
	SFGP	CTC AAG ATA AGT ATG AGT TAA ATG TAA A				
	RpCs.877p	GGG GGC CTG CTC ACG GCG G	citrate synthase (gltA) gene	380	47	Regnery et al. (1991)
	RpCs.1258n	ATT GCA AAA AGT ACA GTG AAC A				
	Rr190.70p	ATG GCG AAT ATT TCT CCA AAA	outer membrane protein A (ompA) gene	632	54	Roux et al. (1996)
	RR190.701R	GTT CCG TTA ATG GCA GCA TCT				
	17 K-5	GCT TTA CAA AAT TCT AAA AAC CAT ATA	17-kDa antigen gene	434	61	
	17 K-3	TGT CTA TCA ATT CAC AAC TTG CC				
	17kD1	GCT CTT GCA ACT TCT ATG TT		434	61	Anstead and Chilton (2013)
	17kD2	CAT TGT TCG TCA GGT TGG CG				
PCR						
Anaplasma/Ehrlichia spp.	16S8FE	AGA GTT KGA TCM TGG YTC AG	16rRNA gene spanning the V1 region	470	57	Bekker et al. (2002)
	B-GA1B	CGA GTT TGC CGG GAC TTY TTC T	16rRNA gene spanning the V1 region			
PCR						
Borrelia burgdorferi sensu lato	Bb23Sf	CGAGTCTTAAAAGGGCGATTTAGT	23S rRNA	77	60	Courtney et al. (2004)
	Bb23Sr	GCTTCAGCCTGGCCATAAATAG				
	Bb23Sp	6-FAM-AGATGTGGTAGACCCGAAGCCGAGTG-TAMRA				
	IGSa	CGA CCT TCT TCG CCT TAA AGC	rrfA-rrlB intergenic spacer (ITS)	225–255	56	Derdáková et al. (2003)
	IGSb	AGC TCT TAT TCG CTG ATG GTA-3				

Table 3 (continued)

Assay (virus and bacteria)	Primer name	Primer sequence (5′- 3′)	Target gene	A. g. (bp)	A. t. (°C)	Reference
PCR						
Bartonella spp.	BA325s	CTT CAG ATG ATG ATC CCA AGC CTT CTG GCG	16S–23S rRNA gene ITS region	420–780	66	Maggi et al. (2009)
	BA1100as	GAA CCG ACG ACC CCC TGC TTG CAA AGC A	16S–23S rRNA gene ITS region			
PCR						
Babesia spp.	BJ1	GTC TTG TAA TTG GAA TGA TGG	18S rRNA	450	55	Casati et al. (2006)
	BN2	TAG TTT ATG GTT AGG ACT ACG	18S rRNA			

transgariepinus (Beaucournu and Clerc 1968; Médard et al. 1997; own unpublished data from Morocco).

Finally, Saliba et al. (1990: 164) reported a record of *Reticulinasus salahi* (as *Ornithodoros salahi*) from Jordan being found on "*Myotis* sp., […] Azraq-Shishan, 2.v.1966." However, the bat specimens originally identified as *Myotis* sp., collected in May 1966 in Azraq-Shishan, represent in fact *Otonycteris hemprichii* Peters, 1859 (see Atallah (1967) and Benda et al. (2010)).

Newly collected specimens of *Reticulinasus salahi* were found on *Rousettus aegyptiacus* examined in two countries of the Middle East, Cyprus, and Oman (Table 1, Fig. 1a, b).

The morphologic characters by Dumbleton (1959) and Sonenshine et al. (1966) mentioned as additional for description and identification of the larvae of *R. salahi*, i.e., the shape of dorsal plate and/or capsule of Haller's organ, are very variable in the Omani specimens (Fig. 1b).

Pathogens

DNA samples were screened for the presence of MHV-68 virus by nested PCR targeting the ORF50. Of the six analyzed samples (3 larvae from Al Hoota cave, 2 larvae from Ain Sahnawt and 1 larva from Wadi Hannah, Oman; Table 1) of the genomic DNA isolated from the larvae of *Reticulinasus salahi*, the presence of the ORF50 sequence of the MHV-68 virus was found in one sample (Al Hotta Cave, Oman). The obtained sequence of the ORF 50 nested PCR fragment showed 100% homology (position from 68,219 to 68,799 nucleotides) only with the major lytic transactivator protein, which is specific for this virus, and 85% homology with wood mouse herpesvirus. In addition, the temperature profile of the PCR reaction was designed in such a way that amplification of partially homologous sequences does not occur. The presence of the MHV-68 strain has been documented for the first time in this part of the Middle East.

On the other hand, all the analyzed samples of *Reticulinasus salahi* larvae were negative for the presence of the DNA of *Rickettsia*, *Bartonella*, and *Babesia* spp. One tick sample (Al Hotta Cave, Oman) was positive for *Borellia burgdorferi* by real-time PCR. However, since the ct value was > 36, it was not successfully sequenced. The DNA extract of the *R. salahi* larva collected from *Rousettus aegyptiacus* in Wadi Hannah (Oman) was PCR positive for the presence of the *Anaplasma*/*Ehrlichia* 16S rRNA. In this sample, the sequences (GeneBank Accession Number OQ466707) were identical for 97.8% to the Candidatus *Ehrlichia shimanensis* (GeneBank Accession Number AB074459).

Discussion

Specimens of the tick *Reticulinasus salahi* were collected in two separate periods. In 1951–1966, the species was described and the first data on its natural history were gathered. Several species of hosts were documented at that time: *Rousettus aegyptiacus*, *Eptesicus serotinus*, *Taphozous perforatus*, *Myotis* sp. [= *Otonycteris hemprichii*], and *Homo sapiens*. The records were made mainly in Egypt and additionally also in Israel, Jordan, and Spain (Hoogstraal 1953; Theodor and Costa 1960; Estrada-Peña et al. 1989; Saliba et al. 1990; Benda et al. 2010). In 2005–2019, new records are available only from one host species, *Rousettus aegyptiacus*. The latter records findings come from specialized trips organized to investigate the bat fauna of the Middle East (Benda et al. 2007, 2010, 2012) (Fig. 3). However, the records of *R. salahi* from the northern Levant (Israel and Lebanon) remain uncertain. On the other hand, we identified a finding from Iran, which represents new extension of the species distribution range. The westernmost record of *R. salahi*, reported from *Eptesicus serotinus* collected in Spain (Estrada-Peña et al. 1989), does not conform to other records considering the host species as well as the distribution area. We suggest to consider it as dubious until it is revised and the species identification confirmed without doubts.

Collections of ticks in free habitats (off the hosts) and checks the tick presence in various habitats still miss, despite the records made in recent time. Considering the primary host range, the available records of *R. salahi* come from just a fragment of the expected distribution range. Based on his personal records, Hoogstraal (1953) regarded *R. salahi* to be by far the most common tick parasite of bats in the downtown of Cairo; its density and abundance thus could be very high. A factor influencing the occurrence of *R. salahi* could be the size colony of the Egyptian fruit bats, the primary host. Already, Hoogstraal (1953: 260) reported that in the course of 2 years, he searched for this tick in tens of potential roosts (caves and artificial spaces) in the Cairo region and surrounding areas of Lower Egypt, but he found of *R. salahi* only in the proper area of Cairo in three sites (Mohammed Ali Mosque in the Citadel, Sultan Hassan Mosque, Fom el Khalig). In all cases, the sites of findings were roosts of very large colonies of *R. aegyptiacus*. Another important factor influencing the obvious presence of ticks in the bat roost could be the day period; Hoogstraal (1953: 261) noted as follows: "Engorged larvae can easily be found among moist bat droppings on the floor at each site where fruit bats rest. Nymphs and adults rest among bat droppings, under rocks, or in lower wall crevices. They commence crawling upwards on walls toward midday." Benda et al. (2011) summarized the records of colonies *R. aegyptiacus* throughout its Palaearctic range; this review could be used for searching of the tick occurrence.

The evidence of the MHV-68 virus in specimens from Oman (Al Hoota Cave) includes *R. salahi* among possible vectors of this pathogen among ticks; the DNA of this virus was detected already in the ixodid species *Dermacentor reticulinasus* and *Ixodes ricinus* (Kúdelová et al. 2015, 2017, 2018). Besides ticks, one of the main reservoirs of this virus is rodents (Blaškovič et al. 1980; Mistríková and Blaskovic 1985; Hricová and Mistríková 2008). However, according to the results of laboratory experiments, *R. salahi* does not parasitize other vertebrates (Lavoipierre and Riek 1955), and this fact suggests that another reservoir of the MHV-68 virus could be bats and/or humans (primates), the only two known groups of hosts of *R. salahi*. In both groups of hosts, this virus was already confirmed (Briestenská et al. 2018; Janíková et al. 2020; Wágnerová et al. 2015). Our new finding of this virus in Oman represents the southernmost known occurrence spot of this pathogen in the Old World. Our results also support the hypothesis that the MHV-68 virus is a globally widespread herpesvirus capable of inter-species transmission, using one of the suitable vectors available on the site. Now is clear that *R. salahi* is another tick species which could serve as a reservoir of the virus and play a certain role in its ecology and epidemiology.

One larva of *R. salahi* collected in Oman (Al Hotta Cave) was positive for *Borellia burgdorferi* s.l. by real-time PCR. Among the soft ticks parasitizing bats, the presence of *B. burgdorferii* s.l. was documented only in *Carios vespertilionis* Latreille, 1796 (Hubbard et al. 1998). This tick species was collected from *Rousettus aegyptiacus* at Lokwi in South Sudan (Hoogstraal 1956), so the parasitation of this bat by *C. vespertilionis* cannot be excluded also in other parts of

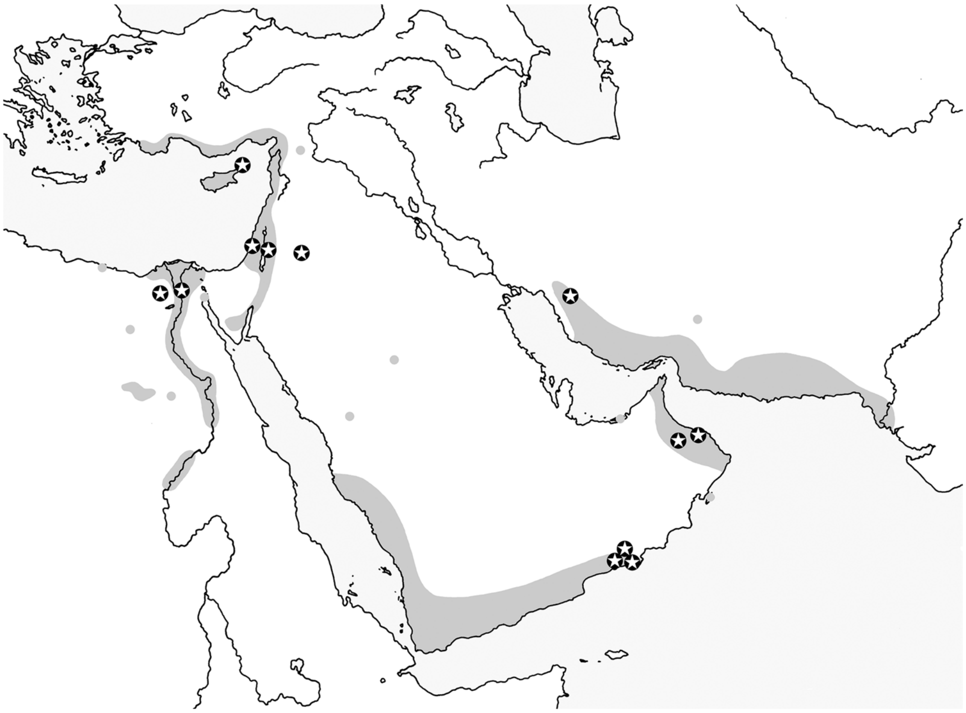

Fig. 3 Map of the records of *Reticulinasus salahi* (star dots) against the Palaearctic distribution range of its principal host, *Rousettus aegyptiacus* (dark gray areas); the gray dots denote the records of *R. aegyptiacus* out of its continuous range. For the parasite records, see text and Tables 1 and 2; the host range is reconstructed after Benda et al. (2011, 2012), Judas et al. (2018), and Benda and Ševčík (2020). The alleged record of *R. salahi* from Spain is not depicted (see text for details)

its range, including the Palaearctic—this tick is a common parasite of the vespertilionid bats in the latter region (Sándor et al. 2021).

The presence of the Candidatus *Ehrlichia shimanensis* DNA was discovered in a larva of *R. salahi* from *Rousettus aegyptiacus* collected at Wadi Hannah in Oman. The Candidatus *E. shimanensis* has been known only from the temperate zone of Central and East Asia, found in game species and small rodents, and also in the hard tick *Haemaphysalis longicornis* Neumann, 1901 (Kawahara et al. 2006; Rar et al. 2008). The vectors of *Ehrlichia* sp. are hard ticks and any connection with the soft ticks has been unknown (Socolovschi et al. 2012). Further studies are needed to describe in detail these agents and determined whether *R. salahi* could really represent their vector and/or reservoir.

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Data availability The sequences obtained in this study are deposited in GenBank. All other relevant data are included in the manuscript and the references are available upon request from the corresponding author.

Declarations

Ethics approval All applicable institutional, national, and international guidelines for the care and use of animals were followed.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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First records of *Secretargas transgariepinus* (Argasidae) in Libya and Jordan: corrections of collection records and detection of microorganisms.

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First records of *Secretargas transgariepinus* (Argasidae) in Libya and Jordan: corrections of collection records and detection of microorganisms

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Abstract

The primarily bat-associated argasid, *Secretargas transgariepinus* (White, 1846), is a member of the Afrotropical and southern Palaearctic fauna. Probably because of its secretive life style, little is known about this species and records of its collection are scant. Based on morphological revisions of the available specimens, we report new Middle Eastern records for this tick species, that had been misidentified as other bat-associated argasid taxa. These specimens are larvae from three localities, and represent the first records of *S. transgariepinus* from two countries: one larva from Sabratha (Libya) was collected from an unidentified bat (possibly *Eptesicus isabellinus*), seven larvae from Azraq-Shishan (Jordan), and 78 larvae from Shamwari (Jordan) were all collected from *Otonycteris hemprichii*. Twenty larvae from Shamwari were also tested for the presence of both, viral or bacterial microorganisms by PCR. Three ticks were found to be infected with the Murine gammaherpesvirus 68, one with *Borrelia burgdorferi* sensu lato, and four with a *Rickettsia* sp. closely related to *Rickettsia slovaca*. The findings represent a first evidence for the occurrence of these possible pathogens in *S. transgariepinus*.

Keywords Secretive bat-argas, hidden tick, arid area, main host, virus, microorganism, Middle East

Introduction

The secretive bat-argas, *Secretargas transgariepinus* (White, 1846) (Ixodida: Argasidae) is a bat ectoparasite, that occasionally parasitizes lizards and, rarely, humans (Reeves et al. 2020, Sándor et al. 2021). It belongs to the Afrotropical and Palearctic fauna and its natural distribution is restricted to arid habitats of the subtropical zone. The geographical range of *S. transgariepinus* covers a belt of deserts and dry steppes extending from Morocco and southern Europe to Afghanistan in the Northern Hemisphere (Roshdy 1961, Sonenshine et al. 1962, Dusbábek 1970, Sándor et al. 2021). In the Southern Hemisphere, it occurs in South Africa and Namibia (White 1946, Neumann 1901, 1906, Howard 1908, Belford 1932, 1934, Pienaar et al. 2018, Hornok et al. 2019, Reeves et al. 2020). Within the northern section of the range, the northernmost records were from France and Switzerland where, however, the tick was considered to be imported by its migratory bat hosts (cf. Aeschlimann et al. 1965, Beaucournu 1966). The southernmost records in the Northern Hemisphere were from Algeria (Bendjeddou et al. 2017). Most records of *S. transgariepinus* are from the western part of the Mediterranean Basin, in Morocco, Algeria, Spain, France, and Italy. In the central and eastern parts of the Mediterranean, abundant findings were reported only from the Cairo area of Egypt (Hoogstraal 1952) and, less frequently, from Greece and Israel (Hoogstraal 1952, 1957, Theodor and Costa 1960, 1967, Sándor et al. 2021).

The rarity of records of *S. transgariepinus* is mainly due to its behavior. Indeed, like in most soft ticks, adults and nymphs parasitize their hosts for a very short time. The two stages are also hard to find because they quest around the bat roosting sites, hiding in small crevices and among rocks (cf. Berlese 1913, Hoogstraal 1952). Larvae have been collected more often, directly from their hosts, because they feed for longer periods. Larval specimens were mostly collected from bats of the genera *Hypsugo* Kolenati, 1856, *Plecotus* Geoffroy, 1818, and *Eptesicus* Rafinesque, 1820 (Mèdard et al. 1997, Medard et al. 2001). Collections from the environment (caves) represent a minority of records. (White 1846, Methuen 1848, Hoogstraal 1957, Pienaar et al. 2018, Reeves et al. 2020).

Because *S. transgariepinus* can occasionally parasitize humans, it is important to establish if it can carry microorganisms of medical interest (Reeves et al. 2020, Sándor et al. 2021). *S. transgariepinus* occurs in sympatry, and even syntopy, with another bat-associated soft tick, *Carios vespertilionis* (Latreille, 1802). In the Old World, *C. vespertilionis* is the soft tick species known to carry the largest number of microorganisms and, possibly, pathogens (Beaucournu and Clerck 1968, Sándor et al. 2021). The two tick species share hosts, and, in particular, Vespertilionidae, a bat family wich has been found to be infected with a very diverse array of bacteria of the genera *Anaplasma* Theiler, 1910, *Bartonella* Strong, Tyzzer, Brues et Sellards, 1915, *Borrelia* Swellengrebel, 1907, *Coxiella* Philip, 1948, *Francisella* Dorofe'ev 1947 , *Leptospira* Noguchi, 1917, *Mycoplasma* Nowak 1929, *Neorickettsia* Philip, Hadlow et Hughesand, 1953, *Rickettsia* Da Rocha-Lima, 1916 (Szentiványi et al. 2023).

The first study of microbes in *S. transgariepinus* was based on materials from the Middle East, more precisely from caves in Ghiza, Egypt (leg. H. Hoogstraal). *Wolbachia* sp. Hertig, 1936 and/or *Rickettsia* were documented from the Malpighian tubules of this tick (Roshdy 1961, 1964). A total of 19 females, collected from one site in Ghiza, were used to test if they could transmit Keterah virus (KTRO, nairoviruses) (Varma and Converse 1976). Additionally, two microbes of unknown pathogenicity, *Rickettsia hoogstraalii*, a spotted fever group bacterium, and *Rickettsiella* sp., were detected with the help of molecular methods in *S. transgariepinus* from the Namib Desert of Namibia (Hornok et al. 2019, Reeves et al. 2020). Recently, interest for Murine gamma herpesvirus 68 (MHV-68) has increased. Antibodies anti-MHV 68 and the DNA of the ORF50 of the MHV-68 virus were detected in domestic and wild mammals, including bats, but also in humans in Europe, Asia, and South America (Wágnerová et al. 2015, Briestenská et al. 2018, Janíková et al. 2020, Kabát et al. 2022). The MHV-68, a prototype strain of *Rhadinovirus muridgamma 4* (muridgammaherpesvirus 4, MuGHV4) from the genus *Rhadinovirus* (Herpesviridae) that is closely related to the human viruses *Lymphocryptovirus* (Epstein-Barrvirus, human gamma herpesvirus 4, HuGHV4) and *Rhadinovirus* (Kaposi's sarcoma-associated virus, the human gamma herpesvirus 8, HuGHV8), were first described by Dong et al. (2017) and Mistríková and Briestenská (2020). It is used as a murid laboratory model for a better understanding of the pathogenesis of similar human infections. In particular, studies have focused on revealing the mechanisms behind the development of malignant forms, like the Burkitt lymfoma, Hodgkin's diseases and/or Kaposi sarcoma (Dong et al. 2017, Mistríková and Briestenská 2020). The role of bats as reservoir host for this virus and the possible part played by ticks in transmitting it remain unexplored (Dietrich et al. 2016).

In this study, we re-determined specimens of soft ticks from Libya and Jordan, that we suspected had been originally misidentified (see Saliba et al. 1990, Benda et al. 2010, 2014). We also screened 20 larval specimens from Jordan for the presence of tick-borne viral and bacterial microorganisms.

Material and Methods

Study material

The examined material included one larval specimen found in a jar containing ticks from different bat species (4 *Eptesicus isabellinus*, 1 *Myotis punicus*, and 4 *Pipistrellus kuhlii*) collected in Sabratha, Libya, on May 28, 2002 and deposited in the Zoological Collection of the National Museum, Prague, Czech Republic, leg. M. Ševčík. It had originally been identified as *Argas* sp., see Benda et al. 2014: 130); 78 larvae, originally identified as *Argas vespertilionis* (Benda et al. 2010: 234), collected from a single female of *Otonycteris hemprichii* (NMP 92824) in the Shawmari Nature Reserve (SNR), Jordan, on July 10, 2010, leg. P. Benda and A. Reiter; and 7 larvae, originally identified as *Ornithodoros salahi* (Saliba et al. 1990: 164) and collected from a bat determined as *Myotis* sp. (later identified as *Otonycteris hemprichii*, see Atallah [1967], Benda et al. [2010], and Ševčík et al. [2023]) of unidentified sex and age in Azraq-Shishan, Jordan, on May 2, 1966, leg. S. Atallah. For a description of the methods used to trap the bats and of other field records, refer to Saliba et al. (1990) and Benda et al. (2010, 2014).

Morphological identification

The ticks were blot-dried on clean filter paper and observed under a stereomicroscope. The re-identification of the taxonomic affiliation of the ticks was carried out using morphological keys by Hoogstraal (1957: 546, Figs 6–9; 548–549), Sonenshine et al. (1962: 205: Fig. 11, 208: Fig. 23 A, B), and Theodor and Costa (1960: 376, Text-fig. 15–16, 377). The following key characters were used to distinguish *S. transgariepinus* from the argasid species at the source of the misidentifications: well-defined dorsal plate, with reticulate pattern consisting of convex and shining meshes; spiracular opening anterior to coxa II, relatively large and oval

with numerous long spines projecting into its lumen; palps with fourth segments, the second segment significantly longer, almost as long as other three segments together, the fourth segment is much thinner than others (cf. Theodor and Costa 1960: 368, Fig. 4; 376–377, Fig. 16b).
Taxonomy and nomenclature of *Secretargas transgariepinus* follow the revision of the family Argasidae by Mans et al. (2021).

Images

The images of the larva specimens of *S. transgariepinus* collected at Azraq-Shishan, Jordan, were taken by using the BK Plus Lab System (Visionary Digital), and stacked with Helicon Focus v. 4.77.

Material depositories

The single larva from Sabratha, Libya, is deposited in the private collection of the first author (CMŠ [alcohol preparations]). Of the 78 larvae from the SNR, Jordan, originally housed at the Department of Zoology and Anthropology, Constantine the Philosopher University in Nitra, Slovakia (cf. Benda et al. 2014), 33 are currently deposited in the private collection of Martin Ševčík, Nitra, Slovakia (CMŠ [alcohol]); 25 in the collection of the National Museum, Prague, Czech Republic (NMP P6A 7529 [alcohol/withered preparations]) and 20 specimens in the collection of the Institute of Virology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia (as a dissoluted DNA sample). The 7 larvae from Azraq-Shishan, Jordan, leg. S. Atallah, are deposited in the United States National Tick Collection, Statesboro, Georgia (USNMENT01786798).

Pathogen screening and phylogenetic analyses

Twenty specimens from the material collected at the SNR, Jordan, were washed with 70% ethanol, then with sterile water, dried, transferred individually to tubes, and fragmented with a sterile Carbon Steel Surgical Scalpel Blade (Surgeon, JAI Surgicals Ltd., India). The DNA from the samples was isolated using the QIAamp DNA Mini Kit (Qiagen, Germany) according to the manufacturer’s instructions. The concentration and purity of the DNA were measured with a NanoPhotometer Pearl (Implen, Germany). The DNA samples were stored at −20°C and later used as templates for the PCR amplifications. Tick samples were tested for the presence of the MHV-68 virus by a nested PCR targeting the ORF50 gene (57). Rickettsial organisms were first detected by real-time PCR with probe SFGP targeting RC0338gene. Primers for *glt*A, *omp*A, and 17-kDa genes fragments (47, 54, 61) were then used to amplify the corresponding gene fragments by conventional PCR. The presence of bacteria *Anaplasma*/*Ehrlichia* spp., *Borrelia burgdorferi* sensu lato, *Bartonella* spp., and the piroplasms *Babesia* spp. (Table S1) were screened by conventional PCR (57, 60, 66, 55). The PCR amplicons were purifed and analyzed by sequencing in both directions in Macrogen Inc. (Amsterdam, The Netherlands). The DNA sequences were compared with those available in GenBank using the Basic Local Alignment Search Tool (Blast; http://blast.ncbi.nlm.nih.gov). A phylogenetic tree was constructed using the Neighbor-Joining method (Saitou and Nei 1987). Evolutionary analyses were conducted in MEGA11 (Tamura et al. 2021). The evolutionary distances were computed using the p-distance method (Nei and Kumar 2000). A concatenated phylogenetic tree inferred from comparison of the *Rickettsia* 17-kDa, *gltA*, *ompA* partial sequences. Partial 17-kDa, *gltA* and *ompA* genes sequences for representative samples were submitted to the GenBank under the accession numbers OR900065–OR900068 for 17-kDa gene, OR900069–OR900071 for the *gltA* gene and OR900072 – OR900074 for the *ompA* gene.

Results

Records

The revision and comparison of the morphological characters of the examined specimens revealed that they all corresponded to *S. transgariepinus.* The engorged larva from Sabratha, Libya was characterized by a typical dorsal plate and the respiratory system at the anterior surface of coxa 2, palps with fourth segments, the second segment significantly long, almost as long as other three segments, the fourth segment is much thinner). Of the original 8 engorged larvae from *Myotis* sp. from Azraq-Shishan, Jordan, 7 specimens were left, of which only one was sufficiently well preserved for examination (see Fig. 1a, b). This specimen shared all relevant morphological characters with *S. transgariepinus.* In few of the 78 larvae (unengorged and engorged) from SNR, Jordan the hypostome was broken and the spiracular plate was not visible. Otherwise, they also all corresponded to the mentioned description.

Pathogens

Of the twenty samples of the *S. transgariepinus* analyzed by nested PCR, the presence of ORF50 sequence was confirmed in 3 of them. The obtained PCR product (580 bp) showed 100% homology to the sequence of the MHV68 WUMS strain (Acc No U97553.2) of the ORF50 gene (position from 68219 to 68799 nucleotides).

One tick sample was positive for *B. burgdorferi* sensu lato by a real-time PCR assay. However, because the ct value was >36, the amplicon could not be successfully sequenced. As for rickettsial testing, while nine tick were positive by real-time PCR with probe SFGP targeting RC0338, only for 3 of them the amplification of *glt*A, *omp*A, and 17-kDa genes fragments was successful. In the phylogenetic reconstruction, the *S. transgariepinus* rickettsial lineages clustered with *R. slovaca* with a branch support of 96–98% (Fig. 2a). This was also confirmed for a fourth specimen for which only the 17kDa gene fragment could be obtained (99.72%; 353/354 bp identity). A comparison of identities of the obtained sequences from *S. transgariepinus* larvae with sequences in GenBank is presented in Fig. 2b; Table S2).

Discussion

Correction of findings

The presence of the desert long-eared bat, *O. hemprichii*, among the hosts of the revised tick material suggesteded that some of the previously listed species could have been incorrectly identified. For the same reason, we questioned the original identification of the ticks.

O. hemprichii is a desert-dwelling species, distributed in arid areas of North Africa and the Middle East (Benda and Gvoždík 2010). It roosts mainly in rock crevices and small cavities which can support the life cycle of a tick that likes to hide, such as *Argas confusus* Hoogstraal, 1955 or *S. transgariepinus* (see Hoogstraal 1956).

The oldest record of *S. transgariepinus* from the Middle East, eight larvae from Azraq-Shishan, Jordan, was originally referred to as *Ornithodoros* (*Reticulinasus*) *salahi* by Saliba et al. (1990). Ševčík et al. (2023: 1274, 1277) reidentified the host species as *O. hemprichii*, instead of the originally reported *Myotis* sp. (see also Atallah 1967 and Benda et al. 2010). This reidentification of the host species, which favors harsh deserts, along with the long distance of Azraq-Shishan from the range of the Egyptian fruit bat, *Rousettus agyptiacus*, a primary host of *Reticulinasus salahi* (more than 100 km to the Rift Valley in western Jordan, see Benda et al. 2010), suggested a possibile concomitant misidentification of the tick species. Indeed, *S. transgariepinus* seemed to be the most probable candidate for the correct identification, as was confirmed by a detailed morphological re-examination of one of the specimens mentioned by Saliba et al. (1990).

The second record from Jordan was originally described as follows: "*Argas vespertilionis*: 78 larvae (UCPN) from 1 fa (NMP 92824), Shawmari Wildlife Reserve, 10 July 2010, from host *Otonycteris hemprichii*" (Benda et al. 2010: 294). The unusually large number of parasites observed on a sigle bat was documented by a photograph showing a very heavy tick infestation (Benda et al. 2010: 291, Fig. 83). The hypothesis that these ticks were also incorrectly identified was supported by our re-examination: all these ticks were in fact *S. transgariepinus.*

The last record comes from Libya, a single specimen of tick was found in a museum jar containing three species of bats (Benda et al. 2014: 130) as follows: "*Argas* sp. (*A. vespertilionis* group): 1 larva (CMŠ [P]) from a jar containing 4 [specimens of] *Eptesicus isabellinus*, 1 *Myotis punicus* and 4 *Pipistrellus kuhlii*, Sabratha, 28 May 2002." The morphological examination of the concerned specimens led to them being reassigned to *S. transgariepinus*. Sándor et al. (2021) suggested a possible occurrence of this tick species in in the western part of Libya, an area included in the distribution range of its main bat hosts, *Eptesicus isabellinus* (Temminck, 1840), *Plecotus gaisleri* Benda, Kiefer, Hanák et Veith, 2004, and *Hypsugo savii* (Bonaparte, 1837). As *H. savii* does not occur in Lybia (Benda et al. 2014) , we can assume that the tick most probably originates from *E. isabellinus* or *P. gaisleri*.

However, the group of main hosts of this tick most probably covers a broad spectrum of bat species and consequently, also its distribution range is probably much large than the currently known. The above mentioned host, *O. hemprichii*, is referred by Sándor et al. (2021) as a secondary host species. However, our evidence, would indicate that it as one of the primary hosts of *S. transgariepinus*. The bats of the genus *Plecotus*, of which three species live in Africa and use a similar roosting strategy, have also been described as primary hosts of *S. transgariepinus*. It's important to mention that ticks, in general, have often been found to be more dependent for their survival on the availability of suitable environments (in this case secluded rock crevices) than on the presence of so-called specific/primary/secondary hosts (Klompen et al. 1996). The available data confirmed the occurrence of this tick also in the tropics of Africa (from *Plecotus balensis* Kruskop et Lavrenchenko, 2000, Desea Forest, Ethiopia, 13°53'N, 39°46'E, 30 October 2012, leg. P. Benda, own unpubl. data).

As a result, the traditionally treated ecological preferences of *S. transgariepinus* have to be re-defined. Originally thought to require very arid environments, such as those encountered in Egypt and the Northern Cape province of South Africa (Hoogstraal 1952, Pienaar et al. 2018), it is now clear that the species occurs also in more humid areas.

Murine gamma herpesvirus 68 (MHV-68), prototype strain

With the help of the PCR assays we confirmed the presence of the MHV-68 virus in four of twenty larva specimens of *Secretargas trangariepinus* collected from a single host specimen of *O. hemprichii* at the SNR, Jordan. The combination of the available data, i.e. collection of ticks from a single host bat individual, the size of the examined sample set (20 samples), and the method used (PCR) do not allow us to speculate on the vectorial capacity of the ticks. Indeed, a virus circulating in the bat blood would be ingested by the ticks (and detected by PCR in the tick DNA samples) even if the ticks might not be able to further transmit the virus to other hosts. Our results, like those of other studies involving bats and ectoparasites, (Briestenská et al. 2018, Janíková et al. 2020, Ševčík et al. 2023) cannot be interpreted for the time being. Laboratory transmission experiments will be required to elucidate whether or not *S. transgariepinus* ticks are competent vectors of MHV-68. Nevertheless, this represents the second geographical record of occurrence of the virus in the Middle East, after its discovery in *Reticulinasus salahi* in the Al Hotta Cave, Oman (Ševčík et al. 2023).

Bacteria

The posititivity for *Borrelia burgdorferi* s.l. was confirmed in a single larva of *S. trangariepinus* from the SNR, Jordan, by real-time PCR. Despite the fact that *Borrelia* was confirmed only in a single larva of the twenty tested, the host tick might really represent a reservoir of this microorganism. The reservoir competence of various vertebrate species for *B. burgdorferi* s.l. is determined by their capacity to effectively infect pathogen-free larval ticks under natural conditions or in xenodiagnostic experiments (Mannelli et al. 2011). The abundance of *B. burgdorferi* s.l. in the larvae of chiropterophilous hard ticks of the genus *Ixodes* predicts that the vespertilionid bats are most probably the reservoir hosts and effective vectors of this bacterium (Michalik et al. 2020). While our results prove the occurrence of a *B. burgdorferi* s.l. genotype in *S. transgariepinus*, they will have to be corroborated by additional studies and, more importantly, the detected spirochete will have to be fully characterized in order to gain any kind of understanding of its epidemiological importance (cf. Obaidat et al. 2020). So far, very little is known about the ecoepidemiology of borrelioses in the Middle East (records are known from Israel and Turkey; Abraham et al. 1991, Polat et al. 1998, Guner et al. 2003). Even less is known about the genetic diversity of *Borrelia* sp. in this area.

The *gltA*, *ompA*, and 17-kDa genes sequences amplified and sequenced from three samples of Jordanian larvae showed that the rickettsial organism in *S. transgariepinus* is a close relative of *R. slovaca*, a member of the spotted fever group (SFG) that was initially isolated in 1968 from the tick *Dermacentor marginatus* Sulzer, 1776 in Slovakia (Rehacek 1984). Although the presence of this pathogen and its role in bats is not known, in humans it can cause tick-borne lymphadenopathy (TIBOLA), also called *Dermacentor*-borne necrosis erythema and lymphadenopathy (DEBONEL) (Lakos 1997, Oteo et al. 2004). The available records of *R. slovaca* are linked to its main vector, *D. marginatus*, but also to other tick species from southern and central parts of Europe (Rehacek 1984, Beati et al. 1993, 1994, Selmi et al. 2008, Raoult et al. 2002, Špitalská et al. 2012) and, less so, from northern Africa and Asia (Shpynov et al. 2006, Sarih et al. 2008, Jiang et al. 2012, Kernif et al. 2012, Tian et al. 2012, Piotrowski et al. 2020). It is interesting to note that *D. marginatus* has sporadically been collected from bats, for instance from *Pipistrellus pipistrellus* (Schreber, 1774) in Iran (Fillipova et al. 1976), from *Myotis blythii* (Tomes, 1857) and *Rhinolophus euryale* Blasius, 1853 in Azerbaijan (Gadžiev and Dubovčenko 1975, Gadžiev et al. 1990). Although rare, these findings can explain how *R. slovaca* might have been introduced into bats. Recently, *R. slovaca* was detected in the visceral organs of Asian bats (Zhao et al. 2020) providing additional support for an epidemiological relationship between this specific rickettsial pathogen and bats. All our records can show, for the time being, is that the bacterium can be found in engorged *S. transgariepinus* and that its distribution range now reaches Jordan.

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Author contributions
Martin Ševčík, conceptualization, study design, methodology, resources, determination of samples, formal analysis, (draft) manuscript writing. Eva Špitalská, methodology, PCR test and sequencing, phylogenetic analyses, Genbank processing, supervision and contribution to molecular analyses, review and editing manuscript, funding acquisition. Michaela Maliterná, PCR test and sequencing. Peter Kabát, methodology, PCR test and sequencing, review and editing manuscript. Petr Benda, sample collection, study design, review and editing manuscript, funding acquisition.

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Data availability
The sequences obtained in this study are deposited in GenBank. All other relevant data are included in the manuscript and the references are available upon request from the corresponding author.

Declarations

Ethics approval
All applicable institutional, national, and international guidelines for the care and use of animals were followed.

Consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Figure captions

Fig. 1 Engorged larva of *Secretargas transgariepinus* found on *Otonycteris hemprichii* at Azraq-Shishan, Jordan, originally identifed as *Ornithodoros salahi* by Saliba et al. (1990). **a** dorsal aspect. **b** ventral aspect.

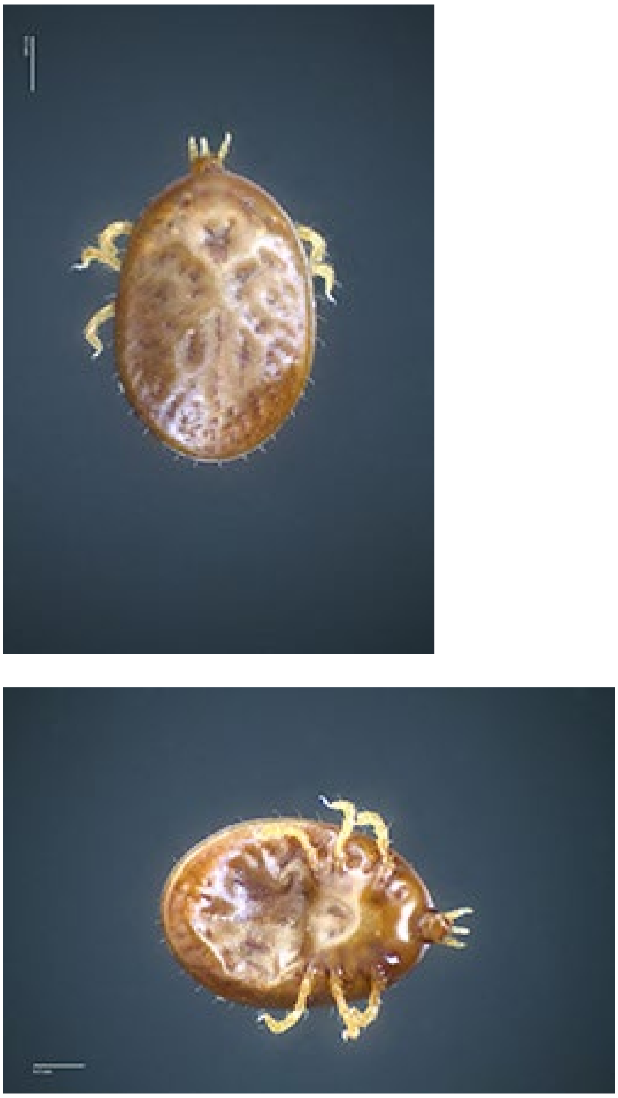
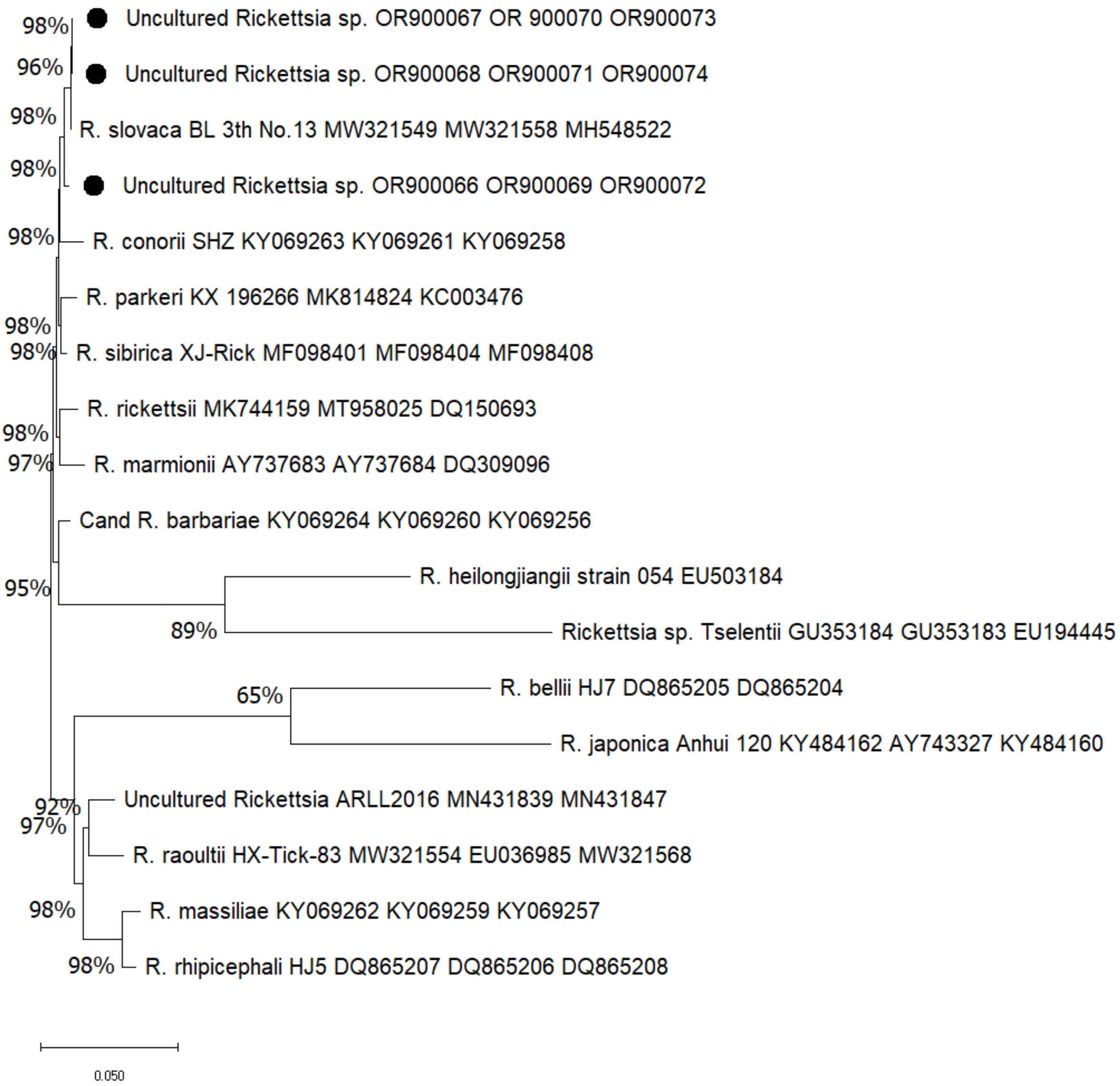
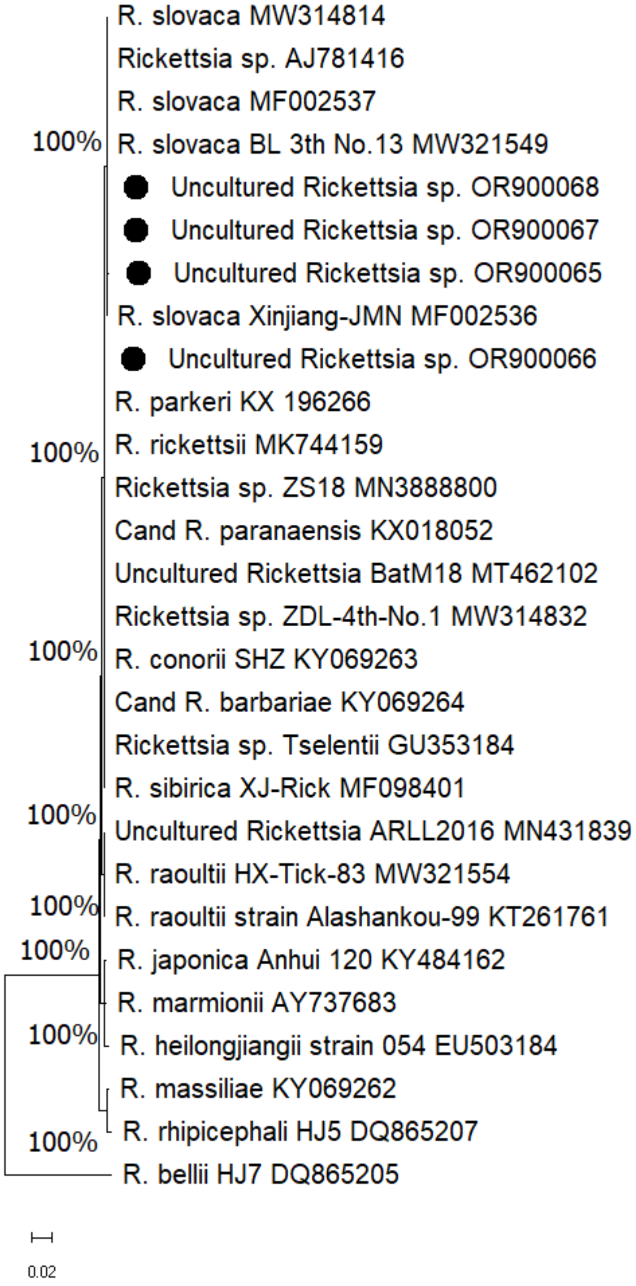


Fig 2 a A concatenated phylogenetic tree inferred from comparison of the *Rickettsia* 17-kDa, gltA, ompA partial sequences including those from *Secretargas transgariepinus* from the Shawmari Nature Reserve (SNR), Jordan, using the neighbor-joining method. **b** A tree inferred from partial sequences of the *Rickettsia* 17-kDa gene including those isolated from *Secretargas transgariepinus* from the SNR, Jordan, using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein et al. 1985). GeneBank accession numbers are included.


98%
● Uncultured Rickettsia sp. OR900067 OR 900070 OR900073
96%
● Uncultured Rickettsia sp. OR900068 OR900071 OR900074
98%
R. slovaca BL 3th No.13 MW321549 MW321558 MH548522
98%
● Uncultured Rickettsia sp. OR900066 OR900069 OR900072
98%
R. conorii SHZ KY069263 KY069261 KY069258
R. parkeri KX 196266 MK814824 KC003476
98%
98%
R. sibirica XJ-Rick MF098401 MF098404 MF098408
R. rickettsii MK744159 MT958025 DQ150693
98%
97%
R. marmionii AY737683 AY737684 DQ309096
Cand R. barbariae KY069264 KY069260 KY069256
R. heilongjiangii strain 054 EU503184
95%
89%
Rickettsia sp. Tselentii GU353184 GU353183 EU194445
65%
R. bellii HJ7 DQ865205 DQ865204
R. japonica Anhui 120 KY484162 AY743327 KY484160
92%
97%
Uncultured Rickettsia ARLL2016 MN431839 MN431847
R. raoultii HX-Tick-83 MW321554 EU036985 MW321568
98%
R. massiliae KY069262 KY069259 KY069257
98%
R. rhipicephali HJ5 DQ865207 DQ865206 DQ865208
0.050


R. slovaca MW314814
Rickettsia sp. AJ781416
R. slovaca MF002537
100%
R. slovaca BL 3th No.13 MW321549
● Uncultured Rickettsia sp. OR900068
● Uncultured Rickettsia sp. OR900067
● Uncultured Rickettsia sp. OR900065
R. slovaca Xinjiang-JMN MF002536
● Uncultured Rickettsia sp. OR900066
R. parkeri KX 196266
100%
R. rickettsii MK744159
Rickettsia sp. ZS18 MN3888800
Cand R. paranaensis KX018052
Uncultured Rickettsia BatM18 MT462102
Rickettsia sp. ZDL-4th-No.1 MW314832
100%
R. conorii SHZ KY069263
Cand R. barbariae KY069264
Rickettsia sp. Tselentii GU353184
R. sibirica XJ-Rick MF098401
100%
Uncultured Rickettsia ARLL2016 MN431839
R. raoultii HX-Tick-83 MW321554
100%
R. raoultii strain Alashankou-99 KT261761
100%
R. japonica Anhui 120 KY484162
R. marmionii AY737683
100%
R. heilongjiangii strain 054 EU503184
R. massiliae KY069262
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R. rhipicephali HJ5 DQ865207
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***Bartonella* in bat flies from the Egyptian fruit bat in the Middle East.**
Manuskript.

Bartonella in bat flies from the Egyptian fruit bat in the Middle East

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Abstract

In the family of fruit bats, Pteropodidae Gray, 1821, as in the third most diverse group of bats (Chiroptera), the bacterium of the genus *Bartonella* was detected in more species as well as in more species of their insect ectoparasites, and in more regions of tropics and sub-tropics of the Old World. The Egyptian fruit bat, *Rousettus aegyptiacus* (Geoffroy, 1810), is one of the most widespread species of the fruit bats, occurring betwen South Africa, Senegal and Pakistan; in this bat species, the presence of Candidatus *Bartonella rousetti* was detected only in three of its African populations, in Nigeria, Kenya, and Zambia. This fruit bat, however, occurs also in the Palaearctic, in an area isolated geographically and phylogenetically from the Afrotropical part of the species distribution. We screened the blood sucking bat flies (family Nycteribiidae) from *R. aegyptiacus* for the presence of the *Bartonella* bacteria. A rich material of bat fly *Eucampsipoda aegyptia* (Macquart, 1850), a monoxenous ectoparasite of the Egyptian fruit bats, was collected at 25 localities in seven countries of the Middle East in 2007–2013. The DNA isolates from the bat flies were subjected to a three-marker (*gltA*, *ssrA*, and intergenic spacer region, ITS) multilocus sequence analysis. Based on the amplification of the fragment of *ssrA* gene by a real-time PCR, 65 *E. aegyptia* samples from 19 localities in all seven countries were positive for the bacteria. One to four *Bartonella*-positive individuals of *E. aegyptia* were collected per one individual of *R. aegyptiacus*. An analysis of the ITS and *gltA* genes indicated the presence of an uncultured *Bartonella* sp., belonging to the *B. rousetti* genogroup. This indicate that the bat fly *E. aegyptia* would act as a vector of *Bartonella* in the Palaearctic (Middle Eastern) populations of the Egyptian fruit bat.

Keywords. *Bartonella*, *Eucampsipoda aegyptia*, *Rousettus aegyptiacus*, Palaearctic, Middle East

Introduction

The genus *Bartonella* Strong, Tyzzer, Brues et Sellards, 1915 (Rhizobiales: Bartonellaceae) comprises phylogenetically diversified facultative intracellular Gram-negative α-proteobacteria that infect mainly the erythrocytes and endothelial cells of mammals (Eicher and Dehio 2012). These bacteria are distributed throughout the world and are transmitted predominantly by blood-feeding arthropods (Chomel et al. 2009).

McKee et al. (2021) suggested bats (Chiroptera) to be a group of mammals that has a crucial role in the origin and spreading of the *Bartonella* bacteria among geographical regions and other mammal groups. In bats, more taxa of blood feeding arthropods can be found which can help in dispersal of this bacterium (Marshall 1982, Szubert-Kruszyńska and Postawa 2008). The available results demonstrated that the genus *Bartonella* use to be found most frequently in one group of these arthropods, in the family of bat flies, Nycteribiidae (Szentiványi et al. 2019). Identical genotypes of *Bartonella* are often reported from bats and their bat flies, what indicates that bat flies act as vectors of spreading of this bacterium among bats and perhaps also other mammals (cf. Morse et al. 2012, Kamani et al. 2014, Brook et al. 2015, Moskaluk et al. 2018, Judson et al. 2015, Dietrich et al. 2016, Qiu et al. 2020).

In the last decade, several new strains/genotypes of *Bartonella* were detected in bats and their flies throughout the whole world (see Han et al. 2021: 2, Fig. 1). The real taxonomic diversity *Bartonella* is only poorly manifested in some bat taxa and/or populations. Due to this, our understanding whether and how are particular species of *Bartonella* shared among related bat species is rather limited.

The family of fruit bats, Pteropodidae Gray, 1821 is one of the richest bat group concerning the species diversity, it is distributed in tropics and subtropics of the Old World. Despite this, reports on prevelence of *Bartonella* in these bats and their bat flies are available only from few localities (Bai et al. 2018, Kamani et al. 2014, Dietrich et al. 2016, Brook et al. 2015, Wilkinson et al. 2016, Qiu et al. 2020, Fagre et al. 2023). The Egyptian fruit bat, *Rousettus aegyptiacus* (Geoffroy, 1810), ranks among fruit bats with very wide geographical distribution – it is an Afro-tropical species with occurrence in most of Africa and in south-western Asia. It occupies areas around the Gulf of Guinea from Senegal to Angola, in southern and eastern Africa from the Cape to Eritrea, and the southwestern part of the Palaearctic, from Egypt, Yemen and Turkey to Pakistan (Kwiecinski and Griffiths 1999, Benda et al. 2011). The distribution range in the Middle East (comprising wholes or parts of Egypt, Sudan, Turkey, Cyprus, Levant, Arabia, Iran, and Pakistan, see Benda et al. 2011 for a review, with additions by Benda et al. 2012, 2023, Judas et al. 2018, Benda and Ševčík 2020) is an occurrence spot isolated both geographically and phylogenetically from the Afro-tropical populations (Stříbná et al. 2019).

The research of *Bartonella* in the populations of *Rousettus aegyptiacus* brought detections of the gentotype *Bartonella rousetti* from the bat fly *Eucampsipoda africana* Theodor, 1955, a frequent ectoparasite of this fruit bat. This strain was was found in this bat fly collected in Nigeria (Bai et al. 2018) and Zambia (Szentiványi et al. 2022), and was also confirmed in the blood of *R. aegyptiacus* from Kenya (Kosoy et al. 2010). The knowledge of *Bartonella* in the Egyptian fruit bat is thus limited to a few points within its distribution range in tropical Africa, while in the Palaearctic populations the screening for this pathogen has not been made.

The absence of any evidence of *Bartonella* in the Palaearctic populations of the Egyptian fruit bat and its monoxenous parasite, respectively, was an impulse for a deeper study of the material of the bat fly *Eucampsipoda aegyptia* (Macquart, 1850), an obligatory ectoparasite of *Rousettus aegyptiacus*. Possibility of the presence of *Bartonella* was studied in almost whole known distribution range of this bat fly in the Middle East, from Egypt to Iran. Our study finally brought new evidence of the *Bartonella* presence from a large part of the Palaerctic range of the Egyptian fruit bat and thus, of its wide and rather common occurrence. Subsequently, the obtained data suggest that the concerned bat fly may represent a vector for this bacterium and the Egyptian fruit bat in the Middle East.

Material and methods

The material examined

Our study comprises the populations of the Egyptian fruit bat, *Rousettus aegyptiacus*, from the Middle East, including its NE African part. The bat flies *Eucampsipoda aegyptia* were collected from the fruit bats at the following 25 sites situated in seven countries (Fig. 1): **Egypt**: (1) Aswan, 24°07'N, 32°54'E, 92 m a. s. l. (24 January 2010, 10 January 2011); (2) Bahariya Oasis, Bawiti, 28°21'N, 28°52'E, 98 m a. s. l. (18 January 2010, 30 December 2010, 2 January 2011); (3) El A'aqab, 24°16' N, 32°54' E, 96 a.s.l. (25 January 2010); (4) El Qahirah, Gezira Island, 30°03'N, 31°13'E, 20 m a. s. l. (29 January 2010); **Iran**: (5) Bishapur, Sasan Cave, 29°47'N, 51°35'E, 860 m a. s. l. (6 October 2011); (6) Jahrom, Sang Eshkan, 28°29'N, 53°35'E, 1102 m a. s. l. (8 October 2011); (7) Zangard, 27°13'N, 54°38'E, 493 m a. s. l. (9 October 2011); **Jordan**: (8) Iraq Al Amir, Wadi As Sir, 31°55'N, 35°45'E, 515 m a. s. l. (2 July 2010); (9) Jufat Al Qafrayn, 31°53'N, 35°37'E, –235 m a. s. l. (15 July 2010); (10) Nahla, 32°17'N, 35°51'E, 728 m a. s. l. (13 July 2010); (11) An Nuzha, Wadi Al Wala, 31°33'N, 35°44'E, 335 m a. s. l. (11 July 2010); **Lebanon**: (12) Dahr El Mghara, Aaonamie Cave, 33°40'N, 35°27'E, 255 m a. s. l. (19 January 2008); (13) Trablous, Matal El Azraq Cave, 34°25'N, 35°50'E, 15 m a. s. l. (21 January 2007); **Oman**: (14) Al War, Wadi Khabbah, 22° 56' N, 58° 51' E, 406 m a. s. l. (5 April 2011); (15) Ain Sahalnawt, 17° 09' N, 54° 11' E, 123 m a. s. l. (27 March 2012); (16) Bidbid, Wadi Dabaum, 23° 25' N, 58° 08' E, 205 m a. s. l. (26 March 2011); (17) Mithqub, Wadi Harabein, 23° 04' N, 58° 59' E, 52 m a. s. l. (2 November 2009); (18) Mudhai, 17° 29' N, 53° 21' E, 542 m a. s. l. (25 March 2012); (19) Shihayt, Wadi Darbat, 17° 09' N, 54° 28' E, 326 m a. s. l. (28 March 2012); **United Arab Emirates**: (20) Al Khari, Shawka Dam, 25°06'N, 56°03'E, 295 m a. s. l. (29 October 2013); **Yemen**: (21) Mashgab, Ash Shamshara, 13°21'N, 43°57'E, 1170 m a. s. l. (26 October 2007); (22) Wadi Zabid, Al Mawkir, 14°10'N, 43°30'E, 270 m a. s. l. (30 October 2007).

The Egyptian fruit bats were caught by standard methods, using mist nets or hand nets. All bodies of the bats were checked for a presence of ectoparasites, and maximum of individuals of the bat flies were removed and collected using tweezers and preserved in the 96% ethanol.

The fixed bat fly specimens were studied with a microscope without additional interventions. The species and sex determinations of the *Eucampsipoda aegyptia* specimens were carried out using the key by Theodor (1967: 413–416), the taxonomy and nomenclature follow those by Maa (1965).

For the detection of pathogens, only a part of the collected bat fly material was used. The examined samples of extracted DNA are stored at the Institute of Virology, Biomedical Research Center, Slovakian Academy of Sciences, Bratislava, Slovakia. The other material of the bat fly *E. aegyptia* that was not screened for a pathogen presence remains stored in a private collection by Martin Ševčík, Nitra, Slovakia.

DNA extraction, molecular genetic and statistic analyses

The ethanol fixed bat fly specimens were washed with sterile water, dried, and crushed with a sterile scalpel, their DNA was extracted using the QIAamp DNA Mini kit (Qiagen) following the manufacturer's protocol. Thirty additional samples from Egypt were stored as dry specimens and their DNA was extracted by chelex (Walsh et al. 1991). The quantity and quality of the DNA were assessed by Nano Photometer Pearl (Implen, Germany) and the extracted DNA was used as a template for the PCR amplification in order to determine the presence of *Bartonella*, with the following species identification (Table 1). The *Bartonella* positive amplicons were purified and then analysed by sequencing in both directions with the same primers as for the PCR amplification by Macrogen Inc. (Amsterdam, The Netherlands). The obtained partial sequences of ITS and *gltA* genes were analysed using the MEGA software, version 11.0.13, and compared with those available in the GenBank using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov).

Phylogenetic trees were constructed using the neighbor-joining method with the Kimura 2-parameter method. Partial *gltA* genes and ITS region sequences for representative samples were submitted to the GenBank under the accession numbers OR553951–OR553952 for the *gltA* gene, and OQ058984–OQ058989 and OR523867–OR523871 for the ITS region, respectively.

Statistical analyses testing the geographical and sexual differences in the presence of *Bartonella* spp. in *Eucampsipoda aegyptia* specimens made using Fisher's exact test with an online calculator (http://www.socscistatistics.com). The p-value<0.05 was considered as a proof of significant difference, 95% confidence intervals (CI) were calculated using an online calculator (http://epitools.ausvet.com.au).

Results

The DNA of *Bartonella* spp. was detected in the samples of *Eucampsipoda aegyptia* collected from *Rousettus aegyptiacus* in Egypt, Jordan, Lebanon, Yemen, Oman, UAE, and Iran, i.e. in all countries from where the samples were examined (Table 2). Based on the real-time PCR analysis, a total of 65 of the bat fly DNA samples (36.9% of the 176 samples analysed; 95% confidence interval (next CI) 29.80–44.06) were found positive for *Bartonella*, and further characterised. The *Bartonella* DNA was found both in females (40.91%; 95% CI 29.05–52.77) and males (34.55%; 95% CI 25.66–43.43), the difference between the presence depending on sex was not significant (p=0.423). The *Bartonella* positive bat flies were collected from 37 individuals of *R. aegyptiacus*, in the frequency between one and five positive bat flies per a fruit bat individual.

In total, 54 good-quality sequences of the ITS region (n=39) and *gltA* (n=15) gene were obtained and analysed, respectively. The sequence analysis of the partial ITS region of all 39 samples revealed the presence of *Bartonella* strains of the *B. rousetti* genogroup (Fig. 2). Twenty samples (four females from four fruit bat individuals from Lebanon; one female and two males from one bat from Yemen; two males from two bats from Jordan; three males from three bats from Egypt; four females and four males from five bats from Iran) were identical with the uncultured *Bartonella* clone 84 deposited in the GenBank (OR523867) – >99% sequence identity to *B. rousetti* (KM382255) detected in the blood of *Rousettus aegyptiacus* from Kenya (Kosoy et al. 2010). Another four male samples of bat flies collected from *R. aegyptiacus* in Jordan, UAE, Egypt, and Iran, were identical with the uncultured *Bartonella* clone 136 deposited in the GenBank (OR523868) – >96% sequence identity to *B. rousetti* (KM382255). Other five samples of *Eucampsipoda aegyptia*, one male and two females from one bat from Egypt, and one male and one female from one bat from in Iran were identical to the uncultured *Bartonella* clone 175 deposited in the GenBank (OR523869) – >97% sequence identity to *B. rousetti* (KM382255). Sequences from one male and one female of the bat fly from two fruit bat individuals from Iran were identical to the uncultured *Bartonella* clone 91 deposited in the GenBank (OR523870) – >98% sequence identity to *B. rousetti* (KM382255). The *Bartonella* ITS region sequences derived from seven individuals of *E. aegyptia*, three males (OQ058984–OQ058986) and three females (OQ058987–OQ058989) from Jordan, and one female from Iran (OR523871) shared >94% similarity with *B. rousetti* (KM382255).

In total, 15 bat flies positive for the *gltA* gene were further analysed and the analysis revealed two types of the *gltA* sequences; the sequence type 1 (OR553951) from a male of *Eucampsipoda aegyptia* collected from the fruit bat from Egypt showed 99% identity to two clones, the uncultured *Bartonella* clone YNBS/BF03 (OP433671) and clone YNBS/BF06 (OP433673) identified previously from *E. africana* from China (Kuang et al. 2022), and 85% identity to *B. rousetti* (HM363764). In total 14 DNA samples of *Bartonella* of the *gltA* genotype 1 were identical each to other and their ITS regions represented three various sequences (OQ058984, OR523870, OR523871). They originated from three females of *E. aegyptia* collected from two fruit bats from Oman, four samples of *E. aegyptia* from Iran, three from Lebanon, and one from Yemen. The nucleotide sequences of the *gltA* genotype 2 originated from a female of *E. aegyptia* collected from a fruit bat from Jordan (OR553952) showed 96% identity to *Bartonella* clone Batfly-3 (LC461051) previously found in *E. africana* from Zambia (Qiu et al. 2020). The sequence of the ITS region from this bat fly was unusable. For the comparison of *Bartonella* sequences amplified from *E. aegyptia* with selected sequences obtained from the GenBank via the BLAST query see Table S1.

The maximum likelihood tree of the genus *Bartonella* based on the *gltA*, *ftsZ*, and *rpoB* genes using the MLSA approach shows strain R-191 closely clustered with the fly-associated strain YNBS/BF03.

Discussion

This study investigated the presence and genetic diversity of *Bartonella* in bat flies captured in the Palaearctic (Middle Eastern) populations of the Egyptian fruit bat. To the best of our knowledge, the presence of *Bartonella* sp. was detected for the first time in the specimens of *Eucampsipoda aegyptia* collected from *Rousettus aegyptiacus* in seven countries of the Middle East, i.e. in almost whole Palaearctic range of this fruit bat with exception of the northern countries of the region, like Turkey, Cyprus, and Syria.

The analysis of the ITS region of *Bartonella* revealed presence of eleven strains of the genogroup of *B. rousetti*. Twenty positive samples coming from a large area comprising Egypt, Lebanon, Jordan, Yemen, and Iran, showed almost hundred percent identity to *Bartonella* R-191 that was identified in the blood of *R. aegyptiacus* in Kenya (Kosoy et al. 2010) and in the bat fly *Eucampsipoda africana* collected from this fruit bat in Nigeria (Bai et al. 2018).

The remaining sequences were highly similar to sequences of the ITS region of *B. rousetti*, and our strains of uncultured *Bartonella* to uncultured *Bartonella* clones identified in *E. africana* from Nigeria (Bai et al. 2018). On the other hand, analysis of the partial *gltA* gene sequences of uncultured *Bartonella* sp. from our study showed almost complete identity to the uncultured *Bartonella* clones identified in *E. africana* from Yunnan, PR China (Kuang et al. 2022).

Eucampsipoda aegyptia is a monoxenous parasite, infesting solely the Egyptian fruit bats (Kock and Nader 1979), and such a host expresses a lower diversity of infection by *Bartonella* over polyxenous species but a higher prevalence of these bacteria (Sándor et al. 2018). As several highly similar *Bartonella* lineages inhabit different geographical regions, the rate of host distribution might not be the major drivers of *Bartonella* diversification. Indeed, it has been observed that *Bartonella* diversity corresponds to host phylogeny, with different pathogen lineages likely occurring within the specific bat suborders or families (McKee et al. 2016). In addition, increasing taxonomic distance in hosts decreased the likelihood of transition rates (McKee et al. 2016). Therefore, the host diversity is more likely to be the determinant of *Bartonella* distribution rather than the host spatial distribution, although there is an increasing evidence of *Bartonella* transmission between phylogenetically distant species, including domestic animals and wildlife (Frank et al. 2018). Our results conform to this hypothesis and the obtained sequences show very high similarity to strains isolated from the host species and/or genus in Africa and China (Kosoy et al. 2010, Bai et al. 2018, Kuang et al. 2022).

As we mentioned above, numerous authors (Morse et al. 2012a, b, Dick and Dittmar 2014, Olival et al. 2015, Wilkinson et al. 2016, Han et al. 2017) suggested that bat flies of the family Nycteribiidae act as vectors of the *Bartonella* bacteria, and some of them (Olival et al. 2015, Wilkinson et al. 2016) suggested these bat flies act as reservoirs of these bacteria. However, such as we have not a direct proof of the survival rate of the bacterium (we did not cultivate it), we do not know the vector capacity of the bat fly tested by an experiment. On the other hand, *E. aegyptia* is a monoxenous parasite of the Egyptian fruit bat, and it indicates that its development and food availability are dependent on this host. Although Kock and Nader (1979) excluded the parasitation of other hosts, Szentiványi et al. (2022) mentioned also accidental host species in other species of *Eucampsipoda*, besides their principal hosts. It could suggest that *E. aegyptia* is a vector guaranteeing a permanent circulation of the *Bartonella* bacterium within population of the fruit bats. An experimental laboratory study of the circa-annual activity of the individuals of *E. aegyptia* taken from a colony of the Egyptian fruit bat demonstrated a reduction of the pupal duration proces in certain periods of a year, but the high feeding frequency starts immediately after the colouring and hardering of newly emerged flies (Hafez et al. 1978). The blood consuption from the host and thus, the circulation of pathogens could be under way during whole year.

In a recent study, a presence of the antibodies against bat associated *Bartonella rousetti* was detected in humans (Bai et al. 2018). It indicates that the bat-associated bacteria can potentially infect humans. However, antibodies against *Bartonella* tend to be highly cross-reactive within the genus and with other non-*Bartonella* agents. The DNA of *Bartonella* spp. was detected in the bat saliva, urine and guano (Dietrich a kol. 2017, Veikkolainen a kol. 2014). Thus, the possibilities of transmission of *Bartonella* to humans do not represent a direct and natural way, on the other hand, potentially dangerous could be only accidental visit of a fruit bat roost. Humans thus can represent one of connecting links of the bacterium transmission and spreading, however, this remains a task for future research.

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Author contribution

EŠ, methodology, PCR test and sequencing, phylogenetic analyses, Genbank processing, supervision and contribution to molecular analyses, manuscript writing, funding acquisition. MŠ, conceptualization, study design, resources, determination of samples, formal analysis, (draft) manuscript writing. YYP, PCR test and sequencing. PB, sample collection, study design, review and editing manuscript, funding acquisition.

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Data availability

Data supporting the conclusions of this article are included within the article. Representative DNA sequences have been deposited in the GenBank database, see Material and methods for the accession numbers.

Declarations

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no competing interests.

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Figure captions

Fig 1 A map of the collection sites of the bat fly *Eucampsipoda aegyptia* used in this study (circles); the pathogen presence is marked by a circle with asterisk, numbers corrspond with the ocality numbers in Material and medthods. The dark grey areas shows the distribution range of *Rousettus aegyptiacus* in the Middle East reconstructed after Benda et al. (2011, 2023).

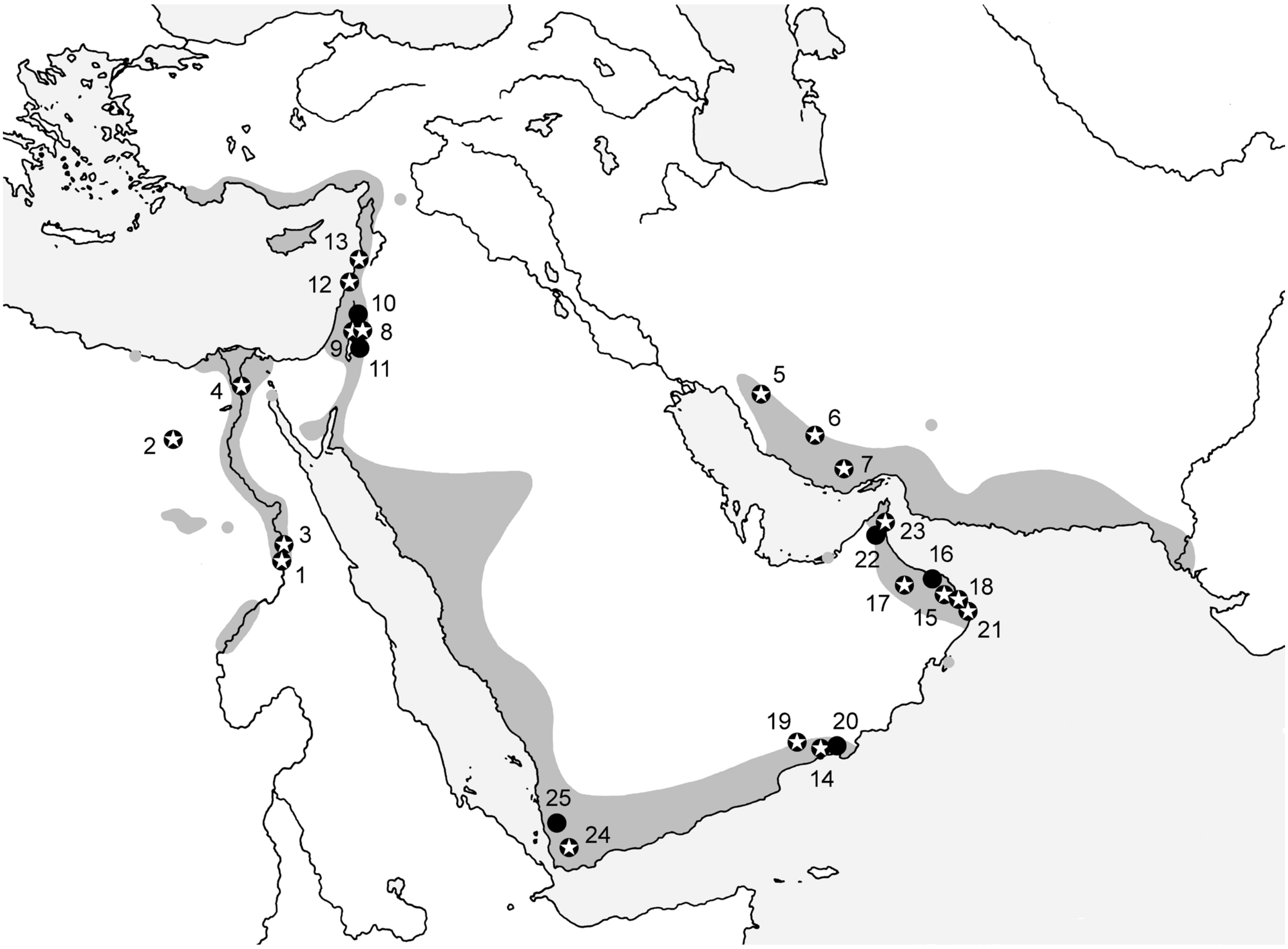

Fig 2 Phylogenetic relationship of *Bartonella* strains based on the internal transcribed spacer sequences (ITS). The neighbor-joining method by Kimura 2-parameter distance and bootstrap calculation was conducted with 500 replicates for phylogenetic analysis. GenBank Accession Numbers are provided for all sequences.

Table 1 Primers used for the detection of the *Bartonella* presence in the examined bat flies collected from the Egyptian fruit bats

Primer name	Primer sequence (5'–3')	Target gene	A. g. (bp)	A. t. (°C)	Reference
ssrA-R1	AAG GCT TCT GTT GCC AGG YG	*ssrA*	124	56.6	Mardosaitė-Busaitiene et al. (2019)
ssrA-F1	AGT TGC AAA TGA CAA CTA TGC GG				
ssrA-P1	FAM-ACC CCG CTT AAA CCT GCG ACG GTT				
BA325s	CTT CAG ATG ATG ATC CCA AGC CTT CTG GCG	16S–23S rRNA gene ITS region	420–780	66	Maggi et al. (2009)
BA1100as	GAA CCG ACG ACC CCC TGC TTG CAA AGC A	16S–23S rRNA gene ITS region			
BhCS 781p	GGG GAC CAG CTC ATG GTG G	*gltA*	357	43	Norman et al. (1995)
BhCS 1137n	AAT GCA AAA AGA ACA GTA AACA				

Abbreviation: A. g. (bp) – Amplicon gene; A. t. (°C) – annealing temperature.

Table 2 Total numbers of the analysed *Eucampsipoda aegyptia* bat flies and the *Bartonella* positive samples (+)

	country	♀♀	♂♂	total	♀♀+	♂♂+	total +	% positive
	Egypt	5	9	14	2	6	8	57.1
(dry)	Egypt	12	18	30	3	7	10	33.3
	Jordan	7	6	13	1	3	4	30.8
	Lebanon	4	1	5	4	0	4	80.0
	UAE	3	2	5	0	1	1	20.0
	Yemen	1	3	4	1	2	3	75.0
	Oman	19	49	68	9	12	21	30.9
	Iran	15	22	37	7	7	14	37.8
	total	66	110	176	27	38	65	36.9

Supplementary material

Table S1 The concordance between sequences of the *gltA* and intergenic spacer region of *Bartonella*obtained from *Eucampsipoda aegyptia* in our study and other sequences extracted in GenBank via BLAST query

ITS region	Country		OQ058984	OQ058985	OQ058986	OQ058987	OQ058988	OQ058989	OR523867	OR523868	OR523869	OR523870	OR523871
B. rousetti KM382255	Kenya	*Rousettus aegyptiacus*	100% (311/311 bp)	94.3% (246/261 bp)	96.7% (289/299 bp)	96.8% (214/221 bp)	95.9% (235/245 bp)	98.3% (228/232 bp)	99.7% (355/356 bp)	96.1% (323/336 bp)	97.1% (338/348 bp)	98.9% (354/358 bp)	76.7% (240/313 bp)
B. sp. Lisso-Nig 922 MN504709	Nigeria	*Myonycteris angolensis*	100% (311/311 bp)	94.3% (246/261 bp)	96.7% (289/299 bp)	96.8% (214/221 bp)	95.9% (235/245 bp)	98.3% (228/232 bp)	99.7% (355/356 bp)	96.1% (323/336 bp)	97.1% (338/348 bp)	98.9% (354/358 bp)	76.7% (240/313 bp)
B. sp. NG13-090-1 MH142638	Nigeria	*Eucampsipoda africana*	97.1% (305/314 bp)	93.7% (237/253 bp)	93.7% (283/302 bp)	96.9% (218/225 bp)	94.7% (234/247 bp)	97.4% (222/228 bp)	97.2% (349/359 bp)	97.6% (328/336 bp)	99.7% (347/348 bp)	96.4% (348/361 bp)	77.0% (261/339 bp)
B. sp. NG13-078-1 MH142635	Nigeria	*E. africana*	96.8% (304/314 bp)	93.3% (236/253 bp)	94.4% (282/302 bp)	96.4% (217/225 bp)	93.9% (234/247 bp)	96.2% (225/234 bp)	96.9% (348/359 bp)	98.2% (330/336 bp)	99.1% (345/348 bp)	96.1% (347/361 bp)	76.4% (259/339 bp)
B. sp. NG13-013-1 MH142632	Nigeria	*E. africana*	99.7% (310/311 bp)	93.9% (245/261 bp)	96.3% (288/299 bp)	96.4% (213/221 bp)	95.5% (234/245 bp)	97.8% (227/232 bp)	99.4% (354/356 bp)	95.8% (322/336 bp)	96.8% (337/348 bp)	99.2% (355/358 bp)	76.4% (239/313 bp)
B. sp. strain R-255 MN258143	Kenya	*Rousettus aegyptiacus*	99.7% (310/311 bp)	93.9% (245/261 bp)	96.3% (288/299 bp)	96.4% (213/221 bp)	95.5% (234/245 bp)	97.8% (227ú232 bp)	99.4% (344/346 bp)	95.8% (319/333 bp)	96.8% (331/342 bp)	98.6% (343/348 bp)	77% (241/313 bp)
B. sp. MF288116	Georgia	*Miniopterus schreibersii*	87.3% (179/205 bp)	92.2% (142/154 bp)	92.2 % (142/154 bp)	93.7% (133/142 bp)	86.7% (170/196 bp)	92.2% (142/154 bp)	86.8% (178/205 bp)	87.6% (169/193 bp)	88.1% (170/193 bp)	87.8% (180/205 bp)	100% (24/24 bp)
B. tribocorum AF312505			93.8% (121/129 bp)	93.8% (121/129 bp)	93.8% (121/129 bp)	93.8% (121/129 bp)	93.0% (120/129bp)	93.8% (121/129 bp)	93.0% (120/129bp)	93.0% (120/129bp)	93.8% (121/129%)	93.2% (123/132 bp)	100% (23/23 bp)
B. grahamii AJ269789	UK		93.0% (120/129bp)	93.0% (120/129bp)	93.0% (120/129bp)	93.0% (120/129bp)	92.3% (119/129 bp)	93.0% (120/129bp)	92.3% (119/129 bp)	92.3% (119/129 bp)	93.0% (120/129bp)	93.0% (120/129bp)	100% (23/23 bp)

*gltA*gene			OR553951	OR553952
OR553951				76.2% (269/353 bp)
B. sp. YNBS/BF03 OP433671	China	*E. africana*	99.2 % (350/353 bp)	76.6% (282/368 bp)
B. sp. YNBS/BF06 OP433673	China	*E. africana*	99.2% (350/353 bp)	76.4% (281/368 bp)
B. sp. Batfly-3 LC461051	Zambia	*E. africana*	75.4% (245/325 bp)	95.8% (319/333 bp)

B. sp. R-191 HM363764	Kenya	*Rousettus aegyptiacus*	85.0% (300/353 bp)	73.0% (259/355 bp)
B. bovis LR15 MN615930	Brazil	cattle	90.9% (321/353 bp)	75.2% (270/359 bp)
B. melophagi R-122-4 MT154631	Peru	*Melophagus ovinus*	90.4% (319/353 bp)	74.6% (264/354 bp)
B. clarridgeiae BCF02 GU056189			90.4% (319/353 bp)	75.0% (276/368 bp)
B. symbiont of *E. theodori* KT751156	Comoros	*Eucampsipoda theodori*	82.5% (273/331 bp)	76.5% (254/332 bp)
B. sp. flyAA033 KR997986	South Africa	*Eucampsipoda* sp.	74.9% (179/239 bp)	96.2% (229/238 bp)
B. sp. GLOSOR-HF16.1-94.1 MH234356	Costa Rica	*Glossophaga soricina*	88.6% (311/351 bp)	77.4% (284/367 bp)