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Anolis sierramaestrae sp. nov. (Squamata: Polychrotidae) of the "chamaeleolis" species group from Eastern Cuba

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Abstract. A new species of anole, *Anolis sierramaestrae* sp. nov., belonging to the "chamaeleolis" species group of the family Polychrotidae, is described from the mountain region in the vicinity of La Mula village, Santiago de Cuba province, Cuba. The species represents the sixth so far known species of the "chamaeleolis" species group. It resembles *Anolis chamaeleonides* Duméril et Bibron, 1837, but differs markedly in larger body size, long and narrow head shape, higher number of barb-like scales on dewlap, small number of large lateral scales on the body and dark-blue coloration of the eyes.

Key words. Taxonomy, new species, herpetofauna, Polychrotidae, *Chamaeleolis*, *Anolis*, Great Antilles, Caribbean, Neotropical region.

INTRODUCTION

False chameleons of the genus *Anolis* Daudin, 1802 represent a highly ecologically specialized and morphologically distinct and unique clade endemic to Cuba Island (Cocteau 1838, Beuttell & Losos 1999, Schettino 2003, Losos 2009). This group has been traditionally recognized as a distinct genus *Chamaeleolis* Cocteau, 1838 due to its multiple derived morphological, ecological and behavioural characters. Recent studies discovering the cladogenesis of anoles have placed this group within the main body of the tree of Antillean anoles as a sister group of a small clade consisting of the Puerto Rican species *Anolis cuvieri* Merrem, 1820 and Hispaniolan *A. barahonae* Williams, 1962 and *A. christophei* Williams 1960 (e.g., Haas et al. 1993, Poe 2004, Nicholson et al. 2005). Consequently, the former genus *Chamaeleolis* is currently considered to be a derived internal clade of the genus *Anolis* sensu lato, and thus, it is treated as a late synonym of *Anolis* (e.g., Losos 2009) The content of the former genus *Chamaeleolis* is obviously monophyletic (see Losos 2009 and the phylogenetic studies cited above) and it is further referred to as "chamaeleolis" species group.

To this date, the following five species of the "chamaeleolis" group are recognized:

(1) *Anolis chamaeleonides* Duméril et Bibron, 1837. Type locality: vicinity of La Habana, La Habana province (cf. Schettino 1999).

(2) *Anolis porcus* (Cope, 1864). Type locality: vicinity of Guantánamo city, Guantánamo (former Oriente) province (cf. Schettino 1999).

(3) *Anolis barbatus* (Garrido, 1982). Type locality: Ojo de Agua, Cinco Pesos, 9 km NW of San Cristóbal, Pinar del Río province (cf. Garrido 1982).

(4) *Anolis guamuhaya* (Garrido, Pérez-Beato et Moreno, 1991). Type locality: between Jibacoa and Topes de Collantes, Sierra de Trinidad (Sierra del Escambray or Macizo de Guamuhaya, Sancti Spiritus province; Garrido et al. 1991).

(5) *Anolis agueroi* Díaz, Navarro et Garrido, 1998. Type locality: La Juba, Bosque Castillo, Meseta de Cabo Cruz, Niquero, Granma province (cf. Díaz et al. 1998).

In this study, we describe a new species from Santiago de Cuba province of Eastern Cuba.

MATERIAL AND METHODS

Besides the designated type specimen (see below under the Results section), we examined 120 adult anoles of the "chamaeleolis" species group of which 77 were live animals or preserved specimens provided by European private breeders (18 *Anolis barbatus*, 23 *A. chamaeleonides*, 19 *A. guamuhaya* and 17 *A. porcus*) and 43 were museum specimens from the herpetological collection of the National Museum in Prague (NMP), Czech Republic (29 specimens of *A. barbatus*: NMP6V 34504, NMP6V 34520/1–11, NMP6V 71872/1–3, NMP6V 71873, NMP6V 73148/1–4, NMP6d 279/03, NMP6j 25/1992, NMP6p; 10 spec. of *A. guamuhaya*: NMP6V 34517, NMP6V 71870/1–8, NMP6V 71871; 3 spec. of *A. chamaeleonides*: NMP6V 34505, NMP6V 34518, NMP6d 81/06; and 1 spec. of *A. porcus*: NMP6V 34519).

The following meristic characters of scalation were counted: the number of (1) barb-like scales on the dewlap, (2) supralabials, (3) infralabials, (4) large lateral scales on the left side in the line leading between front and hind legs, (5) subdigital lamellae on the longest finger of a hind leg, (6) femoral scales in the line oriented from a knee to the base of a hind leg. The scalation patterns of the designated holotype were compared with those counted in the following specimens: *A. barbatus* – 6 females and 4 males from the type locality Ojo de Agua, Pinar del Río province; *A. guamuhaya* – 5 females and 5 males from Topes de Collantes, Sancti Spiritus province; *A. chamaeleonides* – 6 females and 4 males from Viñales, Pinar del Río province; *A. porcus* – 5 females and 5 males from Baracoa, Guantánamo province. These animals were reared in captivity and the localities refer to the origin of the ancestral breeding stocks.

The following measurements of the designated holotype were made with digital calliper to the nearest 0.1 mm: snout-to-vent length (SVL: measured from the tip of the snout to the vent); body lenght (LIE: longitudo interextremitatis – the

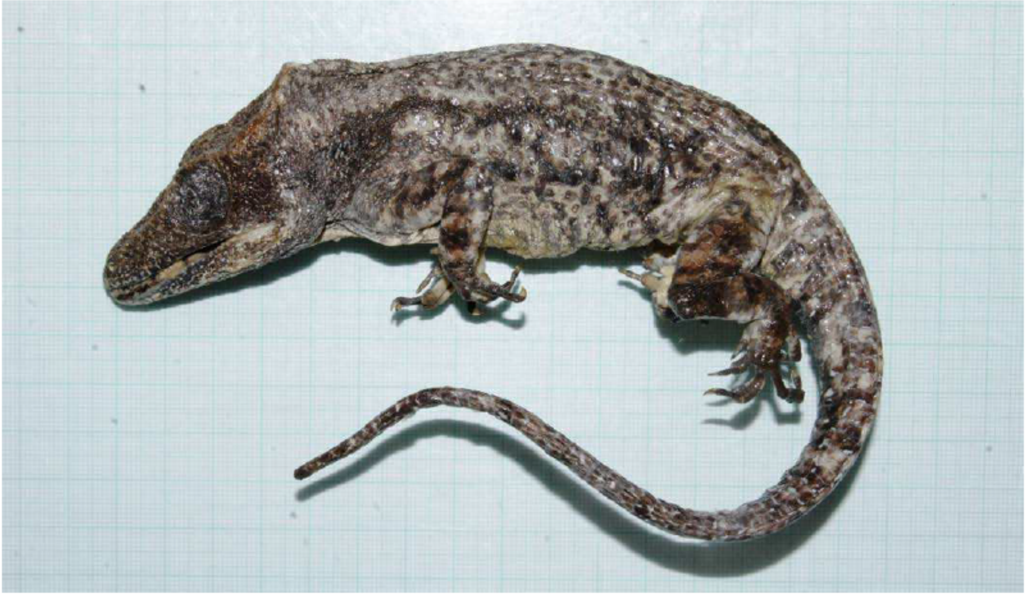


Fig. 1. Holotype of *Anolis sierramaestrae* sp. nov.

distance between front and hind legs); tail length (TL: measured from the vent to the tip of a non-regenerated tail); body height (BH: the greatest height of the body); jaw out-lever distance (JOL: the distance between the jaw articulation and the tip of the jaw); head length (HL: measured from the edge of the head casque to tip of the snout); head width (HW: measured at the intersection with the angle of the jaws); head height (HH: measured just posterior to the orbits); snout-orbit distance (SO: the distance between the tip of the snout to the nearest point of the orbit); snout-nostril distance (SN: the distance between the tip of the snout to the edge of the left nostril); snout-mouth end (SME: the distance from the tip of the snout to the corner of the mouth); lower jaw length (LJL: the distance from the back of the retroarticular process to the tip of the lower jaw); snout length (SL: the length of the snout measured from the back of the jugal bone to the tip of the upper jaw); closing in-lever (CL: the distance between the jaw articulation and the back of the jugal bone; this distance was calculated by substracting the snout length from the distance measured from the jaw articulation to the tip of the jaw = QT); opening in-lever (OL: the distance from the jaw articulation to the back of the retroarticular proces; this distance was calculated by substracting QT from lower jaw length); internasal distance (IN: the distance between the nostrils); orbit-casque distance (OC: the distance between the posterior-most point of the orbit and the highest point of the casque); interorbital distance (IO: the shortest distance between the orbits); ear opening (EO: the maximum vertical length of an ear opening); tibia (TB: the length of the left tibia); femur (FEM: the lenght of the left femur); hind metatarsus (HM: the lenght of the left hind metatarsus); hind finger (HF: the lenght of the longest – the fourth- hind finger excluding the claw); humerus (HU: the lenght of the left humerus); radius (RA: the lenght of the left radius); front metatarsus (FM: the lenght of the left front metatarsus); barb scales (BS: the maximum length of the barb-like scales on a dewlap).

TAXONOMY

Anolis sierramaestrae sp. nov.

(Figs 1, 4 and 6)

Type locality. Vicinity of La Mula village, 19° 57' 45" N, 76° 45' 24" W, Santiago de Cuba province, Cuba (Figs 2, 3).

Type material. **Holotype**. An adult male, catalogue number NMP6V 74453, deposited in the zoological collection of the National Museum in Prague (Fig. 1). Label: "adult male collected by local people in the Vicinity of La Mula village, Sierra Maestra Mts, Santiago de Cuba province, Cuba".

Diagnosis. *Anolis sierramaestrae* sp. nov. is differentiated from all other anoles of the "chamaeleolis" species group by having the following combination of characters: large body size, a long and narrow head shape, a high number of barb-like scales of tiny size, a low number of large lateral scales in between the front and hind legs, and dark-blue coloration of the eyes (Fig. 4).

Description of the holotype. An adult male with the total length of 335 mm and SVL 163 mm; LIE 73.1 mm; tail longer than body TL 172 mm; BH 40.7 mm; JOL 50.0 mm; extremely long head HL 62.2 mm; HW 29.4 mm; HH 23 mm; SO 26.7 mm; SN 6.8 mm; SME 44.8 mm; LJL 53.3 mm; SL 40.8 mm; CL 9.1 mm; OL 5.5 mm; IN 6.4 mm; OC 27.4 mm; IO 18.6 mm; EO 4.3 mm; TB 27.5 mm; FEM 31.8 mm; HM 15.2 mm; HF 22.8 mm; HU 28.5 mm; RA 19.7 mm; FM 6.2 mm; BS 0.9 mm (for definitions of measurements and abbreviations see under the Material and Methods chapter). For scalation patterns, see Table 1; for shape and coloration, see Figs 4 and 5.

Etymology. The specific epithet *sierramaestrae* refers to the mountain area where the type locality is situated.

Collecting circumstances. According to the museum record, the holotype was provided by local people and no further information about its behaviour is available. Another adult male was observed by the first author on a branch of tree about 6 meters above the ground level during a day at the type locality on 14 February 2003. This male was further photographed (see Figs 4 and 5).

Comparisons. *Anolis sierramaestrae* sp. nov. differs from all other species of *Chamaeleolis* group in its large body size (SVL of the designated type specimen is the longest of all examined specimens of the "chamaeleolis" species group), a longer and narrower head, highest number of barb-like scales (70 compared to 60 representing maximum value recorded in a pooled material

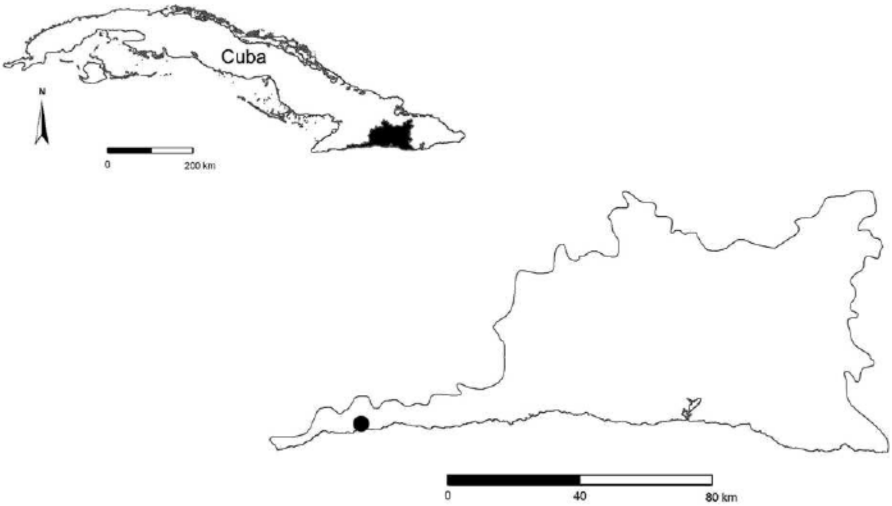


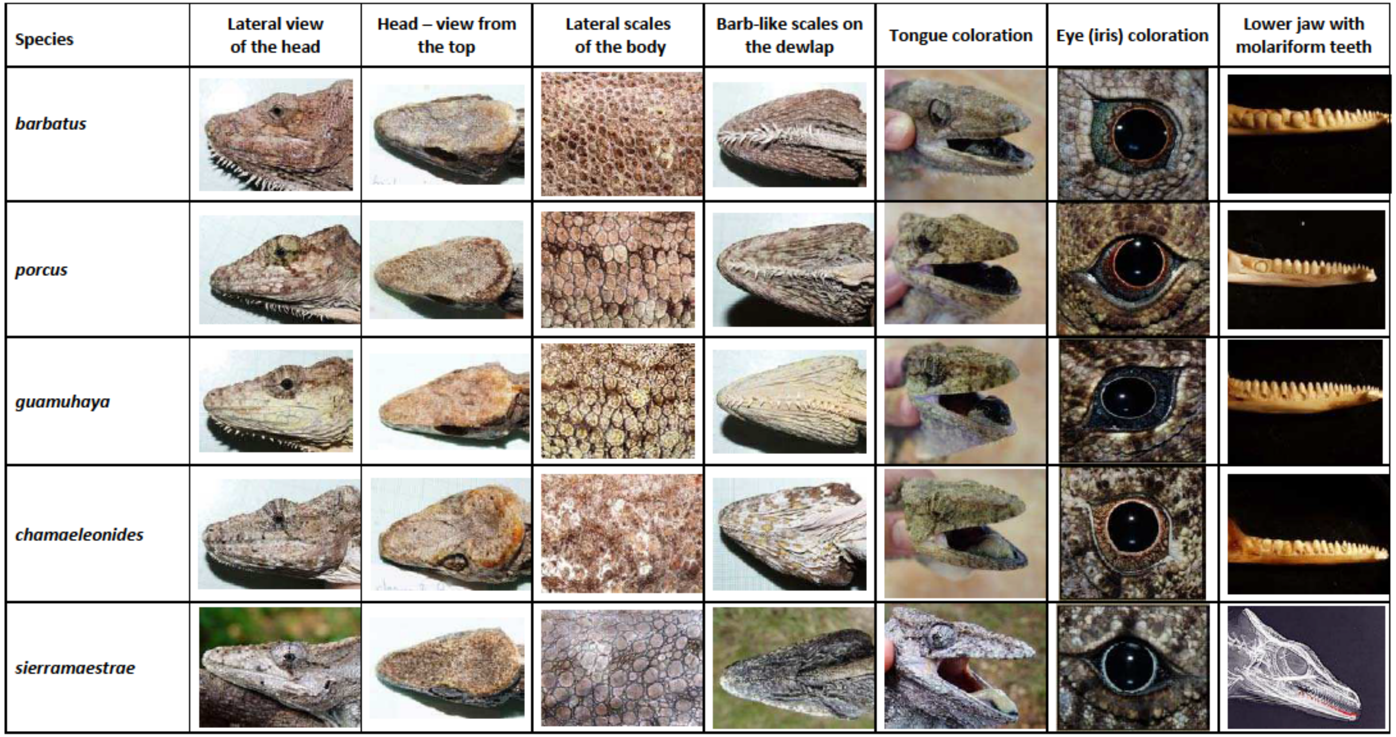
Fig. 2. Sketch map of Cuba showing type locality of *Anolis sierramaestrae* sp. nov.

Fig. 3. Type locality of *Anolis sierramaestrae* sp. nov. in Sierra Maestra Mts (photo by VH on 14 February 2003).

Table 1. Means± standard errors, range and sample sizes for scale counts in six species of Cuban anoles belonging to the "chamaeleolis" species group. Barbs = number of barb-like scales on dewlap, Lateral scales = number of the large lateral scales in line between front and hind legs, Infralabials = number of infralabial scales, Supralabials = number of supralabial scales, Lamellae = number of subdigital lamellas on the longest (fourth) finger of a hind leg, Femoral scales = femoral scales in the line oriented from a knee to the base of a hind leg, Mandibular teeth = number of teeth on half of the mandibula. The data concerning *Anolis agueroi* were adopted from Díaz et al. (1998), in this case counts of labial scales refer to the type specimen only.

Anolis species	*barbatus*	*porcus*	*guamuhaya*	*chamaeleonides*	*agueroi*	*sierramaestrae* sp. nov.
sample size	10	10	10	10	17	1
barbs	42.80 ± 1.15	41.20 ± 1.24	48.80 ± 1.37	54.90 ± 0.81	–	70
	37–50	34–45	41–55	52–60	48–50	–
lateral scales	23.90 ± 0.38	23.00 ± 0.21	20.90 ± 0.35	20.90 ± 0.41	–	17
	22–25	22–24	19–22	19–22	–	–
infralabials	11.40 ± 0.31	12.90 ± 0.23	11.70 ± 0.26	12.70 ± 0.26	11	14
	10–13	12–14	11–13	12–14	–	–
supralabials	10.00 ± 0.21	12.00 ± 0.30	10.10 ± 0.23	13.30 ± 0.33	10	15
	10–13	12–14	11–13	12–14	–	–
lamellae	42.10 ± 0.57	47.70 ± 0.37	45.30 ± 0.58	44.70 ± 0.47	–	46
	10–13	12–14	11–13	12–14	–	–
femoral scales	12.20 ± 0.20	18.40 ± 0.40	11.60 ± 0.16	11.50 ± 0.17	–	11
	11–13	16–20	11–12	11–12	–	–

Fig. 4. Wild adult male of *Anolis sierramaestrae* sp. nov. (photo by VH at the type locality on 14 February 2003).



Species	Lateral view of the head	Head – view from the top	Lateral scales of the body	Barb-like scales on the dewlap	Tongue coloration	Eye (iris) coloration	Lower jaw with molariform teeth
barbatus							
porcus							
guamuhaya							
chamaeleonides							
sierramaestrae							

Fig. 5. Comparison of distinguishing characters in *Anolis barbatus* (Garrido), *A. porcus* (Cope), *A. guamuhaya* (Garrido, Pérez-Beato et Moreno), *A. chamaeleonides* Duméril et Bibron and *A. sierramaestrae* sp. nov.

of the remaining species), lowest number of the large lateral scales in line between front and hind legs (17 compared to the minimum value of 19 recorded in the pooled materials).

A.sierramaestrae sp. nov. resembles *A. chamaeleonides* the most, but, moreover to the differences mentioned above, its tail is longer than the body and the iris of the eye is dark-blue (somewhat resembling that of *A. guamuhaya*). In a sharp contrast, all examined specimens of *A. chamaeleonides* had reddish eyes. *A. sierramaestrae* also lacks yellowish spots on jawline which are the specific sign of *A. chamaeleonides* (see Fig. 5).

Anolis sierramaestrae sp. nov. shares whitish tongue with both *A. chamaeleonides* and *A. agueroi*, while dark coloration of the tongue is shared by *A. barbatus*, *A. porcus* and *A. guamuhaya*.

All anoles of the "chamaeleolis" species group are specialized to malacophagy (molluscivory, cf. Herrel & Holáňová 2008) and their dentition includes enlarged molariform teeth (Estes & Williams 1984) used to crush snail shells. The degree of morphological specialization of the dentition assessed in a designated holotype of *Anolis sierramaestrae* sp. nov. (Fig. 6) is fairly intermediate when compared with that in other species of the "chamaeleolis" species group (cf. Fig. 5). Its dental row consists of 25 teeth like in *A. guamuhaya*, the species with least specialized dentition of the "chamaeleolis" species group.. This fairly conforms to the value 23 reported for *Anolis chamaeleonides*, but sharply contrasts with low teeth counts in *A. barbatus* and *A. porcus* (20 and 16, respectively; teeth counts: Holáňová et al. unpubl. results, see also Holáňová 2009, Zahradníček 2011). Comparisons of *A. sierramaestrae* sp. nov. with the remaining species of the "chamaeleolis" species group are summarized at the Table 1 and Fig. 5.

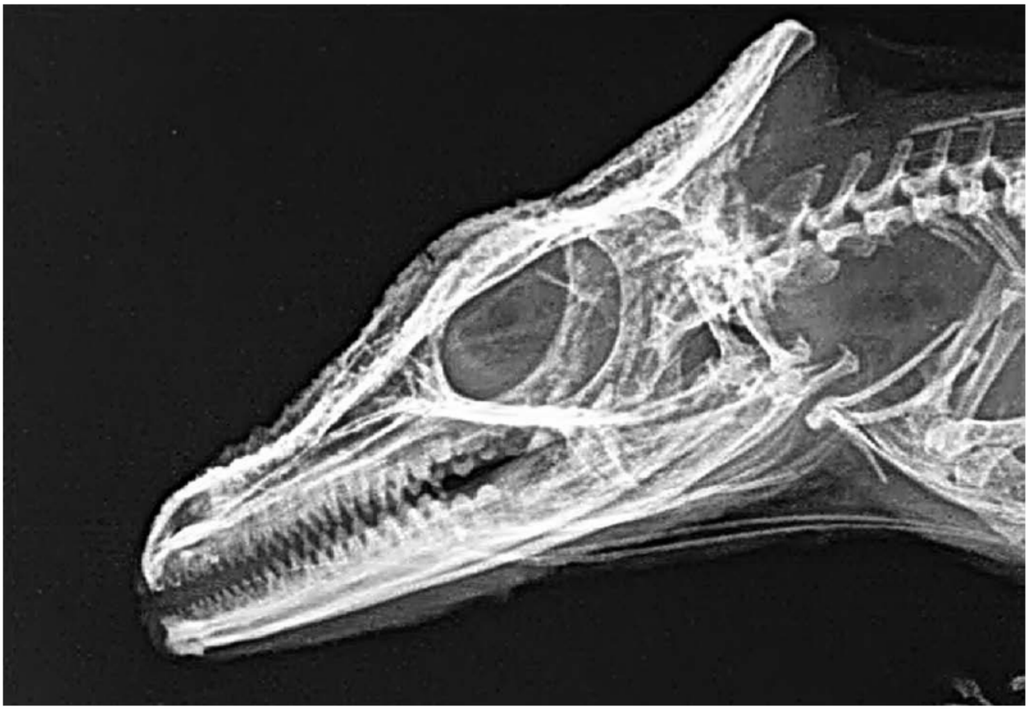


Fig. 6. X-ray picture showing dentition of the holotype (NMP6V 74453) of *Anolis sierramaestrae* sp. nov.

DISCUSSION

The records of *Anolis chamaeleonides* reported by previous authors (Garrido et al. 1991, Díaz et al. 1998, Schettino 1999) from Sierra Maestra mountains come from localities distributed within the range of about 30 km from the type locality of *A. sierramaestrae* sp. nov. Obviously, these records refer to this species. Thus, *A. chamaeleonides* is most probably restricted to the western part of Cuba. The older records from the central part of the island require a revision.

The geographic ranges of the anole species belonging to the "chamaeleolis" species group are apparently mutually exclusive and no case of sympatric occurrence of multiple species has been reported (Garrido et al. 1991, Díaz et al. 1998), for distribution patterns see Schettino (1999). Each species is typically restricted to a local mountainous area and its surroundings. Such distribution pattern provides efficient geographic barriers among the populations/species. The isolation of relatively small areas may explain a considerable degree of morphological divergence we observed. Considering the structural diversity and complex geological history of Cuba as well as the reports of morphologically suspect individuals from other localities (e.g., Rancho Velaz, Villa Clara province, Sierra de Banao, Sancti Spiritus province, Garrido 1982, Garrido et al. 1991), discoveries of additional species of the chamaeleolis group can be expected in the near future.

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Discovering an Antillean *Anolis* (Squamata: Polychrotidae) with contrasting sexual dichromatism in otherwise sexually monomorphic "chamaeleolis" group

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Abstract. The anole (genus *Anolis* Daudin, 1802) dewlap is a rapidly evolving trait. Sexually dichromatic anole species usually occur in the mainland, while the island species display only little dichromatism in particular. The so-called "chamaeleolis" group of anoles endemic to Cuba Island, traditionally classified as the 'twig giant' ecomorph, consists of large, slow and very cryptic species with very similar sexes. Our study describes a new population of "chamaeleolis" anoles which, unlike other related species, display a surprising sexual dichromatis in dewlaps. Males have conspicuously red dewlaps, while the dewlaps of females are whitish. We compared the specimens from the newly discovered populations with related *Anolis barbatus* Garrido, 1982, *A. chamaeleonides* Duméril et Bibron, 1837, *A. guamuhaya* Garrido, Pérez-Beato et Moreno, 1991 and *A. porcus* Cope, 1864 through the means of spectrophotometry, visual modelling, morphology and mtDNA analysis. The results show that the red coloration substantially increases both chromatic and achromatic contrasts, while the dichromatism in the remaining species is only in the achromatic channel, if any. Both genetic and morphometric comparisons suggest distinctness of the dichromatic populations which may represent a separate species. The reason for the unusual dewlap coloration remains unclear, though an ecological explanation is discussed.

Key words. Sexual size dimorphism, body shape, mtDNA, reflectance, *Anolis*, *Chamaeleolis*, Cuba, Neotropical Region.

INTRODUCTION

Coloration of males and females differs considerably in many animal species. This sexual dichromatism is a specific form of sexual dimorphism. Sexual dichromatism has attracted attention of researchers and theoreticians for centuries (e.g., Darwin 1871), but the phylogenetic studies analysing macroevolutionary patterns of sexual dichromatism within major clades of terrestrial vertebrates appeared only recently (frogs: Bell & Zamudio 2012; lizards: Stuart–Fox & Ord 2004; snakes: Shine & Madsen 1994; turtles: Liu et al. 2013; birds: Badayev & Hill 2003, Stoddard & Prum 2011).

The evolution of sexual dichromatism is usually explained by the two following opposing selective forces: (1) sexual selection favouring conspicuous coloration of the sex with higher variance in its reproductive output, typically males; (2) predation pressure favouring cryptic coloration in both sexes, but more strongly in females which are vulnerable to predation during gravidity and/or maternal care (Darwin 1871, for a review see also Stutchbury & Morton 2001). Risks for females associated with mating and male harassment were currently proposed as a driving force leading to the reduction of conspicuous ornamentation in females (Hosken et al. 2016).

Anoles are one of the best model systems for the evolutionary, ecological and phylogenetic studies of morphological traits including sexual dimorphism and dichromatism (for a review see Losos 2009). The most important part of anole body for the study of dichromatism is the dewlap. Dewlap is a flap of skin under the throat of the lizard that is extended and retracted rapidly during signalling and has an important species recognition functions (Ord & Martins 2006, Ng & Glor 2011, Ng et al. 2013). In many anole species females have smaller and/or less conspicuously coloured dewlaps than males (Harrison & Poe 2012). Nevertheless, fundamental sexual differences in dewlap coloration and pattern are rare and such cases are mostly reported from mainland anoles (Köhler 2003, Ugueto et al. 2007, 2009, Köhler et al. 2014).

Intersexual differences in dewlaps could be understood as a result of different pressures on males and females. While the reproductive success of males is determined mostly by success in mate acquisition which is positively associated with signalling, female success depends mainly on egg production and survival. This results in different design of dewlaps between sexes (Vanhooydonck et al. 2009). Females generally use their dewlaps much less than males and rarely in reproductive context (Nunez et al. 1997, Losos 2009, Johnson & Wade 2010). Females also exhibit no relationship between display behaviour and dewlap morphology.

Thus, the use of the dewlap is not associated with the size of the cartilaginous structures that support its movement (Johnson et al. 2011). Harrison & Poe (2012) focused on females dewlap size variation in huge comparative study and found evidence that females have large dewlaps in species with little sexual size dimorphism, while having small or no dewlaps in species with wider sexual size dimorphism. From all Antillean ecomorphs, the largest dewlaps are found in crown-giants and twig anoles. One hypothesis for this phenomenon is that large dewlaps are essential in dense habitats as they would be seen better, and second hypothesis is based on low densities of these two ecomorphs – larger dewlaps facilitate long distance communication (Harrison & Poe 2012).

In this study we focus on the "chamaeleolis" group endemic to Cuba Island (Schettino 2003, Losos 2009). They used to have their own ecomorph class named "twig giants" (Haas et al. 1993) referring to their unique body shape and lifestyle but they were moved to wide-size ecomorph class "twig anoles" (Beuttell & Losos 1999, Mahler et al. 2016). Nevertheless these striking lizards show list of unusual characteristics among other anoles- large body size, short limbs, huge bony casques on heads, lack of tail autotomy (Garrido & Schwartz 1968), independent eye movement, cryptic coloration and extraordinary scalation, molariform teeth in adults (Estes & Williams 1984, Schwartz & Henderson 1991) specialized for crushing snails (Schettino 1999, 2003, Herrel & Holáňová 2008) and cryptic and slow motion lifestyle (Leal & Losos 2000). Consequently, these anoles have been traditionally recognized as a distinct genus *Chamaeleolis* Cocteau, 1838. Because this group forms a derived internal clade of the genus *Anolis* Daudin, 1802 sensu lato, it is usually treated as a junior synonym of *Anolis*. The studies discovering the cladogenesis of the anoles have placed the genus *Chamaeleolis* within the main body of the tree of Antillean anoles. These form a sister group of a clade consisting of the Puerto Rican species *Anolis cuvieri* Merrem, 1820 and Hispaniolan *A. barahonae* Williams, 1962 and *A. christophei* Williams, 1960 (e.g., Haas et al. 1993, Poe 2004, Nicholson et al. 2005, 2012). In this study we discovered a population exhibiting fundamental sexual dichromatism, males possessing red dewlap strongly contrasting with white dewlaps of conspecific females.

The aim of this paper was (1) to describe dewlap coloration and reflectance spectra in the discovered dichromatic population and other four species/populations of anoles belonging to the "chamaeleolis" group; (2) to assess sexual dichromatism and sexual size dimorphism in the studied "chamaeleolis" species; (3) to analyse morphometric and genetic variation in this group and related anoles in order to discuss evolution of the dimorphic traits.

MATERIAL AND METHODS

Species determination

The examined material (see below) was assigned to species according to morphological criteria (Holáňová et al. 2012). We provisionally determined the specimens of dichromatic population from vicinity of San German (Holguín province, Cuba) and Gran Piedra (Santiago de Cuba province, Cuba; Figs. 1–3) as *Anolis porcus*. Considering multiple distinct morphometric and genetic characters of these animals (see under the results), we further refer to this population as *Anolis* sp. In original description of *A. porcus* Cope, 1864 there is no information about dewlap coloration nor about type locality. We avoid taxonomic discussions concerning species identity of these specimens until a thorough revision of *A. porcus* sensu lato including properly localized materials will be performed. Clarification of the geographic origin of the holotype is needed prior to any nomenclatural suggestion.

Spectrophotometry

We measured 3 males and 2 females of *Anolis* sp. from San German population together with *Anolis barbatus* Garrido, 1982 (Soroa), *A. chamaeleonides* Duméril et Bibron, 1837 (Viñales), *A. guamuhaya* Garrido, Pérez-Beato et Moreno, 1991 (Topes de Collantes) and *A. porcus* (Baracoa), each represented by a single male and a single female. All specimens were obtained from collections of private European and Russian breeders. All were captive bred after parental animals with known original localities.

The dewlap colour reflectance was determined between 300 and 700 nm with an OceanOptics USB4000 spectrophotometer, using a PX-2 Pulsed Xenon lamp source. We used the Ocean Optics WS-1 white standard for calibration, which was performed after every three measurements. The probe was held in a constant perpendicular 5mm distance from the gently stretched dewlap skin and the measurements were performed in a darkened room. Each colour patch was measured 3 times and then calculated its mean reflectance value. For visual modelling, we used the photoreceptor sensitivity data for *Anolis lineatopus* Gray, 1840 (Loew et al. 2002, Marshall & Stevens 2014). We calculated both chromatic and achromatic

Figs. 1–3. 1 – Original locality of *Anolis* sp. at Gran Piedra, Santiago de Cuba province, Cuba (photo by VHZ). 2 and 3 – Wild adult male of *Anolis* sp. at locality Gran Piedra, Santiago de Cuba province, Cuba (both photos by VHZ).

Table 1. Genetic samples and their GenBank accession numbers

samples	origin	GenBank Accession Numbers
Anolis barbatus AB 1	Soroa	MF157534
Anolis barbatus AB 2	Soroa	MF157535
Anolis porcus AP 1	El Yunque	MF157536
Anolis porcus AP 2	Duaba	MF157537
Anolis porcus AP 3	Duaba	MF157538
Anolis guamuhaya AG 1	Topes de Collantes	MF157539
Anolis sp. APS 6	Gran Piedra	MF157540
Anolis sp. APS 4	Gran Piedra	MF157541
Anolis sp. APS 5	Gran Piedra	MF157542
Anolis sierramaestrae AS	La Mula	MF157543
Anolis chamaeleonides AC 3	Viñales	MF157544
Anolis chamaeleonides AC 2	Viñales	MF157545
Anolis chamaeleonides AC 1	Viñales	MF157546
Anolis sp. APS 1	San German	MF157547
Anolis sp. APS 2	San German	MF157548
Anolis sp. APS 3	San German	MF157549
Anolis guamuhaya AG 2	Topes de Collantes	MF157550

contrasts between (1) "males and females" dewlaps (the pale colour), and (2) the pale and red colour within the dewlap of the males of *A.* sp. from San German. The chromatic contrast expressed in "just noticeable differences" (JND) was calculated according to Vorobyev & Osorio (1998); values below 1 indicate that two colours are unrecognizable within the particular visual system, values between 1–3 are considered to be distinguishable under ideal lighting conditions and with the increasing value the colours gradually become more distinct. Data for relative photoreceptor densities and the Weber fraction value 0.05 were taken from Marshall & Stevens (2014). The calculation of the achromatic contrast was performed after the model of Siddiqi et al. (2004) and Loyeau et al. (2007). All calculations were performed in Avicol v.6 (Gomez 2006).

Morphometric traits

We examined 128 adult anoles of the "chamaeleolis" group of which 83 were live animals or preserved specimens provided by European private breeders (18 *A. barbatus* from Soroa, Cuba; 23 *A. chamaeleonides* from Viñales, Cuba; 19 *A. guamuhaya* from Topes de Collantes, Cuba; 17 *A. porcus* Baracoa, Cuba and 6 *Anolis* sp. from San German, Cuba) and 44 were museum specimens from the herpetological collection of the National Museum in Prague (NMP), Czech Republic (list of museum specimens in Appendix 1).

The following measurements were made with digital callipers to the nearest 0.1 mm: snout-to-vent length (SVL: measured from the tip of the snout to the vent); body length (LIE: longitudo interextremitatis – the distance between front and hind legs); jaw out-lever distance (JOL: the distance between the jaw articulation and the tip of the jaw); head length (HL: measured from the edge of the head casque to tip of the snout); head width (HW: measured at the intersection with the angle of the jaws); head height (HH: measured just posterior to the orbits); snout-orbit distance (SO: the distance between the tip of the snout to the nearest point of the orbit); snout-nostril distance (SN: the distance between the tip of the snout to the edge of the left nostril); snout-mouth end (SME: the distance from the tip of the snout to the corner of the mouth); lower jaw length (LJL: the distance from the back of the retroarticular process to the tip of the lower jaw); snout length (SL: the length of the snout measured from the back of the jugal bone to the tip of the upper jaw); closing in-lever (CL: the distance between the jaw articulation and the back of the jugal bone; this distance was calculated by substracting the snout length from the distance measured from the jaw articulation to the tip of the jaw = QT); opening in-lever (OL: the distance from the jaw articulation to the back of the retroarticular proces; this distance was calculated by substracting QT from lower jaw length); internasal distance (IN: the distance between the nostrils); orbit-casque distance (OC: the distance between the posterior-most point of the orbit and the highest point of the casque); interorbital distance (IO: the shortest distance between the orbits); ear opening (EO: the maximum vertical length of an ear opening); tibia (TB: the length of the left tibia); femur (FEM: the lenght of the left femur); hind metatarsus (HM: the lenght of the left hind metatarsus); hind finger (HF: the lenght of the longest –the fourth- hind finger excluding the claw); humerus (HU: the lenght of the left humerus); radius (RA: the lenght of the left radius); front metatarsus (FM: the lenght of the left front metatarsus); barb scales (BS: the maximum length of the barb-like scales on a dewlap).

Statistical analysis of morphometric data

Size component of morphometric variation may mask differences in body shape (e.g., Frýdlová et al. 2011). Thus, we performed size adjustment of morphometric traits prior further analyses comparing the sexes and/or populations. For this purpose, we adopted a method published by Somers (1986, 1989) as implemented in the Size analysis v02 (Thompson & Withers 2005a, b, c). This software computes from original untransformed measurements not only generalised (multivariate) isometric size, but also partial isometric size-adjusted measurements. The latter ones were further treated by univariate and/or multivariate statistical procedures. We used STATISTICA, version 6.0, StatSoft Inc., 2001, for these calculations.

Genetic characteristics

We sampled 17 individuals of genus *Anolis* (see Table 1 for GenBank accession numbers of samples). Total genomic DNA was isolated from tissue samples with DNAeasy Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's guidelines. DNA amplification was performed with two previously published primers: L4437 (Macey et al. 1997) and H5730 (Glor et al. 2004). These primers were used to sequence the entire 1035 bp fragment, which includes complete sequence for the gene encoding ND2.

Polymerase chain reactions (PCR) were carried out in 50 µl including 2.5 µl of each 10 µM primer, 5 µl of 10× PCR buffer (Fermentas), 5 µl of 10 mM dNTP, 2.5 µl of 50 mM $MgCl_2$, 0.5 µl of 5 U/ml Fermentas Taq DNA polymerase, 100 ng of DNA and 27 µl of ddH_2O. The PCR amplification protocol consisted of 30 cycles of denaturation at 95 °C for 35 s, annealing at 48–51 °C for 35 s, and extension at 72 °C for 150 s; a further 7 min elongation step at 72 °C followed the last cycle. All PCR products were purified with the Qiaquick® purification kit (Qiagen, Hilden, Germany) and directly sequenced using the same primers as for the amplification.

Bayesian analysis (BA) was conducted with MrBayes 3.2.2 (Ronquist & Huelsenbeck 2003). The optimal model of DNA sequence evolution was selected using the AIC criterion in Modeltest 3.7 (Posada & Crandall 1998). Two independent runs of Bayesian analyses were conducted with a random starting tree and run for 6×10^6 generations, with trees sampled every 100 generations. The burn-in command was used to discard the first 6,000 trees (600,000 generations). Posterior-probability values were used to indicate support for nodes of the Bayesian topology.

The outgroup is composed of ND2 sequences of *Anolis argenteolus* Cope, 1861 (GenBank Accession Number AY296154.1), *A. lucius* Duméril et Bibron, 1837 (GenBank Accession Number AF055962.2), *A. etheridgei* Williams, 1962 (GenBank Accession Number AF055934.2), *A. insolitus* Willians et Rand, 1969 (GenBank Accession Number AF055933.2), *A. cuvieri* (GenBank Accession Number AF055973.2), *A. christophei* (GenBank Accession Number AF055957.2), *A. ricordii* Duméril et Bibron, 1837 (GenBank Accession Number AY367138.1), *A. barahonae* (GenBank Accession Number AF055972.2) and *A. baleatus* Cope, 1864 (GenBank Accession Number AY296155.1).

RESULTS

Sexual dichromatism

Pictorial comparison of heads and dewlaps of males and females in each of five examined species/populations of anoles belonging to "chamaeleolis" group is provided in Fig. 4. Our spectrophotometric measurements confirmed the white/gray/brown character of the dewlaps in *Anolis barbatus* (Soroa), *A. chamaeleonides* (Viñales), *A. guamuhaya* (Topes de Collantes) and *A. porcus* (Baracoa), without any significant peak in the UV (see Table 2). Certain UV reflectance (320–400 nm) was detected in both males and females in these four "non-red" species, but was absent in red-throated *Anolis* sp. from San German (Fig. 5). Chromatic contrast suggested little intersexual differences in the pale colour: 2.09–4.28 JND in "non-red" species; 4.02–6.70 JND in *Anolis* sp. from San German (JND values <1, 1–3 and >3 indicate indistinguishable, barely distinguishable and clearly distinguishable stimuli respectively; see Methods). The only sexual difference in "non-red" species, if present, was manifested mainly through the overall brightness of the pale colour, which was brighter in male dewlaps. In *A. porcus*, the reflectance curve differed at longer wavelengths between males and females, suggesting a possible chromatic difference. It is however hard to evaluate this difference with only a single pair of individuals. The achromatic contrasts between male and female dewlaps spanned from nearly non-dimorphic (3.5 JND in *A. chamaeleonides*) to strongly distinct (22 JND in *A. barbatus*) and the same range was present among the individuals of *Anolis* sp. from San German. The red spot in the males from this popu-

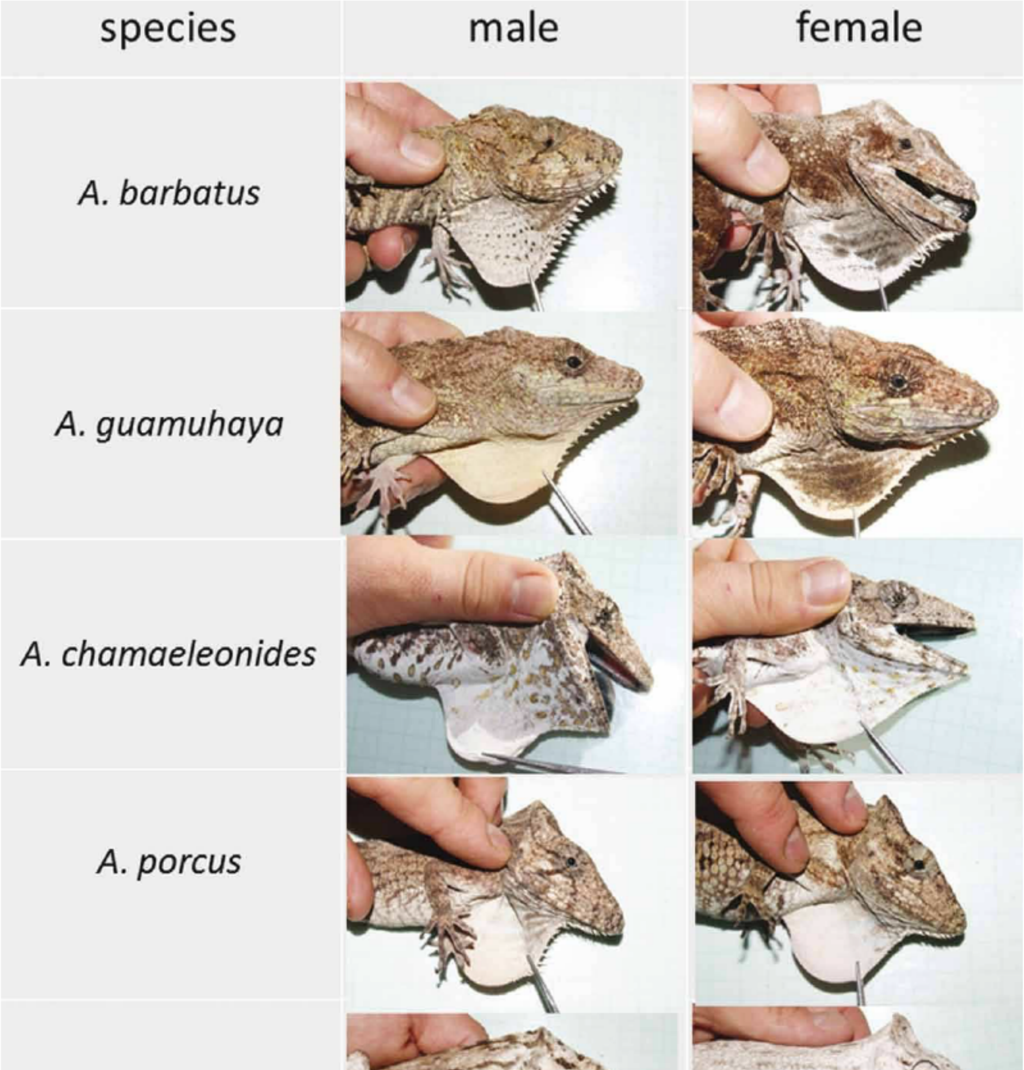

Fig. 4. Lateral view of heads and dewlaps in males and females of five examined *Anolis* species/populations of the "chamaeleolis" group.

lation strongly contrasted with its white background in both chromatic (mean=12.35±0.05 JND) and achromatic (mean=21.40±0.07 JND) visual channels (Table 1).

Table 2. Chromatic and achromatic contrasts between (1) males' and females' dewlaps (the pale color), and (2) the pale and red color within the dewlap of the males of *Anolis* sp. from San German

	chromatic contrast (JND)	achromatic contrast (JND)
Anolis barbatus white M-F	4.193	22.013
Anolis chamaeleonides white M-F	2.094	3.490
Anolis guamuhaya white M-F	4.286	6.759
Anolis porcus white M-F	2.107	20.087
Anolis sp. white M1-F1	6.698	19.956
Anolis sp. white M1-F2	4.496	9.174
Anolis sp. white M2-F1	6.397	9.907
Anolis sp. white M2-F2	4.085	3.467
Anolis sp. white M3-F1	6.071	15.350
Anolis sp. white M3-F2	4.017	4.569
Anolis sp. red-white M1	12.149	20.742
Anolis sp. red-white M2	12.849	21.921
Anolis sp. red-white M3	12.065	21.539

Sexual dimorphism in body size and shape

Multivariate analysis of variance (MANOVA) showed strong effect of species ($F_{100,327}$=6.70, P<0.0001) on 25 size-adjusted morphometric variables, but revealed neither the effect of sex ($F_{25,82}$=1.14, P=0.3221) nor sex*species interaction ($F_{100,327}$=1.26, P=0.0704). This result was confirmed by Analysis of variance examining the effects of species/populations, sex and its interaction on isometric multivariate body size (PC1 produced by the Size analysis v02 software, Thompson & Withers 2005a). Body size differed among species/populations ($F_{4,106}$=8.20, P<0.0001), but not between the sexes (sex: $F_{1,106}$=1.01, P=0.3174; sex*species interaction: $F_{4,106}$=1.39, P=0.2415). Almost the same results were obtained for PC2 and PC3 reflecting body shape (Species: both P<0.0001, for sex and interaction all P>0.20). This allowed us to pool sexes for further analyses.

Morphometric differentiation among populations/species

We performed discriminant function analysis (DFA) to visualize differences among examined species/populations (except *A. sierramaestrae* Holáňová, Frynta et Rehák, 2012 for which only the holotype specimen was available) in size-free morphometric traits. We applied stepwise forward selection method which resulted in inclusion of 21 of variables (four variables were excluded) and high classification success (Wilks' Lambda=0.0161, $F_{84,361}$=7.94, P<0.0001). 108 of 116 specimens (93%) were assigned to proper species/population. All *A. chamaeleonides* (25) and *A. porcus* (17) were reclassified correctly. Two of 40 specimens of *A. barbatus* were misclassified as *A. guamuhaya* while five of 28 *A. guamuhaya* as *A. barbatus*. One of six *Anolis* sp. was misclassified as *A. porcus*. The type specimen of *A. sierramaestrae* was closest to *A. porcus* according to the classification equations derived from DFA.

Cluster analysis (CA) performed by Ward method from the matrix of squared Mahalanobis distances visualized similarities between *A. barbatus* and *A. guamuhaya* as well as between *A. porcus* and *Anolis* sp.; *A. chamaeleonides* was morphometrically least similar to remaining examined species (Fig. 6, Appendix 2).

Canonical analysis revealed four significant (P<0.0005) multivariate axes (Appendix 3). The position of examined specimens in the morphospace of the first two canonical axes is plotted on Fig. 7.

Genetic differentiation among populations/species

The phylogenetic analysis was based on sequences of mitochondrial ND2 gene (alignment consisting of 1035 bp). The sequence divergences between haplotypes of "red dewlap" (here referred to *Anolis* sp.) and "whitish-yellowish dewlap" (*A. porcus*) populations belonging to *A. porcus* sensu lato (*p*-distances ranging from 5.25% to 6.11%, mean=5.62%), is comparable to those among haplotypes of currently recognized species of the "chamaeleolis" group of anoles (i.e.,

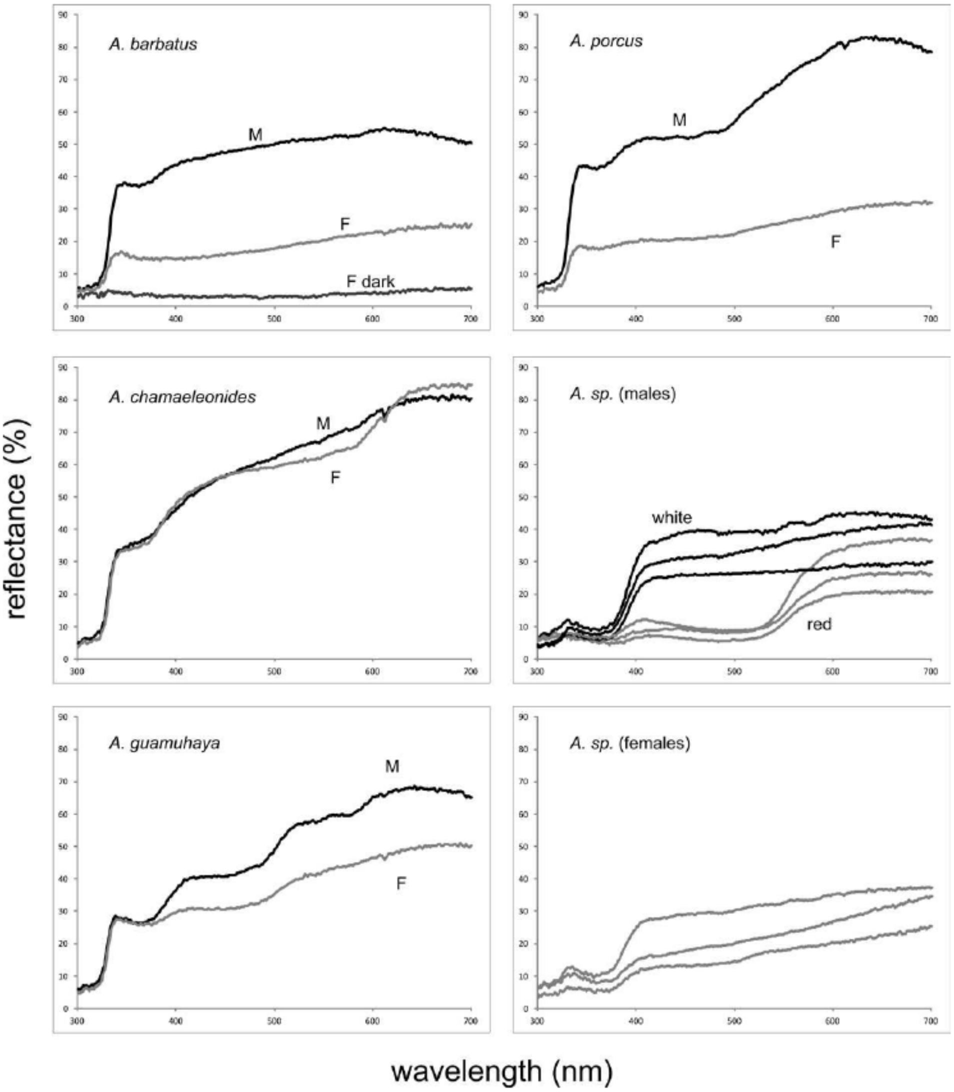

Fig. 5. The dewlap color reflectance between 300 and 700 nm in males and females of five species/populations of anoles of "chamaeleolis" group.

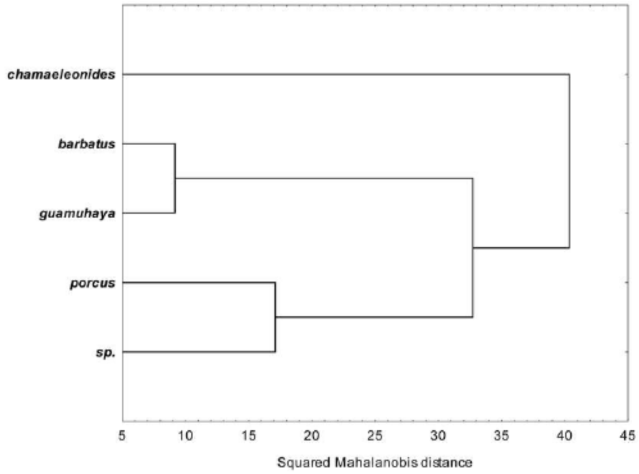

Fig. 6. Phenetic tree of five *Anolis* species based on morphometric data. It is constructed from matrix of Mahalanobis distances clustered by Ward's method.

A. barbatus, *A. chamaeleonides*, *A. guamuhaya*, *A. porcus* and *A. sierramaestrae*). The uncorrected P-distances of these between species comparisons vary within the range of 4.44–10.20%, mean=6.58%, while within species distances are much smaller; maximum values were 0.59, 0.59,

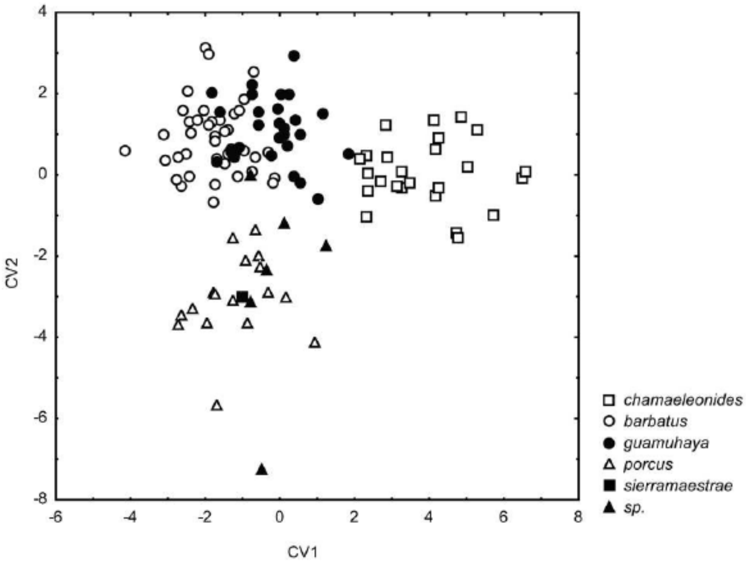

Fig. 7. Position of *Anolis* species/populations of the "chamaeleolis" group in the morphospace of the first two canonical axes (CVA1 and CVA2).

1.19, 0.39, and 2.21 for *A. barbatus*, *A. chamaeleonides*, *A. guamuhaya*, *A. porcus* and *Anolis* sp., respectively. The Bayessian analysis revealed that examined haplotypes belonging to *A. barbatus*, *A. chamaeleonides* and *A. guamuhaya* (all posterior probabilities = 1) form monophyletic groups, but this is not true for *A. porcus sensu lato* including populations of both colour forms. In contrast to this the haplotypes from *A.* sp. (= "red dewlap") and "whitish-yellowish dewlap" (= *A. porcus*) populations formed mutually exclusive monophyletic groups (posterior probabilities were 0.99 and 1.00 respectively). As expected, monophyletic status of entire "chamaeleolis" group of anoles was strongly supported (Fig. 8).

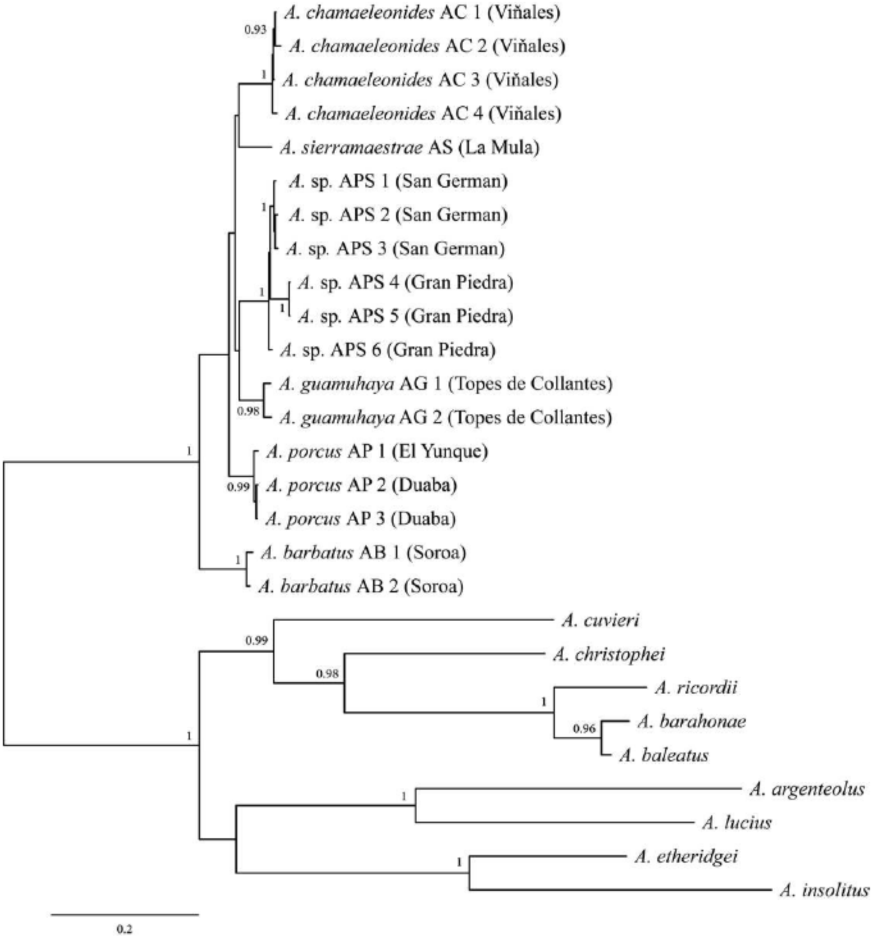

Fig. 8. Bayesian tree of mitochondrial ND2 gene including six species/populations of Cuban anoles belonging to the "chamaeleolis" group as well as nine related anoles introduced as outgroups.

DISCUSSION

Dewlap dichromatism

Our study showed that in comparison to other species/populations of the "chamaeleolis" group, dewlaps of *Anolis* sp. are clearly sexually dichromatic. Taking into account the tetrachromatic vision of the anoles, spectrophotometry and visual modelling revealed no such intersexual difference which would be perceived only by the anoles, but not by humans. While the "dichromatism" in the "non-red" anoles, if present, can be manifested mainly through the lightness of the dewlap, there is both chromatic and achromatic contrast in the red-throated *Anolis* sp., making their dewlaps more conspicuous. Males of the "chamaeleolis" anoles perform the courtship behaviour style as other anoles do, including dewlap displays and head bobs (pers. obs.). The red coloration may therefore represent an honest signal, possibly mediated by carotenoid pigments, which are often involved as indicators of males' quality (Steffen & McGraw 2009, Steffen et al. 2010). The other frequent honest signal in lizards is the ultraviolet colouration (Font et al. 2009, Fleishman et al. 2011), but although our measurements detected some UV reflectance in the "non-red" species (and none in *Anolis* sp.), this did not seem to be the case. Firstly, the UV reflectance was the lowest within the whole spectrum and secondly, when the sexes differed, they did by the overall shape of the reflectance curve, not just by the UV reflectance itself. However, larger samples are needed to determine reliable statistics of possible trends in this or other traits.

Dewlap function and evolution

There are multiple hypotheses about the function and evolution of the dewlap (Losos 2009, Steffen & Guyer 2014, Hagman & Ord 2016). It may play a role in species recognition, territorial signalling (intrasexual selection), honest signalling of male quality, or in predator deterrence.

Huge dewlap evolution in lizards with clearly allopatric distribution, like in the "chamaeleolis" anoles, does not suggest that it should have function in species recognition as it has in sympatrically living anoles of the same ecomorph. Lizards of the "chamaeleolis" group are classified as the "twig anoles" ecomorph which displays active foraging mode. Species belonging to this ecomorph have slow locomotion but can search for usually cryptic prey for greater distances (Irschick & Losos 1996). These authors suppose that it is possible for this reason that twig anoles have wide size habitats and so it is difficult to defend such a large space. That could be the reason why intrasexual selection usually does not impact twig anoles and dimorphism is not noticeable. But this assumption could be applicable in case of low density of the species. In the species Anlis sp., there can be higher local population density which leads to stronger male competition and therefore to stronger intrasexual component of sexual selection. Unfortunately population density has not been measured for this population. The dichromatism subsequently makes the courtship and aggressive communication more unambiguous, unlike in monochromatic species (Regalado 2015).

The habitat type has also considerable influence on the use of the dewlap. Species inhabiting habitats with higher visibility display more frequently. Anole species extend their dewlaps in sunny habitats more often than in shady ones (Ord & Martins 2006), but light conditions appear to be unrelated to the evolution of dewlap colour and signal detectability (Fleishman et al. 2009). Moreover, all "chamaeleolis" anoles live in more or less the same type of habitat (personal observation of the first author), it is therefore unlikely that the dewlap colour is directly associated with their environment.

Different pressures on sexes can also lead to different use and size of dewlaps in some anole species. Johnson & Wade (2010) showed in their comparative study that males have larger dewlaps (and cartilage and muscular components controlling dewlap movement) and use them more frequently than females. Dewlap size is a significant predictor of the winner in male fights in species with low but not high SSD. Neither the dewlap display rate is associated with SSD (Lailvaux & Irschick 2007). But as we detected no significant SSD in *Anolis* sp., this explanation is unlikely as well.

According to the pursuit-deterrence hypothesis, when the prey is detected by the predator, it gives him an "I am aware of you" signal (shows the dewlap), which might deter the predator from pursuing its prey (Caro 1995). This hypothesis predicts that mainland species living under higher predation risk would display more often. Mainland *A. carolinensis* Voigt, 1832 exhibit elevated rate of dewlap use compared to its island relatives (Vanhooydonck et al. 2009, Johnson & Wade 2010). However, this explanation would be doubtful in the case of "chamaeleolis" anoles, as they are not presumably predated much, being considerably cryptic. Moreover, if the conspicuous dewlap was to deter potential predators, it should be present in both sexes and not just the males.

In conclusion, because of lack of data on the population density of *Anolis* sp. and its related species, it is difficult to determine the evolutionary causes of the dewlap dichromatism.

Sexual size monomorphism/dimorphism

The differences between the sexes in morphometric traits have been thoroughly and repeatedly analysed in anoles (see Losos 2009). Sexual differences in body size are positively correlated with those in body shape (Losos et al. 2003). Sexual size dimorphism is traditionally explained by three causes: (1) sexual selection or competition for reproductive success, (2) intersexual resource differences and (3) different reproductive roles (Losos et al. 2003). Nevertheless, the relative clutch size in anoles is considerably reduced compared to the other clades of squamates (Kratochvíl & Kubička 2007). This limits a peak load of maternal investment and thus, the effect of sex differences in reproductive roles have in this particular group of lizards. Contribution of the strength of sexual selection was repeatedly confirmed even by intraspecific comparisons, e.g., sexual size dimorphism increases with population density (Stamps et al. 1997). Empirical studies revealed that habitat use of a particular species belongs to key predictors the sexual size dimorphism in anoles (Butler et al. 2000, Losos et al. 2003). Size of defended habitat, foraging style, food source dispersion – these habitat characteristics influence sexual dimorphism.

In examined material of the "chamaeleolis" anoles, we failed to detect sexual size dimorphism neither in the multivariate body size nor in the body shape. Although, the available sample of the dichromatic Anolis sp. was too small to allow a separate analysis, the sexes were of comparable size and shape even in this population/species. The entire absence of the sexual size and shape dimorphism in this group is not surprising. Lizards of group are arranged to the "twig anoles" ecomorph which displays active foraging mode. Species belonging to this ecomorph have slow locomotion but can search for usually cryptic prey for greater distances (Irschick & Losos 1996). These authors suppose that it is possible for this reason that twig anoles have wide size habitats and so it is difficult to defend such a large space. That could be the reason why intrasexual selection usually does not impact twig anoles and dimorphism is typically not noticeable.

Morphometric and genetic divergence of "chamaeleolis" species

Anoles of the "chamaeleolis" group are slowly moving lizards with limited home ranges. Thus, they are most likely poor dispersers. This suggestion is supported by distribution patterns of these species in Cuba (Garrido et al. 1991, Díaz et al. 1998, Schettino 1999, Holáňová et al. 2012). Each species is typically restricted to a local mountainous area and its surroundings. The isolation of local populations may explain a considerable degree of morphological divergence we observed. Considering the reports of morphologically suspect individuals from other localities (e.g., Rancho Velaz, Villa Clara province, Sierra de Banao, Sancti Spiritus province) (Garrido 1982, Garrido et al. 1991), discoveries of additional species of the chamaeleolis group can be expected in the near future.

Our multivariate analysis of morphometric traits showed that the most distinct species is *A. chamaeleonides*, while *A.barbatus* resembles *A. guamuhaya* and non-dichromatic *A. porcus* is similar to dichromatic *Anolis* sp. In this context it is interesting that a morphotype resembling "chamaeleolis group" evolved entirely independently in Hispaniola in a group of related anole species (Mahler et al. 2016).

Genetically, all studied species/populations seem to differ in mitochondrial DNA. But except of finding that *A. barbatus* is the most distinct species of all we cannot determine their phylogenetic relationship. It seems that in view of the fact that they have low dispersion ability there will be separated species/population on every mountainous locality. It only confirms that there is extensive genetical diversity in Cuba, which is known also in other species (Starostová et al. 2010). The sequence divergence of a mitochondrial gene we found among species/populations of "chamaeleolis" anoles is considerable and this supports the view that each of examined form, including dichromatic *A.* sp., represents distinct species. Nevertheless, recent studies suggest that reproductive isolation and thus speciation process are sometimes not fully completed even between lizards with roughly two times higher genetic distances calculated from the sequence divergence of mitochondrial genes (Jančúchová-Lásková et al. 2015a, b).

CONCLUSIONS

In conclusion, local populations of the "chamaeleolis" groups are distinct genetically as well as morphologically. They are non-dimorphic in size but one population is sexually dichromatic (*Anolis* sp.). Such conspicuous dichromatism is unusual among the island anoles.

Acknowledgements

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APPENDIX 1

List of museum specimens from the herpetological collection of the National Museum in Prague (NMP), Czech Republic used for morphometric examination.

Anolis barbatus, 29 specimens: NMP6j 25/1992, NMP6V 34520/1-11, NMP6V 71872/1–3, NMP6V 71873, NMP6V 73148/1–4, NMP6d 279/03, NMP6V 34504;
Anolis guamuhaya, 10 specimens: NMP6V 71871, NMP6V 71870/1–8, NMP6V 34517;
Anolis chamaeleonides, 3 specimens: NMP6d 81/06, NMP6V 34505, NMP6V 34518;
Anolis porcus, 1 specimen: NMP6V 34519;
Anolis sierramaestrae, 1 specimen: NMP6V 74453.

APPENDIX 2

Matrix of squared Mahalanobis distances between examined species/populations of "chamaeleolis" group revealed by DFA.

	1	2	3	4	5
1 *A. chamaeleonides* : *A. chamaeleonides*	0.000	34.394	23.326	38.285	34.384
2 *A. barbatus* : *A. barbatus*	34.394	0.000	9.163	19.005	24.287
3 *A. guamuhaya* : *A. guamuhaya*	23.326	9.163	0.000	20.939	27.466
4 *A. porcus* : *A. porcus*	38.285	19.005	20.939	0.000	17.099
5 *A. sierramaestrae* : *Anolis* sp.	34.384	24.287	27.466	17.099	0.000

APPENDIX 3

Loadings of roots revealed by canonical analysis subroutine of DFA.

root	1	2	3	4
SVL	–0.136	–0.623	–0.164	–0.185
OL	0.370	–0.132	0.039	0.232
FM	–0.301	–0.085	0.192	0.468
HM	0.026	–0.181	0.053	0.481
IN	0.204	–0.204	0.034	–0.347
HL	0.228	0.071	0.228	–0.183
OC	0.062	0.086	–0.400	–0.129
SN	0.119	0.261	–0.089	0.141
RA	–0.002	0.173	–0.035	–0.197
SL	–0.107	0.002	0.273	–0.267
HF	–0.086	0.107	0.087	0.072
IO	0.207	–0.014	0.187	0.109
HH	0.147	0.220	0.121	0.139
LIE	0.042	0.313	–0.078	–0.065
HW	–0.090	0.122	0.343	–0.385
SO	–0.075	–0.100	–0.139	0.024
JOL	0.077	–0.008	0.080	–0.213
TB	–0.037	–0.060	–0.245	–0.171
LJL	0.102	0.239	–0.065	0.080
HU	–0.064	–0.036	–0.013	–0.020
SME	0.053	0.245	0.096	0.052

Postnatal dentition changes in the Cuban False Chameleons: adaptation to a dietary shift

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Abstract

The ability to form multiple generations of teeth allows for changes in dentition morphology throughout an animal's life. This adaptation can accommodate different dietary strategies at various life stages, most notably between juvenile and adult stages. Common changes between tooth generations include variations in tooth number and morphological parameters. In this study, we compare the dentition morphology of juvenile and adult specimens from four species of Cuban false chameleons in the genus *Anolis* (Chamaeleolis group), within the Iguania clade of squamate reptiles. Unlike most anole lizards, which are primarily insectivorous or saurivorous, these species are specialized feeders on shelled snails and possess distinctive broad molariform teeth at the back of their jaws. The exact dietary preferences of these species remain unclear. Our findings reveal that juveniles differ from adults by the presence of tricuspid teeth at the back of the jaw, and that the examined species exhibit differences in dentition, such as tooth number, shape, arrangement of cusps and crests, presence of molariform teeth, and internal tooth structure. Based on these observations, we discuss how interspecific and age-related changes in dentition may reflect differences in diet.

Keywords

Anolis; teeth; shape; malacophagy; tooth replacement

1. Introduction

The dentition of vertebrates exhibits a wide variety of shapes and sizes, with its morphological complexity reflecting both feeding adaptations and the species' phylogenetic origin (Berkovitz and Shellis, 2016, 2018). In polyphyodont vertebrates—species capable of continuously replacing their teeth throughout their lives—the formation of new tooth generations allows for changes in tooth shape, number, and size, thus enabling dietary adaptations. The relationship between tooth shape complexity and diet was recently examined by Melstrom (2017) in squamates, and differences between juvenile and adult tooth generations have been observed in several distinct groups (Estes and Williams, 1984).

We focused on four of the six described endemic species of molluscivorous Cuban false chameleons (*Anolis barbatus*, *A. chamaeleonides*, *A. guamuhaya* and *A. porcus*), formerly known as *Chamaeleolis* (Cocteau and Bibron, 1838). These species belong to the twig giant ecomorph- large anoles with short limbs that prefer narrow twigs (Beutell and Losos, 1999; Schettino, 2003). Additionally, we studied the Cuban endemic species Baracoa anole (*A. baracoae*), which represents a crown giant ecomorph (Butler et al., 2000; Losos, 2009). Cuban false chameleons undergo significant changes in tooth morphology over their lifetime, whereas *A. baracoae* shows only minimal changes. The Cuban false chameleons examined here are non-dimorphic (Vanhooydonck et al., 2015). One of the most striking morphological characteristics of this group is their large head with a wide casque and molariform dentition in the caudal region of the jaw in adults (Estes and Williams, 1984). The large head and molariform caudal teeth suggest selection for increased bite force (Herrel and Holanova, 2008). These characteristics are crucial for their molluscivorous feeding specialization, which involves crushing snail shells (Estes and Williams, 1984; Lee, 1997; Schettino, 1999, 2003). The blunt molariform teeth in adult Cuban false chameleons are important for preventing tooth breakage during shell crushing and for providing an increased contact area with the food (Herrel and Holanova, 2008). In addition to mollusks, they also feed on insects, fruits, and flowers (Lee, 1997; Schettino, 1999, 2003). In captivity, Cuban false chameleons accept mollusks, arthropods, small vertebrates (lizards, mice), and fruits but prefer mollusks when given a choice (Zahradnickova, personal observation). Despite these observations in captivity, a detailed study of the diet of the species examined here over their lifetime does not exist, and an exact correlation between tooth shape and diet is not yet possible.

In contrast to Cuban false chameleons, *A. baracoae* represents the crown giant ecomorph, which includes large anoles with big heads that inhabit the highest parts of tree crowns (Williams, 1972; Losos, 2009). It is an active predator that prefers to feed on active arthropods, small vertebrates, and occasionally plant material, as observed in the majority of examined anoles (Schoener and Gorman, 1968; Floyd and Jenssen, 1983; Bullock et al., 1993; Herrel et al., 2006; Hernández-Salinas et al., 2016; Narváez et al., 2020). This species possesses tricuspid teeth at the back of the jaws in both juveniles and adults. The presence of tricuspid teeth is a characteristic often found in extant and fossil anoles (e.g., Estes and Williams, 1984; Polcyn et al., 2002; Castaneda et al., 2014; Zahradnicek et al., 2014; Smith et al., 2021).

In this study, we describe the morphology of the juvenile and adult lower jaw dentitions in the aforementioned species and discuss interspecific differences as well as differences between juveniles and adults. We relate these species-specific characteristics to potential changes in feeding preferences throughout the life of Cuban false chameleons.

2. Material and methods

2.1. Animals

Four species of Cuban false chameleons (*Anolis barbatus*, *A. chamaeleonides*, *A. guamuhaya* and *A. porcus*) and one species of Baracoa anole (*A. baracoae*) were used in this study. Supplement 22 shows photos of adult *A. baracoae* and *A. barbatus* as examples of Cuban false chameleons. Two embryos/juveniles and two adults were used from each species. All specimens and skulls originated from the private collection of the first author. All examined specimens died naturally; no specimens were sacrificed for this study. For *A. baracoae*, two late embryos close to hatching were utilized. For *A. barbatus*, one late embryo and one older juvenile were used. For *A. chamaeleonides*, two juveniles that died shortly after hatching were analyzed. For *A. guamuhaya*, two embryos that died close to hatching were studied, and for *A. porcus*, two postnatals that died soon after hatching were analyzed. The exact chronological ages of the animals were not known. Heads of embryos and juveniles were fixed and stored in 70% ethanol. Heads of adult specimens were kept frozen at -20°C before preparation of the jaw bones with dentition. The skulls of these specimens are deposited in the Department of Zoology, Faculty of Sciences, Charles University in Prague, Czech Republic. The length of the tooth row was measured from the first to the last tooth, and the length of the lower jaw was measured from the rostral tip of the jaw to the caudal edge of the articulare (Table 1). Whole specimens were not available for size analysis. The less damaged quadrant of the lower jaw was analyzed after mechanical and chemical preparation. It is important to note that the dental pattern between jaw quadrants was not symmetrical, as is typical for polyphyodont non-mammalian toothed vertebrates (Berkovitz, 2000). No specimens of anoles used in this study came directly from Cuba. All specimens were descendants of animals legally obtained from private breeders, with several generations bred in captivity in the Czech Republic. All methods were carried out in accordance with relevant Czech and EU guidelines and regulations.

2.2. Jaw bones clearing

Soft tissue in the lower jaws of embryos and juveniles was removed mechanically and then chemically using a commercially obtained mixture of sodium hypochlorite (up to 5%) and sodium hydroxide (1%) (Savo, Unilever). For adult specimens, soft tissues from whole heads were initially removed by larvae of the larder beetle *Dermestes sp.*, and the jaws were then treated with a bleaching solution.

2.3. Light microscope

A representative lower jaw from one adult specimen of each species was photographed using a light microscope (Olympus SZX12) and is shown in Fig. 6 and the supplementary material (Supplement 2, 5, 7, 9).

2.4. SEM

Representative lower jaws from two embryos/juveniles and one adult of each species were air-dried and coated with gold. The surface analysis of tooth morphology was performed using scanning electron microscopes (JSM-SEM 6380 LV and JSM IT200, JEOL, USA). One embryo/juvenile from each species is shown in the article, while the additional specimens are provided as supplementary material (Supplement 1, 3, 4, 6, 8).

2.5. Micro CT

High-resolution, non-destructive microstructural analysis was performed using an ex-vivo micro-CT scanner (SkyScan 1272, Bruker, Belgium) and Bruker software (NRecon, DataViewer, CTvox). Samples for μCT were divided into two groups: 1. adults and 2. embryos/juveniles, and scanned according to sample size. Slice images of the teeth and jaw were provided in the mesiodistal plane by DataViewer, with a pixel size of 5.475 μm.

2.6. Analysis of dentition

The shapes of the teeth were analyzed from SEM and light microscopy images. Tooth positions and the number of ankylosed teeth were counted rostro-caudally in the lower jaw. Tooth positions were determined by considering ankylosed teeth, mineralizing teeth between ankylosed teeth, and free interdental spaces, with marks on the dentale indicating lost mineralized teeth during preparation (Supplement 10). For areas where teeth were lost during mechanical and chemical preparation, the number of positions was estimated. Supplement 10 also illustrates the method used to count ankylosed teeth.

For the morphological description of teeth and their positions within the jaws, we used standard anatomical terms for locations and directions (Fig. 1A). Tooth features such as cusps, crests, crenulation, and basic tooth shapes are detailed in Supplement 11. This supplement also explains how angles between the central cusp and accessory cusps in bicuspid and tricuspid teeth were interpreted. The teeth, mostly from adults, were partly damaged from food processing. Supplement 16 provides an example of such damage. The length of the lower jaw, from the rostral tip to the caudal part of the articulare, as well as the length of the dental field, was measured using a digital sliding caliper (Proteco).

2.7. Data availability statement

All data presented here are based on the photos published in this article and the supplementary material.

3. Results

3.1. The number of already formed and forming tooth positions within the tooth row of the juvenile dentition is interspecifically similar in contrast to adult dentitions

To understand the dynamic nature of tooth number, and its relation to jaw size, the number of teeth that were present at embryonic/juvenile and adult stages within the lower jaw quadrant was assessed in all species. In all examined embryonic and juvenile lower jaws, the number of already formed and forming tooth positions (based on the presence of tooth germs at different stages of mineralization) was 18–22 (N = 10). The number of ankylosed teeth at these early stages ranged between 8–18 (Table 1, Supplement 12).

In contrast to juvenile dentition, the adult specimens had a greater range from 17 to 31 tooth positions and ankylosed teeth from 16 to 30 Table 1, Supplement 13) (N = 10). The highest number of tooth positions was observed in the adult *A. baracoae* specimens, which had 29 and 31 tooth positions and 27 and 3 ankylosed teeth. The examined adult specimens had the shortest lower jaw. Their tooth row was also short in comparison to the most of Cuban false chameleons except A.

porcus which had the nearly same size of length of the tooth row. Similarly, adult *A. guamuhaya* and *A. chamaeleonides* had a high number of tooth positions and ankylosed teeth, but their lower jaw and tooth row were long (Table 1, Supplement 13). Adults from the two other Cuban false chameleon species, *A. barbatus* and *A. porcu*s had fewer ankylosed teeth (Table 1, Supplement 13). From all tested Cuban false chameleons, adult *A. porcus* had the lowest number of ankylosed teeth and also shortest tooth row and lower jaws from all of the examined species (Table 1, Supplement 13). *A. barbatus* had oppositely long tooth row and lower jaw (Table 1).

One specimen of *A. chamaeleonides* and one specimen of *A. barbatus* had ankylosed teeth from two functional generations located at a few positions within caudal area of the jaw. However, the teeth of the former generation were almost resorbed and only the rests of the tooth crowns remained present (Fig. 2F and 6A, C).

When all samples were plotted, there was a trend for more tooth positions to be associated with a longer tooth row (Table 1, Supplement 14). Overall, we observed the greatest relation of the number of teeth on the length of the tooth row in *A. baracoae*, and among the Cuban false chameleons it was in *A. guamuhaya,* and to a reduced extent *A. chamaeleonides*. Interestingly, *Anolis barbatus* and *A. porcus* showed only low relation between the number of tooth positions and tooth row length (Supplement 14). Both these species had very large caudal teeth in the adult compared to *A. baracoae* (Fig. 2 and 3)., suggesting a trade-off between tooth number and tooth size in the adults.

3.2. Tooth shape changes over time with distinct morphologies in different parts of the jaw

Interspecific differences and age-related differences in tooth morphology in four Cuban false chameleon species and *Anolis baracoae* from crown giant group were compared in order to predict possible feeding preferences.

3.2.1. Unicuspid teeth

In all the examined anoles, unicuspid teeth with conical or nearly conical shape were the simplest teeth observed (Fig. 2A–F and 3A–D). The majority of unicuspid teeth were positioned within the rostral region of the lower jaw. The caudal, and in some cases also middle regions, lacked teeth of this type (Fig. 2A–F and 3A–D). The majority of unicuspid teeth were positioned side by side to form a row of unicuspid teeth in both members of crown and twig giants (Fig. 2A–F and 3A–D). In the middle jaw regions of juvenile dentitions, the unicuspid teeth mixed with bicuspid or tricuspid teeth (Fig. 2A, C, E and 3A, C). In several cases of embryonic dentition, miniature rudimentary teeth of the null generation were found to be ankylosed to the edge of the dentale (Supplement 15). Number of ankylosed unicuspid teeth in the lower jaw of embryos/juveniles ranged between 6–12 in Cuban false chamaeleons and *Anolis baracoae* (Table 2) and no distinctive interspecific difference was found (N = 10). In examined adults, differences in the number of unicuspid teeth appeared to exist (N = 10). Adult *A. baracoae* had a large number of unicuspid teeth (Table 2) while adult Cuban false chameleons tended to have fewer unicuspid teeth, except for the one specimen of *A. guamuhaya* (Table 2 Fig. 3B). The conical shape of unicuspid adult teeth was slightly modified in some cases, with mesial and distal expansions of the tooth sides. These expansions were formed by an enamel crest running mesiodistally over the tooth. The expansions were placed in similar locations as accessory cusps (Fig. 4E, Supplement 11). These expansions could almost take the shape of accessory cusps (Supplement 11). The top of the unicuspid teeth was sharp or slightly rounded in adults. Along the top of both juvenile and adult teeth one or two enamel crests ran mesiodistally in examined Cuban false chamaeleons and *A.*

baracoae (Fig. 4B–C, E–F, H–I, K–L, O, Q–R and 5B–C, F, I, L). When two crests were present, a shallow groove was apparent between them in the area of the tooth tip (Fig. 4B–C, E–F, Q–R, I, L). The lingual part of the tooth crown was often contoured with crenulations in the form of low vertical ridges. The degree of their visual clarity was stage and species-dependent (Fig. 4B, E–F, L, O, Q–R and 5C, L).

3.2.2. Teeth with accessory cusps

The majority of teeth with accessory mesial and distal cusps were tricuspid and less frequently bicuspid. The bicuspid teeth, when formed, were in most cases found in the middle jaw region, tricuspid teeth were found within the caudal and middle jaw regions.

Juvenile dentitions lacked or possessed only a few bicuspid teeth (Table 2; Fig. 2A, C, E and 3A, C). In adults, only *A. baracoae* (Fig. 2B; Table 2) and one specimen of *A. guamuhaya* (not figured specimen, Table 2) had bicuspid teeth within the tooth rows. One adult specimen had two bicuspid teeth within its jaws, each with tips composed of two cusps of equal size (Supplement 17). Similar teeth were also found in the opposite lower jaw, but not in the upper jaws.

Tricuspid teeth (2–5) were found in all embryos/juveniles (Fig. 2A, C, E and 3A, C). In adults were tricuspid mostly absent (Fig. 2D, F and 3B, D; Table 2) with the exception of *A. baracoae* (Fig. 2B).. When present they were found within the caudal and middle jaw regions. Intraspecific and interspecific differences in the numbers of tricuspid teeth in embryos/juveniles were small (Table 2).

The morphology of bicuspid and tricuspid teeth in embryos/juveniles varied between species (Fig. 2A, C, E and 3A, C). In *A. baracoae* the central cusp of these teeth was the most protruding cusp from the tooth crown when compared to Cuban false chameleons. The central cusp in juvenile bicuspid/tricuspid teeth of Cuban false chameleons was usually contoured by crenulation (Fig. 4 G–H, M–N and 5A–B, G–H). In *A. porcus* the crenulation was less apparent (Fig. 5G–H) and was lacking in cuspidate teeth of *A. baracoae* (Fig. 4A–B).

The angle between the central cusp and accessory cusps in *A. baracoae* tended to be obtuse or right angled in rostrally placed teeth and more acute in caudally placed teeth (Fig. 2A, and 4A). In embryos/juveniles of Cuban false chameleons a few caudal teeth with accessory cusps had wide central cusps, which proportionally dominated the accessory cusps (Fig. 2C, E; 3A, C; 4G, M and 5A, G). The central cusp of their more rostrally placed cuspidate teeth was usually narrower and also smaller in comparison to caudal teeth (Fig. 2C, E; 3A, C; 4H, N and 5B, H). Cuban false chameleons had right or acute angles between the central cusp and accessory cusps in more rostrally placed teeth and acute in the caudally placed teeth (Fig. 2C and 3A, C), except in *A. chamaeleonides* where mostly obtuse and rarely acute angles were found between the central cusp and accessory cusps in the caudal multicuspid teeth (Fig. 2E). Embryos/juveniles of *A. barbatus* and *A. porcus* had the most pointed accessory cusps (Fig. 2C; 3C; 4G and 5G). The accessory cusps of the juvenile caudal teeth of *A. barbatus* and also in some caudal teeth of *A. chamaeleonides* were placed high in the tooth crown close to the tip of the central cusp (Fig. 2C and 2E).

In adults, only *A. baracoae* fully forms accessory cusps and in Cuban false chameleons only *A. guamuhaya* and *A. porcus* form small tubercles or collar-like structures, but accessory cusps are not well defined (Fig. 3B, D and 5K). *Anolis chamaeleonides* and *A. barbatus* lack accessory cusps in teeth at all. The size proportions between accessory cusps and the central cusp in adult teeth of *A. baracoae* were different in adults when compared to juvenile teeth. In adults, the central cusp was much bigger compared to accessory cusps (Fig. 4D) and the situation resembled teeth with accessory cusps in embryos/juveniles of Cuban false chameleons where the central cusp was wide, accessory cusps were proportionally smaller and placed high in the tooth crowns (Fig. 2B and 4D).

3.2.3. Molariform teeth in adults

Robust broad molariform teeth were found in all adult Cuban false chameleons in the caudal jaw region (Fig. 2D, F; 3B, D; 4J, P and 5D, J; Table 2). The tooth crowns of these teeth are rounded in most species, with the exception of *A. guamuhaya*. This species developed an intermediate state of teeth between cuspidate and molariform shape (Fig. 3B and 5D). The lingual side of molariform tooth crowns of Cuban false chameleons was contoured by very low ridges running centrifugally from the top of the tooth crown (Fig. 4J, P and 5D, J). The number of molariform teeth in Cuban false chameleons was 3–7 (Table 2). One adult specimen of *A. barbatus* and one specimen of *A. chamaeleonides* had teeth of two functional generations located in several positions. The teeth of the former generation were at different degrees of resorption (Fig. 2F and 6A, C). The tops of the tooth crowns of successive teeth did not reach the tops of resorbing teeth (Fig. 2F and 6A–C) and between the tooth base and bone of attachment was an apparent thin line (Fig. 6C). The SEM observations of adults showed that not fully growed teeth were already ankylosed, but the bone of attachment and the edge of the tooth base were still active in mineralization (Supplement 18, 19). The bone of attachment continued to grow, eventually positioning the top of the tooth to the level of the neighboring teeth. The same mechanism was observed in adults of *A. baracoae* (Supplement 18). We did not observe such ankylosis formation prior to the tooth being positioned correctly in juvenile dentitions. Not all Cuban false chameleon caudal teeth were large. Most adult specimens had a few small teeth right at the back of the jaw (Figs. 2B, D, F and 3B, D; Table 2). The absence of such teeth in some adults was possibly caused by their loss during mechanical preparation.

3.3. The thickness of the dentin depends on the position of the tooth in the jaw and probably also on the food specialization

To understand the likely relationship between dentin organization and function of the teeth the samples were μCT scanned and the hard tissue layers observed. The μCT mesiodistal and transversal sections trhough teeth positioned in rostral, middle and caudal jaw area show Supplement 20 and 21. The μCT mesiodistal and transversal sections through teeth positioned in the rostral, middle, and caudal jaw areas are shown in Supplements 20 and 21.

The major mass of mineralized dental tissues was formed by dentin in all examined species. Dentin was covered by a thin layer of enamel (white) in the crown area (Fig. 7A–F and 8A–D). The width of the dentin gradually decreased from the top of the tooth to the apical end of the tooth base in both embryos/juveniles and adults. Tooth shape did not affect this dentin distribution pattern (Fig. 7A–F and 8A–D; Supplement 20 and 21). The dentin was generally thinner in the rostral teeth compared to the caudal teeth (Fig. 7A–F and 8A–D; Supplement 20 and 21).

In embryos/juveniles within unicuspid teeth was the widest layer of dentin was localized at the tips of unicuspid teeth. In teeth with accessory cusps, the widest layer of dentin was found at the tip of the central cusp with slightly thinner dentin at the tips of the mesial and distal cusps (Fig. 7A, C, E and 8A, C; Supplement 20A1-C2, G1-I2, M1-02). In mesiodistal section, the embryos/juveniles shown a high correlation between the shape of the dental papilla and the shape of the tooth. However, the cuspidate shape of the papilla in cuspidate teeth was less distinctive. The noticeable interspecific differences in the pattern of thickness of dentin and the shapes of the dental papilla of teeth along the lower jaws were not apparent in embryos/juveniles (Fig. 7A, C, E and 8A, C).

The teeth of adult *A. baracoae* possessed a wide dentine layer in the tooth crowns and a much thinner dentine layer in the tooth bases. The shape of the dental papilla resembled the shape of the teeth (Fig. 7B, Supplement 20D1-F2). In contrast to *A. baracoae*, molariform teeth of the other species from the middle and caudal lower jaw regions were characterised by stronger dentine deposition also at the tooth base (Fig. 7D, F and 8B, D; Supplement 20J1-J2, P1-P2 and Supplement 21D1-D2 and J1-J2).

4. Discussion

The morphology of heterodont dentitions and their postnatal changes were investigated in four twig giant species of Cuban false chameleons (*Anolis barbatus*, *A. chamaeleonides*, *A. guamuhaya* and *A. porcus*) and compared with the crown giant Baracoa giant anole (*A. baracoae*), also from Cuba. We discuss how the observed dental characters and postnatal changes in dental morphology might reflect the degree of molluscivory, saurivory, and insectivory in the context of the twig giants and crown giants of the genus *Anolis*. Our focus was on the following characteristics: the number of tooth positions, tooth shape, and differences in dentine deposition.

4.1. Increasing tooth number throughout life may be related to diet

In lizards, the number of teeth within a tooth row can partially reflect their shape, diet, jaw length, and phylogenetic relationships. *Anolis baracoae* represents the crown giant ecomorph of anole, feeding primarily on invertebrates and small vertebrates, such as small lizards or birds. This species exhibits a significant increase in the number of tooth positions throughout its life. A similar increase in tooth positions was observed only in *A. guamuhaya* among the twig giants. Fully grown adults of this species have been described as having the longest jaws among the Cuban false chameleons assessed here, measured from the back of the retroarticular process to the tip of the lower jaw (Holanova Zahradnickova et al., 2017). However, the length of the jaws and tooth row in the examined specimens was nearly the same as in adult *A. barbatus* and *A. chamaeleonides*. We hypothesize that the significant increase in tooth positions in *A. guamuhaya* resembles the pattern found in *A. baracoae*, suggesting that this species might also feed on a higher percentage of arthropods and small vertebrates, similar to *A. baracoae*. In contrast, the other species of Cuban false chameleons (*A. barbatus*, *A. chamaeleonides* and *A. porcus*) showed no or minimal increase in the number of tooth positions throughout life, which is likely indicative of a molluscivorous diet. *A. porcus* has a shorter lower jaw and tooth row compared to all other examined Cuban false chameleons, making it more comparable to the adult *A. baracoae*. However, unlike *A. porcus*, *A. baracoae* exhibits a significantly increased number of tooth positions.

4.2. The shape of teeth as a predictor of diet

Tooth shape reflects the function of the teeth, diet, and the phylogenetic background of the species. Rostral teeth are used for prey capture, while more caudally placed teeth are utilized for further food processing. The evolution of tooth structures, such as enamel crests, cusps, and crenulations, plays an important role in diet specialization in lizards (discussed in Melstrom, 2017).

4.2.1. Unicuspid teeth, their structure and possible relation to diet

Surprisingly, the unicuspid teeth of juvenile Cuban false chameleons exhibit mixed characteristics, suggesting that in addition to their usual diet of arthropods, these anoles might also feed on hard-shelled mollusks. One notable feature found in *A. chamaeleonides* and, to a lesser extent, in *A. porcus* is the expanded lingual side of the tooth base. This expansion may enhance the robustness of the unicuspid teeth and potentially reduce their susceptibility to breakage. Additionally, juvenile unicuspid teeth of *A. chamaeleonides* have well-developed crenulations. In the durophagous Varanus niloticus, it has been suggested that crenulations help prevent the slipping of hard shells and may disperse pressure across the tooth crown during crushing (Rieppel and Labhardt, 1979).

Conversely, the presence of both labial and lingual enamel crests, which divide the tooth tip of unicuspid teeth, is often associated with insectivorous lizards (e.g., Delgado et al., 2003; Zahradnicek et al., 2012). However, this is not an exclusive characteristic, as there is substantial overlap in tooth complexity across dietary groups in saurians, and insectivorous lizards may have dental complexities similar to those of omnivores (Melstrom, 2017). Labial and lingual crests were present in all examined embryos and juveniles, except for the most rostral teeth of *A. barbatus*, which had only a labial enamel crest. The presence of both crests could indicate that juveniles predominantly feed on small arthropods, as is common among juvenile anoles (Losos, 2009). The absence of the lingual enamel crest in the most rostral teeth of the juvenile dentition of *A. barbatus* could be an adaptation for molluscivory. The lack of the lingual enamel crest is also typical of the unicuspid teeth in adult Cuban false chameleons, except for *A. guamuhaya*, which has tooth tips divided into labial and lingual enamel crests similar to those of *A. baracoae*. This suggests that adults of *A. guamuhaya* may also consume a significant amount of arthropods.

Our results highlight that the labial enamel crest may be a more evolutionarily stable feature within the genus *Anolis* compared to the lingual crest. Another indicator of feeding on hard-shelled mollusks is the slightly rounded tooth tops in adult Cuban false chameleons (excluding *A. guamuhaya*), which are likely less prone to mechanical damage when interacting with snail shells (Herrel and Holanova, 2008).

4.2.2. Tricuspid/bicuspid teeth and possible relation to diet

The presence of tricuspid and bicuspid teeth with mesial and distal accessory cusps is commonly found in lizard species that feed on arthropods (e.g., Montanucci, 1968; Christensen and Melstrom, 2021) and in larger *Anolis* lizards that also consume smaller vertebrates such as fish, frogs, lizards, and birds (De Armas and Iturriaga, 2019; Fong and Blanco Ojeda, 2002; Schettino, 1999, 2003). Bicuspid teeth were present only in small numbers within the middle area of the juvenile lower jaw and often alternated with unicuspid and/or tricuspid teeth. This indicates the presence of zones within the jaw where different tooth types can appear simultaneously.

We hypothesized that the organization, form, and position of accessory cusps in the juvenile dentition of Cuban false chameleons, as well as in both age cohorts of *A. baracoae*, reflect their diet. Tricuspid teeth in the juvenile dentition of *A. baracoae* seem well adapted for feeding on small arthropods. These teeth have the most protruding central cusp above the accessory cusps, facilitating the penetration of the cuticle of arthropods. However, the accessory cusps were proportionally larger compared to the central cusp, and the angle between the central cusp and the accessory cusps was mostly acute (though in more rostrally placed teeth, it could be obtuse or right-angled). In contrast, the tricuspid teeth of adult A. baracoae typically show a large central cusp that proportionally dominates the accessory cusps. We hypothesize that such teeth with a dominant central cusp are well adapted to a broader spectrum of food, including both arthropods and small vertebrates. Consistent with this, *A. baracoae* has been observed feeding on both snails and hummingbirds (Schettino, 1999).

The tricuspid teeth of juvenile Cuban false chameleons usually feature wide, large central cusps that are taller than the accessory cusps, resembling the tricuspid teeth of adult *A. baracoae*. These teeth in juveniles are likely less prone to breakage and suitable for processing both arthropods and snails. The lingual side of the central cusp in Cuban false chameleons is ornamented with crenulations, which again suggests a diet including mollusks. In more rostrally placed tricuspid teeth, the central cusp is typically narrower and reminiscent of the juvenile caudal tricuspid teeth of *A. baracoae*. Dietary adaptations are more evident in the most caudal tricuspid teeth. This aligns with the general pattern in lizards, where bite force is typically strongest at the back of the jaws compared to the rostral part (Anderson et al., 2007).

The more rostrally placed teeth with accessory cusps show a pattern similar to the tricuspid teeth observed in juvenile *A. baracoae*. The angle between the accessory cusps and the central cusp in juvenile Cuban false chameleons was acute in the caudal tricuspid teeth (and either right or acute between the central cusp and accessory cusps in more rostrally placed teeth), except in *A. chamaeleonides*, where the angles were mostly obtuse and rarely acute. The most pointed accessory cusps were observed in the juvenile dentition of *A. barbatus* and *A. porcus*. We propose that the obtuse angle in *A. chamaeleonides* might correlate with molluscivory, while the pointed accessory cusps of *A. barbatus* and *A. porcus* suggest a diet focused on arthropods. The relatively high positioning of the accessory cusps in the tooth crowns of *A. barbatus* and *A. porcus* may aid in cracking mollusk shells and minimize the potential for shells to slip from the teeth. Thus, the juveniles of the Cuban false chameleon species examined utilize different adaptations in their tricuspid teeth to crush snail shells. Overall, juvenile Cuban false chameleons exhibit a developmental pattern in their tricuspid teeth that is consistent with what is observed in adult anoles.

4.2.3. Molariform teeth of Cuban false chameleons and relation to diet

Molariform teeth, adapted for durophagy, have evolved multiple times throughout reptilian adaptive radiation and evolution. These teeth are typically large and broad with rounded tops, and often feature low enamel ridges on the sides of the tooth crowns (crenulation). Molariform teeth have been documented in various extinct (Estes, 1961; Estes, 1983; Estes and Williams, 1984; Bardet et al., 2005) and extant (Lönnberg, 1903; Mertens, 1942; Dalrymple, 1979; Rieppel and Labhardt, 1979; Estes and Williams, 1984; Pregill, 1984; D’Amore, 2015) reptilian species.

In Cuban false chameleons, molariform teeth were first described by Edmund (1969) in *A. chamaeleonides*. The odontogenic shift from tricuspid teeth to molariform (bulbous or rounded) teeth was noted by Estes and Williams (1984) in *A. porcus* and *A. chamaeleonides*. In our study, we found that all adult Cuban false chameleons examined, except *A. guamuhaya*, developed broad molariform teeth instead of tricuspid teeth. In *A. guamuhaya*, the tricuspid teeth exhibited a shape intermediate between tricuspid and broad rounded teeth, with no clear size-based distinction between the caudal teeth and more rostral adjacent teeth. In contrast, the broad molariform teeth of *A. barbatus*, *A. chamaeleonides* and *A. porcus* were distinct from neighboring teeth due to their larger size and rounded shape. The lingual side of the tooth crowns of molariform teeth in these species was ornamented with crenulations. Previously, weak striation or crenulation in molariform teeth was observed in *A. porcus* and *A. chamaeleonides* (Estes and Williams, 1984).

Based on our results, we hypothesize that the presence of broad molariform teeth along with crenulation indicates an adaptation to molluscivory in the adults of *A. barbatus*, *A. chamaeleonides* and *A. porcus*. It appears likely that *A. guamuhaya* also consumes a significant percentage of arthropods and lizards.

4.3. Dentine thickness and possible relation to diet

In all examined specimens, the thickness of dentine varies depending on tooth position within the jaw, age cohort, and likely diet specialization. Thinner dentine was observed in the smaller rostral teeth used for prey capture, while thicker dentine was found in the larger, more complex caudal teeth used for prey processing (Fig. 7, 8). The maximum dentine thickness in juvenile teeth was located at the tip of unicuspid teeth and at the tips of the central cusps of bicuspid and tricuspid teeth. This thickened area, which makes first contact with food, helps protect the teeth from breakage.

Adults of *A. baracoae* possessed a wider dentine layer within the tooth crowns and a thinner layer at the tooth bases. The arrangement of the dentine layer in the rostral unicuspid teeth of Cuban false chameleons was similar to that of *A. baracoae*, with the exception of *A. porcus*, which had a wider dentine layer in its rostral teeth. In the middle and caudal segments of the jaw across all species of Cuban false chameleons, the dentine layer was thick in both the crown and base areas. This robust dentine deposition likely correlates with the requirements for crushing snail shells. Whether the extensive dentine deposition observed in adults of Cuban false chameleons occurs after ankylosis, as described in adult chameleons where dentine nearly obliterates the dental papilla (Dosedělová et al., 2016), or if it results from changes in the developmental program of mineralization during tooth germ development was not evident from the examined material using the methods employed.

5. Conclusion

Overall, our study has shown that not all Cuban false chameleons exhibit the same dental patterns with respect to tooth shape changes over time, highlighting potential variations in diet among the species. Some juveniles displayed intermediate characteristics, suggesting a transition from insectivory-saurivory to molluscivory. In addition to larger, more robust teeth, the shift to molluscivory was associated with a proportional decrease in the size of accessory cusps, the formation of rounded tooth crowns, and the development of crenulations that increase the tooth's surface area. Three of the four Cuban false chameleons studied exhibited no or minimal increase in tooth number from late fetal/hatchling stages to adulthood, suggesting that changes in tooth shape and proportions are more significant for molluscivory than changes in tooth number. Additionally, larger teeth adapted for molluscivory may occupy more space in the jaw, limiting the space available for the development of new tooth positions. Consistent with this, the species showing the greatest increase in tooth number over time had fewer molariform-shaped caudal teeth as adults. As in other species, there appears to be a trade-off between tooth morphology and tooth number, as discussed by Catón and Tucker (2009).

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Author contributions

V. Zahradnickova was responsible for conceptualization, provision of materials, methodology, investigation, data collection, and writing the original draft. M. Hovorakova and A. S. Tucker contributed to writing the original draft and reviewing and editing. M. Bartos handled the micro-CT portion of the methodology. I. Rehak contributed to reviewing and editing. O. Zahradnicek supervised the research project, validated the results and methodology, conducted formal analysis, provided visualization, and wrote the original draft. All authors reviewed previous versions of the manuscript and approved the final version.

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Figure legends

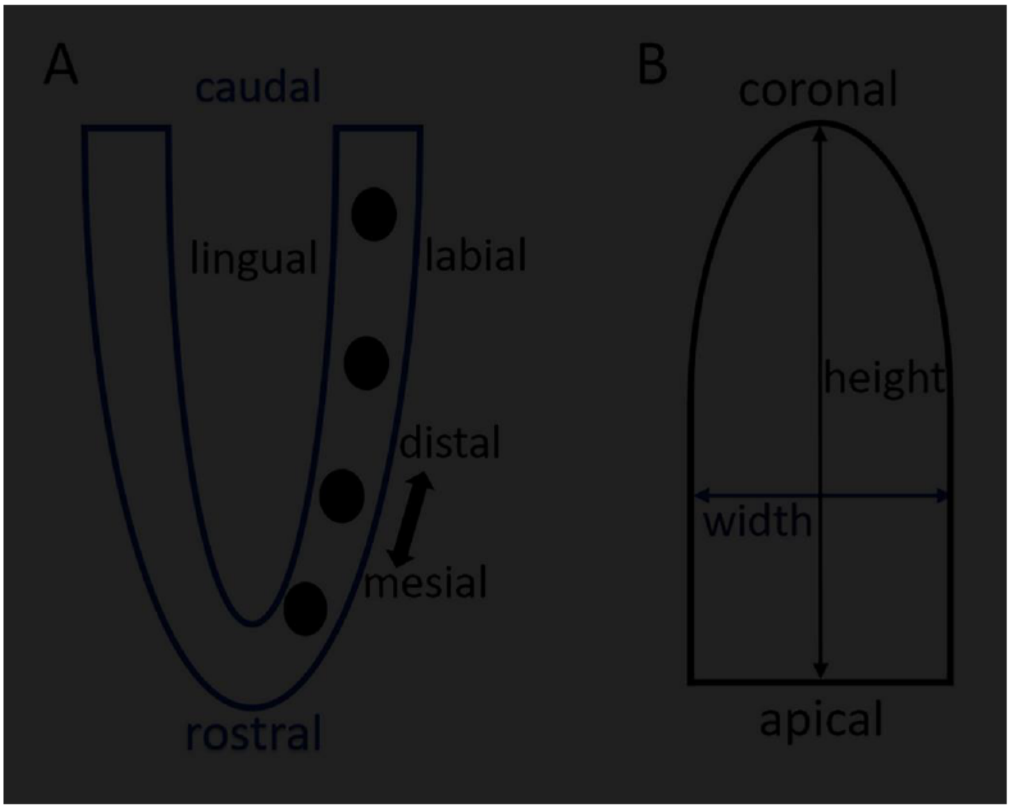

Fig 1: Diagrams depicting specimen orientation: A) shows the axes in the context of the lower jaws and teeth, with teeth marked by black ellipsoids. B) illustrates the apical and coronal axes in relation to the tooth.

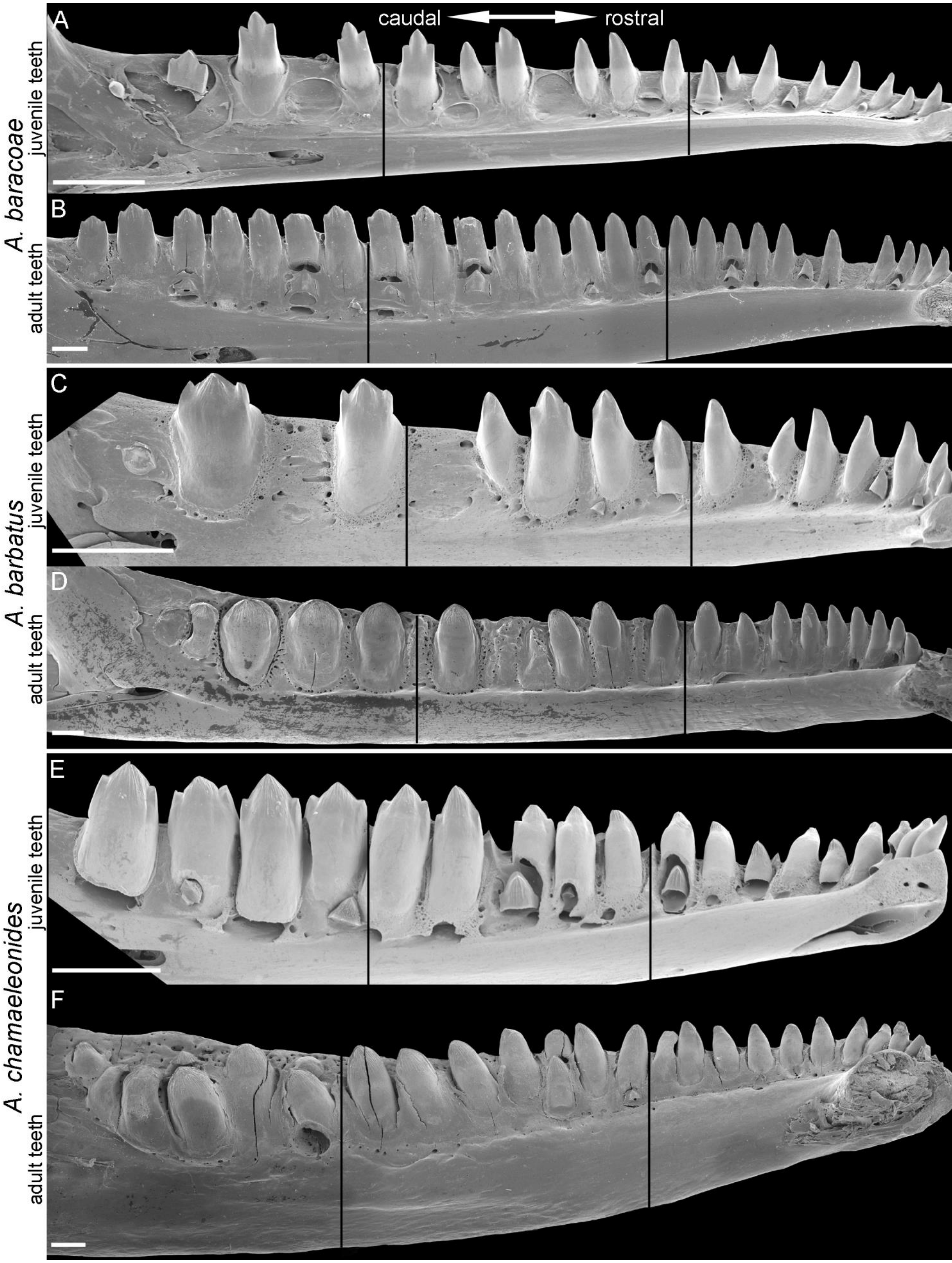
A
caudal
rostral
B
C
D
E
F
A. baracoae
juvenile teeth
adult teeth
A. barbatus
juvenile teeth
adult teeth
A. chamaeleonides
juvenile teeth
adult teeth

Fig 2: SEMs of the juvenile and adult dentitions of *A. baracoae, A. barbatus* and *A. chamaeleonides*. Images (A, C, E) show the morphology of the juvenile dentition, while (B, D, F) show the adult dentition. In the presented specimens, rostral juvenile and adult unicuspid teeth are conical. In juvenile *A. chamaeleonides*, the lingual side of the unicuspid teeth is expanded (E). Bicuspid and tricuspid teeth are found in the middle and caudal segments of the juvenile lower jaw (A, C, E). Adult *A. baracoae* is the only species with well-formed accessory cusps (B), while adults of *A. barbatus* and *A. chamaeleonides* do not have teeth with accessory cusps (D, F). Black vertical lines separate the lower jaw into rostral, middle, and caudal segments. The abbreviation "bt" denotes a bicuspid tooth in adult *A. baracoae*. In the juvenile dentition of *A. baracoae*, "aa" between the central and accessory cusps marks an acute angle, while "oa" indicates an obtuse angle. The scale bar is 1 mm.

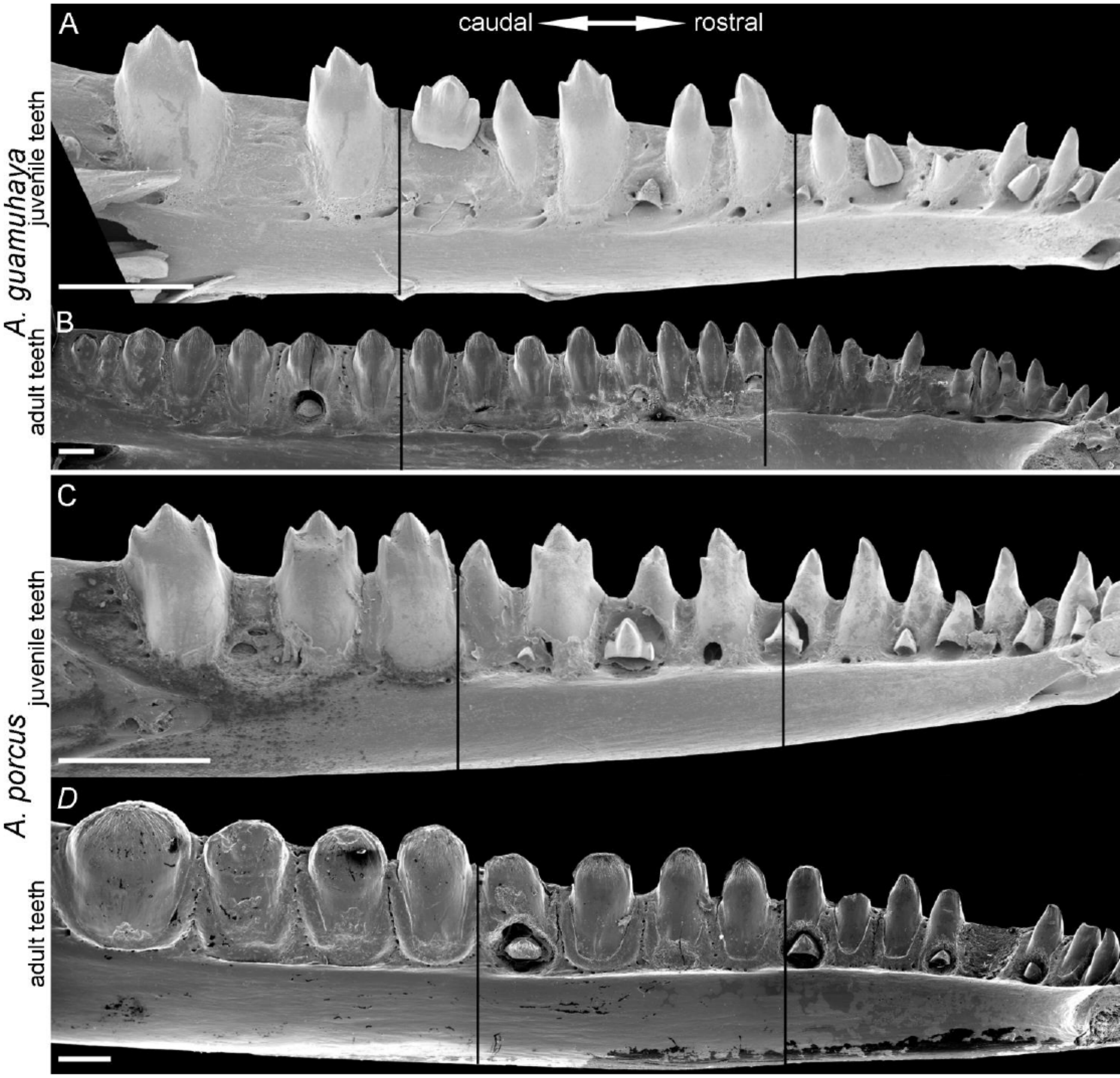

Fig 3: SEMs of the juvenile and adult dentitions of *A. guamuhaya* and *A. porcus*.

Images (A, C) show the morphology of the juvenile dentition, while (B, D) show the adult dentition. Juvenile unicuspid teeth of *A. guamuhaya* are nearly conical (A), whereas the most rostrally placed juvenile teeth of *A. porcus* are slightly expanded on the lingual side (C). The rostral teeth of adults have a conical-like shape (B, D). In the juvenile dentitions, teeth with accessory cusps are located in the middle and caudal segments (A, C). Adults of both species do not have teeth with well-defined accessory cusps (B, D). Black vertical lines separate the lower jaw into rostral, middle, and caudal segments. The scale bar is 1 mm.

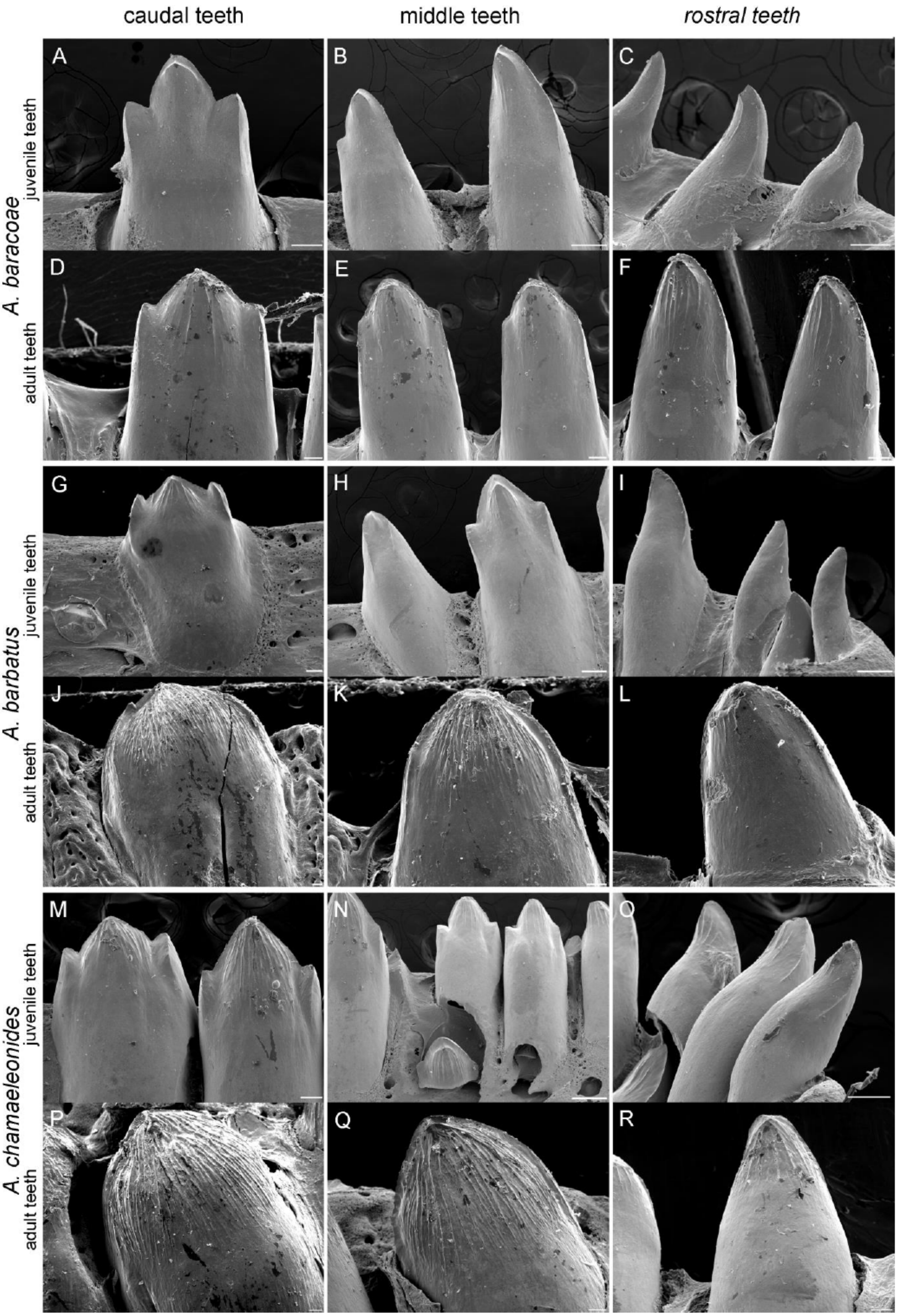
caudal teeth
middle teeth
rostral teeth
A. baracoae
juvenile teeth
adult teeth
A. barbatus
juvenile teeth
adult teeth
A. chamaeleonides
juvenile teeth
adult teeth
A
B
C
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Fig 4: Details of juvenile and adult teeth of *A. baracoae*, *A. barbatus* and *A. chamaeleonides*. Juvenile teeth of *A. baracoae* had tooth tips divided by labial and lingual enamel crests (A–B). The lingual side of the teeth was smooth or had only subtle crenulations (B–C). Juvenile unicuspid teeth of *A. barbatus* had well-formed labial enamel crests at the tooth tips (H–I), while their lingual sides were smooth (I). In this species, both labial and lingual enamel crests were present on teeth with accessory cusps (G–H), and the lingual side of the tooth crown was ornamented with subtle crenulations (G–H). Juvenile teeth of *A. chamaeleonides* had robustly formed crenulations on the lingual side (M, O). A well-defined lingual enamel crest was absent at the tooth tips, while the labial enamel crest was present (M–O). In adults, the shape of unicuspid teeth was conical-like (F, L, R), except for the unicuspid teeth in the middle jaw segment of *A. baracoae*, which were expanded mesially and distally, taking the form of partial accessory cusps (E). The caudal teeth in adult *A. baracoae* were tricuspid (D), while in *A. barbatus* and *A. chamaeleonides*, the teeth were molariform with rounded crowns (J, P). Lingual crenulations were weakly formed in adult *A. baracoae* (D) and robustly formed in caudal molariform teeth in *A. barbatus* and *A. chamaeleonides* (J, P). The scale bar size is 100 µm.

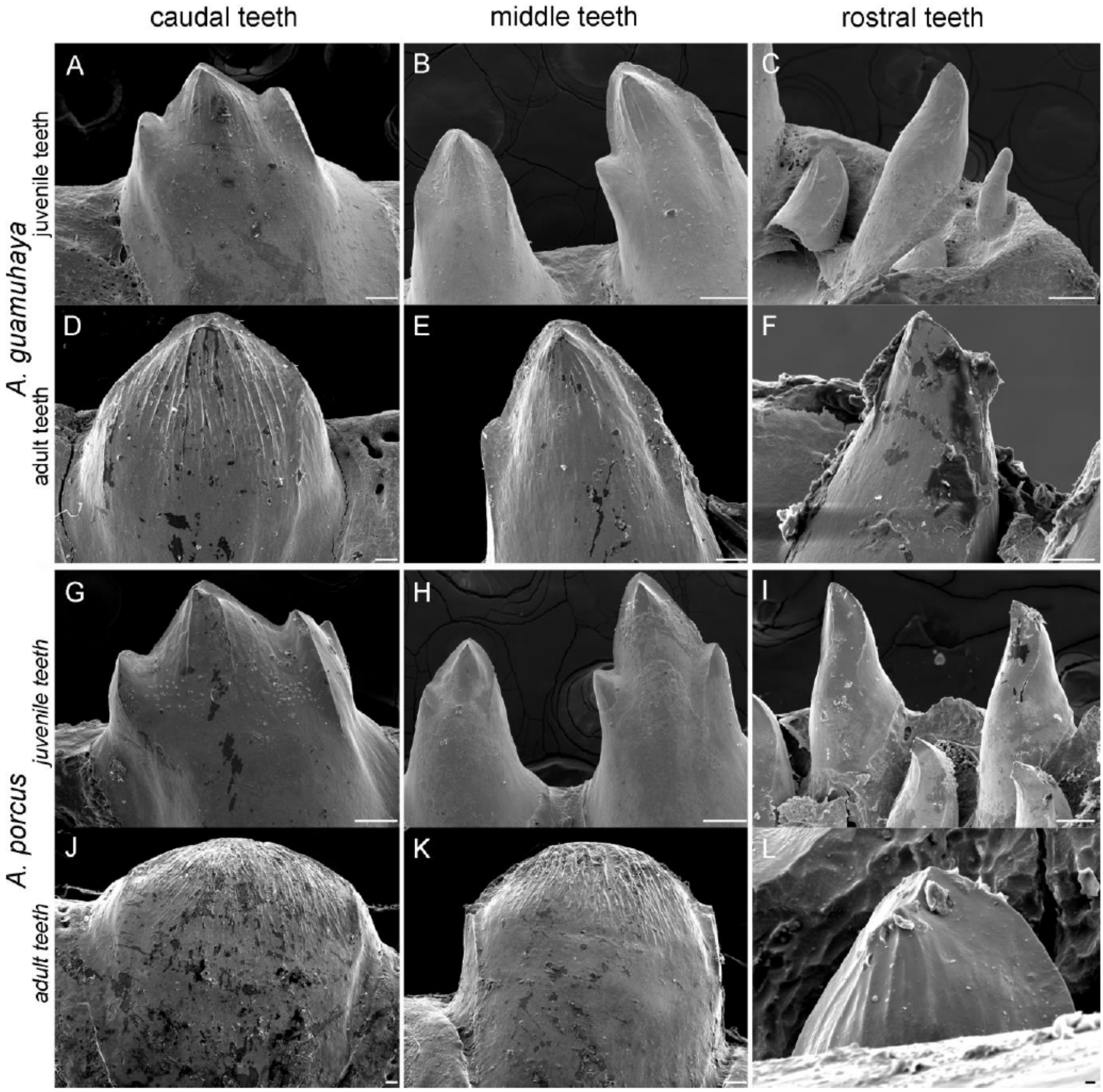


Fig 5: Details of juvenile and adult teeth of *A. guamuhaya* and *A. porcus*.

The tips of the juvenile teeth of *A. guamuhaya* had well-formed labial enamel crests, while the lingual crests were either not apparent or only weakly formed (A–C). Crenulation was not evident in the rostral teeth (C), but it was pronounced in the caudal teeth (A). The rostral unicuspid juvenile teeth of *A. porcus* possessed only labial enamel crests (I), while the more caudally located unicuspid teeth, including those with accessory cusps, also had lingual enamel crests, though these were less distinct than the labial crests (G–H). Crenulation in this species was weakly formed in the caudal teeth and absent in the more rostral teeth (G–I). Adult *A. guamuhaya* had unicuspid teeth with distinct labial enamel crests (F). Lingual crenulations were not observed in the rostral teeth (F) but were well-formed in the caudal teeth (D). Teeth from the middle segment showed an intermediate state of crenulation (E). Adult unicuspid rostral teeth of *A. porcus* had well-formed labial enamel crests (L). The molariform teeth were massive with rounded crowns (J–K). Crenulation was evident not only in the molariform teeth (J–L) but also in the rostral unicuspid teeth (K). The scale bar size is 100 μm.

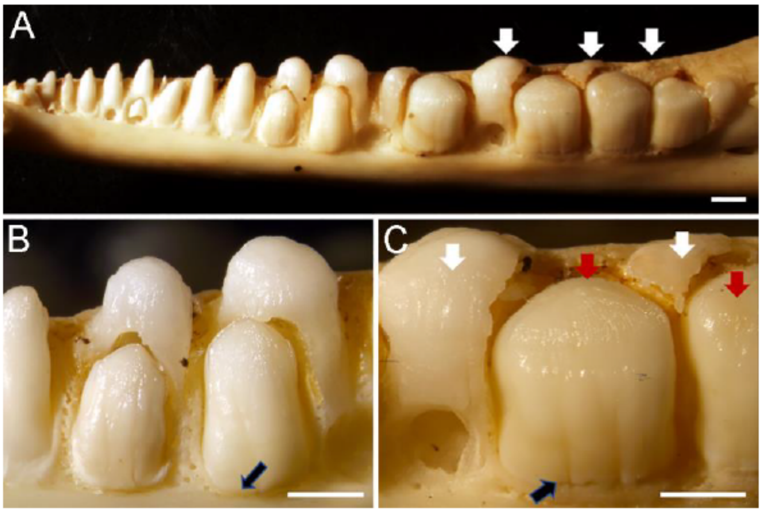


Fig 6: Dentition of the lower jaw of adult *A. barbatus*. A) The whole lower jaw of an adult A. barbatus. The functional teeth from two generations are present in the caudal part of the jaw. Teeth of the former generation (white arrows) are resorbed to varying degrees, while the teeth of the successive generation (red arrows) have not yet reached the tops of their predecessors, despite being already ankylosed (A-C). The tooth base-bone of attachment interface indicated by black arrows shows that the teeth are still growing. The scale bar is 1 mm.

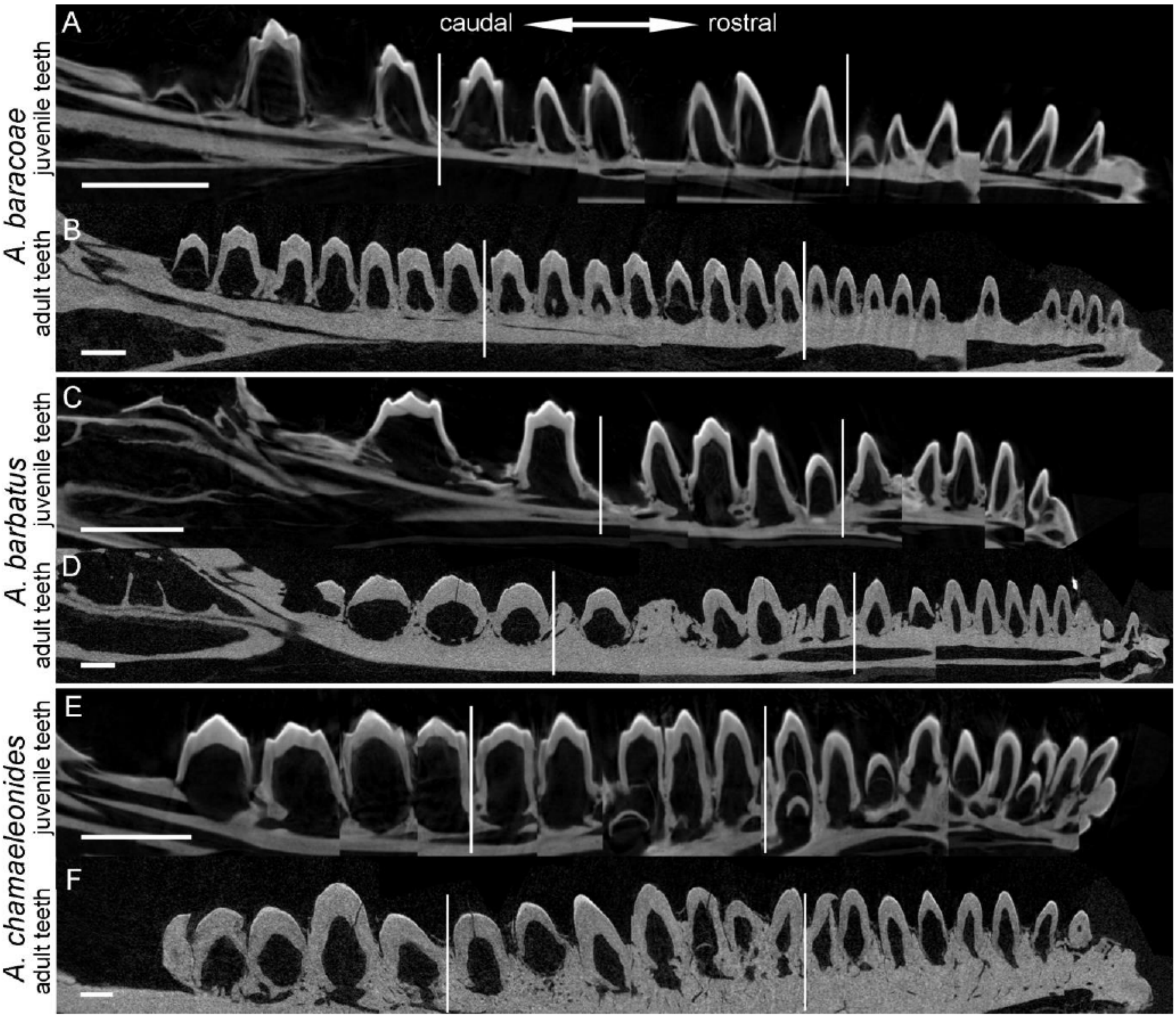


Fig 7: Mesiodistal longitudinal cross-sections through the juvenile and adult dentition of *A. baracoae*, *A. barbatus*, and *A. chamaeleonides* are shown. Panels (A–F) illustrate the thickness of dentine and enamel in the juvenile and adult teeth along the lower jaw. Dentine constitutes the majority of the tooth mass, with only a thin layer of enamel covering the tooth crown in both juvenile and adult teeth of all species shown. The dentine layer is thickest at the tips of unicuspid teeth and at the cusp zones of bicuspid and tricuspid juvenile teeth. In *A. baracoae*, this thick dentine layer is also present in the adult teeth (A–C, E). In the molariform adult teeth of *A. barbatus* and *A. chamaeleonides*, the dentine layer is very thick, comprising the majority of the tooth crown (D–F). The dentine layer at the base of the adult teeth of these two species is thinner compared to the crown region, but still thicker than in the caudal tricuspid teeth of adult *A. baracoae* (B, D, F). The scale bar size is 1 mm.

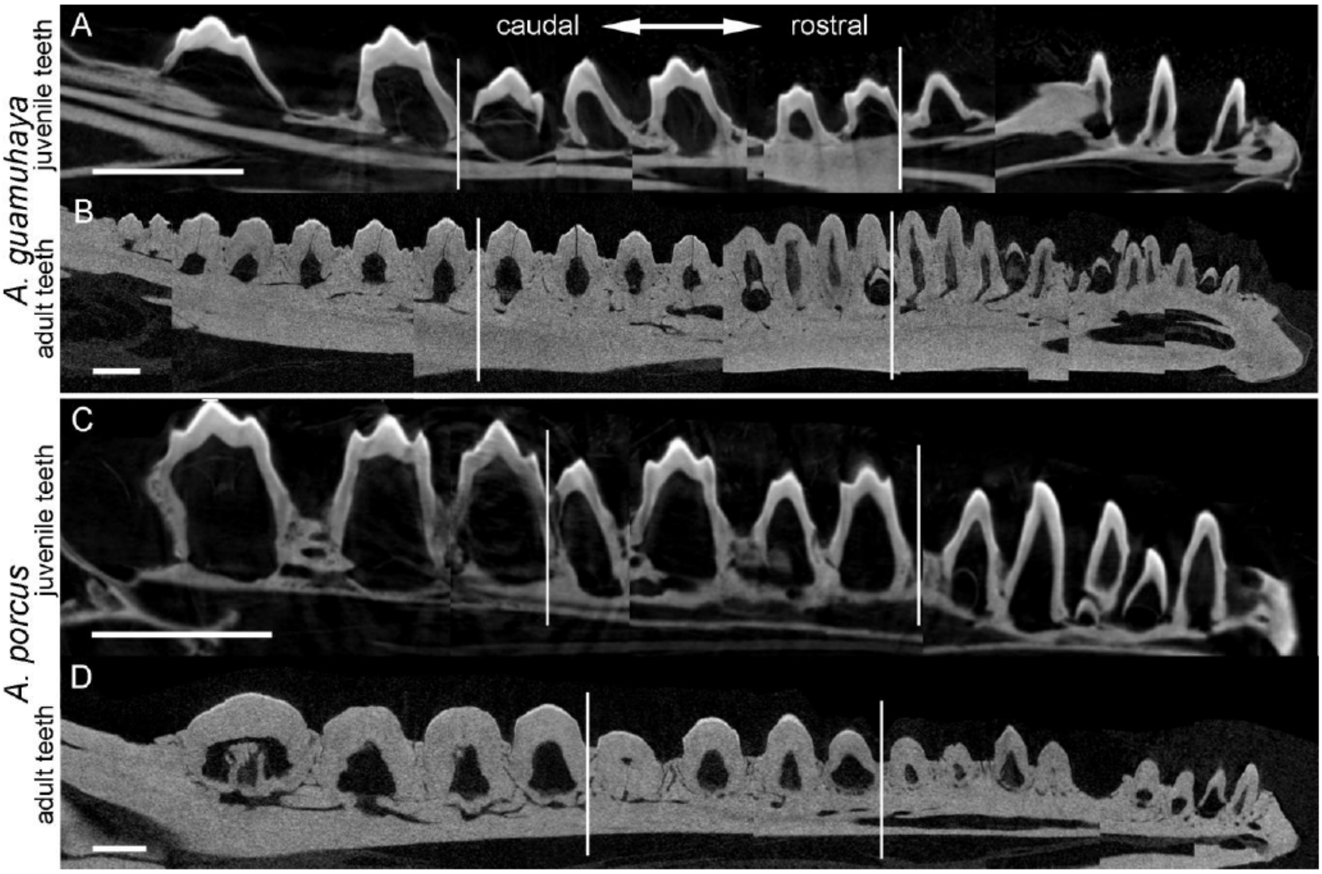


Fig 8: Mesiodistal longitudinal cross-sections through the juvenile and adult dentitions of *A. guamuhaya* and *A. porcus*. Panels (A–D) show the thickness of dentine and enamel in juvenile and adult teeth along the lower jaw. The dentine layer is thickest at the tips of unicuspid teeth and at the cusps of bicuspid and tricuspid juvenile teeth (A, C). In adults, the thickest dentine layer is observed in the crown area (B, D). The tooth base also shows a thick dentine layer in both unicuspid and molariform teeth (B, D). In *A. guamuhaya*, the dental papilla is extensively filled with dentine in both unicuspid and molariform teeth. The scale bar size is 1 mm.

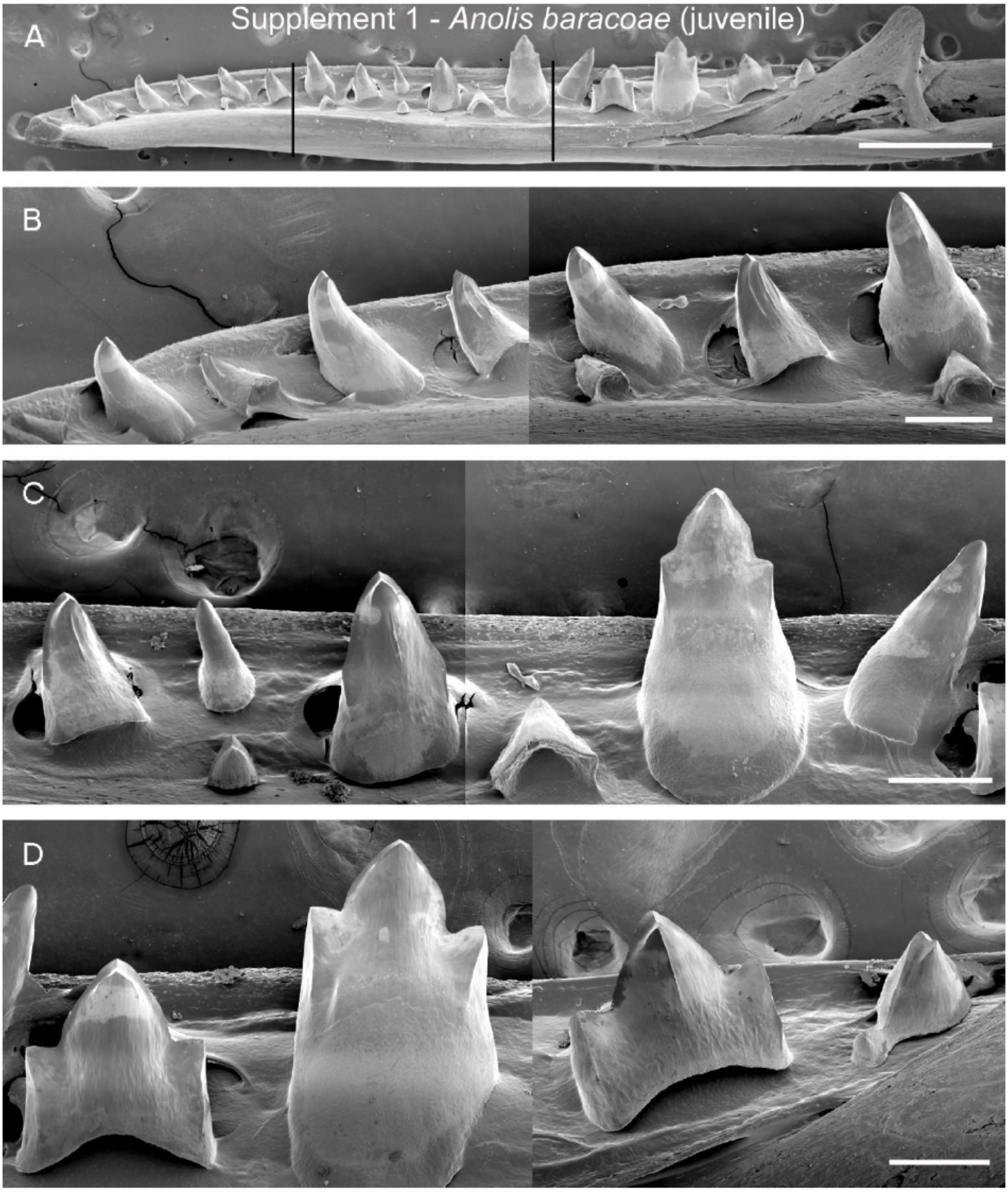
Tab1: Number of tooth positions, ankylosed teeth and dynamics of tooth replacement determined by signs of tooth resorption and the presence of successive generations of teeth.

Species	*A. baracoae*		*A. barbatus*		*A. chamaeleonides*		*A. guamuhaya*		*A. porcus*	
Age cohorts	juveniles	adults	juveniles	adults	juveniles	adults	juveniles	adults	juveniles	Adults
Length of the lower jaw in mm	14.5(7.6)	37(42)	13(18.6)	50(50)	14.3(15.6)	48(52)	11.4(13)	54(45)	10.5(11.8)	44(44.5)
Length of the tooth row in mm	8.5(6)	23,5(25.5)	7.3(10.7)	27(29)	8(9.3)	27(29)	8.3(8)	30(27.5)	7(7)	22(22.5)
Number of tooth positions	22(18)	29(31)	18(19)	20(19)	18 (20)	23(21)	20(18)	29(25)	17(16?)	18(17)
Number of ankylosed teeth	14(8)	27(30)	12 (15)	17(20)	15(18)	25(23)	13(15)	26(24)	14 (12?)	17(16)
Number of teeth with signs of resorption	0(0)	8(9)	0(8)	5(9)	10(11)	7(10)	0(0)	9(8)	6(6+?)	6(4)
Number of teeth of successive generation	7(13)	9(9)	8(10)	4(9)	13(9)	4(12)	8(4)	8(8)	11(8)	4(5)
Number of rudimentary teeth	0(1)	0(0)	0(0)	0(0)	1(0)	0(0)	2(0)	0(0)	1(0)	0(0)

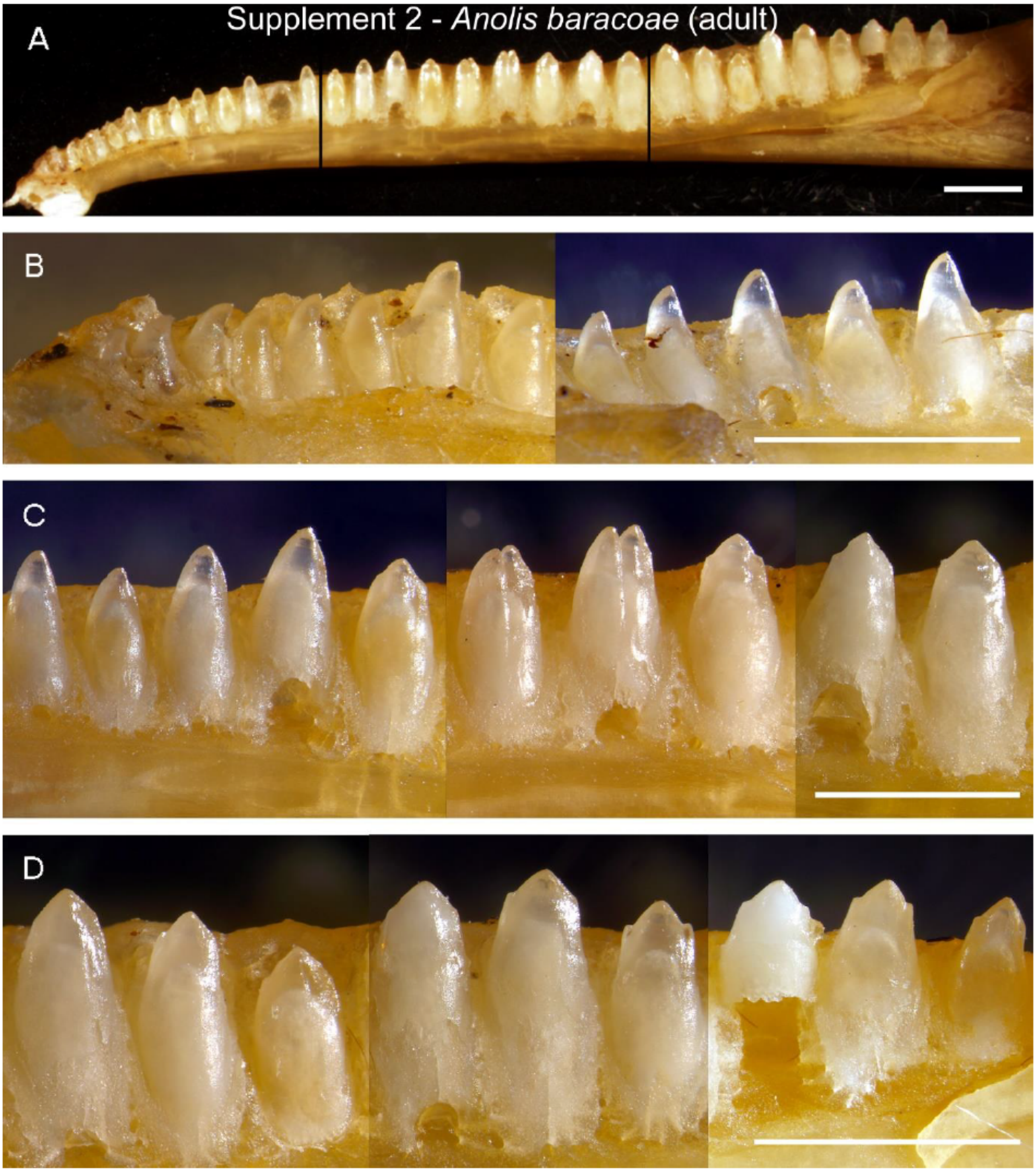
Table 1: Length of the tooth row, number of tooth positions, ankylosed teeth and dynamics of tooth replacement determined by tooth resorption and the presence of successive generations of teeth. The number of ankylosed teeth in resorption and the number of mineralizing successive teeth highlighted the dynamics of tooth replacement and provided an estimate of age at the time of death. Numbers were counted for embryos/juveniles (juv) and adults (adu). The first number before the brackets is related to the specimens that are shown in the figures here. The second number within brackets is related to specimens shown in supplementary material, with the exception of the adult *A. barbatus*, which is shown also here in figure 6.

Tab. 2: Number of teeth defined by their morphological characteristics										
Species	*A. baracoae*		*A. barbatus*		*A. chamaeleonides*		*A. guamuhaya*		*A. porcus*	
Age cohorts	juveniles	adults	juveniles	adults	juveniles	adults	juveniles	adults	juveniles	Adults
Number of unicuspid teeth	10(6)	16(22)	9(6)	7(7)	8(12)	13(13)	9(9)	16(4)	9(7?)	9?(9)
Number of bicuspid teeth	1(0)	2(6)	0(5)	0(0)	2(2)	0(0)	1(2)	0(9)	0(0)	0(0)
Number of tricuspid teeth	3(2)	8(3)	3(4)	0(0)	4(5)	0(0)	3(4)	0(2)	5(4)	0(1)
Number of teeth with shape intermediate between unicuspid and molariform	0(0)	0(0)	0(0)	5?(5)	0(0)	6(2)	0(0)	1(0)	0(0)	4(3)
Number of molariform teeth	0(0)	0(0)	0(0)	4(7)	0(0)	5(5)	0(0)	7(6)	0(0)	4(3)
Number of small teeth at back of tooth row	0(0)	1(0)	0(0)	1(1)	0(0)	1(1)	0(0)	2(3)	0(0)	0(0)

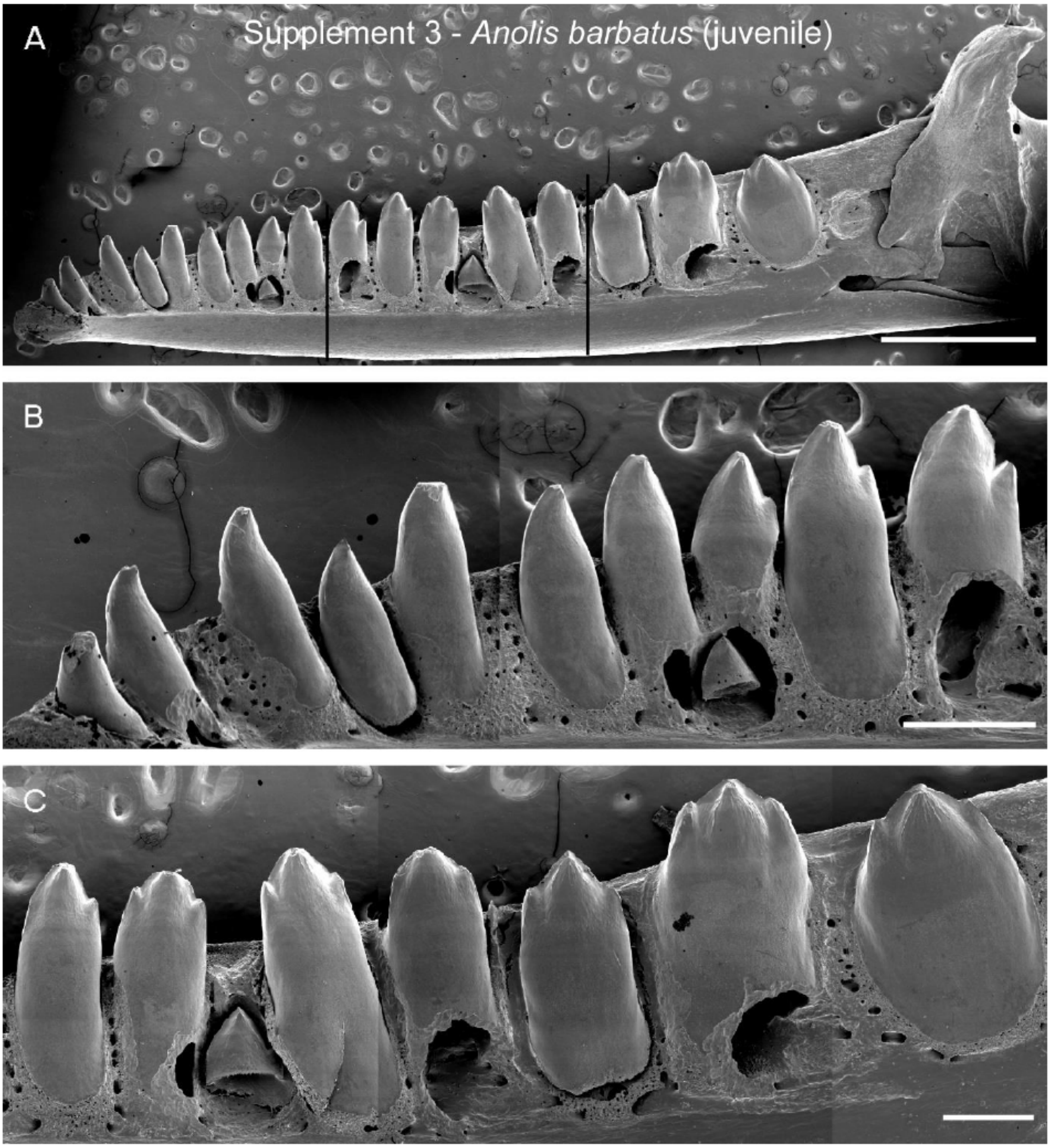
Table 2: Number of teeth defined by their morphological characteristics. The table shows the numbers of teeth defined by different morphological characteristics. Only ankylosed teeth were counted. Counting was carried out for embryos/juveniles (juv) and adults (adu). The first number before the brackets is related to the specimens that are shown in the figures here. The second number within brackets is related to specimens shown in supplementary material, with the exception of the adult *A. barbatus*, which is shown also here in figure 6.



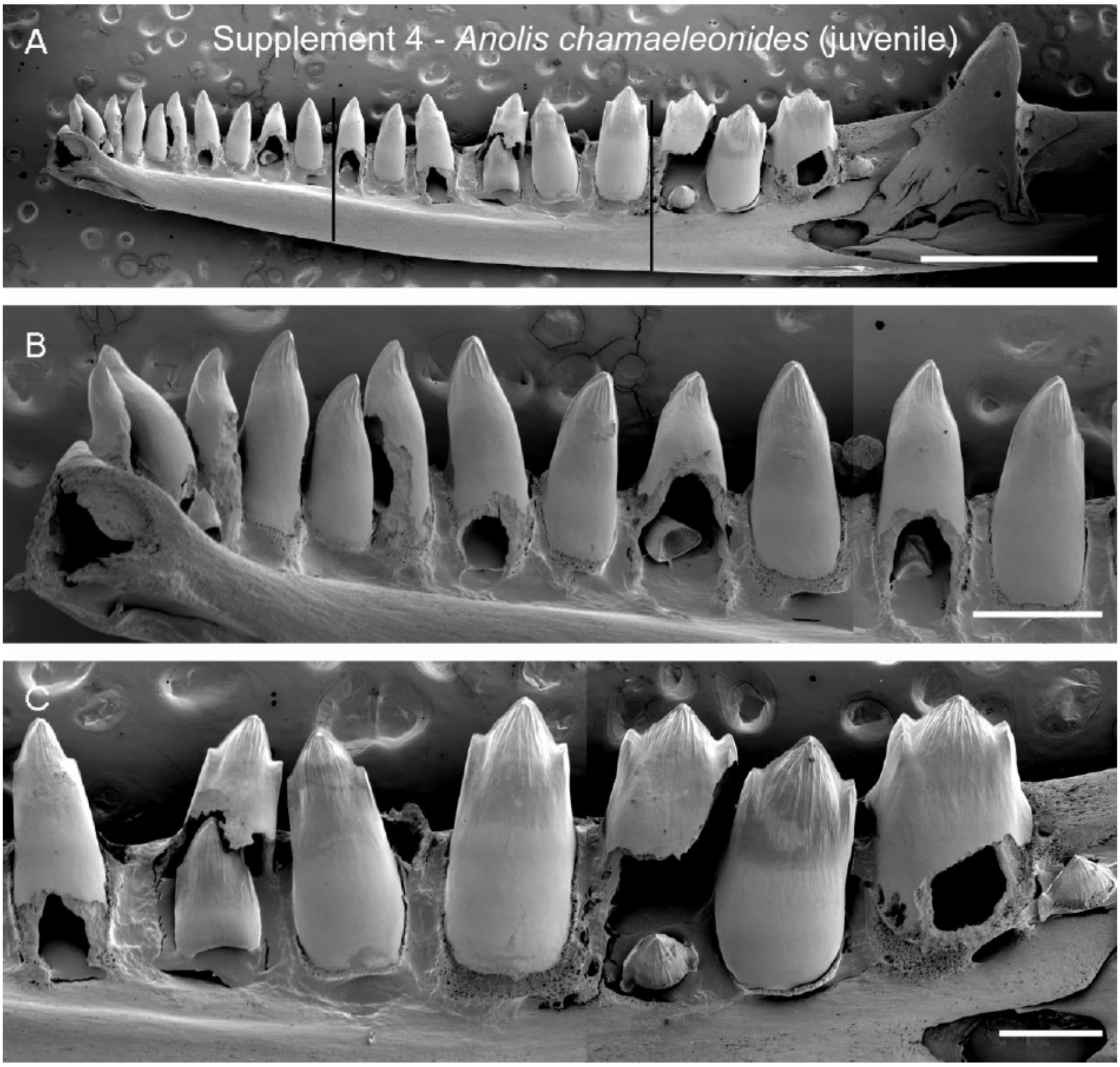
Supplement 1: SEMs of the juvenile dentition of *A. baracoae*. Image (A) shows the lower jaw from the lingual side with a scale bar of 1 mm. The black vertical lines divide the lower jaw into rostral, middle, and caudal segments. Images (B, D) provide an enlarged view of the dentition with scale bars of 0.2 mm.



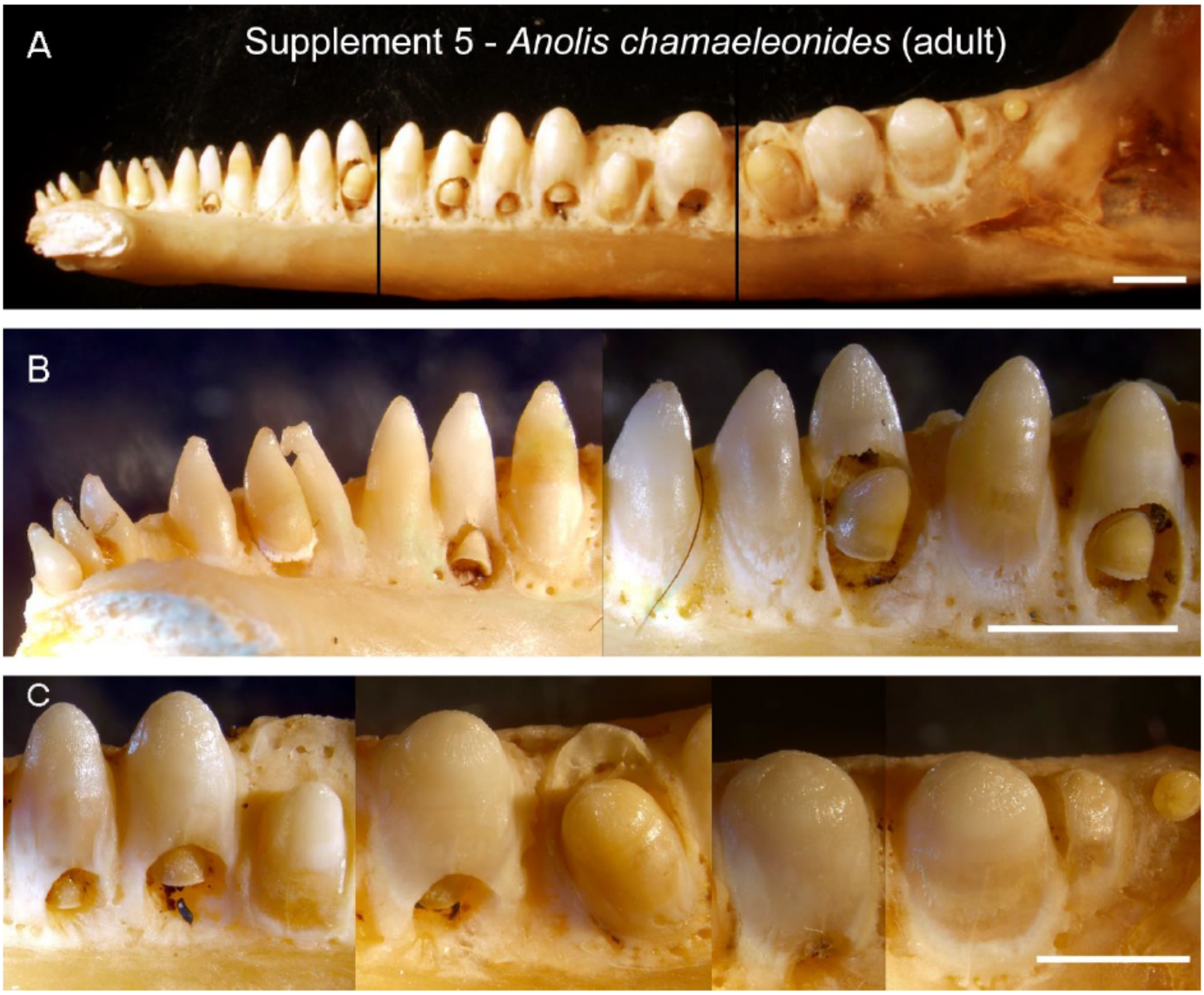
Supplement 2: Light microscope photograph of the adult dentition of *A. baracoae*. Image (A) shows the lower jaw from the lingual side. The black vertical lines divide the lower jaw into rostral, middle, and caudal segments. Images (B, D) provide an enlarged view of the dentition. The scale bars are 2 mm.



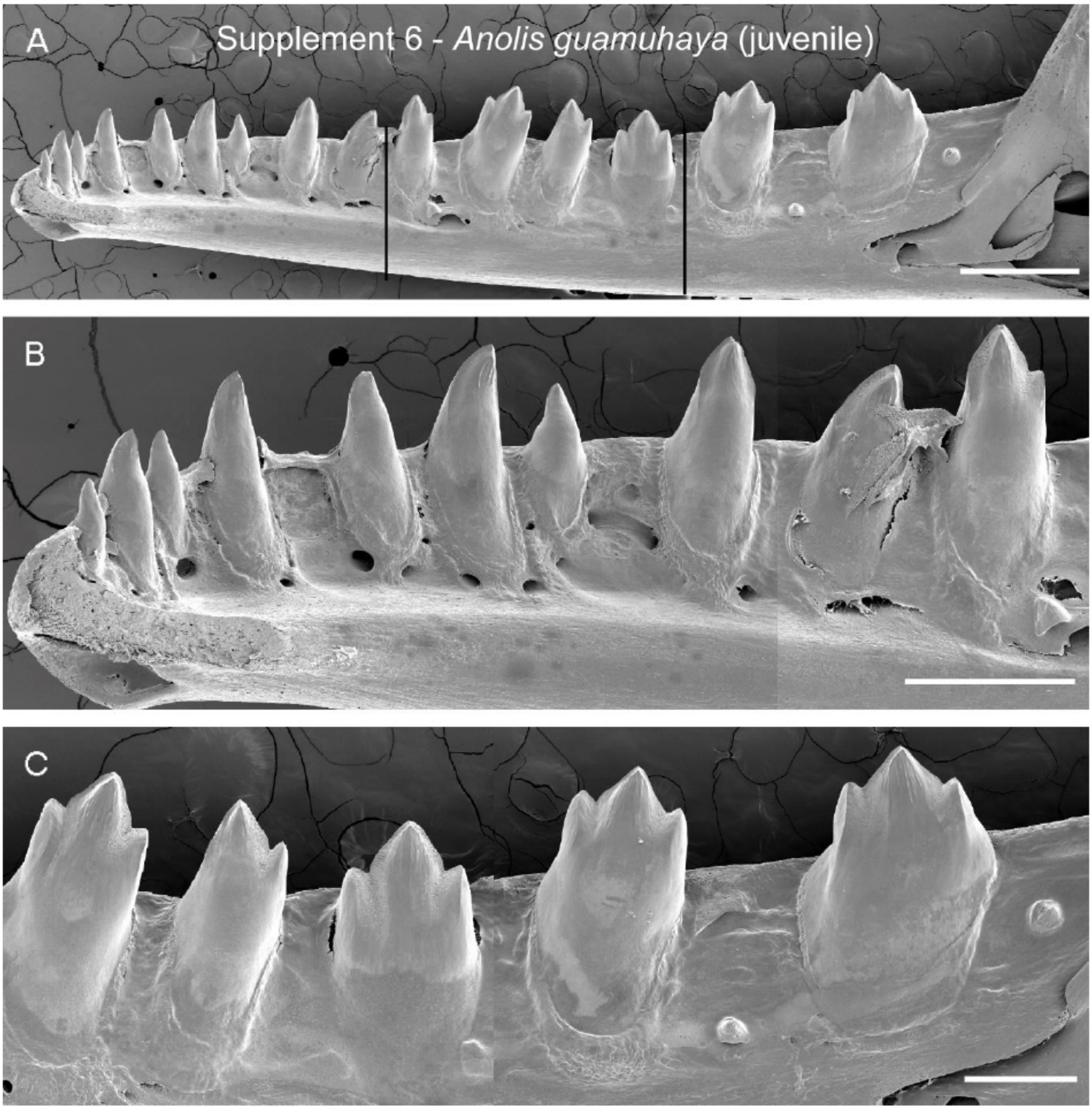
Supplement 3: SEMs of the juvenile dentition of *A. barbatus*. Image (A) shows the lower jaw from the lingual side, with a scale bar of 2 mm. The black vertical lines divide the lower jaw into rostral, middle, and caudal segments. Images (B, D) provide an enlarged view of the dentition, with scale bars of 0.5 mm.



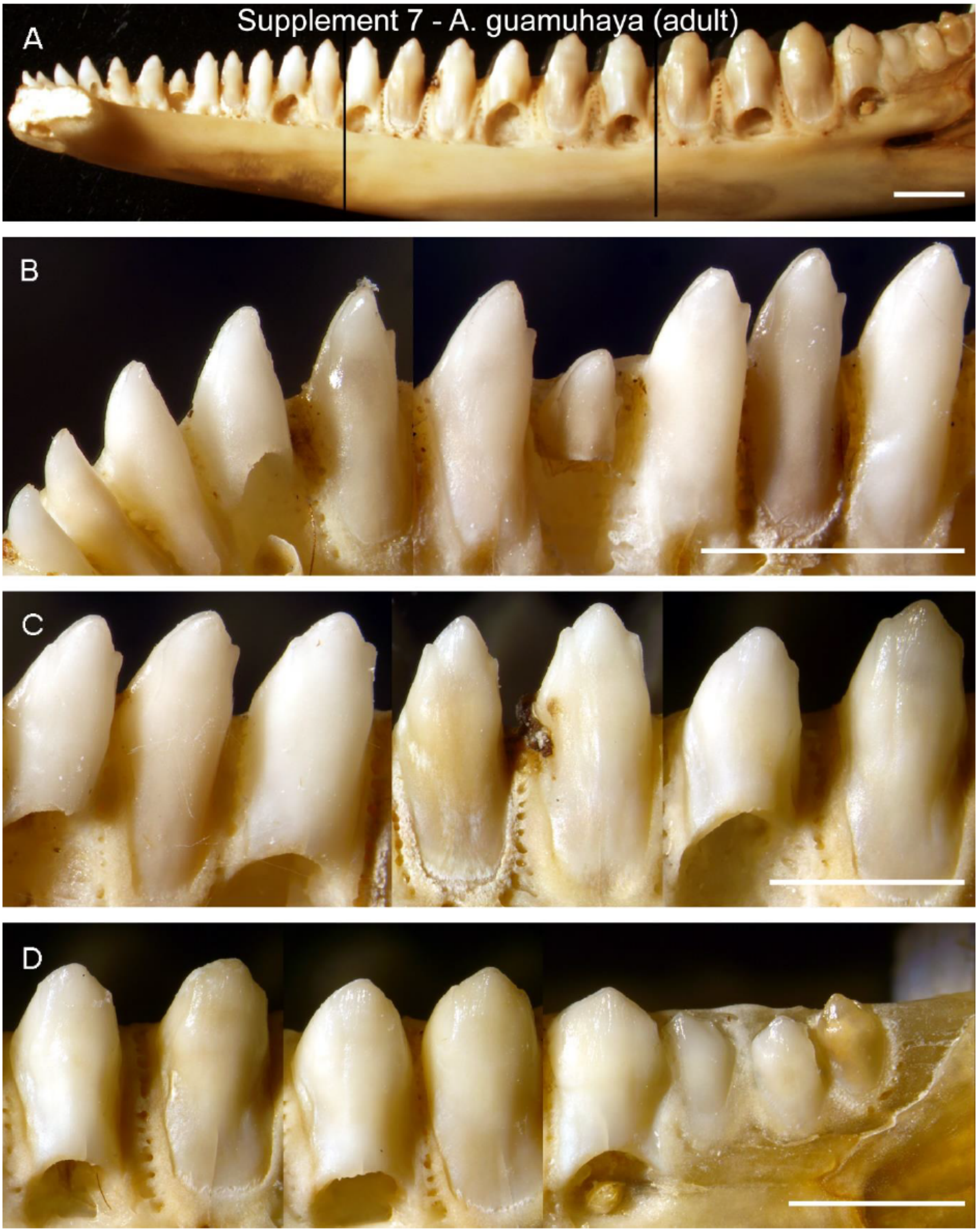
Supplement 4: SEMs of the juvenile dentition of *A. chamaeleonides*. Image (A) shows the lower jaw from the lingual side, with a scale bar of 2 mm. The black vertical lines divide the lower jaw into rostral, middle, and caudal segments. Images (B, D) provide an enlarged view of the dentition, with scale bars of 0.5 mm.



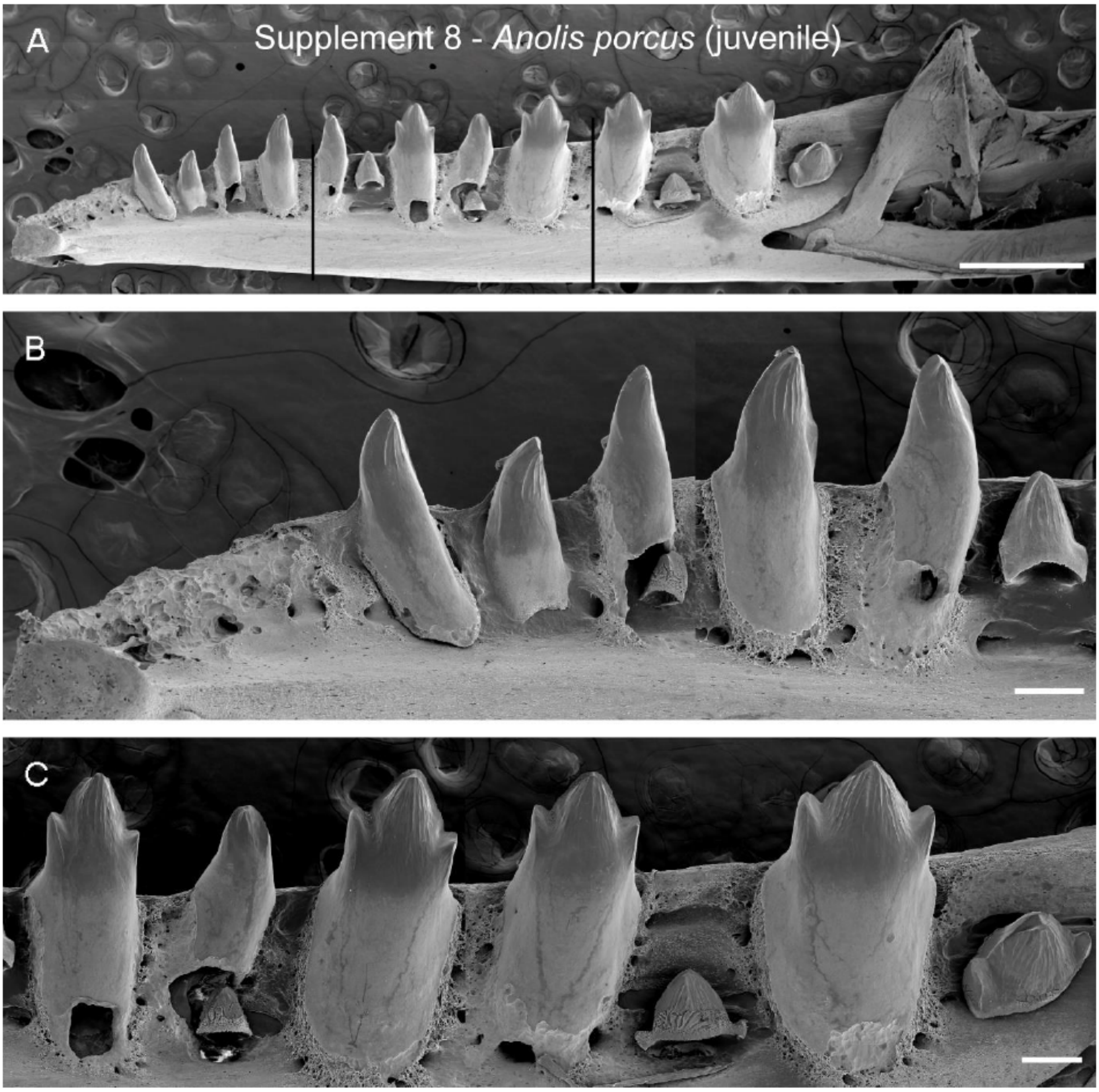
Supplement 5: Supplement 2: Light microscope photograph of the adult dentition of *A. chamaeleonides*. Image (A) shows the lower jaw from the lingual side. The black vertical lines divide the lower jaw into rostral, middle, and caudal segments. Images (B, D) provide an enlarged view of the dentition. The scale bars are 2 mm in size.



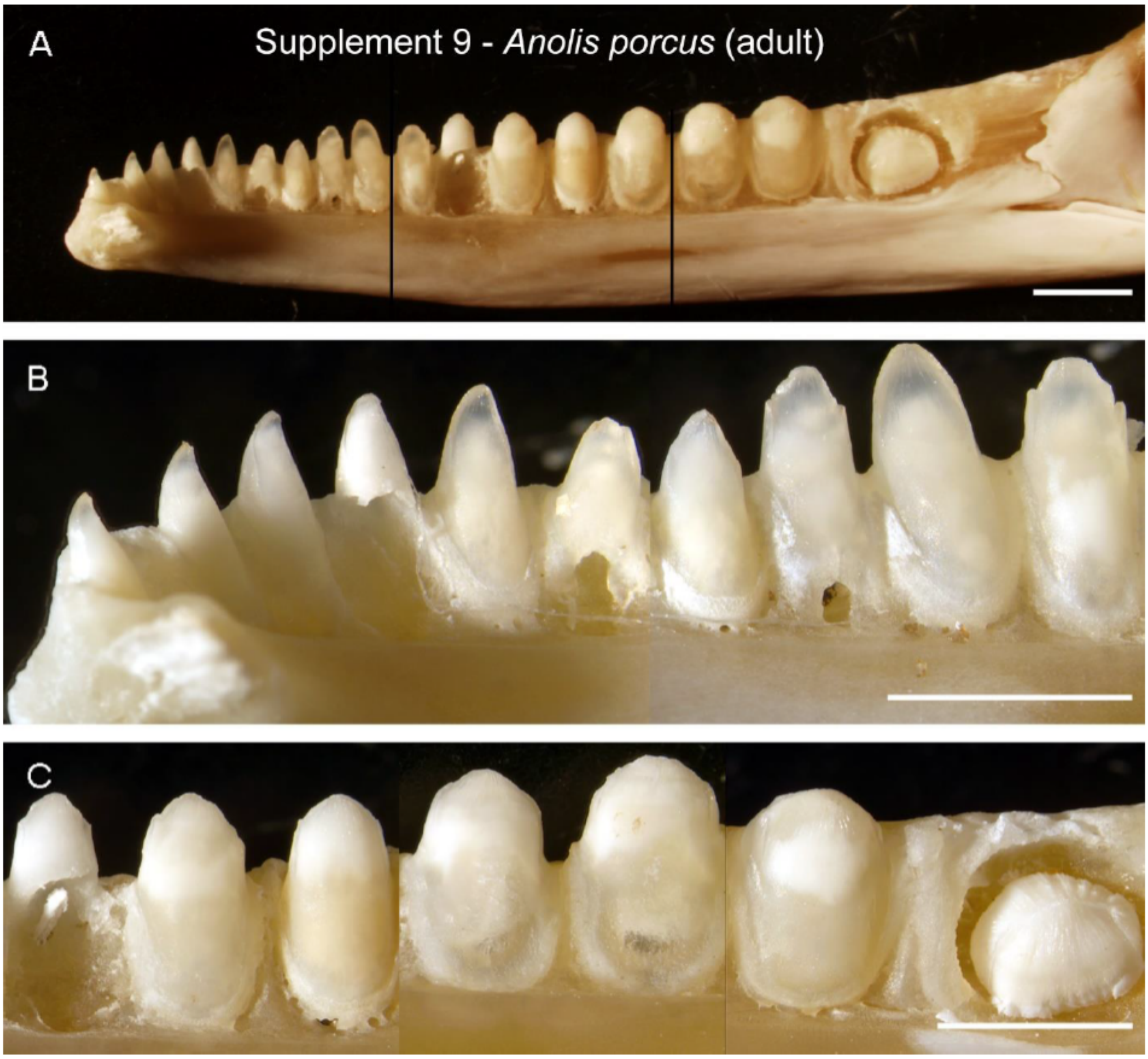
Supplement 6: SEMs of the juvenile dentitions of *A. guamuhaya*. Image (A) shows the lower jaw from the lingual side with a scale bar of 1 mm. The black vertical lines divide the lower jaw into rostral, middle, and caudal segments. Images (B, D) provide an enlarged view of the dentition with scale bars of 0.5 mm.



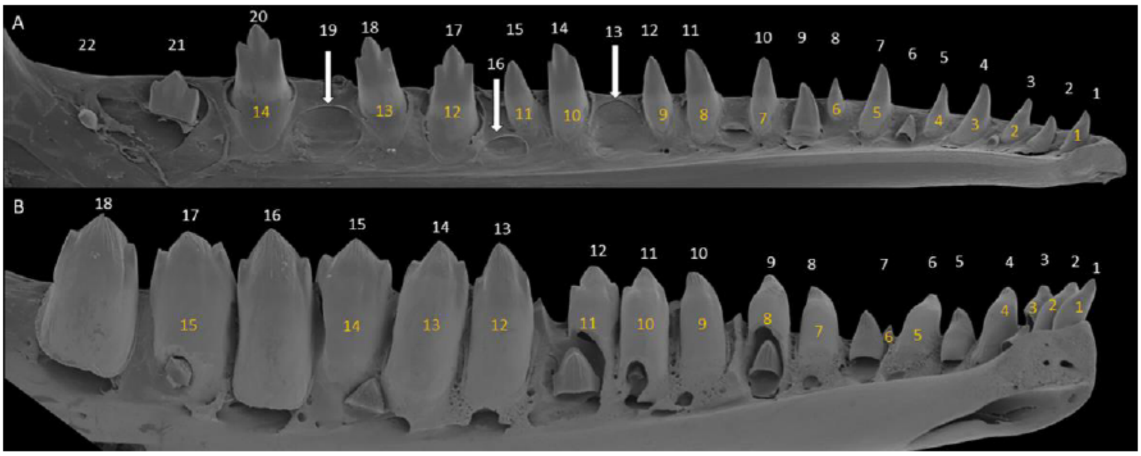
Supplement 7: Light microscope photograph of the adult dentition of *A. guamuhaya*. Image (A) shows the lower jaw from the lingual side. The black vertical lines divide the lower jaw into rostral, middle, and caudal segments. Images (B, D) provide an enlarged view of the dentition. The scale bars are 2 mm in size.



Supplement 8: SEMs of the juvenile dentitions of *A. porcus*. Image (A) shows the lower jaw from the lingual side with a scale bar of 1 mm. The black vertical lines divide the lower jaw into rostral, middle, and caudal segments. Images (B, D) provide an enlarged view of the dentition with scale bars of 0.2 mm.

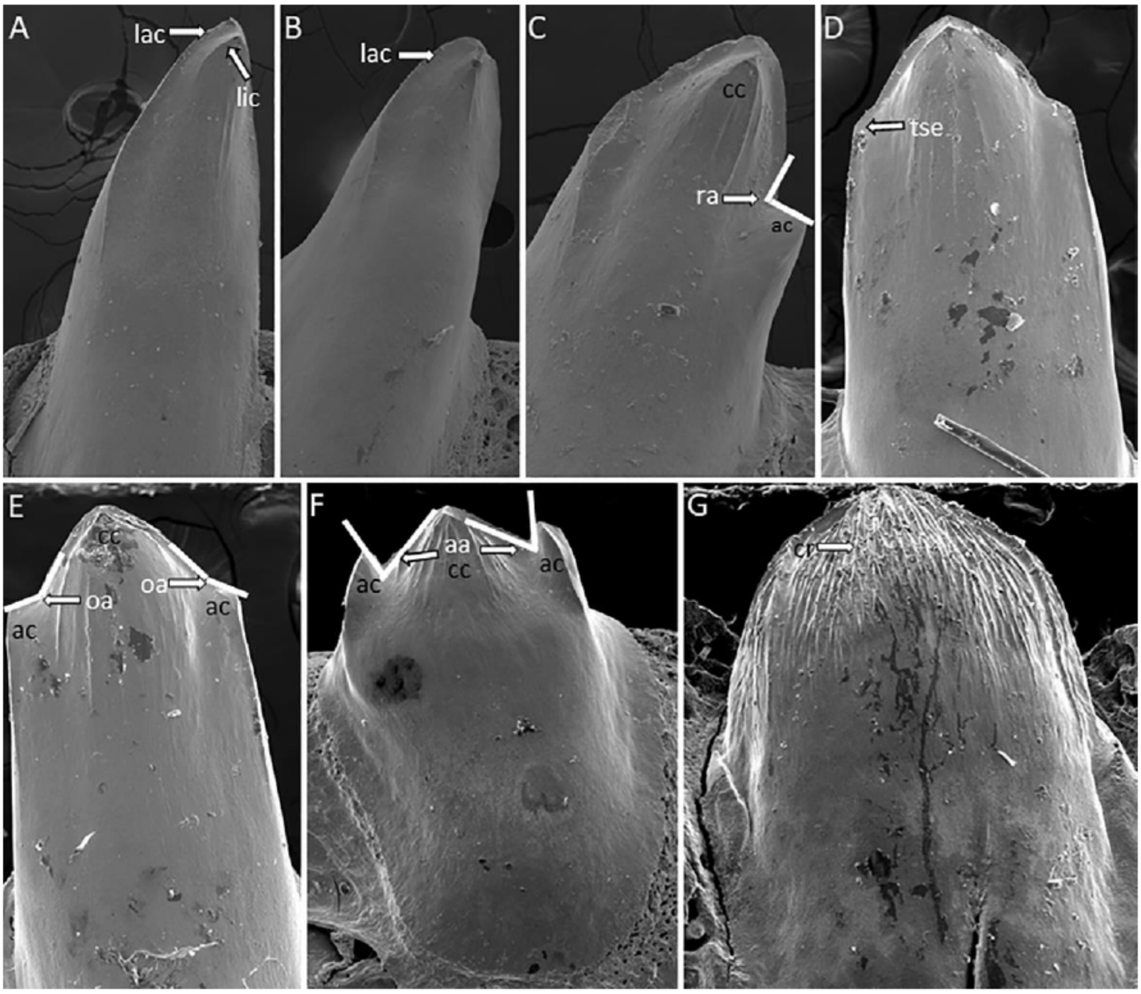


Supplement 9: Light microscope photograph of the adult dentition of *A. porcus*. Image (A) shows the lower jaw from the lingual side. The black vertical lines separate the lower jaw into rostral, middle, and caudal segments. Images (B, D) show enlarged views of the dentition. The scale bars are 2 mm.

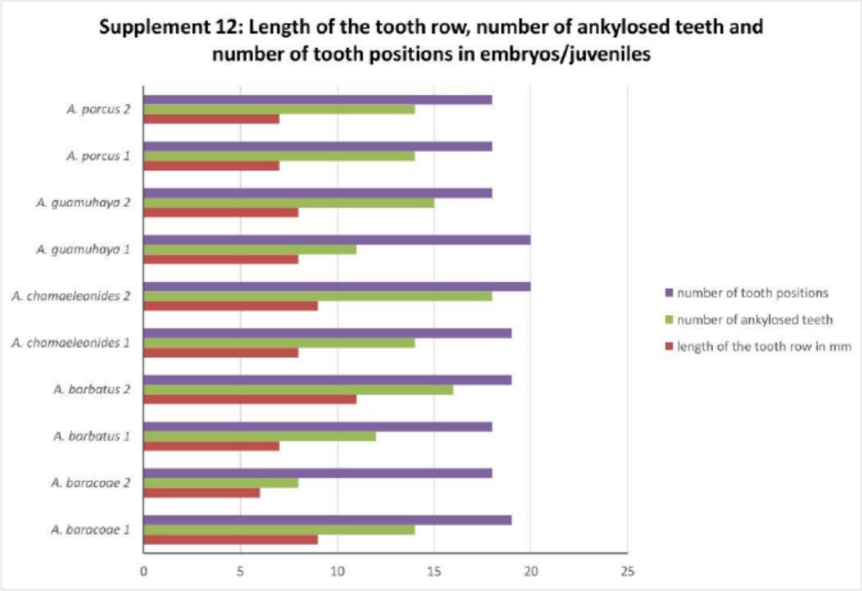


Supplement 10: SEM micrographs showing how tooth positions and the number of ankylosed teeth were counted. Positions and ankylosed teeth were counted from the rostral tip of the jaw. A) shows the situation in a late embryo, while B) shows it in a juvenile. White numbers indicate tooth

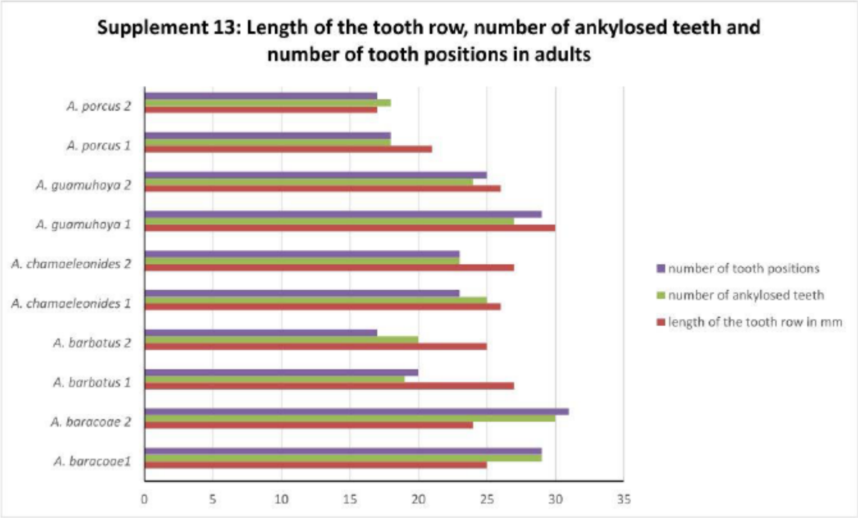
positions, while yellow numbers indicate ankylosed teeth.



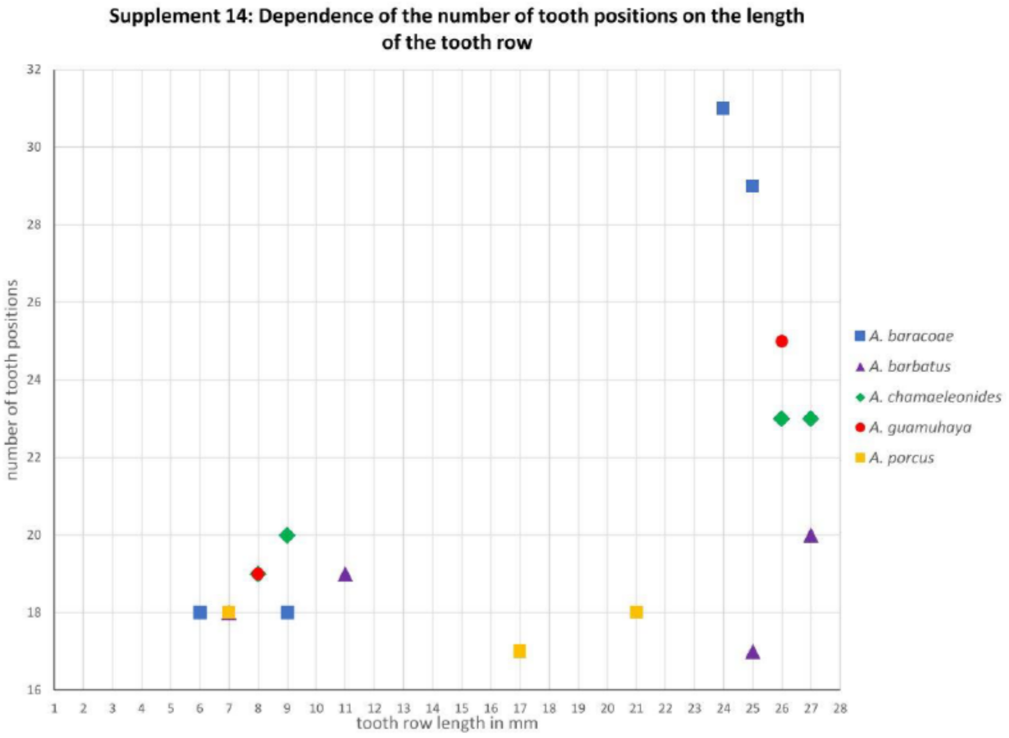
Supplement 11: Figures show tooth shapes and tooth crown features. A-B and D) depict unicuspid teeth. Unicuspid tooth A) has a tip divided into labial (lac) and lingual enamel crests (lic), unicuspid tooth B) shows wear on only the labial enamel crest, and D) the unicuspid tooth possesses side expansions (tse) that nearly take the shape of accessory cusps. Figure C) shows a bicuspid tooth with a central cusp (cc) and an accessory cusp (ac) forming a right angle (ra). Figures E-F) depict tricuspid teeth. The tricuspid tooth in E) has a central cusp and two accessory cusps forming an obtuse angle (oa), while in F) the cusps form a right angle (ra). Figure G) shows a molariform tooth ornamented with enamel crenulation.



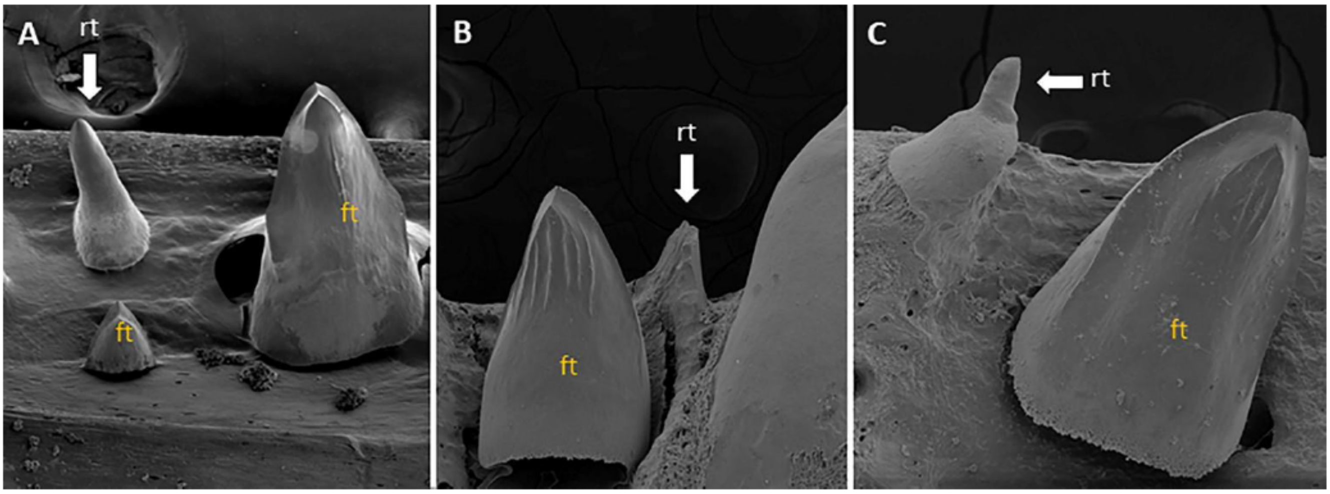
Supplement 12: The chart shows the length of the tooth row, the number of ankylosed teeth, and the number of tooth positions examined in embryos and juveniles.



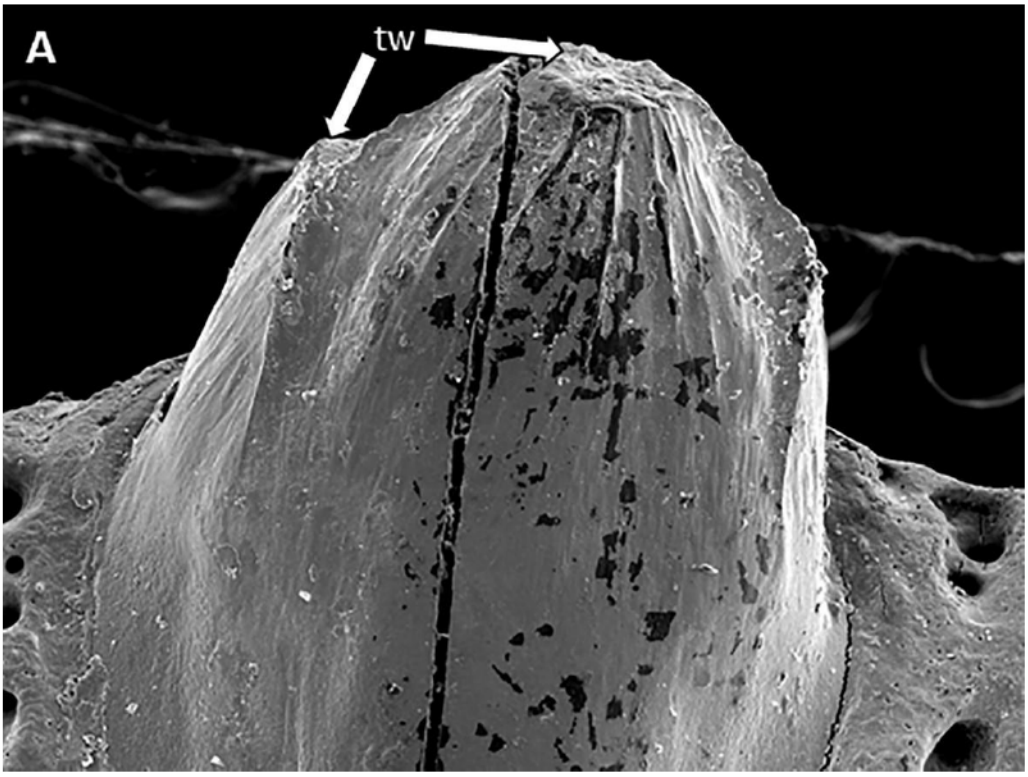
Supplement 13: The chart shows the length of the tooth row, the number of ankylosed teeth, and the number of tooth positions examined in adults.



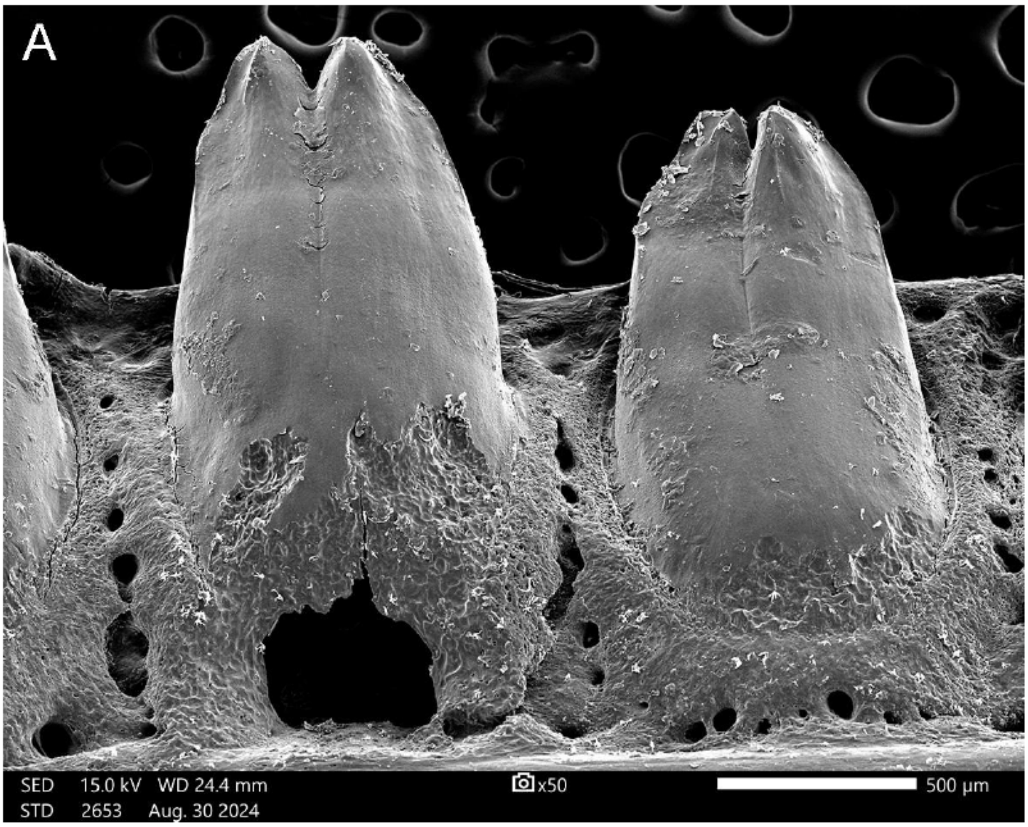
Supplement 14: The chart shows the correlation between the number of tooth positions and the length of the tooth row in the examined anole species.



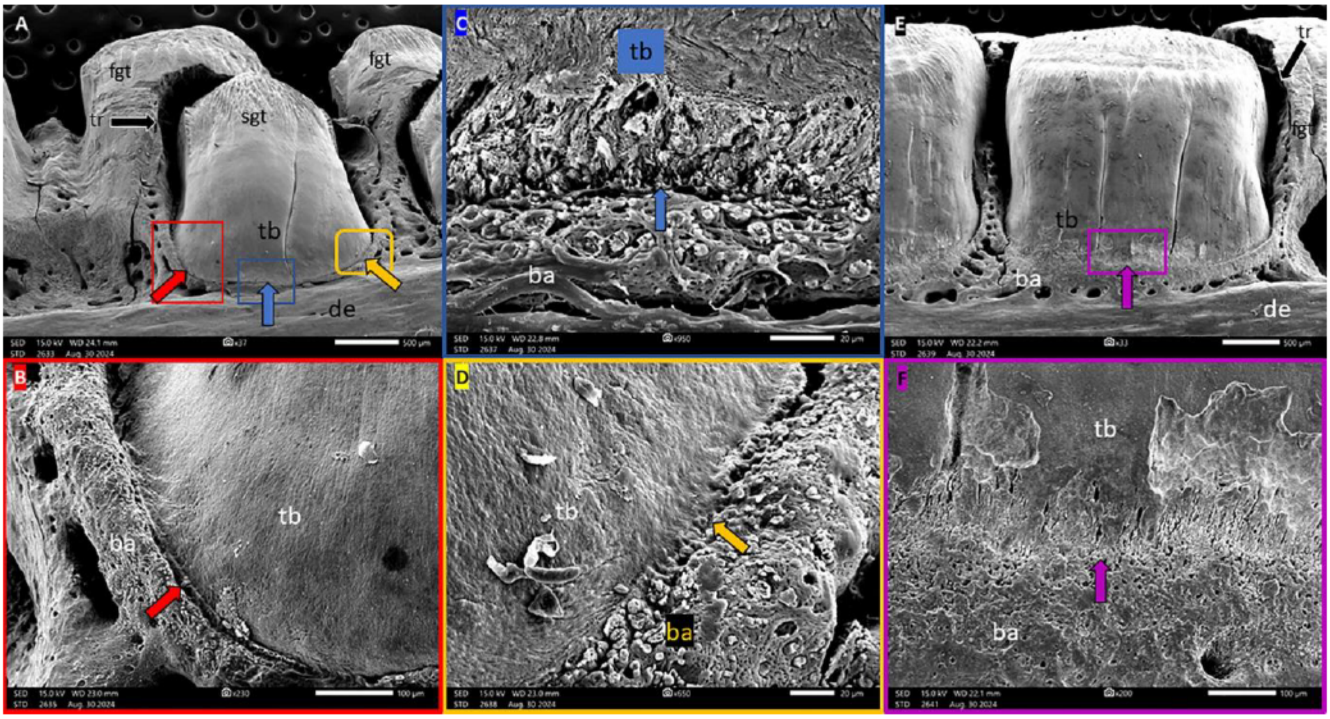
Supplement 15: SEM micrographs show teeth of the rudimentary generation. A-C) Rudimentary teeth (rt) are miniature mineralized teeth situated between the teeth of the first functional generation (ft).



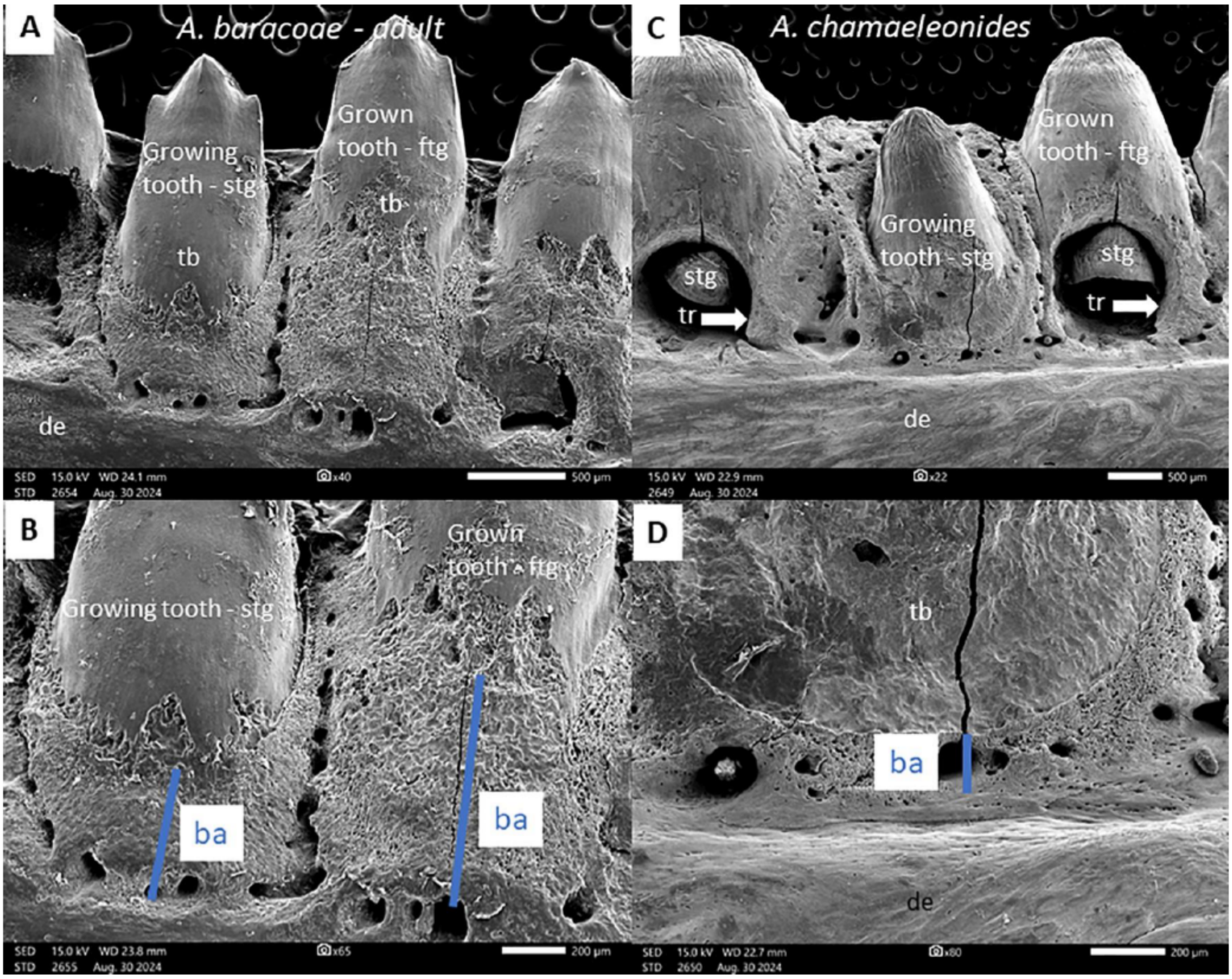
Supplement 16: The figure shows tooth wear (tw). The enamel is damaged in the marked areas of the tooth crown.



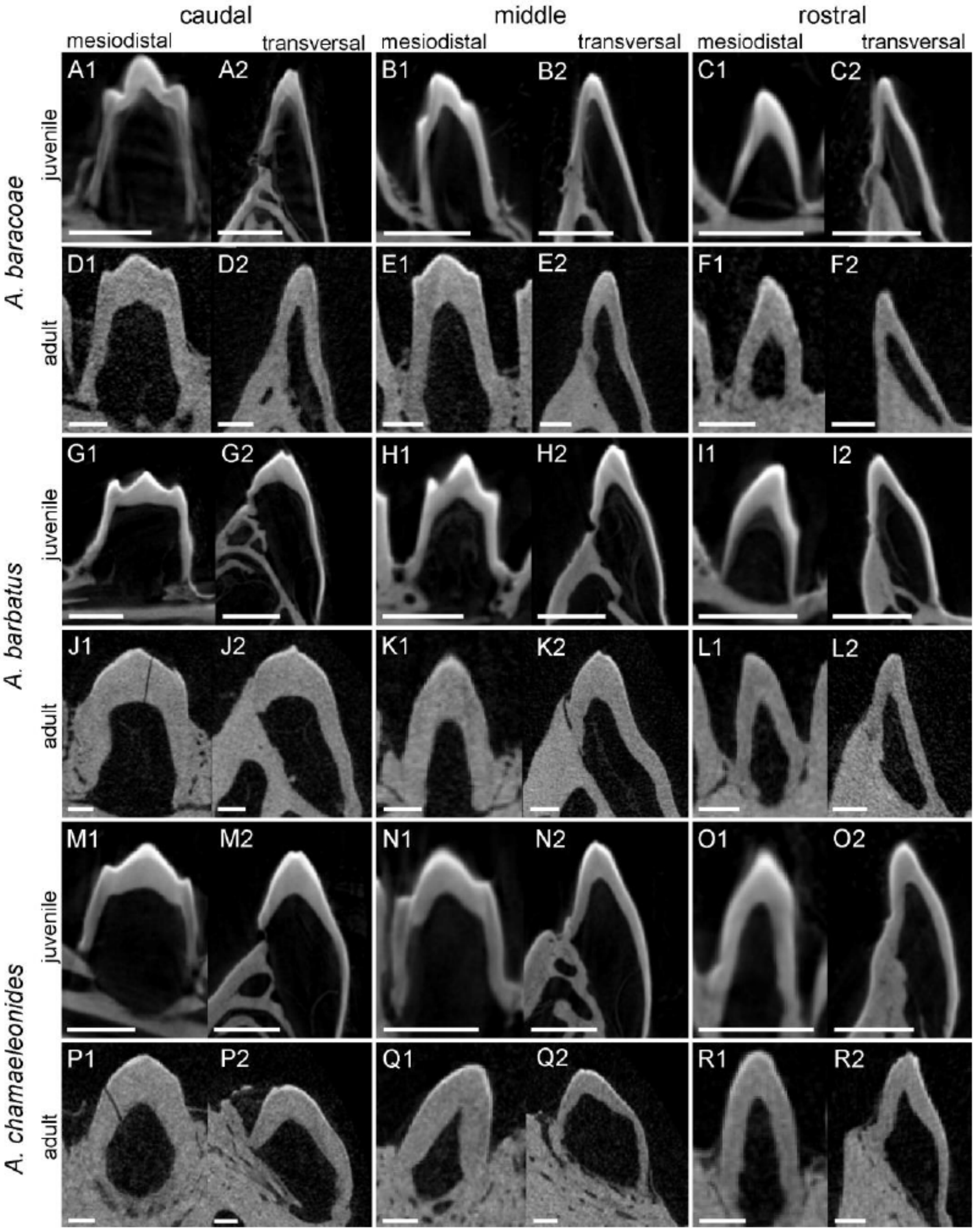
Supplement 17: This micrograph shows bicuspid teeth found in one adult *A. baracoae*. These teeth differ from other bicuspid teeth as their cusps are the same size, suggesting the possibility that they formed via the fusion of two tooth germs. This phenomenon has been previously described in egg teeth. In this case, it could be a developmental disorder.



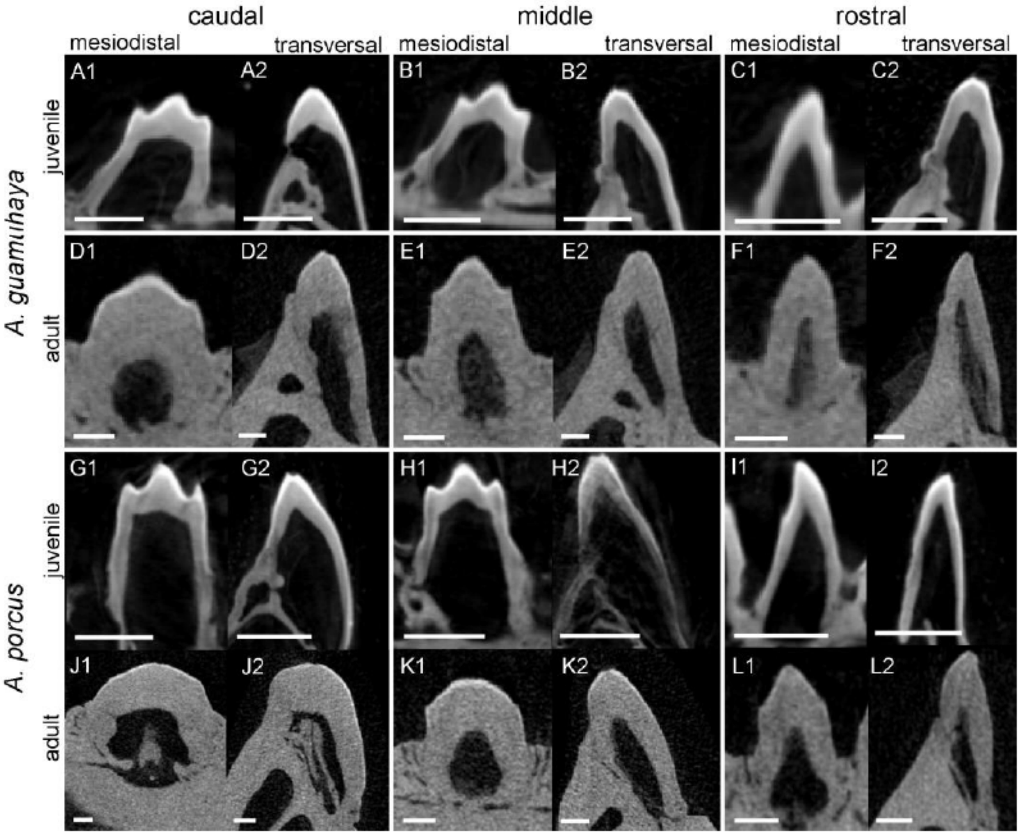
Supplement 18: Shows SEM micrographs of ankylosed teeth that are still growing and those that are no longer growing, attached to the dentale (de) in an adult *A. barbatus*. A) Shows a still-growing ankylosed tooth (sgt) adjacent to a tooth of a former generation (fgt) that is resorbed (tr). B-D) Provide higher magnification of the tooth base (tb)/bone of attachment (ba) interface, which appears very active in the mineralization process and contributes to positioning the tooth. E) Shows an ankylosed tooth in its final position. F) Provides higher magnification of the tooth base/bone of attachment interface, which no longer appears active in the formation of new mineralized tissue.



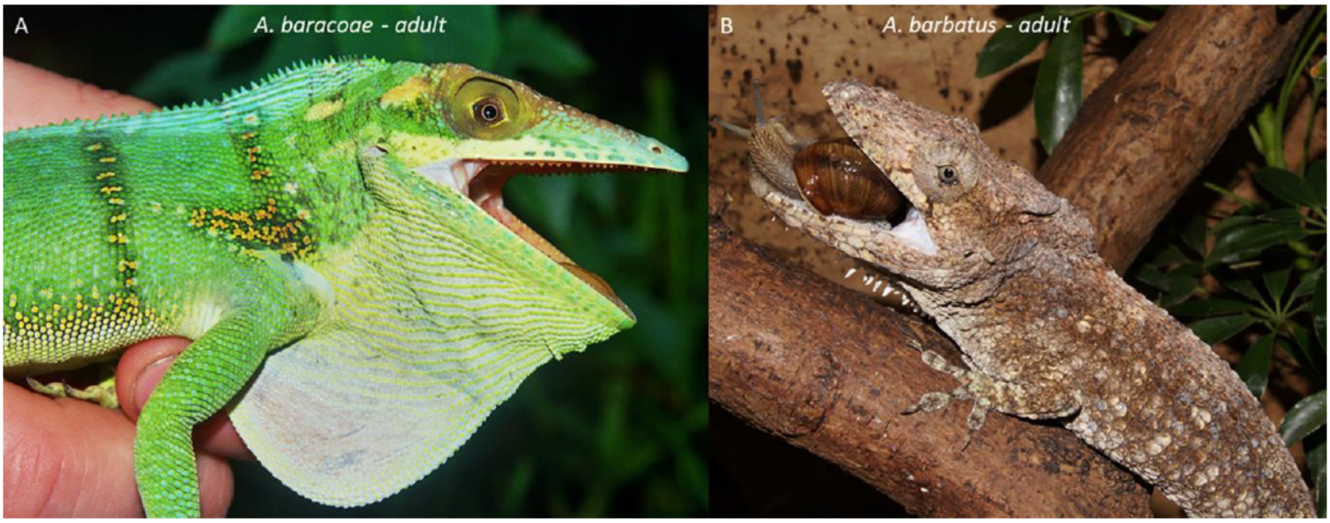
Supplement 19: Shows SEM micrographs of ankylosed teeth that are still growing and those that are no longer growing, attached to the dentale (de) in A-B) an adult *A. baracoae* and C-D) *A. chamaeleonides*. A) Shows a still-growing ankylosed tooth (sgt) adjacent to a tooth from a previous generation (fgt). B) Provides a higher magnification of the tooth base (tb)/bone of attachment (ba) interface in both the still-growing and fully growed teeth. The blue lines mark the width of the bone of attachment. This micrograph clearly shows that the bone of attachment continues to grow after initial ankylosis to shift the tooth into its final position. C) Also shows an ankylosed growing tooth next to teeth in their final position, whose tooth bases are already resorbing (tr). Mineralizing teeth of the successive generation (stg) are visible inside the resorbing teeth. D) Provides a higher magnification of the tooth base/bone of attachment interface in an ankylosed and still-growing tooth. The blue line marks the width of the bone of attachment.



Supplement 20: The figures show micro-CT mesiodistal and transverse sections through selected teeth from the rostral, middle, and caudal segments of the lower jaw in juvenile and adult *A. baracoae*, *A. barbatus* and *A. chamaeleonides*. A1-F2) depict the dentine deposition in *A. baracoae*, G1-L2) in *A. barbatus*, and M1-R2) in *A. chamaeleonides*.



Supplement 21: The figures show micro-CT mesiodistal and transverse sections through selected teeth from the rostral, middle, and caudal segments of the lower jaw in juvenile and adult *A.guamuhaya and A. porcus*. A1-F2) depict the dentine deposition in *A. guamuhaya*, and G1-L2) in *A.porcus*.



Supplement 22: Shows pictures of adult *A. baracoae* (A) and *A. barbatus* (B), a member of the Cuban false chameleons. *A. baracoae* has a slender head and long limbs (A), while *A. barbatus*, shown eating a snail, has massive jaws and short limbs (B).

RESEARCH ARTICLE

Built to Bite: Feeding Kinematics, Bite Forces, and Head Shape of a Specialized Durophagous Lizard, *Dracaena Guianensis* (Teiidae)

JOURNAL OF EXPERIMENTAL ZOOLOGY
PART A
ECOLOGICAL GENETICS AND PHYSIOLOGY
A Journal of Integrative Biology

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ABSTRACT

Most lizards feed on a variety of food items that may differ dramatically in their physical and behavioral characteristics. Several lizard families are known to feed upon hard-shelled prey (durophagy). Yet, specializations toward true molluscivory have been documented for only a few species. As snails are hard and brittle food items, it has been suggested that a specialized cranial morphology, high bite forces, and an adapted feeding strategy are important for such lizards. Here we compare head and skull morphology, bite forces, and feeding kinematics of a snail-crushing teiid lizard (*Dracaena guianensis*) with those in a closely related omnivorous species (*Tupinambis merianae*). Our data show that juvenile *D. guianensis* differ from *T. merianae* in having bigger heads and greater bite forces. Adults, however, do not differ in bite force. A comparison of feeding kinematics in adult *Dracaena* and *Tupinambis* revealed that *Dracaena* typically use more transport cycles, yet are more agile in manipulating snails. During transport, the tongue plays an important role in manipulating and expelling shell fragments before swallowing. Although *Dracaena* is slow, these animals are very effective in crushing and processing hard-shelled prey. *J. Exp. Zool. 00:1–11, 2012.* © 2012 Wiley Periodicals, Inc.

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Food acquisition is very important for the daily survival and fitness of individuals and has been studied in a wide variety of animals (for vertebrates, see Schwenk, 2000 for an overview). Morphological and behavioral specializations related to prey manipulation may provide some species access to food resources that may be unavailable or unmanageable for closely related species (e.g., Aguirre et al., 2003). Indeed, there is a strong precedent for predicting a relationship between an organism's functional capacities and its potential resource use (Grant, '85; Fisher Huckins, '97; Herrel et al., 2002a, b).

In lizards, true feeding specialists are rare. Indeed, most lizards feed on a wide variety of food items that often differ in their physical and behavioral characteristics (Greene, '82; Schaerlaeken et al., 2007, 2008; Metzger, 2009; Montuelle et al., 2009). If prey properties impose specific mechanical demands on the feeding system of the predator, then these will likely

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influence the efficiency of prey capture and transport (Meyers and Herrel, 2005). Consequently, most lizards are capable of adjusting their feeding behavior in accordance to prey characteristics that may allow them to optimize food processing (e.g., Bels and Baltus, '88; Herrel et al., '96; Herrel and De Vree, '99; Urbani and Bels, '99; Schwenk, 2000; Ross et al., 2007; Schaerlaeken et al., 2008).

Several lizard families are known to feed upon hard-shelled prey (durophagy). Yet, for only a few species true molluscivory has been documented. As snails are hard and brittle food items, it has been suggested that lizards that eat snails should display a specialized cranial morphology with more massive cranial muscles (Dalrymple, '79; Rieppel and Labhardt, '79) and greater bite forces. Among lizards, *Chamaeleolis* lizards (Herrel and Holanova, 2008), some amphisbaenids (*Amphisbaena ridleyi*; Pregill, '84), and nile monitors (*Varanus niloticus*; Lonnberg, '03; Rieppel and Labhardt, '79) are known to include a considerable amount of hard-shelled prey such as snails in their diet. Although relatively little is known about these animals, enlarged posterior teeth are often observed. Such blunt and rounded teeth are assumed to be important to avoid tooth breakage and to increase the contact area with the food. However, only in a few species do the blunt molariform teeth occur in juveniles (e.g., *Dracaena guianensis*; some species of the genus *Tiliqua;* Estes and Williams, '84) suggesting that in these species, juveniles may already be specialized for molluscivory.

Feeding on hard or tough prey puts demands on increased bite force. Such an increase in bite force can be achieved in multiple ways. For example, one way is to increase overall body size, or head size relative to body size. Additionally, an increase in the mass, changes in the architecture of the jaw adductors (i.e., more pennate muscles with shorter fibers), or changes in the mechanics of the lever system (i.e., increasing jaw closing in lever relative to the jaw out lever) could improve bite performance (Herrel et al., 2002a, b, 2007; Herrel and Holanova, 2008). Consequently, lizards feeding on hard prey are expected to show one or several of these specializations.

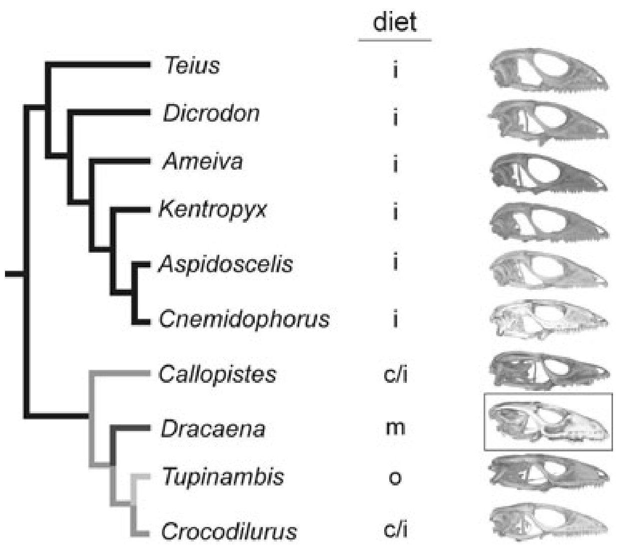

Figure 1. Phylogenetic relationships of teiid lizards (Giugliano et al., 2007). To the right, the predominant diet within the genus is indicated. Branch colors indicate the results of an ancestral character state reconstruction of diet using parsimony implemented in Mesquite. Whereas the basal diet is clearly insectivory (i), the Tupinambinae are characterized by the evolution of derived diets including carnivory (c) and omnivory (o). Yet, dedicated molluscivory (m) has originated only once within the group. To the right lateral views of the skulls of representative species of each clade are shown to illustrate how divergent *Dracaena* is compared to other teiids. The skull of *Dracaena* is highlighted in the box. Images derived from CT scans.

In the present study, we compare a true molluscivorous lizard, *D. guianensis*, with a closely related but omnivorous lizard, *Tupinambis merianae*. Whereas lizards from the Teiinae subfamily are typically insectivorous (Presch, '74; Vitt et al., '95, '97, 2000), Tupinambinae are characterized by a derived diet that encompasses the inclusion of vertebrates and fruits into the diet in some species (Dessem, '85; Castro et al., '91; Mercolli and Yanosky, '94; Kiefer and Sazima, 2002; Martins, 2006; see Fig. 1). Yet, only in *Dracaena* is a true durophagous diet observed. It has been suggested that the inclusion of hard-shelled prey in this species is associated with its semiaquatic lifestyle (Vanzolini, '61; Duellman, 2005). Although both *Tupinambis* and *Dracaena* are characterized by well-developed jaw adductors with complex tendinous systems, *Dracaena* stands out in having more developed pseudotemporalis profundus muscle and a greater degree of muscle pennation (Dalrymple, '79; Rieppel, '80). This suggests that *Dracaena* may have greater bite forces allowing them to crush hard food items such as snails and crustaceans (Vanzolini, '61; Duellman, 2005).

Here, we focus on differences in cranial size and shape that could improve bite force in both juveniles and adults. Given their specialized teeth, we expect juvenile *D. guianensis* to have relatively bigger heads and changes in the mechanics of the lever system allowing them to generate high bite forces allowing them to crush hard prey such as snails. As adults of both *D. guianensis* and *T. merianae* reach large adult body size (over 300 mm snout-vent length), selection on bite force generation might be relaxed. We also test for differences in head shape and skull shape that could lie at the basis of a high bite force capacity. Finally, we test for behavioral differences in prey manipulation, prey transport, and swallowing in adults of both species. Given the largely aquatic lifestyle of *Dracaena*, we expect careful manipulation of food items to avoid the loss of prey when manipulating and crushing at the water's edge or in water.

MATERIAL AND METHODS

Specimens

Data for *D. guianensis* were obtained from both adults ($N = 4$; SVL: 389.0 ± 38.1 mm; mean ± standard deviation) and juveniles ($N = 24$; 156.4 ± 10.2 mm) housed at the Prague Zoo. As these animals are extremely rare in captivity, this comprises an exceptionally large sample size. The adults were housed in a large glass vivarium on a 12 hr:12 hr light:dark cycle and were maintained on a diet of snails only. The juveniles were housed in smaller glass vivaria also on a 12 hr:12 hr light:dark cycle but were offered mostly slugs. The environmental temperature varied from 22 C during daytime to 12 C at night. An incandescent bulb provided the animals with a basking spot at a higher temperature.

For *T. merianae* bite forces from adults ($N = 35$; 375.6 ± 21.1 mm) and juveniles ($N = 25$; 163.3 ± 13.4 mm) were obtained from animals housed at the Jacarezário at the Universidade Estadual Paulista (Rio Claro, São Paulo, in south-eastern Brazil; see Herrel et al., 2009). At this laboratory, the lizards are kept in small groups (5–10 individuals) in outdoor pens and are provided with water and ground shelters, as well as shade and sunny areas for thermoregulation. In spring and summer, the animals are fed three times a week with ground beef, fruits, and/or 1-day-old chickens.

Kinematic data on feeding in *T. merianae* were obtained from two adults (383.33 ± 20.82 mm) kept at the laboratory of the University of Antwerp. These animals were housed separately in cages (120 × 80 × 80 cm) on a 12 hr:12 hr light:dark cycle and provided with snails, dog food, fruit, and mice ad libitum. The environmental temperature varied from 28 C during daytime to 22 C at night. An incandescent bulb provided the animals with a basking spot at higher temperature.

Morphometrics

Head dimensions and snout-vent length (SVL) were measured in all individuals. Head length was taken as the distance between the back of the parietal bone and the tip of the upper jaw; head width was measured at the widest part of the head; head height was measured as the highest point of the head just posterior to the orbits; lower jaw length was taken as the distance between the back of the retroarticular process and the tip of the lower jaw. Additionally, two morphological variables related to the biomechanics of the jaw system were estimated by measuring: (1) the distance from the jaw articulation to the tip of the lower jaw (i.e., the jaw outlever) and (2) the distance from the posterior edge of the jugal to the tip of the lower jaw (snout length). First, the length of the jaw-in-lever for opening was calculated by subtracting the jaw outlever from the lower jaw length. Second, by subtracting snout length from the jaw outlever, the in-lever for jaw closing was calculated. Measurements were taken using digital callipers (Mitutoyo CD-20DC, England; precision: 0.01mm).

In addition, we measured the skulls of 70 individuals belonging to 20 species of teiids representing all known genera using digital callipers (Mitutoyo CD-20DC). Measurements were based on museum specimens located in the collections of the Museum of Comparative Zoology at Harvard, the Field Museum of Natural History in Chicago, the American Museum of Natural History, the Muséum National d'Histoire Naturelle in Paris, and one specimen of *C. tigris* from the private collection of A.H (nine *Ameiva ameiva*, two *Aspidoscelis deppei*, one *A. exsanguis*, two *A. gularis*, one *A. motaguae*, one *A. neomexicanus*, one *A. sexlineata*, four *A. tigris*, three *Callopistes flavipunctatus*, one *C. maculatus*, two *Cnemidophorus murinus*, two *Crocodilurus lacertinus*, three *Dicrodon guttulatum*, one *Dicrodon Holmbergi*, three *D. paraguayensis*, one *D. guianensis*, five *Kentropyx calcaratus*, two *Teius teyou*, two *Tupinambis nigropunctatus*, 23 *Tupinambis teguixin,* and one *Tupinambis rufescens*). Note that *Dracaena* are rare in collections and only two intact skulls (MCZ, *D. paraguayensis* and MNHN, *D. guianensis*) were available for measurements. Data for two others were extracted from μCT data of intact specimens (both *D. paraguayensis*). On each skull, we measured skull length from the back of the parietal to the tip of the premaxillary, the length of the skull between the posterior edge of the jugal and the tip of the premaxillary bone (muzzle length), the height of the skull at the level of the frontoparietal suture, the height of the skull at the level of the orbit, the length of the lower jaw from the symphysis to the back of the posterior most tooth, the length of the lower jaw from the symphysis to the anterior edge of the jaw articulation, the length of the lower jaw from the symphysis to the posterior edge of the jaw articulation, the length of the lower jaw from the symphysis to the back of the retroarticular process, and the length of the lower jaw from the symphysis to the tip of the coronoid bone (see also Metzger and Herrel, 2005).

Bite Forces

Isometric force transducers (Kistler, types 9203: ± 500 N and 9207: ± 5000 N) mounted on purpose-built holders and connected to a Kistler charge amplifier (type 5995A, Kistler Inc., Winterthur, Switzerland) were used to measure in vivo bite forces (see Herrel et al., '99). When placing the free end of the holder between the teeth of the animal, prolonged and repeated biting occurred. The place of application of bite forces was standardized for all animals and occurred at the tip of the jaws. Measurements were repeated five times for each animal. The maximal value recorded of the five trials was considered to be the maximal bite force for each animal. Although snails are crushed at the back of the jaws, it was impossible to obtain data on bite forces at the back of the tooth row.

Feeding Behavior and Video Analysis

Adult individuals of both species were filmed in lateral view while eating intact snails (*Cornu aspersa*). Data on feeding behavior for two adult *D. guianensis* were collected at the Prague Zoo. Data for *T. merianae* were recorded for two individuals housed at the University of Antwerp. Feeding behavior was recorded at 50 Hz using a digital camcorder (Sony DCR-HC94, Sony, Tokyo, Japan). Video recordings were reviewed using Midas Player software (Xcitex, Cambridge, MA; version 2.1.5). A total of 30 (15 and 15 for each individual) and 22 (10 and 12 for each individual) feeding sequences were recorded for *D. guianensis* and *T. merianae,* respectively. Based on these recordings, the transport and swallowing stage duration, the number of crush bites during transport, the number of transport and swallowing cycles, the average transport cycle duration, and the average swallowing cycle duration were extracted for both species. In the number of transport cycles, we included the inertial transport movements, the repeated attempts to pick up the snail, and the movements of the jaws while removing shell fragments after dropping the snail (only observed in *T. merianae*).

Data on feeding kinematics for *T. merianae* were collected at the University of Antwerp. For these animals, small lead markers were inserted at the anterior tip of the upper and lower jaw before videofluoroscopic recordings were made. Before implantation of these markers, the animals were anaesthetized using Ketamine (100 mg/kg; ketamine hydrochloride, 50 mg/mL, Parke-Davis, Brussels, Belgium). Marker placement was checked using dorsoventral and lateral radiographs. A Redlake MotionPro2000 digital high-speed camera attached to a Philips 14-inch image intensifier was used to record parts of the feeding event at 250 Hz. X-rays were generated using a Philips Optimus M200 X-ray generator (Philips, Eindhoven, The Netherlands). Videofuoroscopic recordings were reviewed using the Midas Player software (Redlake, San Diego, CA; version 2.1.7).

For selected transport and crush cycles where animals remained in lateral view, two externally visible landmarks (the anterior tip of the upper and lower jaws) in the case of *Dracaena*, or the two implanted markers near the tip of the jaws in the case of *Tupinambis*, were digitized frame by frame using Didge (Image Digitizing Software version 2.2.0; Alistair Cullum). Based on the X-Y coordinates of these markers, gape distance was calculated. A total of 15 cycles (10 transport and 5 crush cycles; evenly divided across individuals) were analyzed for each species. Raw displacements were smoothed using a zero-phase shift, fourth-order low-pass Butterworth filter.

Feeding events were divided into three stages: prey capture, intraoral prey transport, and swallowing. In the transport stage, we made a distinction between two cycle types, a transport cycle without crushing and a crush bite. In accordance with Bramble and Wake ('85), we divide transport and crush cycles into four phases based on the changes in velocity of the jaws: slow open (SO), fast open (FO), fast close (FC), and slow close (SC).

All procedures were approved by the animal ethics committee at the University of Antwerp (*Tupinambis*) and the Prague Zoo (*Dracaena*).

Statistical Analysis

To meet the assumptions of homoscedascity and normality for parametric analyses (Sokal and Rohlf, '81; Kachigan, '91), all morphometric and kinematic data were $\log_{10}$-transformed prior to analysis.

To investigate the differences in head shape and bite forces between *T. merianae* and *D. guianensis,* we first tested whether adult and juvenile individuals of both species differed in overall body size (snout-vent length, SVL) using an ANOVA. Next, we tested for differences in head dimensions for both age classes using a MANCOVA with SVL as covariate. As the species by age class interaction effect was highly significant, morphometric data were further analyzed for juveniles and adults separately using MANCOVAs. Finally, we tested for differences in bite force between species and age classes using an ANCOVA. As interaction effects were significant, we subsequently tested for differences in bite force for both age classes separately.

To explore differences in skull shape between representatives of the different teiid genera, we performed a factor analysis with varimax rotation on the skull data. First, we regressed all Log_{10}-transformed skull dimensions against the geometric mean of all the skull measures and extracted unstandardized residuals. Next, we used residual data in a factor analysis with varimax rotation and extracted factors with eigenvalues greater than one and plotted the position of *D. guianensis* relative to other individuals. Next, we ran a multivariate analysis of variance (MANOVA) testing for differences between diet groups coupled to univariate ANOVA's and Bonferroni post-hoc tests on the factor scores to test whether the molluscivorous *Dracaena* were different from other diet groups.

Kinematic data were used to test for differences between species and cycle type (transport or crush bite). A MANOVA was performed to test for species, cycle type, and interaction effects with individual entered as a random factor in the analyses. As the interaction effects were highly significant, species effects were analyzed separately for transport and crush cycles using MANOVA's. Finally, differences in overall feeding behavior (number of cycles of each type used and overall duration of feeding event) were tested using a MANOVA.

RESULTS

Morphometrics and Bite Forces

An analysis of variance indicated no differences between species in body size ($F_{1,84} = 0.04$; $P = 0.84$) or in the interaction between body size and age ($F_{1,84} = 3.3$; $P = 0.08$). Differences between age classes were, however, significant ($F_{1,84} = 1782.6$; $P < 0.001$).

Table 1. Summary table representing means and standard deviations of the morphological and bite force data in juvenile and adult *T. merianae and D. guianensis.*

	Adults		Juveniles	
	T. merianae ($N = 35$)	*D. guianensis* ($N = 4$)	*T. merianae* ($N = 25$)	*D. guianensis* ($N = 24$)
SVL (mm)	375.6 ± 21.1	389.0 ± 38.1	163.3 ± 13.4	156.4 ± 10.2
HL (mm)	78.0 ± 7.2	81.2 ± 6.6	37.3 ± 2.6	40.6 ± 2.2
HW (mm)	53.7 ± 6.1	69.9 ± 8.2	23.4 ± 2.1	28.6 ± 1.8
HH (mm)	45.6 ± 5.4	55.5 ± 6.0	18.5 ± 1.4	25.2 ± 1.5
LJL (mm)	96.6 ± 9.8	100.0 ± 8.5	42.0 ± 3.2	47.1 ± 3.8
Snout (mm)	61.4 ± 5.4	70.5 ± 4.2	28.1 ± 1.9	34.8 ± 2.1
Open in (mm)	7.6 ± 2.4	14.7 ± 3.6	3.3 ± 1.1	6.9 ± 2.0
Close in (mm)	27.6 ± 4.8	14.8 ± 5.9	10.6 ± 1.0	5.4 ± 0.8
Outlever (mm)	89.1 ± 8.7	85.3 ± 10.1	38.7 ± 2.6	40.2 ± 2.6
Bite force (*N*)	334.8 ± 99.6	383.3 ± 88.2	35.5 ± 10.1	56.0 ± 10.2
Close/out	0.45 ± 0.07	0.21 ± 0.07	0.38 ± 0.03	0.15 ± 0.02

SVL, snout-vent-length; HL, head length; HW, head width; HH, head height; LJL, lower jaw length; snout, distance from the back of the jugal to the tip of the jaw; open in, jaw in lever for opening; close in, jaw in lever for closing; outlever, distance from the quadrate to the snout tip; close in/out, jaw in lever for closing relative to jaw out lever.

A MANCOVA testing for differences in head shape indicated significant species (Wilks' lambda $= 0.078$; $F_{8,76} = 112.53$; $P < 0.001$) and species by age class interaction effects (Wilks' lambda $= 0.55$; $F_{8,76} = 7.72$; $P < 0.001$). Differences in head shape between age classes were, however, not significant after taking into account variation in body size (Wilks' lambda $= 0.94$; $F_{8,76} = 0.59$; $P = 0.78$). For juveniles, a MANCOVA performed on the morphometric data indicated significant differences in head shape between species (Wilks' lambda $= 0.018$; $F_{8,39} = 266.54$; $P < 0.001$). Subsequent univariate ANCOVAs indicated that differences in head shape were significant for most variables with *Dracaena* having generally bigger heads. However, the in-lever for jaw closing and the lever ratio were significantly greater in *Tupinambis* (Table 1). For adults, significant differences in head shape were also present (Wilks' lambda $= 0.12$; $F_{8,29} = 25.64$; $P < 0.001$). However, the univariate ANCOVAs indicated that species were not different in head length, lower jaw length, and the length of the jaw outlever after Bonferroni correction (Table 1). Although adult *Dracaena* had wider and taller heads (Fig. 2), the closing inlever and the lever ratio were greater for *Tupinambis* compared to *Dracaena*.

An ANCOVA performed on the bite force data for juveniles indicated significant differences between species with *Dracaena* biting much harder for a given body size than *Tupinambis* ($F_{1,46} = 160.45$; $P < 0.01$; Fig. 3). However, adults of both species did not differ in bite force ($F_{1,36} = 0.30$; $P = 0.59$; Fig. 3).

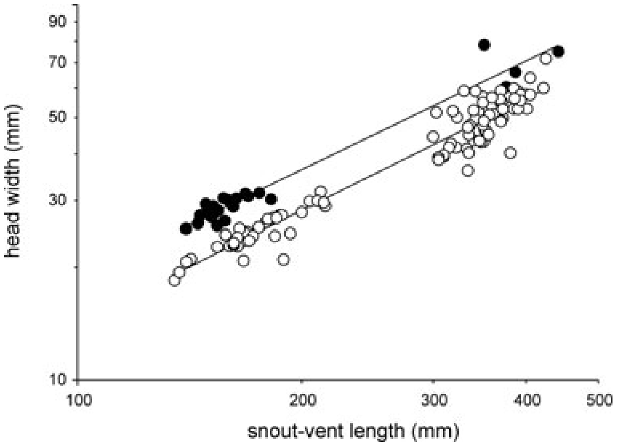

Figure 2. Plot of head width against snout-vent length for juvenile and adult *D. guianensis* (filled circles) and *T. merianae* (open circles) showing that *D. guianensis* has a wider head for its body size compared to *T. merianae*. Note the log scale on both X- and Y-axes.

Skull Shape

A factor analysis performed on the size-free skull and lower jaw dimensions retained three factors jointly explaining 73.14% of the total variance in the dataset. Whereas the first factor was strongly correlated with relative jaw length and the distance between the symphysis and the jaw articulation, the second one was strongly positively correlated with muzzle length and negatively with frontoparietal skull height. The third axis was correlated with the distance from the symphysis to the most posterior tooth and to the coronoid (Table 2). A MANOVA detected

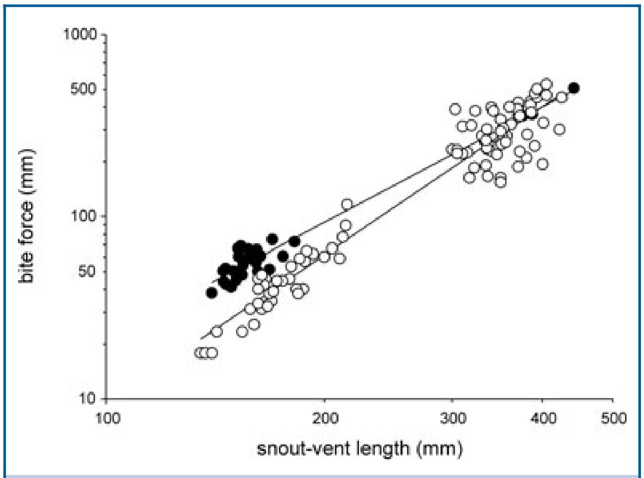

Figure 3. Plot of bite force against snout-vent length for juvenile and adult *D. guianensis* (full circles) and *T. merianae* (open circles). Whereas juveniles *D. guianensis* bite harder than juvenile *T. merianae*, this is not the case for adults. Note the log scale on X- and Y-axes.

Table 2. Results of a factor analysis with varimax rotation performed on the size free morphometric data of the skull and mandible in Teiid lizards (see Material and Methods for details about sample).

	Factor 1	Factor 2
Eigenvalue	2.65	2.98
Percentage variance explained	29.43	22.00
Residual distance symphysis – posterior tooth (mm)	0.327	0.201
Residual distance symphysis – anterior articulation (mm)	**0.856**	0.075
Residual distance symphysis – posterior articulation (mm)	**0.869**	0.184
Residual distance symphysis to back retroarticular process (mm)	**0.745**	0.164
Residual distance symphysis to tip of coronoid process (mm)	0.138	0.007
Residual skull length (mm)	0.248	0.630
Residual muzzle length (mm)	0.307	**0.780**
Residual frontoparietal skull height (mm)	0.197	**0.775**
Residual mid-orbital skull height (mm)	0.532	0.517

Bolded values represent loadings greater than 0.7.

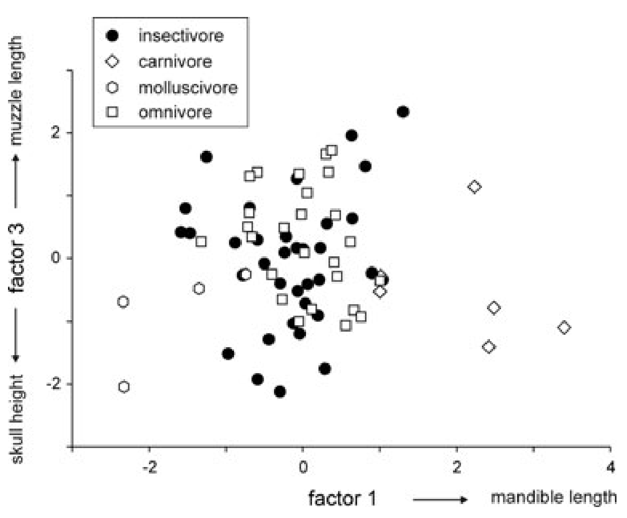

Figure 4. Scatter plot illustrating the results of a principal component analysis performed on linear dimensions describing skull shape in a wide range of teiid lizards encompassing all genera represented in Figure 1. *Dracaena* is characterized by a tall skull with a relatively short mandible and muzzle relative to other teiids. Open symbols represent individuals belonging to the Tupinambinae; dietary groups (see Fig. 1) are indicated by different symbols. Note that whereas the two clades do not differ from one another in skull shape, dietary groups do.

significant differences in skull shape between diet groups (Wilks' Lambda $= 0.369$; $F_{9,\ 155.91} = 8.78$; $P < 0.001$). Subsequent univariate *F*-tests indicated that effects of diet were significant on factor one ($F_{3,66} = 28.93$; $P < 0.001$) and approached significance on factor two ($F_{3,66} = 2.25$; $P = 0.09$). Differences between dietary groups were nonsignificant on factor 3 ($F_{3,66} = 1.28$; $P = 0.29$). Bonferroni post-hoc tests indicated that insectivores and omnivores were not different from one another ($P > 0.05$). Yet, all other dietary groups, including the molluscivorous Dracaena were different from one another (all $P < 0.001$). An examination of the plot of factor one against factor two shows that *Dracaena* is characterized by having a short mandible, a short muzzle, and a tall skull compared to other teiids (Fig. 4).

Feeding Behavior

Dracaena guianensis approached snails immediately when offered and picked them up with their jaws. Next, the head was rotated dorsally and the jaws opened slightly causing the snail to roll toward the back of the tooth rows. Snails were repositioned in the oral cavity using the tongue before being crushed. Multiple bites were used to crush snails and repositioning took place in between crushing. Shell fragments were expelled from the mouth by the tongue before swallowing (Dalrymple, '79) as has been observed in other snail-eating lizards (Herrel and Holanova, 2008). During the manipulation, snails were never dropped.

In contrast, although *T. merianae* also used the jaws to grab the snails, it used kinetic inertial food transport. During inertial transport, the jaws are opened rapidly and simultaneously the head-neck system is drawn back, initiating the backward displacement of the prey after release from the jaws. At maximal gape, the entire head and body move forward and the jaws are closed (Gans, '69; Smith, '82; Schwenk, 2000; Montuelle et al., 2009). *Tupinambis merianae* used mostly inertial movements to reposition the snails for crushing in contrast to the tongue-based repositioning observed in *Dracaena*. *Tupinambis* lizards often needed several attempts and snails were dropped repeatedly. After crushing, shell fragments were removed by the tongue, by shaking the head, but also by dropping the snail followed by wiping movements of the jaws on the bottom of the cage resulting in the removal of shell fragments.

Video Analysis

A MANOVA performed on the kinematic dataset of transport cycles and crush bites revealed significant species (Wilks' lambda $= 0.13$; $F_{6,21} = 23.81$, $P < 0.001$) and cycle type effects (transport/crush cycle; Wilks' lambda $= 0.061$; $F_{6,21} = 54.26$, $P < 0.001$). Interaction effects were also significant (Wilks' lambda $= 0.34$; $F_{6,21} = 6.93$, $P < 0.001$) suggesting that the changes in kinematics with cycle type are species dependent. Owing to the significance of the interaction effects, transport and crush cycles were analyzed separately for *T. merianae* and *D. guianensis.*

For pure transport cycles significant species effects were observed (Wilks' lambda $= 0.10$; $F_{6,13} = 19.07$, $P < 0.001$) with differences in gape distance ($F = 5.04$, $P = 0.038$), the duration of the slow open phase (dSO; $F = 6.8$, $P = 0.02$), the duration of the fast open phase (dFO; $F = 39.09$, $P < 0.001$) and the duration of fast close phase (dFC; $F = 25.49$, $P < 0.001$) being significant. Transport of snails in *D. guianensis* is associated with greater gape distances and shorter slow open phases than in *T. merianae* (Fig. 5). The duration of the fast open and fast close phases is, however, longer in *D. guianensis* (Table 3). These kinematic differences reflect the difference in feeding strategy contrasting tongue based with inertial transport and repositioning.

For crush bites, we also detected a significant species effect (Wilks' lambda $= 0.035$; $F_{6,3} = 13.65$, $P = 0.028$) with significant differences in the duration of the slow open phase ($F_{1,8} = 9.36$, $P = 0.02$), the duration of the fast open phase ($F_{1,8} = 49.81$, $P < 0.001$), the duration of the slow close phase ($F_{1,8} = 19.84$, $P = 0.002$), and the total duration of a crush cycle ($F_{1,8} = 16.59$, $P = 0.004$). Crushing of snails in *D. guianensis* is associated with shorter slow open phases, longer fast open phases, longer slow close phases, and a greater total crush cycle duration compared to *T. merianae* reflecting a careful handling and pronounced crushing of snails (Fig. 5; Table 3).

An analysis performed on variables related to the entire feeding event revealed a significant species effect (Wilks' lambda $= 0.38$; $F_{7,44} = 10.08$, $P < 0.001$) with significant differences in the total number of transport cycles ($F_{1,50} = 61.11$, $P < 0.001$), the total number of crush bites ($F_{1,50} = 34.28$, $P < 0.001$), the total duration of transport ($F_{1,50} = 40.58$, $P < 0.001$), and the total number of swallowing cycles ($F_{1,50} = 12.97$, $P = 0.001$). The total number of transport and swallowing cycles, the number of crush bites, and the total transport duration are greater in *D. guianensis* (Table 4; Fig. 5). The duration of the swallowing stage is, however, not different between species ($F_{1,50} = 1.49$, $P = 0.23$) suggesting a more careful handling of snails during intraoral transport rather than an intrinsic limitation on the speed of jaw movement.

DISCUSSION

Although both species include snails in their diet, *D. guianensis* is a true molluscivorous lizard. *Tupinambis merianae* is omnivorous and eats a wide variety of foods including fruits, vertebrates, insects, and molluscs (Dessem '85; Castro et al., '91; Mercoli and Yanosky, '94; Kiefer and Sazima, 2002; Martins, 2006). Our data demonstrate that *D. guianensis* is morphologically and behaviorally different from *T. merianae* and that these differences appear related to their specialized diet.

Our data on head morphometrics in *D. guianensis* and *T. merianae* show that juvenile *D. guianensis* have longer, wider, and taller heads allowing for bigger jaw muscles and resulting in a greater bite force. Unexpectedly, the in-lever for closing is smaller in *D. guianensis* compared to *T. merianae* in both juveniles and adults. Yet, juvenile *D. guianensis* do bite harder than juvenile *T. merianae* which may be due to their relatively bigger heads. Our data on skull morphology show that the mandible of *Dracaena* is relatively short, yet the skull is tall and the muzzle short. Although one would expect this to result in a greater bite performance, this is not the case. Consequently, the relative decrease in bite force in adult relative to juvenile *D. guianensis* in comparison to *T. merianae* suggests growth-related differences in the jaw adductor musculature, yet this remains to be tested explicitly.

The size-dependent decrease in bite force suggests that selection on bite-force capacity may be stronger in juvenile *D. guianensis* or, alternatively, that selection on relative bite force capacity is high in adult *T. merianae.* As adults of both *D. guianensis* and *T. merianae* have large bite forces in absolute terms because of their large body and head size, hard-shelled prey become potential dietary items. Juvenile lizards have smaller heads resulting in lower absolute bite forces thus preventing them from crushing hard or tough prey items. The enlarged heads of juvenile *D. guianensis* may, however, allow them to exploit this food resource as has been suggested for other specialized

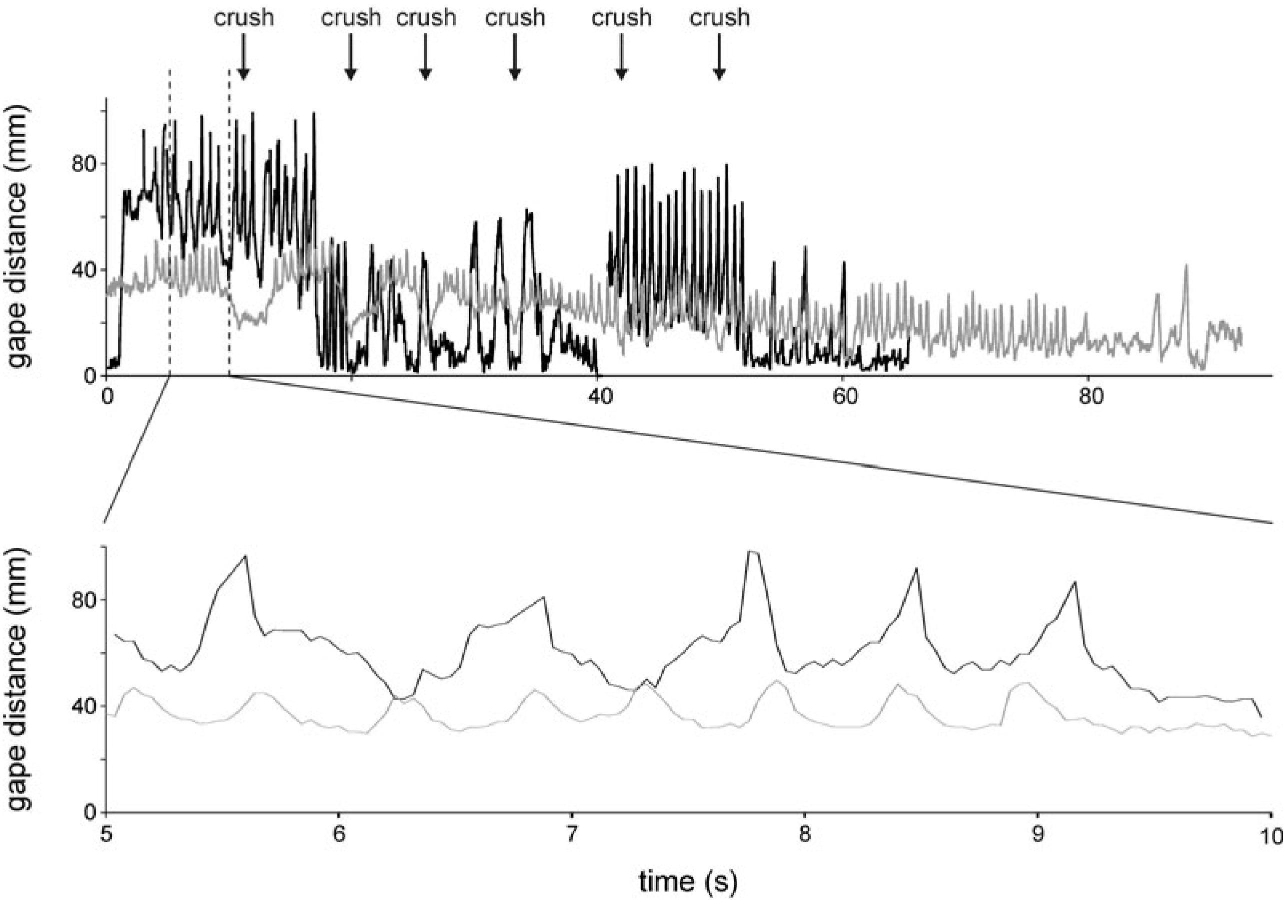

Figure 5. Top: plot of gape distance against time for a total feeding event in *D. guianensis* (gray line) and *T. merianae* (black line). Bottom: blow-up of 5 sec of the sequence to illustrate individual gape cycles in both species. Selected crushing events for *D. guianensis* are indicated by arrows.

molluscivores such as *Chamaeleolis* (Herrel and Holanova, 2008). Unfortunately, no quantitative data on the diet of juvenile *D. guianensis* are available, and we can only speculate about the relative importance of snails in the diet of the juveniles. However, one argument for the importance of snails in the diet of juveniles is that, unlike in *Tupinambis* and many other lizards, molariform teeth are present in juveniles.

Given the semiaquatic lifestyle of *D. guianensis,* we predicted a specialized mechanism for food transport allowing precise manipulation and repositioning of snails during intraoral transport. Whereas *D. guianensis* uses its tongue and gravity to reposition snails, *T. merianae* uses predominantly inertial movements to reposition and transport snails. Consequently, snails were often dropped by *T. merianae* during transport and repositioning (on average 4 ± 2 times in a single sequence). Given the aquatic lifestyle of *Dracaena*, dropping snails could involve losing them in the water and thus an alternative transport method is used that assures accurate transport and repositioning and is dependent on the use of the tongue. Another striking difference in the manipulation of snails was associated with the use of the tongue to expel snail fragments in *Dracaena*. Whereas this has been observed in other specialized molluscivorous lizards (Herrel and Holanova, 2008), *T. merianae*, in contrast, tends to drop the snail to wipe the snail fragments from its mouth after which the snail is picked up again. Whether these differences in tongue use are reflected in tongue morphology remains unknown as data on tongue structure in *Dracaena* are not available in the literature.

During transport cycles, the gape distance in *T. merianae* is smaller than that observed in *D. guianensis*. This can be explained by the differences in feeding behavior between the two species whereby *D. guianensis* flexes the head dorsally and lets the snail roll to the back of the tooth rows while opening the

Table 3. Summary table representing the means and standard deviations of the kinematic data of transport and crush cycles in adult *T. merianae* and *D. guianensis*.

	Transport		Crushing	
	T. merianae	*D. guianensis*	*T. merianae*	*D. guianensis*
Gape distance (mm)	**37.23 ± 8.78**	**43.07 ± 2.02**	46.77 ± 9.32	38.86 ± 6.54
Duration slow opening (s)	**0.29 ± 0.09**	**0.20 ± 0.08**	**0.29 ± 0.09**	**0.18 ± 0.02**
Duration fast opening (s)	**0.07 ± 0.02**	**0.13 ± 0.02**	**0.06 ± 0.01**	**0.12 ± 0.01**
Duration fast closing (s)	**0.12 ± 0.03**	**0.18 ± 0.02**	0.09 ± 0.02	0.18 ± 0.11
Duration slow closing (s)	0.23 ± 0.06	0.18 ± 0.06	**0.73 ± 0.23**	**1.64 ± 0.44**
Gape cycle duration (s)	0.71 ± 0.13	0.69 ± 0.11	**1.16 ± 0.30**	**2.12 ± 0.48**

Significant differences between the species are indicated in bold.

Table 4. Summary table representing the means and standard deviations of variables describing the entire feeding event in adult *T. merianae* and *D. guianensis*.

	T. merianae (*N* = 22)	*D. guianensis* (*N* = 30)
Number of transport cycles	**59.73 ± 17.07**	**99.47 ± 22.03**
Number of crush cycles	**4.27 ± 1.12**	**6.93 ± 1.96**
Number of swallowing cycles	**13.05 ± 4.18**	**18.93 ± 7.08**
Duration of intraoral transport stage (s)	**52.21 ± 16.84**	**86.03 ± 23.40**
Duration of swallowing stage (s)	17.83 ± 5.33	19.58 ± 5.76

Significant differences between species are indicated in bold. *N*, number of feeding events analyzed.

jaws widely. In contrast, *T. merianae* transports the snail using inertial movements. The shorter fast opening and fast closing phase durations in *T. merianae* can also be explained by the differences in feeding behavior with rapid inertial movements being associated with short FO and FC durations (Montuelle et al., 2009).

Although we expected the SO duration to be shorter in *T. merianae* compared to *D. guianensis*, this was not the case and may be explained by the extensive positioning of the snail in *T. merianae* before initiating inertial transport and repositioning. Moreover, our data indicated a difference in the duration of the slow closing phase with *D. guianensis* having longer slow closing phase durations than *T. merianae* that may be associated with extensive crushing. In addition, a significant difference between transport and crush cycles was observed with crush cycles being associated with longer SC phases than transport cycles as predicted (Table 3). *Dracaena guianensis* also used a larger number of transport, crush, and swallowing cycles resulting in an increase in total transport duration. Thus, the feeding behavior in *D. guianensis* seems more specialized for eating snails and is associated with a greater overall duration of food manipulation. This can be explained by the more accurate and secure handling of snails and expelling of the shell fragments by the tongue during transport and crushing (Dalrymple, '79).

In summary, our data demonstrate morphological and behavioral differences between *D. guianensis* and *T. merianae*. Although feeding upon snails takes longer in *D. guianensis*, their feeding behavior seems to be better suited to handle this hard and brittle food type while minimizing prey loss. Our results suggest, moreover, that selection may operate on juvenile life-history stages in *D. guianensis*, resulting in large heads and large bite forces. Since adults do not differ in bite force from adult *T. merianae*, but do have larger heads, this suggests that the adult phenotype may be simply the result of selection on juveniles. In contrast, adult *T. merianae* have greater jaw in-levers for closing suggesting selection on bite performance in adults which may be related to their territorial or antipredator behavior (Herrel et al., 2009).

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Differential growth of naturally and sexually selected traits in an *Anolis* lizard

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Abstract

Sexual dimorphism may be indicative of differential natural and/or sexual selection pressures acting on males and females. Although similar degrees of adult sexual dimorphism may be observed among populations and species, underlying physiological, developmental and ecological processes driving growth during the juvenile stages may vary. Unraveling how and when differences arise during ontogeny is important to our understanding of the evolutionary patterns observed among adults. In addition, growth patterns may differ among traits within a single individual. *Anolis* lizards vary greatly in the degree of adult sexual size and shape dimorphism. Ultimate hypotheses (e.g. sexual selection, habitat partitioning) have been put forward to explain this variation, but proximate mechanisms operating during the juvenile stages remain largely unknown. Herein, we aim at elucidating the proximate mechanisms of sexual dimorphism in head and limb shape, performance and dewlap size in *Anolis baracoae*, a crown-giant anole. We used a longitudinal approach and raised 23 captive-bred juvenile *A. baracoae* lizards through adulthood. By quantifying body size, head and limb dimensions, dewlap size and bite performance, we obtained growth trajectories that were compared between the sexes and among traits. Our results show that the growth of head and limb shape is similar in males and females, whereas dewlap size and bite performance diverge at a specific age. Based on these results, we hypothesize that, whereas selection favors a similar head and limb shape in males and females, selection pressures on dewlap size and bite performance likely differ between the sexes. In addition, dewlap size starts diverging around the time the animals reach sexual maturity, whereas bite performance diverges much earlier in (sexually) immature males.

Introduction

Phenotypic differences between males and females have attracted the attention of evolutionary biologists for over a century (Darwin, 1871). Sexual dimorphism has been invoked as indicative of differential sexual and natural selection pressures, including inter- and intrasexual selective pressures, intersexual resource partitioning and/or different reproductive roles of males and females (Andersson, 1994; Losos, Butler & Schoener, 2003). Apart from shedding light on the ultimate causes of sexual dimorphism, however, our understanding of how and when the differences develop during ontogeny is essential to understand evolutionary patterns observed among adults (Badyaev, 2002; Cox & John-Alder, 2007; Howard, 2009). Sexual dimorphism can arise because of different growth trajectories in males and females before and after maturation, different size at birth and/or differential survival or dispersal (Badyaev, Hill & Whittingham, 2001*a*; Badyaev, Whittingham & Hill, 2001*b*; Badyaev, 2002; Haenel & John-Alder, 2002). Intersexual differences in size at birth and in growth trajectories are typically caused by differences in developmental or physiological pathways (e.g. Cox & John-Alder, 2007). Differential survival and dispersal are linked to ecological factors that may also influence males and females differently (e.g. predation, competition; Badyaev *et al*., 2001*b*). To complicate matters even further, sexual dimorphism can be plastic and the degree to which it is expressed often depends on environmental conditions, such as food availability, predation pressure, humidity and density (e.g. Madsen & Shine, 1993; Stamps, Losos & Andrews, 1997; Stamps, 1999; Bronikowski, 2000; LeGalliard *et al*., 2006; Bonneaud *et al*., in press). Because males and females may differ in their response to limiting resources, their growth rates may differ, potentially resulting in sexual size dimorphism at adulthood (e.g. Niewiarowski & Roosenburg, 1993; Perry, 1996; Stamps *et al.*, 1997; Haenel & John-Alder, 2002; LeGalliard *et al.*, 2006; Lailvaux, Gilbert & Edwards, 2012).

Whereas most studies have compared growth patterns of a single trait, that is body size, between sexes of different species or populations, growth patterns may also differ between traits within a single individual (e.g. Humphrey, 1998; Badyaev *et al.*, 2001*a*,*b*; Howard, 2009) and between size and shape variables (Butler & Losos, 2002; Schwarzkopf, 2005; Kaliontzopoulou, Carretero & Llorente, 2008; Hasumi, 2010). Differences in growth patterns among traits result from differences in the allocation of energy toward each trait, which in turn depends on the immediate functional importance of the trait at a certain age. For instance, within one population of house finches, the heterochrony between body and bill traits seemed related to the onset of various life-history events, such as (early) fledging and self-feeding (Badyaev *et al.*, 2001*a*).

In this study, we document patterns of sexual dimorphism in body and limb dimensions, bite performance and dewlap size in an *Anolis* lizard. *Anolis* lizards are a textbook example of an adaptive radiation and the different ecomorphs –species which inhabit similar microhabitats and which resemble each other behaviorally and phenotypically – evolved independently on each of the bigger Caribbean islands (Losos & Chu, 1998; Losos *et al.*, 1998). It has been documented previously that ecomorphs vary in degree of adult sexual dimorphism in body size and shape (Butler, Schoener & Losos, 2000; Butler & Losos, 2002; Losos *et al.*, 2003; Butler, 2007), with microhabitat partitioning and sexual selection as the most likely candidate selective pressures explaining this variation (Butler *et al.*, 2000). The habitat hypothesis links habitat visibility to sexual size dimorphism in such a way that sexual size dimorphism is predicted to be greater in open (i.e. high visibility) habitats as intruders will more easily see the territory holder and will be more effectively deterred than in closed (i.e. low visibility) habitats (Butler *et al.*, 2000).

In addition, *Anolis* lizards are also sexually dimorphic in dewlap size (Losos *et al.*, 2003; Vanhooydonck *et al.*, 2005*a*; Johnson & Wade, 2010). Most *Anolis* species possess a dewlap, that is an extendable throat fan, which varies greatly in size, shape and pattern among species, populations or individuals (Vanhooydonck *et al.*, 2005*a*,*b*, 2009; Nicholson, Harmon & Losos, 2007). In most island species, females also possess a dewlap, but it is generally smaller and less frequently used during displays than in males (Johnson & Wade, 2010, but see Harrison & Poe, 2012). Although the function of the dewlap is still not fully understood, it has been shown to be an important signal during social inter- and intrasexual interactions. In addition, it may also serve as species recognition signal during encounters with congenerics and as a pursuit deterrence signal in encounters with predators (e.g. Echelle, Echelle & Fitch, 1978; Fitch & Hillis, 1984; Losos & Chu, 1998; Leal, 1999; Jenssen, Orrell & Lovern, 2000; Lailvaux & Irschick, 2007; Nicholson *et al.*, 2007). Some ultimate hypotheses, such as sexual selection, species recognition or ecomorph convergence, have been put forward to explain the diversity in male dewlap design and dewlap dimorphism across *Anolis* species (Nicholson *et al.*, 2007; Harrison & Poe, 2012). However, the proximate mechanisms shaping the dewlap during ontogeny, driving sexual dimorphism in adult dewlap size, have not been elucidated. It is, however, known that the growth of the dewlap is maintained under conditions of dietary restriction in males (Lailvaux *et al.*, 2012).

In this study, we document the patterns in sexual dimorphism in shape, performance (bite force) and dewlap size in *Anolis baracoae*, a crown-giant anole from Cuba. The crown-giant ecomorph as a group has been previously shown to be dimorphic in both size and shape (Butler *et al.*, 2000; Butler & Losos, 2002; Losos *et al.*, 2003). We use a longitudinal approach and raised *A. baracoae* juveniles through adulthood under homogeneous laboratory conditions to avoid a potential differential response of males and females to limited resources (but see Lailvaux *et al.*, 2012). By quantifying body size, head and limb dimensions, dewlap size and bite performance at set time intervals, we determine whether growth trajectories differ between males and females, and if so, at what point in time the sexes diverge. In addition, we compare the growth trajectories of head and limb dimensions, which are generally to be thought of as naturally selected traits, with the growth trajectory of dewlap size, a primarily sexually selected trait, and bite performance (an ecologically relevant performance trait that is of crucial importance in male–male interactions and may allow individuals to increase their dietary breadth).

Material and methods

Study animals

We obtained 23 captive-bred juvenile and subadult *A. baracoae* ($N_{males} = 14$, $N_{females} = 9$) through a breeder in October 2007. These animals are the first and second generation offspring from wild caught, non-related adult individuals. Details on the breeding conditions are described in Holáňová & Hribal (2005). Upon arrival in the laboratory in Antwerp, the lizards were kept in individual cages ($h \times w \times d_{small} = 0.35 \times 0.60 \times 0.60$ m and $h \times w \times d_{large} = 1.00 \times 0.40 \times 0.60$ m) in a climate-controlled room with a day : night cycle of 10:14 h and room temperature set at 28°C. Each cage was equipped with a light bulb (8W), providing extra heat and light for 10 h per day, some branches and leaf litter scattered on the floor. We fed the lizards with calcium-dusted crickets, waxworms and banana twice a week. In addition to spraying the lizards three times a week, water was provided *ad libitum*.

Upon arrival in the laboratory, we measured bite force, body, head and limb dimensions of all individuals and took a digital picture of the dewlap for the first time (measurement 0; measurement details indicated in the succeeding text). Subsequently, these measurements were repeated 11 times at varying time intervals spanning a total of 3.5 years. Measurements 1–5 were taken at a 4-week interval; measurements 6–7 at a 6-week interval; measurements 8–10 at a 5–6-month interval and the last measurement 20 months after measurement 10.

Measurements

All measurements were carried out in a climate-controlled room set at 28°C during daytime hours.

We measured *in vivo* bite force for all individuals using an isometric Kistler force transducer (type 9203) mounted on a purpose-built holder and connected to a Kistler charge amplifier (type 5995; see Herrel *et al.*, 1999 for a detailed description of the set-up). A lizard was taken from its cage and immediately induced to bite the force transducer by tapping it on the side of the mouth. The tapping typically resulted in a threat response in which the jaws are opened maximally. The free ends of the holder (bite plates) were then placed between the jaws, which resulted in prolonged biting. Each bite was scored as 'good' or 'bad' based on the willingness of the lizard to bite the plates and the vigor with which it bit. This was repeated five times in a row. As an estimate of maximal bite-force capacity, we used the highest bite force of the five bites for each individual. Because of logistical problems (i.e. unavailability of the set-up), we were unable to execute the third bite-force measurement, that is 8 weeks after the arrival of the animals in the laboratory.

Subsequent to the bite-force measurements, we took the following morphological measurements using digital calipers (Mitutoyo CD-15DC, Mitutoyo Europe GmbH, Neuss, Germany; accuracy of 0.01 mm) for each individual: snout-vent length (SVL), head length, femur length, tibia length, metatarsus length and length of longest toe of the hind foot. Head length was measured from the tip of the snout to the posterior edge of the parietal bone. We summed the four segments of the hind limb and used them as a measure of total hind limb length in subsequent analyses.

Lastly, we positioned the lizards sideways on a paper grid (grid cells = 10 mm × 10 mm) and pulled the base of the second ceratobranchial gently forward with a pair of forceps to obtain a measure of dewlap size. As the dewlap consists of a skin flap attached to the lizard's throat on the one side and to the hyoid bone on the other, the dewlap becomes maximally extended when the ceratobranchial is pulled forward completely (Font & Rome, 1990). After positioning the lizard in such a way that its extended dewlap was parallel to the lens (Tamron SP DI AF 90 mm; TAMRON Europe GmbH, Cologne, Germany) of the camera (Nikon D70S with a Sigma EM-140DG ring flash; Nikon Europe BV, Amsterdam, The Netherlands), we took a digital picture. Using the program TPSDIG v1.40 (J. Rohlf, SUNY, Stonybrook, NY, USA), we digitized the outer edge of the dewlap and calculated total dewlap size for each individual. This method of measuring dewlap size yields highly repeatable results (Vanhooydonck *et al.*, 2005*a*).

Analyses

We compared the associations between the response variables dewlap size, head length, hind limb length and maximal bite force and the explanatory variable SVL between male and female lizards using mixed models. Both SVL and SVL^2 were added as continuous covariates (after centering on zero by subtracting mean SVL). Sex and the two-way interactions between sex and both SVL and SVL^2 were added to compare associations between males and females. To incorporate the repeated measures structure of the data in the analyses, we added individual and its interactions with SVL and SVL^2 as random effects. We used an analysis of variance (ANOVA) approach to test for the significance of the fixed effects and Satterthwait's method (lmerTest in R v. 2.15; free software to be downloaded from: http://www.r-project.org/) to determine the degrees of freedom. Parameter estimates and their statistical significant are reported and estimates are used to visualize the model fit in the graphs. Model fit was explored by testing normality of the residuals and inspecting associations between the fitted and residual values.

Results

ANOVA results, the parameter estimates and the statistical significance of the factors in the mixed models comparing the association between head length, hind limb length, dewlap size and maximal bite force with SVL between males and females are given in Tables 1 and 2. Most importantly, two interactions appeared to be statistically significant, suggesting a sexual growth dimorphism in dewlap size and bite force (Table 1). For head length and hind limb length, these interactions were not statistically significant, suggesting that growth in these traits is similar in males and females (Table 1, Fig. 1a and b).

For dewlap size, we found a difference in the quadratic term between males and females (significant SVL^2 × sex interaction, Table 2). In males, there was a slight, albeit not significant,

Table 1 Analysis of variance results for the mixed models comparing the associations between head length, hind limb length, dewlap size and bite performance with snout-vent length (SVL; centered around zero) between male and female *Anolis baracoae* lizards

Source	Head length	Hind limb length	Dewlap size	Bite performance
SVL	**$F_{1,67} = 17050$*****	**$F_{1,17} = 4046$*****	**$F_{1,17} = 324$*****	**$F_{1,21} = 123$*****
SVL^2	$F_{1,41} = 0.10$	$F_{1,41} = 1.8$	$F_{1,26} = 0.14$	**$F_{1,39} = 5.1$***
Sex	**$F_{1,236} = 6.6$***	**$F_{1,140} = 8.2$****	$F_{1,210} = 0.31$	**$F_{1,191} = 17.6$*****
SVL × sex	$F_{1,67} = 2.0$	$F_{1,17} = 0.0$	$F_{1,19} = 2.27$	**$F_{1,21} = 6.9$***
SVL^2 × sex	$F_{1,236} = 0.4$	$F_{1,140} = 1.2$	**$F_{1,210} = 7.4$****	$F_{1,191} = 0.1$

Asterisks refer to the level of significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Statistically significant effects are shown in bold.

Table 2 Overview of parameter estimates (±SE) and associated significance tests of the mixed models comparing the association between snout-vent length (SVL) and head length, hindlimb length, dewlap size and bite performance (centered around zero) between male and female *Anolis baracoae*

Effect	Head length	Hind limb length	Dewlap size	Bite performance
Intercept[a]	**28.4 ± 0.11*****	**62.2 ± 0.40*****	**416 ± 16*****	**11.1 ± 0.60*****
SVL[a]	**0.31 ± 0.003*****	**0.74 ± 0.013*****	**10.6 ± 0.70*****	**0.30 ± 0.03*****
SVL^{2a}	0.0002 ± 0.0001	**0.0016 ± 0.0005****	0.026 ± 0.015	**0.0026 ± 0.0008****
Sex[b]	0.07 ± 0.17	0.76 ± 0.63	9.27 ± 25.1	**2.13 ± 0.95***
SVL × sex[c]	0.0064 ± 0.0046	0.0042 ± 0.020	1.62 ± 1.08	**0.12 ± 0.04***
SVL^2 × sex[c]	0.0001 ± 0.0002	0.0010 ± 0.0009	**0.07 ± 0.025****	0.0004 ± 0.0013

The bottom two rows provide tests for differences between males and females in the linear and quadratic terms, respectively (i.e. two-way interactions). Asterisks refer to the level of significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Parameter estimates significantly different from zero are shown in bold.

[a]Parameter estimate describing the association for males.

[b]Difference in intercept between males and females.

[c]Difference in slope between males and females.

positive quadratic effect of SVL, whereas in females the quadratic term was significantly smaller (negative difference in slopes, Table 1) and appeared to be significantly negative (slope = 0.04, SE = 0.02, t = 2.04, $P = 0.04$). A graphic exploration showed a very comparable increase of dewlap area with SVL for animals with SVL smaller than 100. When growing larger, however, the estimated curves diverged between males and females (Fig. 1c). In females, the growth after reaching a SVL of 100 mm was less pronounced compared with males, leading to a male biased sexual dimorphism in larger/older individuals (Fig. 1c).

For bite force, the linear term differed between males and females (significant SVL × sex interaction, Table 2). In males, there was a slight, albeit not significant, negative linear effect of SVL, whereas in females the linear term was significantly smaller (negative difference in slopes, Table 2) and appeared to be significantly negative (slope = 0.29, = 0.12), t = 2.40, $P = 0.02$. A graphic exploration showed a very comparable increase of maximal bite force with SVL for animals with SVL smaller than 80 mm. When growing larger, however, the estimate curves diverged between males and females (Fig. 1d). In females, the increase in maximal bite force after reaching a SVL of 80 mm was less pronounced compared with males, leading to a male biased sexual dimorphism in larger/older individuals (Fig. 1d).

For all models, residuals did not show signs of deviations from normality (Shapiro Wilks' W > 0.95) and there were no associations between the fitted values and the residuals, suggesting appropriate model fit.

Discussion

Our results show differences between the sexes in the growth patterns of different traits in *A. baracoae*. Whereas head length and hind limb length grow in a similar fashion in males and females, dewlap size and bite performance diverge between males and females during growth. Even more so, the timing at which dewlap size and bite performance start diverging differs. Whereas growth in dewlap size is similar until the animals reach a SVL of 100 mm, bite-force growth curves start diverging much earlier (i.e. at a SVL of 80 mm). No data on age or size at sexual maturity in *A. baracoae* lizards are currently available in the literature or from professional breeders. However, to estimate body size and age at sexual maturity of our animals, we made use of such data available for a wide range of *Anolis* species, including other crown-giants (Ruibal, Philibosian & Adkins, 1972; Andrews & Rand, 1974; Trivers, 1976; Stamps & Krishnan, 1997). Size at sexual maturity in *Anolis* lizards can be expressed as a proportion of maximum adult body size in males and females. Based on actual data of 33 *Anolis* species, the ratio of size at sexual maturity to maximum body size averages 0.73 (range: 0.68–0.81) in males and 0.77 (range: 0.64–0.82) in females (Stamps & Krishnan, 1997). Using the average ratio and maximum body size of *A. baracoae* as reported in Rodriguez-Schettino (1999; 150.4 mm in males, 135.5 mm in females), we estimate males reach sexual maturity at a SVL of 110.4 mm and females at 103.8 mm. Our results thus suggest that dewlap size diverges around the time the animals become sexually mature, whereas bite performance diverges already in (sexually) immature animals.

We hypothesize that the difference in timing of the sexual divergence in dewlap size and bite performance during growth may be related to a difference in the functional role of the two traits at a certain age (cf. Badyaev & Martin, 2000). For dewlap size, our data suggest the dewlap in males starts to grow faster than the dewlap in females around the time they reach sexual maturity. In *Anolis* lizards, dewlap extensions are part of the typical displays performed by males during fights with conspecific males, during interactions with females and in antipredator displays (Williams & Rand, 1977; Leal & Rodriguez-Robles, 1997*a*,*b*; Losos & Chu, 1998; Leal, 1999; Jenssen *et al.*, 2000; Tokarz, Paterson & McMann, 2003; Johnson & Wade, 2010). Females typically use dewlap extensions less frequently (Jenssen *et al.*, 2000; Orrell & Jenssen, 2003; Johnson & Wade, 2010), as do juveniles (Lovern & Jenssen, 2001). It can be expected that dewlap size is of high functional importance to male *A. baracoae* upon reaching

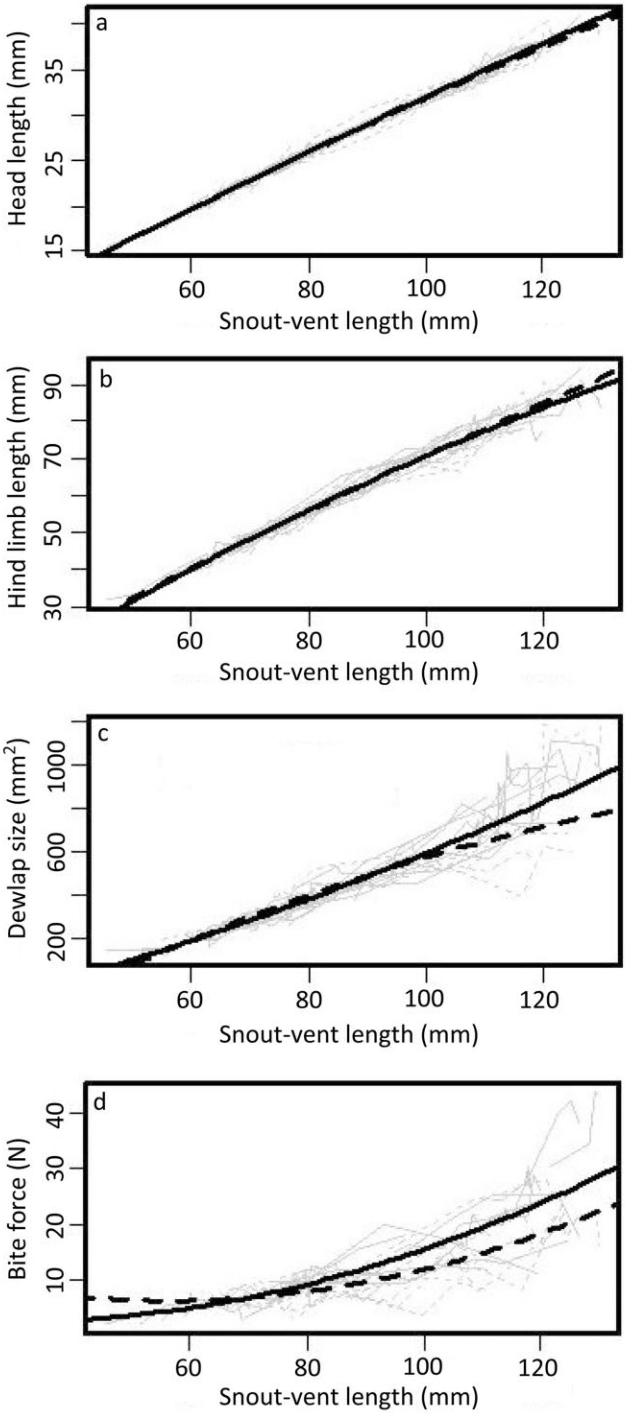

Figure 1 Association between snout-vent length (SVL) and head length (a), hind limb length (b), dewlap size (c) and bite performance (d) in *Anolis baracoae* males and females. Grey lines represent the individual-specific measurements. Estimates curves, based on a mixed model analysis (see text and Table 1 for details), are shown in black. Solid lines represent males, dashed lines females.

sexual maturity, as this may correspond to the point in a male's life when it becomes important to fight conspecifics and occupy high-quality territories. Possibly, displays which involve extending the dewlap are intense and crucial in males needing to gain access to a territory and/or to guard established territories from intruders imposing strong constraints on the growth of the dewlap at this point.

Bite performance, on the other hand, diverges when animals are still (sexually) immature. Based on our repeated SVL measurements, we estimate the delay between the divergence in dewlap size and bite performance to be between 6 to 12 months. Although it has been shown previously that dewlap size is a good predictor of bite performance (Vanhooydonck *et al.*, 2005*a*,*b*; Irschick *et al.*, 2006; Lailvaux & Irschick, 2007) and bite performance may determine fighting success in (some) male *Anolis* lizards (Lailvaux *et al.*, 2004; Lailvaux & Irschick, 2007), our findings do not corroborate the idea that bite performance is (solely) under sexual selection, at least not in *A. baracoae* lizards. On the contrary, our data suggest bite performance may be at least partly under natural selection given that bite force starts diverging before sexual maturity is reached in either sex. The divergence in bite performance early on (i.e. before reaching sexual maturity) may be related to a sexual difference in ecological factors, such as diet, to avoid intersexual competition over limited resources between subadult males and females (Schoener, 1967; Herrel *et al.*, 2006; Vanhooydonck *et al.*, 2010; Lopez-Darias *et al.*, in press). Because detailed ecological data are missing, we were unable to explicitly test this idea in this study.

Notably, the differences in growth of both dewlap size and bite performance occur without the influence of environmental factors, such as predation pressure and/or interindividual competition, as the animals were raised under identical laboratory conditions. Contrary to field studies that have shown that growth rates in males and females differ, resulting in sexual dimorphism at adulthood, because of a differential response to limiting resources (e.g. Niewiarowski & Roosenburg, 1993; Perry, 1996; Stamps *et al.*, 1997; Haenel & John-Alder, 2002, LeGalliard *et al.*, 2006) as well as laboratory studies showing that bite force is dependent on resource availability (Lailvaux *et al.*, 2012), this cannot be argued in this study. Instead, differential growth in the dewlap and bite performance appears to be 'intrinsic' to the animals and may be physiologically (e.g. hormonally) and/or genetically determined (Irschick *et al.*, 2006; Husak *et al.*, 2007).

Lastly, head length and hind limb length relative to body size (i.e. shape) grow in a similar way and at a similar speed in both male and female *A. baracoae*, suggesting that selection pressures favor similar trait expression in either sex (Howard, 2009). As in other ecomorphs, the degree of shape dimorphism (in adults) seems to be positively correlated to the variation in structural habitat (Butler & Losos, 2002; Losos *et al.*, 2003); our result suggests a very restrictive use of the available microhabitat by the two sexes in *A. baracoae*. In addition, Butler & Losos (2002) predicted a great limb shape dimorphism in crown-giant anoles because of the need for the highly territorial male anoles to display in open microhabitats, thus being more vulnerable to predation. They hypothesize that high sprint speeds, and thus long limbs, are selected for under these circumstances in male crown-giant anoles. However, our data on *A. baracoae* do not corroborate this idea. Because quantitative data on microhabitat use and display behavior in this species, and crown-giants in general (see Losos *et al.*, 2003), are largely lacking, we are currently unable to explicitly test the validity of these hypotheses for the species under study. In addition, we cannot exclude the possibility that raising the animals under laboratory conditions in a predator- and competitor-free environment may have resulted in similar growth rates in head and limb dimensions in male and female *A. baracoae* whereas this may not have been the case in the wild.

In conclusion, growth patterns differ between the sexes and among traits in laboratory-raised *A. baracoae* lizards. The similar growth of head and limb shape possibly suggests that selection favors similar trait expression in males and females. The opposite is true for dewlap size and bite performance. Moreover, as dewlap size diverges around the time the animals reach sexual maturity, this suggests that dewlap size functions as a signal during intra- and intersexual interactions. Bite performance, on the other hand, starts diverging between males and females at a much earlier, that is the immature stage. This may suggest that bite performance is, at least partly, under natural selection with young males and females potentially already specializing on different food resources. We acknowledge, however, that based on the data presented in this study, we can only put forward evolutionary hypotheses without being able to test them. To actually test adaptive and/or selective explanations, data from field selection experiments and/or a comparative approach are needed.

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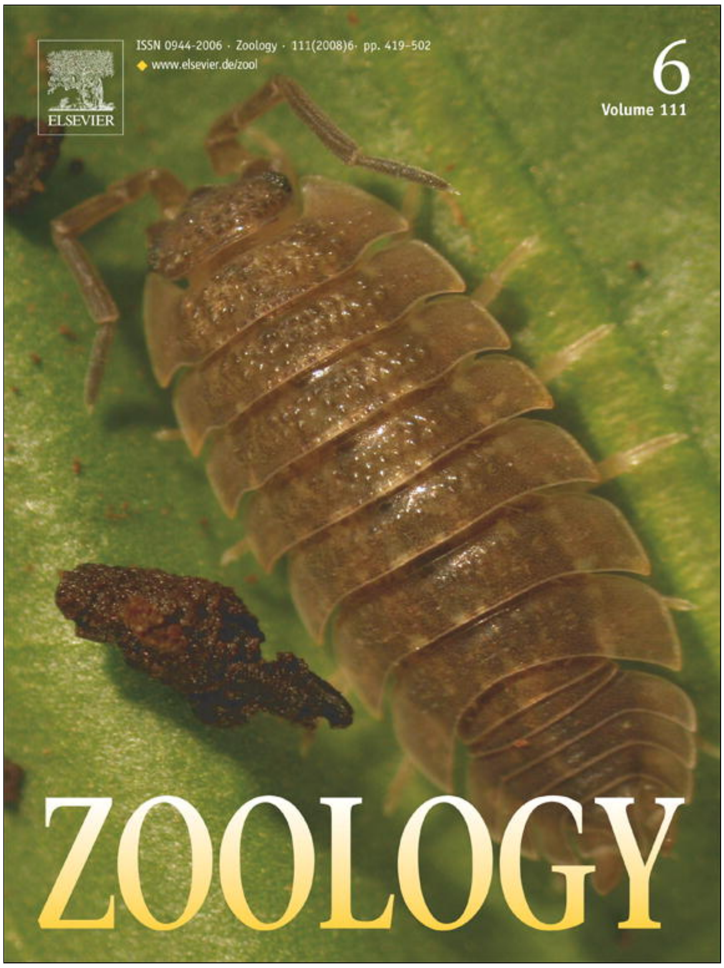

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Cranial morphology and bite force in *Chamaeleolis* lizards – Adaptations to molluscivory?

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Abstract

Anolis lizards have become a model system for the study of adaptive radiations as species with similar morphologies occupying similar habitats have arisen independently on all the larger islands in the Caribbean. However, on both, Cuba and Hispaniola unique forms have evolved that seemingly have no counterparts on any of the other Caribbean islands. Anoles of the genus *Chamaeleolis* comprise such a unique form and have been termed ‘twig giants’ because of their cryptic life style, slow locomotor mode, and short limbs. However, some of the most unusual features of these lizards are their large heads and molluscivorous diet. Here, we compare head shape, bite force, and muscle structure among sexes and age classes of *Chamaeleolis* lizards with *Anolis* crown giants. Our data show that *Chamaeleolis* lizards have a dramatically different head shape characterized by tall heads with a pronounced temporal ridge and long snouts. Analyses of bite force, surprisingly, show no differences between adult *Chamaeleolis* and *Anolis* crown giants. Juveniles of *Chamaeleolis*, however, have very tall heads for their size and bite harder than *Anolis* juveniles do. This can be related to the propensity of juveniles of this genus to eat snails, food items for which high bite forces are crucial. This observation is corroborated by the presence of well-developed jaw adductors in juveniles. Thus, our data suggest that the unusual phenotype of adults with large and tall heads may be due to selection on the juvenile life history stages.

Keywords: Head shape; Bite force; Feeding; *Anolis*

Introduction

In the past couple of decades, *Anolis* lizards have become one of the model systems to study adaptive radiations and evolutionary diversification (Schluter 2000). On the different islands of the Greater Antilles, similar forms that occupy similar habitats (termed ecomorphs) have arisen independently (Williams 1972; Losos 1995; Losos et al. 1998; Langerhans et al. 2006). *Anolis* lizards are a taxonomically (±370 species; Poe 2004) and phenotypically diverse group of lizards that have occupied a great diversity of niches in the Caribbean, ranging from terrestrial over arboreal to even aquatic species (Schwartz and Henderson 1991). Interestingly, while morphological convergence across islands is great for ground-dwelling and arboreal forms other ecotypes such as the aquatic anoles appear to

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show little or no convergence in morphology (Leal et al. 2002).

Interestingly, Cuba and Hispaniola are characterized by the presence of unique anoles typically not found on any of the other Greater Antillean islands (Rodriguez-Schettino 1999; Beuttell and Losos 1999). A striking example is the lizards of the genus *Chamaeleolis* which have been characterized as twig giants (Hass et al. 1993; but see Beuttell and Losos 1999). *Chamaeleolis* lizards are animals of large body size that move slowly and deliberately around their habitat (Leal and Losos 2000). These animals are typically cryptic, slow, and have relatively short limbs (Wilson 1957; Beuttell and Losos 1999). Unexpectedly, and despite their unique morphology, this monophyletic clade is deeply nested within the genus *Anolis* and is most closely related to a group including a series of crown giants among which are *Anolis cuvieri* from Puerto Rico and *Anolis barahonae* from the Dominican Republic (Hass et al. 1993; Nicholson et al. 2005).

Besides their short limbs, one of the most striking features of these lizards is their head morphology (Rodriguez-Schettino 1999). Not only do they have relatively big heads, they also have a casque-like extension at the back of their head and molariform teeth in adults (Estes and Williams 1984; Schwartz and Henderson 1991). These features have often been associated with their reported snail-eating habits (Estes and Williams 1984; Rodriguez-Schettino 1999, 2003; Lee 1997, 2003).

As snails are hard and brittle food items, changes in cranial morphology leading to increased bite forces have been suggested to be important features for lizards exploiting snails as a dietary resource (Dalrymple 1979; Rieppel and Labhardt 1979). For instance, the snail-crushing teiid lizard genus *Dracaena* is characterized by enlarged blunt molariform teeth and enlarged jaw muscles (Dalrymple 1979). Similar features have been reported for a snail-eating amphisbaenid lizard (*Amphisbaena ridleyi*; Pregill 1984) and Nile monitors (*Varanus niloticus*) which as adults incorporate snails into their diet (Lonnberg 1903; Mertens 1942; Rieppel and Labhardt 1979). Clearly, blunt molariform teeth are important to avoid tooth breakage and increase the contact area with the food. An increase in bite force, however, could be achieved in multiple, not mutually exclusive ways. The easiest way to increase bite force would be to increase overall body size, or head size relative to body size. Additionally, an increase in the mass or architecture (i.e. more pennate muscles with shorter fibers) of the jaw adductors, or changes in the mechanics of the lever system (i.e. increased jaw closing in-lever relative to the jaw out-lever) could improve bite performance (Herrel et al. 2007).

Here we compare *Chamaeleolis* lizards with *Anolis* crown giants to test for differences in cranial size and shape that could improve bite performance and may allow these lizards to exploit hard dietary items such as snails. Where possible we specifically chose *A. barahonae* to compare *Chamaeleolis* lizards to as *A. barahonae* is of similar body size and is also a member of the sister group to the *Chamaeleolis* clade, making it an ideal species for comparison. Because sample sizes of juveniles for this species were small, we decided to add data for other crown giants (see Statistical analyses). In addition to comparing morphological traits, we also measured bite forces in lizards of different sex and age classes to test for differences in performance. Finally, we observed feeding behavior and quantified cranial morphology to investigate how *Chamaeleolis* lizards handle snails.

Materials and methods

Specimens

Anolis equestris specimens ($N = 13$) were captured on the grounds of the University of Miami and surrounding neighborhood. The *A. cuvieri* specimens were captured in Cambalache ($N = 17$) and El Yunque ($N = 2$) National Forests in Puerto Rico, and the *A. barahonae* specimens ($N = 39$) were captured in the vicinity of Polo on the Barahona peninsula in the Dominican Republic. *Anolis baracoae* ($N = 3$), *A. equestris persparsus* ($N = 6$), *Anolis luteogularis* ($N = 3$), *Anolis noblei* ($N = 2$), *Anolis smallwoodi* ($N = 6$), *Chamaeleolis barbatus* ($N = 13$), *Chamaeleolis chamaeleonides* ($N = 5$), *Chamaeleolis guamuhaya* ($N = 6$), and *Chamaeleolis porcus* ($N = 17$) specimens were measured in the private collection of Veronika Holanova and Jan Hribal in Prague, Czech Republic. Although *Chamaeleolis* should technically be merged with the genus *Anolis*, we will use the genus name *Chamaeleolis* to indicate members of this clade throughout the manuscript for the sake of clarity.

Morphometrics

The snout vent length (SVL) of all individuals was measured from the tip of the snout to the posterior edge of the anal scale; head length was measured from the back of the parietal bone to the tip of the upper jaw; head width was measured at the widest part of the head (at the level of the jugal bones); head height was measured just posterior to the orbits; lower jaw length was measured from the back of the retroarticular process to the tip of the lower jaw (Fig. 1). Bony elements used to delineate morphological segments could be detected easily through palpation. Additionally, three morphological variables (Fig. 1) reflecting the biomechanics of the jaw system were estimated by

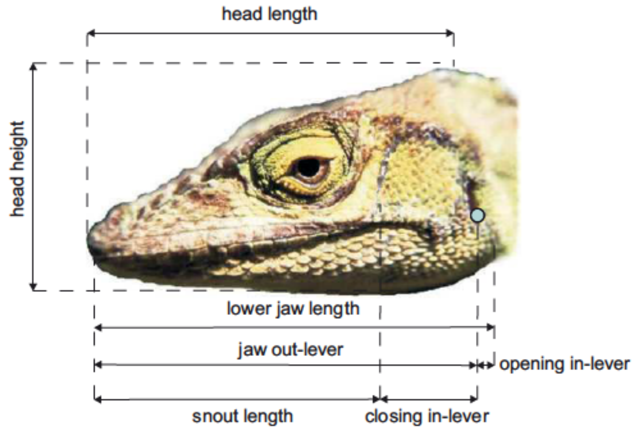

Fig. 1. Picture of the head of an adult *Anolis cuvieri* to illustrate the morphometric data gathered. See Materials and methods for a detailed explanation. The circle represents the quadrato-mandibular joint.

measuring: (1) the distance from the jaw articulation to the tip of the lower jaw, and (2) the distance from the posterior edge of the jugal (as an indicator of the position of the coronoid) to the tip of the lower jaw. By subtracting distance (1) from the lower jaw length we calculated the length of the jaw in-lever for opening (i.e. distance from the articulation to the back of the retroarticular process where the jaw-opener muscles insert). Subtracting distance (2) from distance (1) gave the in-lever for jaw closing (i.e. the distance from the articulation to the coronoid where the jaw-closing muscles attach). Distance (1) from the articulation to the tip of the jaw is the jaw out-lever. All measurements were taken using digital callipers (Mitutoyo CD-20DC, Sakato, Japan; precision: 0.01 mm).

Muscle mass

Jaw muscles were removed unilaterally in one adult male specimen of *A. cuvieri*, one adult female of *A. barahonae*, one adult and one juvenile of *A. garmani*, and one adult female and one hatchling of *C. chamaeleonides*. All specimens used for the analysis of muscle mass were preserved in a 10% aqueous formaldehyde solution for 24–48 h, depending on the size of the specimen. After fixation, specimens were rinsed in water and transferred to a 70% aqueous ethanol solution. The *Chamaeleolis* were preserved directly in a 70% aqueous ethanol solution. All specimens were kept in 70% ethanol for at least 2 months before dissection thereby assuring a similar degree of dehydration of tissue. All cranial muscle bundles were removed individually from specimens and stored in 70% ethanol until weighed. Muscles were blotted dry and weighed on a Mettler MT5 electronic balance (accuracy: ±0.01 mg).

Bite force

In vivo bite forces were measured using an isometric Kistler force transducer (type 9203, range ±500 N; Kistler, Switzerland) mounted on a purpose-built holder and connected to a Kistler charge amplifier (type 5995A, Kistler, Switzerland; see Herrel et al. 1999, for a more detailed description of the setup). When the free end of the holder was placed between the jaws of the animal, prolonged and repeated biting resulted. The place of application of bite forces was standardized for all animals. Gape angle was standardized by moving the bite plates away from each other for larger animals. Measurements were repeated five times for each animal, with an inter-trial interval of at least 30 min. The maximal value obtained during such a recording session was considered to be the maximal bite force for that individual.

Feeding behavior

Juvenile and adult *C. chamaeleonides*, *C. porcus*, *C. barbatus*, and *C. guamuhaya* as well as one adult *A. baracoae* were filmed with a Redlake Imaging MotionPro 500 camera set at 200 frames s^{-1} and a Sony camcorder while eating snails. Recordings were reviewed to identify bite positions and the way animals handled and crushed snails.

Statistical analyses

All data were $\log_{10}$-transformed before analyses. To explore overall head shape differences between *Chamaeleolis* lizards and crown giants in general, we assembled a data set including all the species listed above. Data on head dimensions in these animals were recorded and used as input for a factor analysis. However, as head dimensions co-vary with body size, and the first factor would therefore be simply an indicator of overall size, all variables were regressed against snout–vent length and unstandardized residuals were saved. These were used as input for a factor analysis with Varimax rotation and factor scores were saved (Table 1). A multivariate analysis of variance was conducted on the factor scores to test for overall differences in head shape between *Chamaeleolis* lizards and *Anolis* crown giants.

Next, the data set was separated according to age and sex classes (including both *Anolis* and *Chamaeleolis*). For adults, comparisons of head dimensions and bite force were restricted to a comparison of the different *Chamaeleolis* species lumped together (due to limited within-species sample sizes) to *A. barahonae*. For juveniles, additional data on *A. cuvieri* and *A. equestris* were added to the data set to increase the sample size. Head

Table 1. Results of a factor analysis performed on the residual cranial morphometric data

Residual cranial morphometric data	Factor		
	1 (60.11)	**2** (14.60)	**3** (14.04)
Residual head length	**0.934**	0.004	0.124
Residual head width	**0.839**	0.235	0.176
Residual head height	0.693	0.149	0.242
Residual lower jaw length	**0.968**	0.085	0.157
Residual out-lever	**0.972**	0.086	0.141
Residual opening in-lever	0.134	0.016	**0.981**
Residual snout length	**0.919**	0.306	0.109
Residual closing in-lever	0.078	**0.991**	0.016

Loadings higher than 0.7 are indicated in bold. The percentage of variance explained by each factor is indicated in brackets.

dimensions and bite forces were compared between the two groups using analyses of co-variance. Stepwise multiple regression models within each sex and age class were run with $\log_{10}$-transformed bite force as the dependent variable and the $\log_{10}$-transformed morphometric variables as independent variables to explore which cranial variables best explained variation in bite force.

Results

Head shape

All head dimensions were highly correlated with snout vent length (all $P<0.001$) across the entire data set as well as within groups. A factor analysis on the residual morphometric variables yielded three axes together explaining 88.7% of the variation in the data set. While the first factor was most strongly correlated with residual head length and residual head width, the second factor was determined by the residual in-lever for jaw closing and the third axis by the residual in-lever for jaw opening (Table 1). A MANOVA on the factor scores indicated significant differences between the two groups, one group containing all *Chamaeleolis* individuals and another one containing the crown giants (Wilks lambda $= 0.5$, $F = 55.62$, $P<0.001$; see Fig. 2). Subsequent univariate F-tests indicated that differences were significant on the first axis only ($F_{1,169} = 162.63$, $P<0.001$) with *Chamaeleolis* lizards having significantly longer and wider heads. Stepwise multiple regression with residual bite force as independent variable and residual morphometric variables as dependent factors/variables yielded a model with residual head height as only predictor. Thus, lizards with relatively taller heads bite harder compared to their body size.

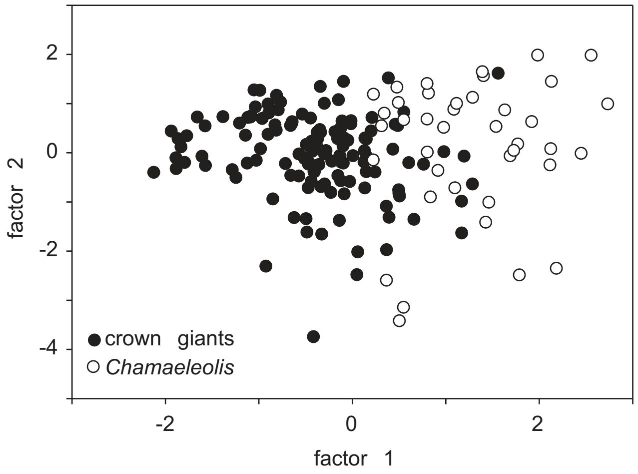

Fig. 2. Results of a factor analysis performed on the residual head measures. Note how the two groups are separated on the first factor indicating strong differences in head shape between *Chamaeleolis* and *Anolis* crown giants. Filled circles represent *Anolis* crown giants, and empty circles represent *Chamaeleolis* lizards.

As a MANOVA on the raw morphometric variables indicated significant differences between the sexes (Wilks lambda $= 0.71$, $F = 5.15$, $P<0.001$) and age classes (Wilks lambda $= 0.34$, $F = 30.35$, $P<0.001$), data were further analyzed by sex and age class separately.

Bite force and head shape

A MANCOVA on the morphometric data for males indicated significant differences in head shape between *A. barahonae* and *Chamaeleolis* (Wilks lambda $= 0.13$, $F = 21.61$, $P<0.001$). Univariate ANCOVAs indicated that these differences were significant for all variables (Figs. 3 and 4) with the exception of the residual in-lever for jaw opening ($F_{1,40} = 1.66$, $P = 0.21$) and head height ($F_{1,40} = 2.95$, $P = 0.09$). In general, *Chamaeleolis* lizards had bigger heads than *A. barahonae*. A univariate analysis of variance indicated no differences in bite force between the two groups ($F_{1,40} = 2.39$, $P = 0.13$). Stepwise regression with bite force as independent variable yielded a significant model with head height and snout length as only predictors ($r = 0.90$, $P<0.001$) indicating that male lizards with greater head height and longer snouts bite harder.

Results for females were similar to those for males; whereas a MANCOVA indicated significant differences in head shape (Wilks lambda $= 0.09$, $F = 9.55$, $P = 0.002$), univariate ANCOVAs indicated that the in-lever for jaw opening was not different between *Chamaeleolis* and *A. barahonae* ($F_{1,16} = 0.63$, $P = 0.44$). As in males, female *Chamaeleolis* have relatively bigger heads than female *A. barahonae*. A univariate ANCOVA indicated that there were no significant differences in bite force between groups ($F_{1,16} = 0.09$, $P = 0.78$). Stepwise regression with bite force as independent variable and the morphometric variables as dependent factors yielded a significant model with head height as only predictor ($r = 0.82$, $P < 0.001$) indicating that female lizards with greater head height bite harder.

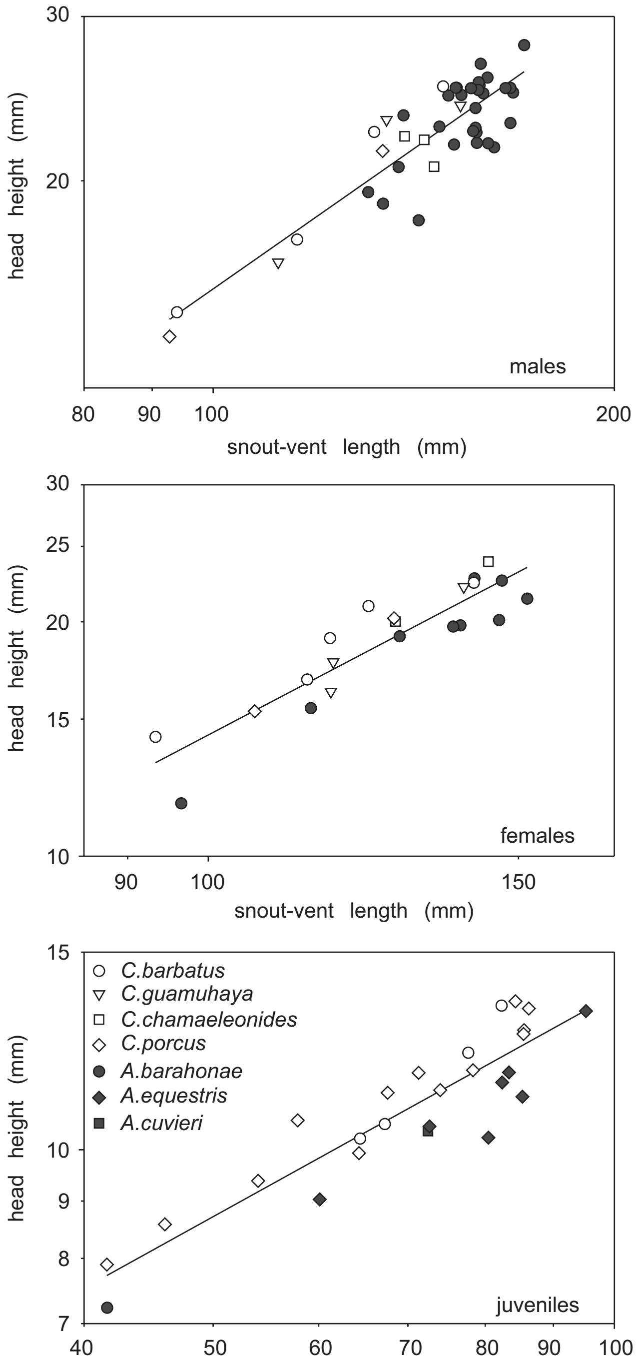

Fig. 3. Plots of head height against snout–vent length for male (top), female (middle), and juvenile (bottom) lizards. Filled symbols represent *Anolis* crown giants, empty symbols represent *Chamaeleolis* lizards. Note how especially juvenile *Chamaeleolis* have much taller heads than *Anolis* crown giants. Different symbols represent different species. Note the log-scale on *X*- and *Y*-axes.

Juveniles of both groups were also significantly different in overall head shape (Wilks' lambda = 0.07, $F = 22.87$, $P < 0.001$). Subsequent ANCOVAs showed that differences were significant in all head dimensions (Figs. 3 and 4) with the exception of the in-levers for jaw opening ($F_{1,23} = 0.03$, $P = 0.86$) and jaw closing ($F_{1,23} = 2.72$, $P = 0.11$). In contrast to the data for adults, an ANCOVA on residual bite force demonstrated significant differences ($F_{1,23} = 23.26$, $P < 0.001$). Thus, juvenile *Chamaeleolis* have relatively bigger heads and also bite harder than *Anolis* crown giants. Stepwise multiple regression yielded a model with snout length as only predictor ($r = 0.95$, $P < 0.001$). Thus, juveniles with longer snouts bite harder.

Cranial morphology

Inspection of the tooth rows in juvenile and adult *Chamaeleolis* indicates that in accordance with previous

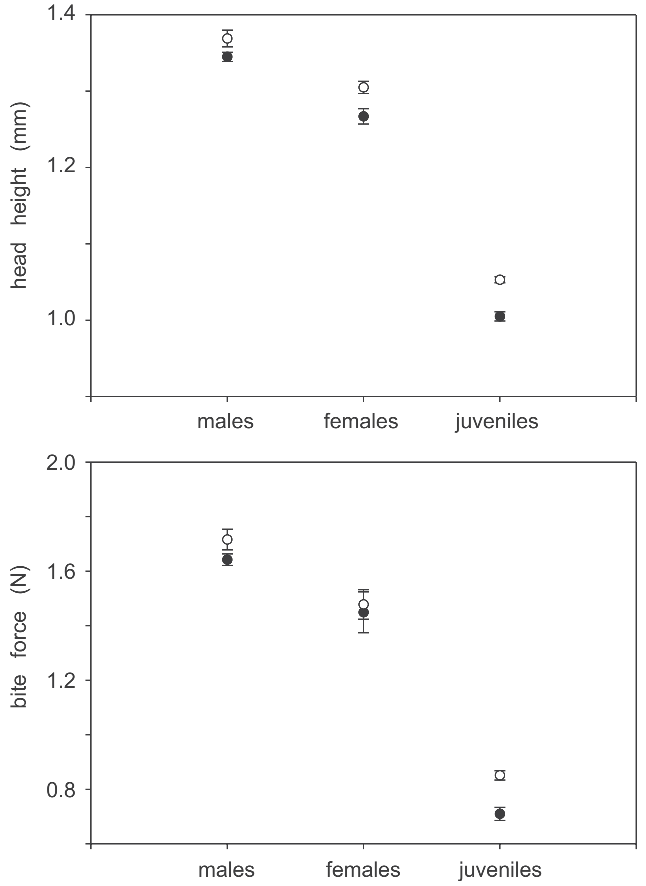

Fig. 4. Plots showing the adjusted means of the $\log_{10}$-transformed head height and bite force for adult males, adult females, and juveniles. Error bars represent one standard deviation. Filled circles: *Anolis* crown giants, empty circles: *Chamaeleolis* lizards.

reports (Estes and Williams 1984) the tooth morphology changes from typical tricuspid anteriorly to blunt and molariform at the posterior part of the tooth row. None of the crown giants examined showed the presence of molariform teeth, neither in juveniles nor in adults. The cranial morphology of *Chamaeleolis* lizards changed dramatically throughout ontogeny. Whereas an open upper temporal window allowing the jaw adductors to bulge and attach to the parietal crest is typical of the skulls in juveniles, this window closes completely in adults (Fig. 5). In *Anolis* crown giants no marked changes in cranial morphology appeared to take place and the upper temporal window remains open throughout life (Fig. 5).

An analysis of the jaw closer muscle mass shows that juvenile *Chamaeleolis* lizards have a relatively large jaw adductor for their size compared to *Anolis* crown giants. Whereas a juvenile *C. chamaeleonides* with a head length of only 17.76 mm had a total adductor mass of 36.52 mg, *A. garmani* with a head length of 21.29 mm had a comparatively small adductor mass of 41.39 mg (Fig. 6). This is most pronounced in the extension of the musculus pseudotemporalis pars superficialis, which takes up most of the upper temporal window and attaches to the enlarged parietal crest. As a consequence of the change in cranial morphology characterized by the closing of the upper temporal window, adult *Chamaeleolis* lizards do not have disproportionately large jaw adductors compared to closely related *Anolis* crown giants (Fig. 6).

Feeding behavior

Both adult and juvenile *Chamaeleolis* readily approached snails when presented with them and picked them up from the substrate using their tongue. One-week-old juveniles already recognized and ate snails of appropriate size when presented with them (Fig. 7A). Hatchlings could not be tested due to the lack of snails small enough for feeding them. Snails were manipulated in the oral cavity and repositioned several times before being crushed. Crushing took place either unilaterally at the posterior aspect of one of the mandibles or bilaterally with the snails being positioned in between the tooth rows of the lower jaw (Fig. 7B). Multiple bites (five to six) were often used to crush the shell. Shell fragments were removed from the mouth by the tongue before swallowing. Animals were able to crush snails with a diameter of up to roughly half the length of the

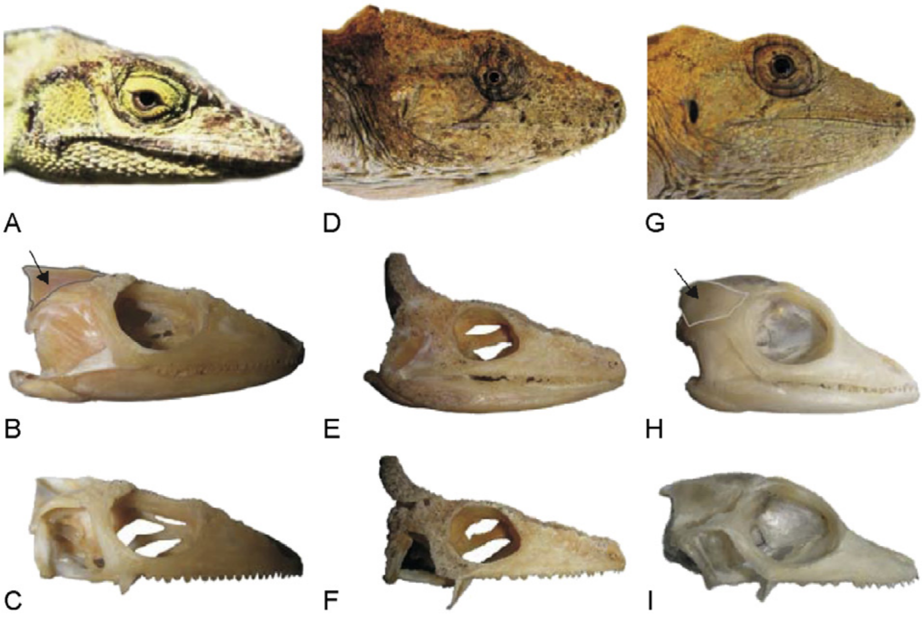

Fig. 5. Photographs of the head and skull of a typical crown giant and a *Chamaeleolis* lizard. (A) Lateral view of the head of *A. cuvieri*; (B) lateral view of the jaw adductor musculature in *A. cuvieri*. Note how the jaw adductors run under the upper temporal bar and attach at the parietal crest (arrow); (C) lateral view of the skull of *A. cuvieri*; (D) lateral view of the head of *C. chamaeleonides*; (E) lateral view of the jaw adductor muscles in *C. chamaeleonides*. Note how the upper temporal window has been covered completely by the outgrowth of the parietal; (F) lateral view of the skull of *C. chamaeleonides*; (G) lateral view of the head of a juvenile *C. chamaeleonides*; (H) lateral view of the jaw adductor musculature in a hatchling *C. chamaeleonides*. In contrast to the adults, juveniles have an open temporal fenestra and pronounced parietal crest allowing for the attachment of the jaw adductors; (I) lateral view of the skull of a hatchling *C. chamaeleonides*. The arrows in (B) and (H) indicate the adductor musculature which can be seen bulging through the upper temporal window in both juvenile *C. chamaeleonides* and adult *A. cuvieri*. The window has been outlined to improve clarity.

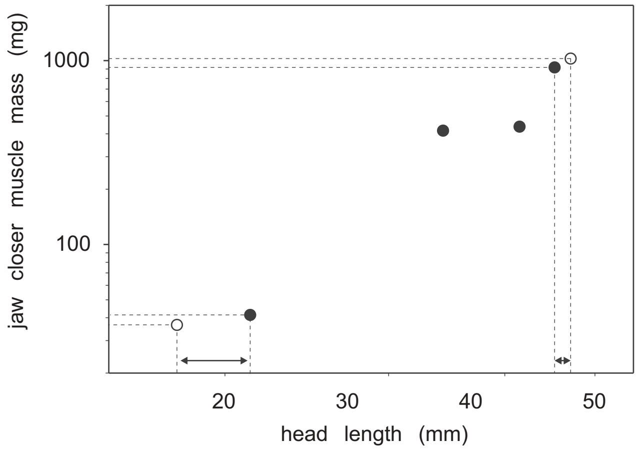

Fig. 6. Plot of jaw closer muscle mass against head length illustrating how juvenile *Chamaeleolis* lizards (empty symbols) have relatively large jaw adductors compared to *Anolis* crown giants (filled symbols) for their head size. This appears not to be the case for adults.

tooth row. One *A. baracoae* which was willing to eat a snail crushed the snail once at the posterior tooth row and swallowed it whole without removing the shell fragments.

Discussion

The radiation of *Anolis* lizards has been characterized by recurrent adaptive changes in morphology in response to the invasion of novel niches (Losos et al. 1998). The demands imposed by the micro-habitats occupied have led to strong natural selection on limb morphology such that animals perform best in the habitat they live in (Irschick and Losos 1999). For example, although short-limbed anoles are relatively slow on all substrates, they perform relatively better on the narrowest perches (Losos and Sinervo 1989; Vanhooydonck et al. 2006; Calsbeek and Irschick 2007). Interestingly, it has recently been demonstrated that convergence in habitat specialists is not restricted to limb size and shape, but also occurs in other aspects of morphology such as sexual size dimorphism and head shape (Harmon et al. 2005). Although it has been suggested that the functional demands imposed by the prey (i.e. prey hardness and elusiveness) may select for certain head shapes (Herrel et al. 2006) in different sexes and age classes, it remains currently unknown which aspects of the trophic niche may select for convergence in head shape in different ecomorphs.

Our data for *Chamaeleolis* lizards show that they are morphologically different from closely related crown giants by having bigger and differently shaped heads. Given the supposed molluscivorous diet of *Chamaeleolis* lizards, it is tempting to interpret these head shape differences as the result of natural selection for improved bite force capacity. Indeed, having longer and wider heads should allow for more jaw muscle and should thus increase bite force as has been demonstrated for lizards and other vertebrates that specialize on molluscs (Dalrymple 1979; Rieppel and Labhardt 1979; Pregill 1984; Fisher Huckins 1997; Herrel et al. 2002). However, our results are paradoxical as the difference in head morphology in adult *Chamaeleolis* is not translated into a difference in bite performance. Thus, these data would, at first sight, suggest that the observed changes in head size and shape are non-adaptive and may be the result of, for example, sexual selection on head size for display purposes. Juvenile *Chamaeleolis*, on the other hand, have bigger heads and do have bigger bite forces. This suggests that selection on bite force capacity may be stronger in juveniles.

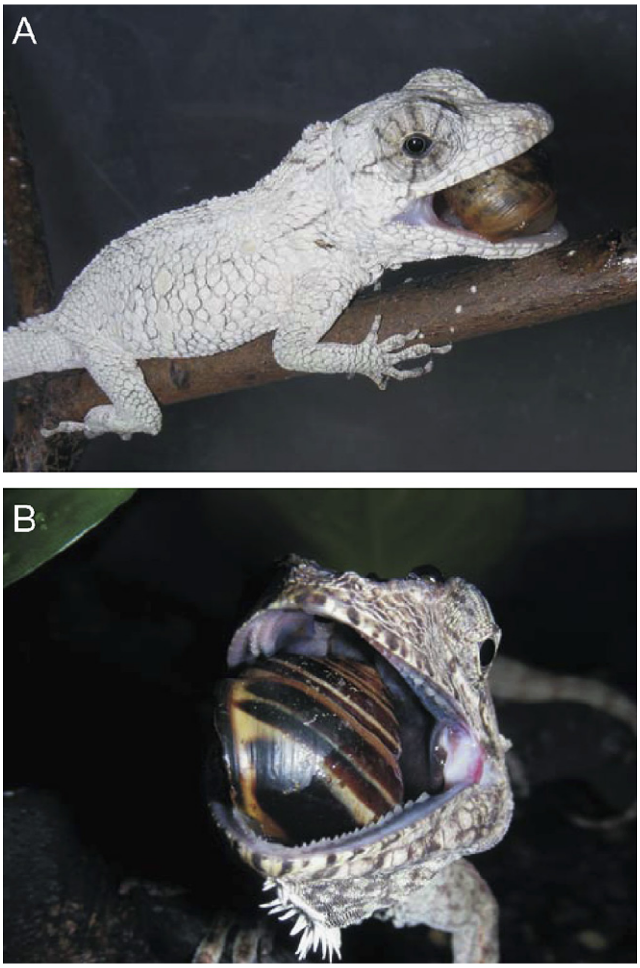

Fig. 7. (A) Ten-day-old *Chamaeleolis porcus* eating a snail. (B) Adult *C. guamuhaya* crushing a snail in between the mandibular tooth rows.

Unfortunately, no ecological data on the diet of *Chamaeleolis* are available, and we can only speculate about the relative importance of hard prey such as snails in the diet of both juvenile and adult lizards. One argument against the importance of snails in the diet of juveniles is the lack of molariform teeth, often considered crucial in allowing lizards to exploit hard and brittle prey (Estes and Williams 1984). However, behavioral experiments with just 1-week-old juvenile lizards indicate that they readily recognize and capture snails if presented with them. Moreover, and in contrast to other anoles that occasionally eat snails (e.g. *A. baracoae* will swallow snails whole and never remove shell fragments before swallowing), juvenile *Chamaeleolis* crush the snails and remove shell fragments from the mouth before swallowing. This suggests the presence of an innate behavioral pattern specifically associated with eating snails. Clearly, there is a great need for quantitative dietary data in these animals to verify the importance of snails in their diet (see also Estes and Williams 1984).

Interestingly, our data thus suggest that selection has operated on the juvenile life-history stages in *Chamaeleolis* lizards, resulting in large heads and large bite forces. Since adults do not differ in bite force from *Anolis* crown giants but do have larger heads this suggests that the adult phenotype may be the result of selection on juveniles. Adults have large bite forces in absolute terms which may allow them to crush snails without the need for an additional increase in head dimensions and bite force. Although adult crown giants do not appear to specialize on hard prey, they do eat relatively large prey such as other vertebrates and fruits (Brach 1976; Dalrymple 1980; Schwartz and Henderson 1991; Bowersox et al. 1994; Herrel et al. 2004; Holanova and Hribal 2005) and do include a significant proportion of hard prey such as large beetles into their diet (Herrel, personal observation). Our morphological data show why adult *Chamaeleolis* lizards do not bite harder than *Anolis* crown giants: the upper temporal window is completely covered by bone, thus preventing the jaw adductors from growing into that space. Juvenile *Chamaeleolis*, on the other hand, have an open temporal window, an enlarged parietal crest and a strongly hypertrophied m. pseudotemporalis. These features explain why juvenile *Chamaeleolis* lizards have stronger bites than juvenile *Anolis* crown giants. Why the upper temporal window is covered during ontogeny remains unclear at this point and needs to be investigated further.

In summary, our data suggest morphological and behavioral specializations of *Chamaeleolis* lizards to a diet of snails. As these lizards are cryptic, have short limbs and move about slowly, snails may be a profitable prey resource. As adult *Chamaeleolis* have large bite forces in absolute terms because of their large heads, hard prey such as snails are potential dietary items. Juvenile lizards in general, however, have small heads and thus low bite forces. In *Chamaeleolis*, selection appears to have operated on juveniles and has resulted in an increase in head size and bite force compared to closely related *Anolis* crown giants. This may provide juvenile *Chamaeleolis* with a relatively unexploited dietary resource as most other small lizards are not able to crush these prey items. Interestingly, twig *Anolis* lizards such as *Anolis sheplani* also have a relatively strong bite force and appear to include hard prey into their diet (i.e. beetles; see Huyghe et al. 2007). Although it is tempting to interpret the presence of big heads and high bite forces as a convergent trait in slow moving and cryptic anoles, this needs to be tested. At least in *Chamaeleolis* lizards, the small size of juveniles appears to have led to strong selection on head size to increase bite force and to allow these animals to exploit a free niche. The adult phenotype in these animals, on the other hand, appears largely the result of selection on juveniles rather than adaptively related to diet.

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IRCF REPTILES & AMPHIBIANS
CONSERVATION AND NATURAL HISTORY

Copulation and Oviposition in the Green-blotched Giant Anole, *Anolis smallwoodi* (Squamata: Dactyloidae), from southeastern Cuba

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Abstract.—Crown-giant anole ecomorphs have evolved independently across the Greater Antilles (Cuba, Hispaniola, Jamaica, and Puerto Rico), but only a few of these species have well documented life histories. Herein we report previously undocumented observations of copulation, oviposition, and hatchlings for the Green-blotched Giant Anole (*Anolis smallwoodi*) of southeastern Cuba. We observed eight mating events (three with *A. s. smallwoodi* and five with *A. s. palardis*) along the coastline between Santiago de Cuba and Guantánamo Province during June 2010 and September 2011. One female *A. s. palardis* mated with two males in the same territory. Females dig a hole with their snouts, oviposit single eggs, and cover them with substrate. We monitored eight eggs incubated in slightly moist vermiculite. The minimum incubation times were 54–69 days (varying with temperature). Juveniles usually are colored like adults, bright green with white spots on the body, pale postlabial bands, and suprascapular stripes.

Key words: crown-giant, copulation, oviposition, hatchling

Studies of *Anolis* lizards have indicated an important role for natural selection acting on morphology (Losos and de Queiroz 1997, Calsbeek and Smith 2007) and physiology (Calsbeek and Irschick 2007, Calsbeek et al. 2008). Differences in body size are thought to reflect differences in microhabitat use, and are important traits in the ecomorphological diversification of anoles (Butler and Losos 2002, Butler et al. 2007). Environmental factors such as temperature, photoperiod, precipitation, relative humidity, and food availability are important in the coordination of seasonal reproductive processes and also may help ensure that anoles will hatch at the most appropriate time of year to give them their best chance at survival (Domínguez et al. 2010).

Anolis lizards have low fecundity, with one egg per oviposition and several clutches per season (reviewed in Henderson and Powell 2009). However, of the 64 currently recognized species of Cuban anoles (Rodríguez-Schettino et al. 2013), the reproductive cycle is known for only seven (*A. allisoni, A. allogus, A. homolechis, A. bartschi, A. lucius, A. porcatus, A. sagrei*; see Domínguez et al. 2010 and references therein). Regarding oviposition, some authors have described intraspecific (Novo Rodríguez 1985, Estrada and Novo Rodríguez 1986; Silva et al. 1982; Estrada 1987, 1993; Robinson 2014) and interspecific (Alfonso et al. 2012) communal sites.

Crown-giant anole ecomorphs have evolved multiple times across the Greater Antilles (Cuba: *Anolis baracoae, A. equestris, A. luteogularis, A. noblei, A. pigmaequestris, A. smallwoodi*; Hispaniola: *A. baleatus, A. barahonae, A. ricordii*; Jamaica: *A. garmani*; and Puerto Rico: *A. cuvieri, A. roosevelti*; see Rodríguez-Schettino 1999, Henderson and Powell 2009, Losos 2009). *Anolis smallwoodi* Schwartz 1964 (Fig. 1A–C) has been found inhabiting both lowland and upland areas, semi-deciduous, evergreen, gallery, and rain forests, mangroves, and coffee plantations (Rodríguez-Schettino 1999, Henderson and Powell 2009). This species has large home ranges and multiple individuals are rarely observed together (Fläschendräger and Wijfels 1996, 2009); however, males and females can be observed together during the breeding season (VH, pers. obs.; Fig. 1D). Coloration in nature can vary individually and geographically (Rodríguez-Schettino 1999), and occasional deep-green individuals have blue spots on the dorsum (Fig. 1E).

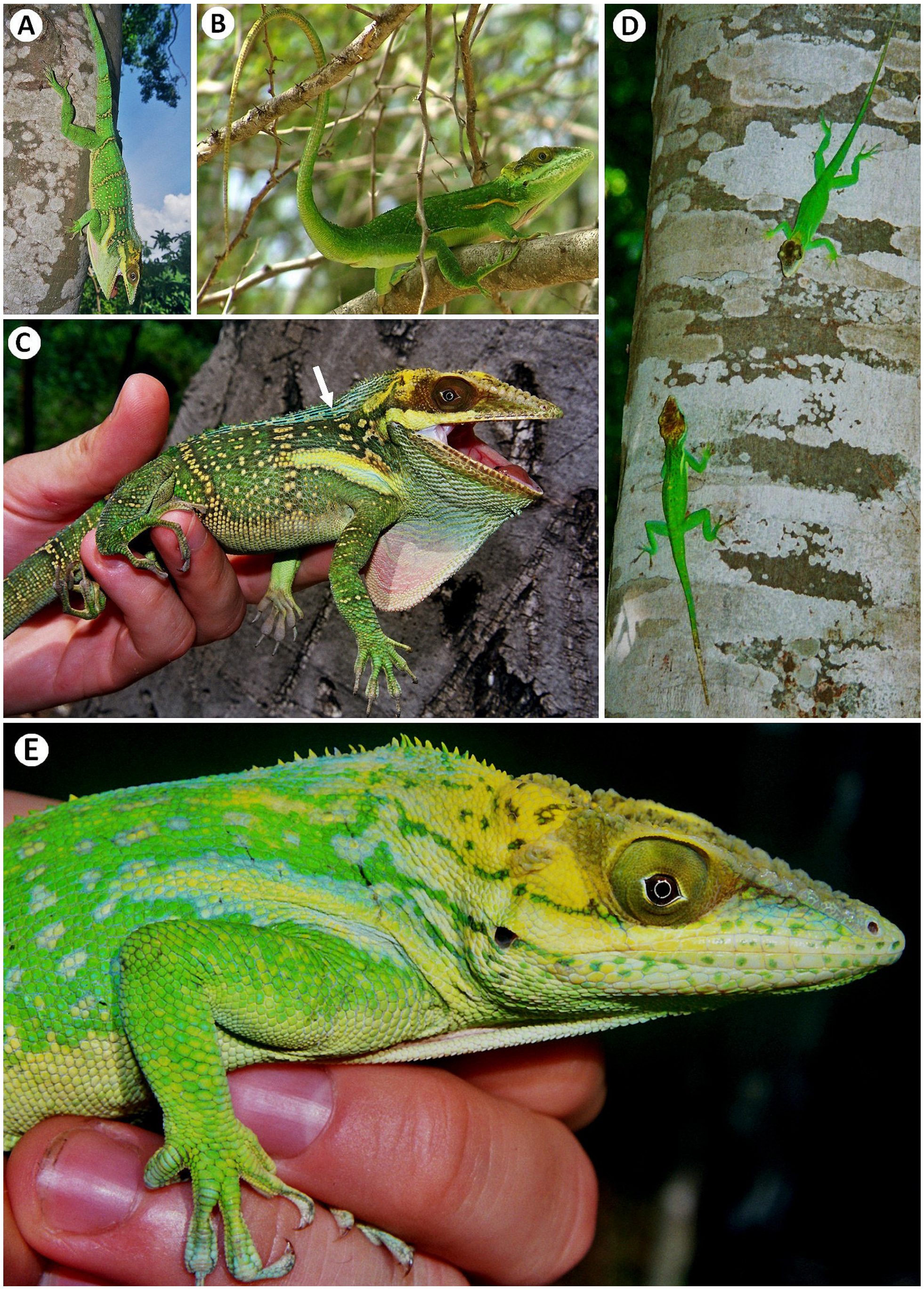

Fig. 1. *Anolis smallwoodi smallwoodi* on the southeastern coast of Cuba. A: Adult female perching face-down on a tree trunk. B: Adult male jumping on a branch of *Dichrostachys cinerea*. C: Adult female captured on a tree trunk, arrow indicate the blueness of the nuchal crest. D: paired couple during the breeding season. E: Adult female, note the strong yellow coloration on the head, blue coloration around the suprascapular stripe, and blue spots alternating with the bright green coloration on dorsum. Photographs from southeastern Santiago de Cuba province: Verraco (A, C–E) and Siboney-Jutici (B). Photographs by Veronika Holanova (A, C–E) and Yasel U. Alfonso (B).

Copulation time, oviposition, or hatching have been reported for some Greater Antillean crown-giant species (summarized in Henderson and Powell 2009). In this paper, our aim is to describe these natural history data for *Anolis smallwoodi* from semi-arid microhabitats in southeastern Cuba.

Materials and Methods

Study area and focal populations.—We conducted fieldwork from June 2010 to September 2011 at 1000–1500 h along the coastline between Santiago de Cuba and Guantánamo Provinces in southeastern Cuba. Dominant vegetation consisted of xerophytic scrub, microphyllous evergreen forest, dry microphylous forest, and coastal hyperxeromorphic semidesert scrub (Capote and Berazaín 1984, Reyes 2006). We surveyed four localities for mating behavior (copulation) in *Anolis smallwoodi* (*A. s. smallwoodi* in Santiago de Cuba Province: Juraguá, 19.936805° N, 75.670188° W, 36 m asl; Verraco, 19.907291° N, 75.575775° W, 23 m asl; *A. s. palardis* in Guantánamo Province: Baitiquirí, 20.030244° N, 74.880822° W, 25 m asl; Yacabo Abajo, 20.052266° N, 74.699741° W, 11 m asl).

In most instances, we observed copulation while using a monocular Nikon Fieldscope at distances of 13–37.4 m. We describe mating behavior involving three phases: precopulation, copulation, and postcopulation. We measured the duration of each stage with a digital chronometer (± 0.1 s). Means are presented ± one standard deviation.

Ovipositioning behavior in captivity.—Captive individuals observed during oviposition belong to the private collection of Veronika Holanova in Prague, Czech Republic. Anoles were maintained in pairs inside terraria (50 x 50 x 80 cm) with approximately 5 cm of mixed lignocel and sand (proportion 2:1) as a substrate. Branches were placed vertically and horizontally inside terraria. Giant anoles prefer wide, vertical branches for daily activities and thin, horizontal branches for sleeping at night (VH, pers. obs.). Live plants (e.g., *Ficus benjamina*) from the leaves of which anoles lick water also were placed in terraria. The back wall of each terrarium was covered with cork bark.

Temperature was regulated with 40-watt bulbs in ceramic shades. Light was provided with fluorescent tubes (2 x 25 watt) above the terraria. Daytime temperatures ranged between 26–28 °C (35 °C directly under the bulb) and nighttime temperatures never dropped below 22 °C. Terraria were misted daily with water. Each oviposited egg was weighed (Ew) and measured (EL: egg length; EW: egg width). After hatching, neonates were measured (TL: total length, SVL: snout-vent-length, HL: head length) and weighed (NW: neonatal weight). Measurements were taken with a digital caliper (Mitutoyo CD-20DC, Sakato, Japan; precision, ± 0.01 mm) and mass with a Kern 440-33N electronic balance (± 0.01 mg).

Results

Copulatory behavior.—We observed eight mating events, three involving *A. s. smallwoodi* and five *A. s. palardis*. During the May-to-September breeding season, most observations were of pairs (usually a single male and female on the same tree). Males copulated exclusively with those females located within their territories (YUA and VH, pers. obs.). Only one female *A. smallwoodi palardis* mated with multiple (two) males in the same territory (see details below).

Males initiated the precopulatory phase by closely following receptive females that usually submitted to a male's advances when he climbed onto her back. At that point, males made a sudden lateral movement and grasped the female, effectively rendering her immobile (Fig. 2A). The male's grip on the female's dorsum differs between the two subspecies; it is middorsal for *A. s. smallwoodi* (Fig. 2B) and more anterior for *A. s. palardis* (Fig. 2C). Subsequent preparation for penetration was expeditious, with male tail and hindlimb overlapping the female's tail (Fig. 2B–C). Precopulation took 7.11 ± 3.78 min (*A. s. smallwoodi*) and 5.47 ± 3.5 min (*A. s. palardis*). The male's grasp of the female was maintained throughout copulation.

The copulatory phase began when females responded to intromission by making an abrupt movement. Mating was similar between subspecies; neither moved the body, although females performed some slow lateral head swings. Copulatory duration was similar in both subspecies (*A. s. smallwoodi*: 44.2 ± 23.5 min; *A. s. palardis*: 40.6 ± 20.1 min). The postcopulatory phase began with the separation that occurs without any sudden movements. Females initiated separation with elevation of their hindlimbs to facilitate extraction of the hemipenis. After separation, lizards invariably moved upward until one or both were concealed among the leaves.

We observed one case of multiple copulation of *Anolis smallwoodi palardis* involving a pair 223 cm high on a tree trunk. Mating behavior and duration were similar to others observed for this subspecies. However, after separation, the adult male, seemingly unaware of the presence of another adult male, moved higher into the canopy while the female remained in the same initial position without any male protection. A nearby male (ca. 420 cm away and 137 cm above the ground in a bush) opportunistically entered the first male's territory and successfully copulated with the female that apparently remained receptive. The period between separation after the first copulation and the beginning of the second copulation was 123 min. Mating behavior for the second mating was similar to others observed for the subspecies, but durations were relatively short for both precopulation (2.5 min) and copulation (29.7 min). After separation, the female climbed upward into the canopy and the second male jumped from the trunk to another tree.

Oviposition behavior in captivity.—Paired males and females (all *Anolis smallwoodi smallwoodi*) initiated courtship behavior in March. After a short "chase," copulation lasted 10–30 min. (Fig. 3A–B). Females ready to lay eggs descended from elevated perches to the substrate, where they dug a few "test" holes apparently searching for a site with optimal temperature and humidity. When satisfied with a location, a female digs a hole with her snout, lays an egg and rolls it in substrate material (Fig. 3C). When the egg is covered, she buries it using her snout and forelimbs before climbing onto an elevated perch. The entire process

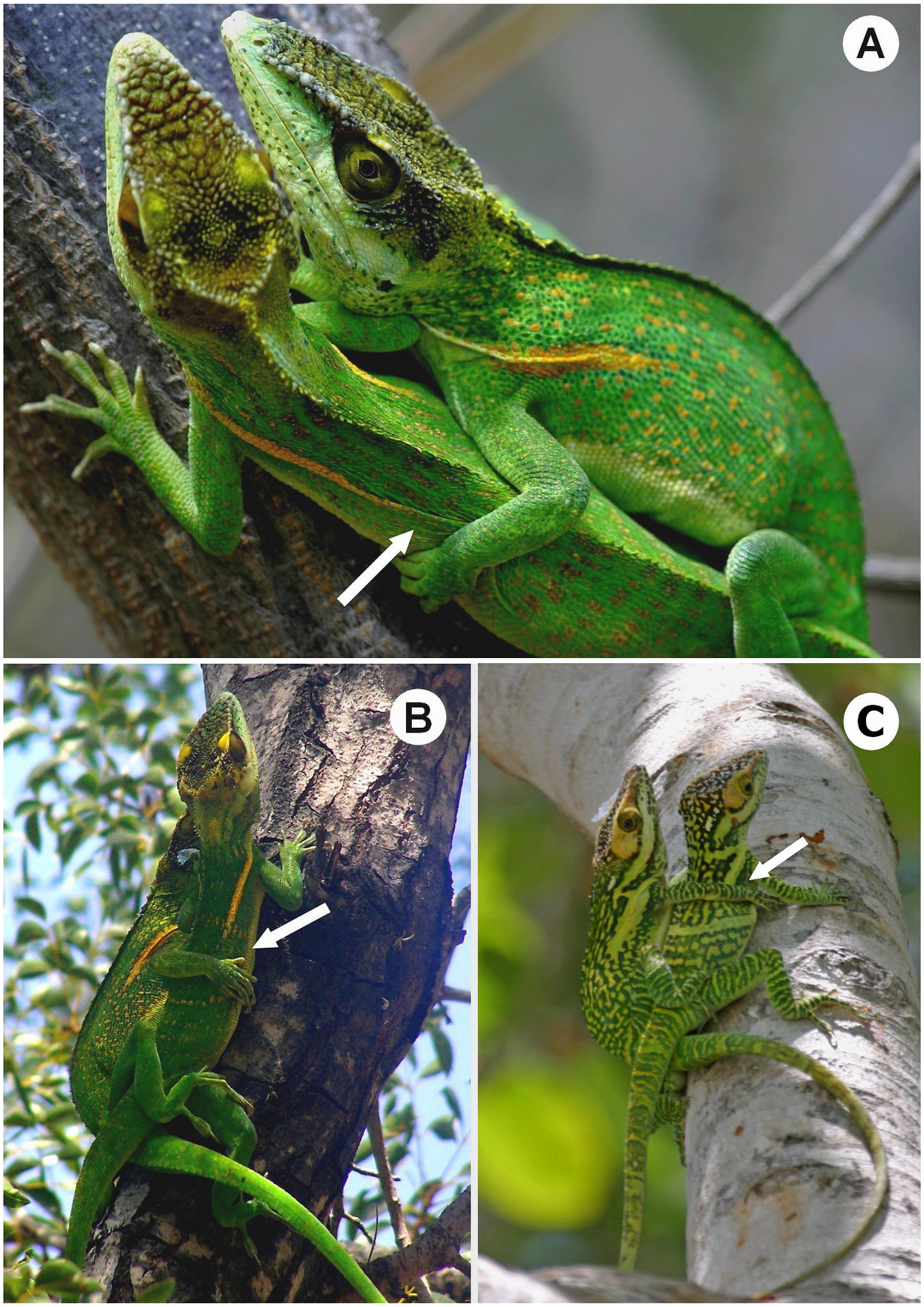

Fig. 2. Mating behavior in *Anolis smallwoodi* in southeastern Cuba. Arrows represent the male grasp position. A–B: *A. smallwoodi smallwoodi* and C: *A. smallwoodi palardis*. Photographs by Yasel U. Alfonso (A–B) and Joseph Burgess (C).

takes about 20 min. Males usually copulate with females the same day she lays an egg. Females always lay a single egg (Fig. 3C). Measurements for eight eggs were 2.59 ± 0.45 g and 2.54 ± 0.12 cm by 1.33 ± 0.08 cm. Well-fed females can lay an egg every seven days during the March–September breeding season.

Eggs were incubated in slightly moist vermiculite at temperatures of 27–29 °C. The minimum incubation time was 54–69 days (varying with temperature). Three eggs were infertile, but measurements for four hatched neonates were 2.6 ± 0.11 g and 13.8 ± 0.32 cm (SVL= 4.61 ± 0.15 cm, HL= 1.54 ± 0.03 cm). Juveniles usually are colored like adults, bright green with white spots on the body, pale postlabial bands, and suprascapular stripes. We began feeding neonates two days after hatching. They accepted a wide range

Fig. 3. A–B: Positions during mating in *Anolis smallwoodi* in captivity. C: Female burying a recently laid egg (arrow) using her forelimbs and snout. Note the blue coloration of this adult female. Photographs by Veronika Holanova.

of insects (e.g., crickets, grasshoppers, flies, and beetle larvae (Fig. 4C–D).

Discussion

Copulatory behavior.—Reproductive behavior is not well documented for Greater Antillean crown-giant anoles. Reviews by Rodríguez-Schettino (1999), Henderson and Powell (2009), and Losos (2009) included pertinent information for only some species (i.e., *Anolis baleatus*, *A. baracoae*, *A. barahonae*, *A. cuvieri*, *A. equestris*, *A. garmani*, and *A. pigmaequestris*). The mating behavior of three Cuban crown-giants for which we have some information (*A. baracoae*, *A. luteogularis*, and *A. noblei*) is similar in some aspects to that of *A. smallwoodi*. All have been observed in pairs (usually one male and one female on the same tree) and precopulation usually is initiated when a male follows a female from behind and ends with the male grasping and largely immobilizing the female (YUA, pers. obs.).

Our observations also suggest that *Anolis smallwoodi* exhibits behavior similar to that of other Greater Antillean crown-giants (Losos, 2009 and references therein). Rios-López and Puente-Colón (2007) observed multiple sleeping pairs of *A. cuvieri* and suggested that this might be indicative of pair-bonding. Rodríguez-Schettino (1999) noted that courtship and mating in *A. equestris* and *A. luteogularis* occurred in tall trees, and that only one or two individuals of the latter occupy a single tree. Apparently, crown-giants have large home ranges and two individuals are rarely seen together (Fläschendräger and Wijfels 1996, 2009).

The duration of copulation varies considerably among some species of anoles (Jenssen 1996). It can be lengthy in many species and, in at least some species, duration seems to vary consistently among males (Crews 1973, Tokarz 1988) and appears to be greater later in the breeding season (Stamps 1975, Tokarz 1999). In Jamaican *A. garmani*, Trivers (1976) recorded an average duration of 25 min. For other Greater Antillean anoles, mean duration varies from a few seconds to more than an hour (e.g., *A. sagrei*, 3.8 min; *A. bremeri*, 64.9 min; *A. valencienni*, 2 min; *A. websteri*, ±1 sec; Regalado and Garrido 1993; Henderson and Powell 2009; Losos 2009 and references therein).

Male anoles (non-crown-giant species) usually mate with multiple females in their territories, and those males with larger territories (presumably supporting more females) suffered more paternity loss to extra-pair copulations (Losos 2009 and references therein). Our observation of multiple copulations in one female *A. smallwoodi palardis* might be an example of cryptic mate choice, wherein females mate multiple times over an extended period and subsequently select a specific male's sperm to fertilize the egg (Losos 2009). Female *A. garmani* position themselves in exposed places, perhaps rendering mating males highly visible to other nearby males or to solicit males so that the female may choose among them (Trivers 1976, Hicks and Trivers 1983). Extra-pair copu-

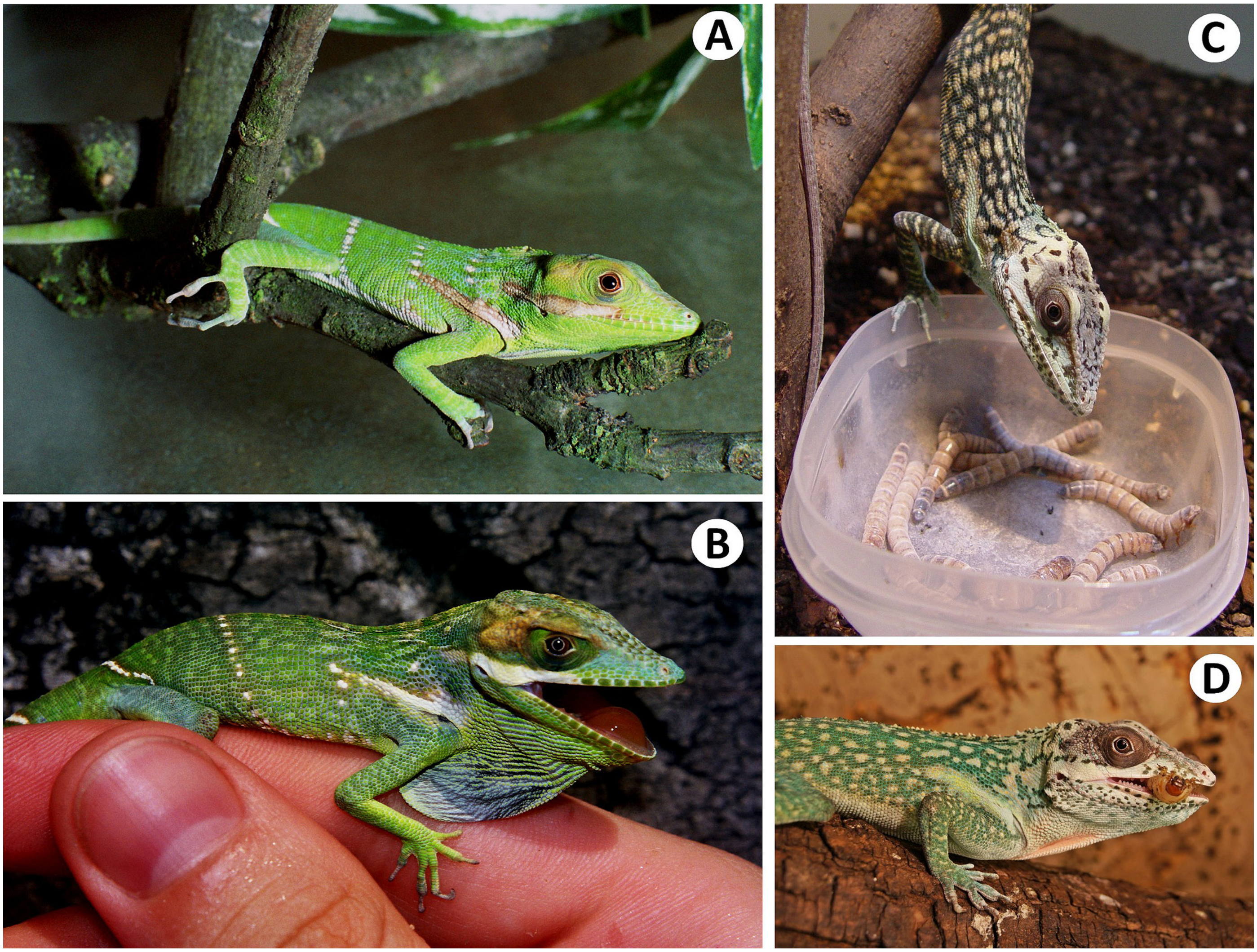

Fig. 4. A: Neonate *Anolis smallwoodi smallwoodi.* B: Holding a neonate outside the terrarium. C: Adult feeding on larvae of *Zophobas morio* (Tenebrionidae). D: Adult feeding on larvae of *Pachnoda marginata* (Scarabaeidae) inside the terrarium. Photographs by Veronika Holanova.

lations have been reported at least in one crown-giant species (*A. garmani*; Trivers 1976). Hicks and Trivers (1983) observed 49 copulation events in *A. garmani*, including both resident males and neighboring males (i.e., not with the main resident male within the territory).

Ovipositioning behavior in captivity.—Courtship behavior in captivity has been reported for some Greater Antillean crown-giants (Henderson and Powell 2009). *Anolis baracoae* can mate 2–3 times per week, and courtship involves head-bobbing, dewlap extension, and paired couples facing down on vertical branches (Holanova and Hribal 2004). Appler (1963) recorded the duration of copulation for *A. cuvieri* in captivity; the range of 20–90 min is more variable than our observations of *A. smallwoodi.* Female anoles produce only one egg at a time with an average of 5–25 days between eggs (Andrews and Rand 1974, Andrews 1985). Only a single egg, usually from alternating ovaries, is ovulated at a time, but females can retain eggs during times of drought and thus sometimes carry as many as three eggs, two shelled and one in the oviduct (Stamps 1976).

Egg deposition reported herein for *Anolis smallwoodi* is similar to that described for other anoles. Females usually begin by selecting a potential site, probing the substrate with the snout, digging a hole into which an egg is deposited, and finally covering the egg with the substrate (Losos 2009 and references therein). For crown-giants, Rodríguez-Schettino (1999) observed a female *A. equestris* ovipositing in a hole near the base of a tree, and Panton (1928) described a female *A. garmani* descending a tree at midday, digging a small hole with her snout, and laying an egg.

Egg and hatchling sizes, incubation temperatures, and minimum incubation times have been reported for some crown-giants (Table 1). Incubation time for several Caribbean species can range from 3.5–6 weeks under laboratory conditions (Greenberg and Hake 1990, Sanger et al. 2008). Our

Table 1. Mean oviposition data of crown-giant anoles in the Greater Antilles. MIT = minimum incubation time, IT = incubation temperature, SVL = snout-vent length. An asterisk indicates data obtained from our study. Neonate SVLs are given based on total length. Remaining data are from Losos (2009) and Henderson and Powell (2009), which should be consulted for the original sources in each case.

	Eggs					Hatchling
Species	**Length (mm)**	**Width (mm)**	**Mass (g)**	**MIT (days)**	**IT (°C)**	**SVL (mm)**
*Anolis smallwoodi**	23–27	12–14	1.7–3.1	54–69	27–29	134–142
Anolis baracoae	23–25	6–7	—	74–81	26	130
Anolis cuvieri	21–23	11–12	—	95	20–25	41
Anolis equestris	23–25	12.8–18	—	60–92	18–30	80–140
Anolis garmani	12–15	8–11	—	60–75	24–26	27
Anolis pigmaequestris	23.5	13.3	—	—	—	—

results are generally similar to those previously reported for Caribbean species, although Bech (1986) observed one captive female *A. equestris* laying 12 eggs in one year (compared to one egg every seven days during breeding season in *A. smallwoodi*).

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