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At home at least: the taxonomic position of some north African *Xerocrassa* species (Pulmonata, Geomitridae)

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Abstract

In order to clarify the systematic position of *Helix latastei* Letourneux in Letourneux & Bourguignat, 1887, and *Helix latasteopsis* Letourneux & Bourguignat, 1887, a comprehensive approach using morphological and molecular methods is presented. The investigation of the genital organs of both species showed that they belong to the genus *Xerocrassa* Monterosato, 1892 with two very small dart sacs and a few tubiform glandulae mucosae. In our phylogenetic analysis using the mitochondrial markers COI, 16S and the nuclear cluster 5.8-ITS2-28S, the results of the anatomical research were confirmed. Thus, the genus *Ereminella* Pallary, 1919, which is based on *H. latastei*, becomes a junior synonym of *Xerocrassa*. A review of the genus-level taxa *Xerobarcana* Brandt, 1959, and *Xeroregima* Brandt, 1959, showed that these should also be considered as synonyms of *Xerocrassa*. A third species, *Helix lacertara* Bourguignat, 1863 from Algeria was found to be closely related to *X. latastei* based on its shell morphology. A map showing the distribution of the three species treated is supplied.

Résumé

Une étude basée sur des approches morphologiques et moléculaires a été réalisée dans le but de clarifier la position systématique de deux espèces *Helix latastei* Letourneux 1887 et *Helix latasteopsis* Letourneux & Bourguignat, 1887. L'examen des organes génitaux a montré des critères typiques du genre *Xerocrassa* Monterosato, 1892 avec la présence de deux petits "Dart Sac" et des glandes digitiformes à mucus. Les résultats de l'analyse phylogénétique de deux gènes mitochondriaux (COI et 16S) et un gène nucléaire 5.8S-ITS2-28S ont confirmé les résultats de l'étude anatomique. Par conséquent, le genre *Ereminella* Pallary, 1919, qui a

été basé sur *Helix latastei* est donc un synonyme du genre *Xerocrassa*. La révision de deux genres *Xerobarcana* Brandt, 1959 et *Xeroregima* Brandt, 1959, suggère que ces deux genres sont aussi des synonymes du genre *Xerocrassa*. L'examen de la coquille de l'espèce Algérienne *Helix lacertara* Bourguignat, 1863 a montré une forte ressemblance avec *X. latastei*, ce qui nous a permis, ainsi, de la classer dans le genre *Xerocrassa*. Une carte montrant la distribution des trois espèces a été fournie.

Keywords
Algeria, anatomy of genital organs, systematics, Tunisia, *Xerocrassa latastei*, *Xerocrassa latasteopsis*, COI, 16S, 5.8S-ITS2-28S

Mots clés
Algérie, anatomie de l'appareil génital, systématique, Tunisie, *Xerocrassa latastei*, *Xerocrassa latasteopsis*, COI, 16S, 5.8S-ITS2-28S

Introduction

The systematic position of most taxa described by Letourneux and Bourguignat, 1887 in their "Prodrome" on the Tunisian malacofauna is under debate since their description. This holds true for *Helix latastei* as well as for *Helix latasteopsis*. Their generic status was maintained until Pallary (1919) erected the new genus *Ereminella* based on *H. latastei*, but without giving any descriptive characters that could discriminate this taxon from others. The first researcher intensively dealing with *H. latastei* was Brandt (1959: 113), who, deducing from an anatomical drawing by Bisacchi (1932: 363–364, figs 2–4), perceived *H. latastei* to be a member of his *Trochoidea* sensu lato (which at that time included what is separated today as *Xerocrassa*). Bisacchi erroneously identified the Libyan specimens he dissected as *Helix* (*Xerophila*) *pseudosimulata* Germain, 1921 from Alexandria, Egypt. However, Forcart (1976: 152) recognized this Egyptian taxon as a synonym of *Xerocrassa simulata* (Ehrenberg 1831) (for further discussion of this name refer to Forcart, loc. cit.). Jaeckel (1963) repeated Brandt's generic affiliation while recording the species from Djerba. Finally, Frank (1988) mentioned *X. latastei* from northern Tunisia, a record which is out of the recently known range of this species and needs to be verified. A comparison of Tunisian species with a selection of *Xerocrassa* species from the radiation of this genus on the Island of Crete (Sauer and Hausdorf 2009) and from western Europe including Spain and the Balearic Islands (Chueca et al. 2017) is provided.

Materials and methods

Sampling

Living specimens were collected from several localities in Tunisia during two periods: spring 2014, and winter 2015/2016. Geographic coordinates were recorded using GPS (see Table 1). For subsequent molecular analysis, specimens were preserved and stored in 80% ethanol until dissection and DNA extraction.

Table 1. List of localities of live collected specimens used in this study.

Species	Locality name, all Tunisia	Latitude	Longitude
X. latasteopsis	Sidi Aich 1, Gafsa	34.667881°	8.824673°
X. latasteopsis	Sidi Aich 2, Gafsa	34.706090°	8.797217°
X. latasteopsis	Henchir El Zitouna, Medenine	33.353749°	10.236242°
X. latastei	El Djorf (=Jorf), Medenine	33.696428°	10.729867°
X. latastei	Boughrara, Medenine	33.544044°	10.672908°
C. virgata	Ain Bitar, Bizerte	37.249618°	9.907816°
T. pyramidata	Djebal Recas, Ben Arous	36.608323°	10.327392°
T. elegans	Ghar el Melh, Bizerte	37.170999°	10.206831°

Empty shells were also collected (see section material under the species description) in order to complete the distributional record of the species. Specimens used in this study (both shells and preserved animals) are housed in the voucher collections of the High Institute of Biotechnology of Monastir and the Natural History Museum Bern; all sequenced specimens are deposited in the museum's collection.

Morphological and anatomical studies

First assessments of the shell morphological characters were done by using simple magnifying glasses. Preserved animals were dissected under LEICA M212 stereo-microscope using thin tweezers. The genital organs of the specimens were removed from the body, the genital situs (i.e. the outer morphology of the complete hermaphroditic genital organ) and further morphological details were investigated. After that, shells, genital situs, and details of the genital organs were photographed with a LEICA DFC 425 camera combined with a LEICA M205 C. The multifocal images were processed by using an imaging software (Imagic Switzerland).

Abbreviation of museum's acronyms

MVHN Museu Valencià d'Historia Natural;
MHNG-MOLL Museum d'Histoire Naturelle de Genève, malacological collection;
NMBE Naturhistorisches Museum der Burgergemeinde Bern;
ZMH Zoological Museum of the University of Hamburg.

Abbreviations of shell measurements

D: shell diameter; H: shell height; PD: peristome diameter; PH: peristome height; W: number of whorls.

Molecular study

Fourteen specimens of *Xerocrassa* from southern Tunisia could be used in this study, originating from five localities. Sequenced specimens are housed in the voucher collection of the NMBE (Table 2). In the analysis, sequences of four Cretan *Xerocrassa* species were also included (Sauer and Hausdorf 2009), and eleven Spanish and Balearic *Xerocrassa* species from the work recently published by Chueca et al. (2017).

As outgroup species *Cernuella virgata*, *Trochoidea elegans,* and *Trochoidea pyramidata* were used. All three species are each represented by one specimen from Tunisian localities, and complemented by one specimen of *Hygromia limbata*, one *Xerosecta adolfi,* and one *T. elegans* (Razkin et al. 2014). All specimens used to produce phylogenetic trees are listed in Table 2. Specimens where nuclear markers are not available were excluded from the analysis of the concatenated mitochondrial // nuclear dataset. Thus, all Cretan *Xerocrassa* specimens, except two specimens of *X. cretica* (recently collected by Neubert), and the Tunisian *Trochoidea* and *Cernuella* species were not used in this type of analysis.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from the foot muscle tissue using the standard phenol chloroform method (Estoup et al. 1996). Two mitochondrial gene fragments and one rDNA region were chosen to be analysed in the current study. Mitochondrial markers were consisting of Cytochrome c oxidase subunit I (COI) and the 16S ribosomal RNA subunit (16S) gene. The nuclear marker was formed by the 3' end of the 5.8s ribosomal RNA, the complete ITS2 region and the 5'end of the large subunit of the 28S rRNA. Polymerase chain reactions (PCR) were performed in a reaction mixture, containing 15 ng of DNA template, 1×1.5 mM buffer reaction, 0.1 mM of each selected couple primers, 0.2 mMdNTPs, Taq polymerase (1.25U) and adjusted till a total volume of 25 µl with DNAase free water/sterilized water (UNIMED) (H2O). PCR reactions were run under following conditions: 3 min at 95°C, followed by 35cycles of 1 min at 95°C, 1 min at 40°C and 1 min at 72°C and finally, 5 min at 72°C for COI. For 16S the amplification conditions were: 3 min at 95°C, followed 35 cycles of 1 min at 95°C, 1 min at 50°C and 1 min at 72°C. To amplify the ribosomal cluster, two pairs of primers were used to get a sequence of 1300 bp: the standard LSU1/LSU3 and the 28SF/28SR (see Table 3). PCR reactions were run under the following conditions: 3 min at 96°C, followed by 35 cycles of 1 min at 94°C, 1 min at 50°C and 1 min at 72°C and finally, 5 min at 72°C for LSU1/LSU3 and 5 min at 95°C, followed by 35cycles of 1 min at 95°C, 30 s at 62°C and 1 min at 72°C and finally, 10 min at 72°C for 28SF/28SR. PCR products were sequenced using automated and standardised ABI 3730 XL sequencing run with a read length up to 1100 bp (PHRED20 quality) and using the same primers as for the PCR (Table 3).

Table 2. Taxa used: Species, localities, and voucher and GenBank accession numbers for the mitochondrial genes COI and 16S and the nuclear ribosomal 5.8S-ITS2-28S region.

Species	Locality	Voucher number	GenBank accession numbers		
			COI	16S	5.8-ITS2-28S
X. latastei	El Djorf, Medenine, Tunisia	NMBE 541956	KY706528	KY747539	MF687913
	Boughrara, Medenine, Tunisia	NMBE 549851	KY706529	KY747540	MF687914
	Boughrara, Medenine, Tunisia	NMBE 549852	KY747533	KY747541	MF687915
	Boughrara, Medenine, Tunisia	NMBE 549853	KY706530	KY747542	MF687916
X. latasteopsis	Sidi Aich 1, Gafsa, Tunisia	NMBE 549847	KY706527	KY747536	MF687903
	Sidi Aich 1, Gafsa, Tunisia	NMBE 549848	KY747531	KY747537	MF687904
	Sidi Aich 1, Gafsa, Tunisia	NMBE 548449	KY747532	KY747538	MF687905
	Sidi Aich 2, Gafsa, Tunisia	NMBE 541954	KY747534	KY747543	MF687906
	Sidi Aich 2, Gafsa, Tunisia	NMBE 549846	KY747535	KY747544	MF687907
	Henchir el Zitouna, Medenine, Tunisia	NMBE 549854	MF678555	MF683092	MF687908
	Henchir el Zitouna, Medenine, Tunisia	NMBE551288	MF678556	MF683093	MF687909
	Henchir el Zitouna, Medenine, Tunisia	NMBE 551289	MF678557	MF683094	MF687910
	Henchir el Zitouna, Medenine, Tunisia	NMBE 551290	MF678558	MF683095	MF687911
	Henchir el Zitouna, Medenine, Tunisia	NMBE 551291	MF678559	MF683096	MF687912
X. frater frater [Chueca et al. 2017]	Cala Romantica, Baleares, Spain	EHUMC-1327	KT968955	KT969152	KT969343
	Cala Romantica, Baleares, Spain	EHUMC-1328	KT968956	KT969153	KT969344
	Tossals Verds, Baleares, Spain	EHUMC-1329	KT968957	KT969154	KT969345
X. majoricensis [Chueca et al. 2017]	Illetes Calvià, Baleares, Spain	EHUMC-1317	KT968945	KT969142	KT969333
	Illetes Calvià, Baleares, Spain	EHUMC-1318	KT968946	KT969143	KT969334
	Bunyolí Establiments, Baleares, Spain	EHUMC-1319	KT968947	KT969144	KT969335
X. ferreri ferreri [Chueca et al. 2017]	Path to French's monument Baleares, Spain	EHUMC-1295	KT968924	KT969121	KT969312
	Peguera Baleares, Spain	EHUMC-1296	KT968925	KT969122	KT969313
X. prietoi prietoi [Chueca et al. 2017]	Bunyolí, Establiments Baleares, Spain	EHUMC-1399	KT969024	KT969221	KT969392
	Sont Cotoneret Baleares, Spain	EHUMC-1400	KT969025	KT969222	KT969393
	Inca Baleares, Spain	EHUMC-1401	KT969026	KT969223	KT969394

Species	Locality	Voucher number	GenBank accession numbers		
			COI	16S	5.8-ITS2-28S
X. ponsi [Chueca et al. 2017]	Path to French's monument,Baleares, Spain	EHUMC-1387	KT969012	KT969209	KT969386
	French's monument Baleares, Spain	EHUMC-1388	KT969013	KT969210	KT969387
	French's monument Baleares, Spain	EHUMC-1390	KT969015	KT969212	KT969388
X. nyeli [Chueca et al. 2017]	Ses Mongetes, Baleares, Spain	EHUMC-1361	KT968987	KT969184	KT969374
	Ses Mongetes, Baleares, Spain	EHUMC-1362	KT968988	KT969185	KT969375
	Alaior, Baleares, Spain	EHUMC-1366	KT968991	KT969188	KT969376
X. cisternasi cisternasi [Chueca et al. 2017]	Illa de Santa Eulalia Baleares, Spain	EHUMC-1279	KT968908	KT969105	KT969297
X. caroli caroli [Chueca et al. 2017]	Cap des Jueu Baleares, Spain	EHUMC-1259	KT968888	KT969085	KT969277
	Cap des Jueu Baleares, Spain	EHUMC-1260	KT968889	KT969086	KT969278
	Cap des Jueu Baleares, Spain	EHUMC-1261	KT968890	KT969087	KT969279
X. ebusitana [Chueca et al. 2017]	Cap de Barbaria Baleares, Spain	MVHN-281009TF02	KT969064	KT969260	KT969416
	Racó des Forat Baleares, Spain	EHUMC-1241	KT968870	KT969067	KT969262
	Cap de Barbaria Baleares, Spain	EHUMC-1242	KT968871	KT969068	KT969263
X. barceloi [Chueca et al. 2017]	Orihuela, Alicante, Spain	EHUMC-1413	KT969038	KT969235	KT969406
X. subrogata | [Chueca et al. 2017]	Serra de la Borja, Tarragona, Spain	EHUMC-1412	KT969037	KT969234	KT969405
	Serra de la Borja, Tarragona, Spain	EHUMC-1411	KT969036	KT969233	KT969404
X. amphiconus [Sauer and Hausdorf 2009; Sauer and Hausdorf 2012]	Kato Zakros, Crete, Greece	ZMH 36820-606	FJ627140	JN701872	–
	Kato Zakros, Crete, Greece	ZMH 36820-452	FJ627076	JN701834	–
	Moni Toplou, Crete, Greece	ZMH 36606-473	FJ627090	JN701848	–
X. grabusana [Sauer and Hausdorf 2009; Sauer and Hausdorf 2012]	Kaliviani, Crete, Greece	ZMH 29885-465	FJ627089	JN701847	–
X. mesostena [Sauer and Hausdorf 2009; Sauer and Hausdorf 2012]	Agia Galini, Crete, Greece	ZMH 36790-638	FJ627160	JN701877	–
	Gerakari, Crete, Greece	ZMH 29631-636	FJ627158	JN701876	–
	Theriso, Crete, Greece	ZMH 29807-524	FJ627117	JN701866	–
X. cretica [Sauer and Hausdorf 2009; Sauer and Hausdorf 2012]	Moni Gorgolani, Crete, Greece	ZMH 36304-423	FJ627055	JN701813	–
	Palekastro, Crete, Greece	ZMH 50000-671	FJ627168	JN701878	–
	Palekastro, Crete, Greece	ZMH 50121-620	FJ627150	JN701874	–

Species	Locality	Voucher number	GenBank accession numbers		
			COI	16S	5.8-ITS2-28S
X. cretica [coll. Neubert [2017]]	Plateau between Lithines and Perivolakia, Crete, Greece	NMBE 550935	MF678560	MF683097	MF687917
		NMBE 550936	MF678561	MF683098	MF687918
X. ripacurcica [Chueca et al. 2017]	Circo de Armeña, Huesca, Spain	EHUMC-1416	KT969041	KT969238	KT969409
	Congost de Ventamillo, Huesca, Spain	MVHN-210813FS03	KT969057	KT969253	KT969411
X. montserratensis [Chueca et al. 2017]	Monistrol de Montserrat, Barcelona, Spain	EHUMC-1414	KT969039	KT969236	KT969407
	Castellar del Vallès, Barcelona, Spain	EHUMC-1415	KT969040	KT969237	KT969408
"X. meda" [Chueca et al. 2017]	Mosta, Malta	MVHN-230412LR01	KT969058	KT969254	–
T. elegans	Ghar el Melh, Bizerte, Tunisia	NMBE 549908	KY706532	KY747546	–
T. elegans [Razkin et al. 2014]	L'Alcudia, Valencia, Spain	MVHN 1310	KT969047	KJ458564	KJ458642
T. pyramidata	Djebal Recas, BenArous, Tunisia	NMBE 549882	KY706531	KY747545	–
C. virgata	Ain Bitar, Bizerte, Tunisia	NMBE 549850	KY706533	KY747547	–
Xerosecta adolfi [Razkin et al. 2014]	Nijar, Almeria, Spain	EHUMC 1036	KT968868	KJ458567	KJ458645
H. limbata [Razkin et al. 2014]	Queralbs, Daió, Girona, Spain	EHUMC 1027	KT968867	KJ458529	KJ458616

Table 3. List of primers used for PCR and sequencing.

Gene	Name	Sequence	Reference
COI	COIF	5'-ACTCAACGAATCATAAAGATATTGG -3'	Folmer et al. 1994
	COIR	5'-TATACTTCAGGATGA CCAAAAAATCA-3'	Folmer et al. 1994
16S	16Sar	5'-CGCCTGTTTATCAAAAACAT-3'	Palumbi et al. 1991
	16Sbr	5'-CCGGTCTGAACTCTGATCAT-3'	Palumbi et al. 1991
5.8S-ITS2	LSU-1	5'-CTAGCTGCGAGAATTAATGTGA-3'	Wade et al. 2000
	LSU-3	5'-ACTTTCCCTCACGGTACTTG-3'	Wade et al. 2000
28S	28S F	5'-AACGCAAATGGCGGCCTCGG-3'	Koene and Schulenburg 2005
	28SR	5'-GAAGACGGGTCGGGTGGAATG-3'	Koene and Schulenburg 2005

Sequence alignment

Forward and reverse sequences were assembled, checked for ambiguities and aligned using default settings of "Clustal W" implemented in Bioedit V 7.2.5 (Hall 1999). Aligned sequences of Tunisian *Xerocrassa* species were analysed using DnaSP v5.10.01 software (Librado and Rozas 2009) to estimate number of informative sites and nucleotide diversity for each marker used. The p-distance values within Tunisian samples were estimated using Mega v.6 (Tamura et al. 2013). The relationships of inferred haplotypes of mitochondrial nuclear and concatenated dataset of Tunisian *Xerocrassa* species were estimated using the TCS method (Clement et al. 2002) implemented with Popart software v1.7 (Leigh et al. 2015).

Phylogenetic analysis

Our data consist of two mitochondrial markers and one nuclear ribosomal cluster. The data was partitioned used the PartitionFinder software v1.1.1 (Lanfear et al. 2012), in six partitions: three codon positions of the COI, the 16S the rRNA 5.8S and 28S were considered as a single partition and finally the ITS2.

For the mitochondrial dataset as well as for the concatenated data, we produced two phylogenetic trees within the Mediterranean *Xerocrassa* species using the Maximum Likelihood (ML) and the Bayesian inference (BI). The ML analyses were conducted using RAxML v7.2.6 (Boc et al. 2012, Stamatakis 2006) under the GTRGAMMA model, with 1000 nonparametric bootstrap replicates to estimate node support. For the Bayesian Inference, we used Mr Bayes v3.2.2 (Ronquist and Huelsenbeck 2003) using partition scheme and substitutions models suggested by PartitionFinder v1.1.1 (Lanfear et al. 2012). Four independent runs were conducted for 10^6 generations, sampling every 1000. The first 25% trees were discarded as default burn-in and a majority rule consensus tree was calculated from the remaining trees. The topology obtained, and the posterior probabilities of each node were displayed on Figtree V1.4.0 (Rambaut 2012).

Results

Taxonomy

Both, the results of our morphological research on the genital organs as well as the molecular study, prove the affiliation of *Helix latastei* and *Helix latasteopsis* to the genus *Xerocrassa* Monterosato, 1892. For the subgeneric placement refer to the chapter "Discussion,"

Xerocrassa (*Xerocrassa*) *latastei* (Letourneux in Letourneux & Bourguignat, 1887)

Figs 1, 2, 3

1887 *Helix latastei* Letourneux in Letourneux & Bourguignat, Prodrome de la malacologie terrestre et fluviatile de la Tunisie: 63 [Ketenna et dans le vallon de l'Oued El-Ftour, ainsi qu'à l'oasis du Hammam de Gabès. Plaine entre Ras-el-Aïn et Sidi-Salem-Bouguerara. Bir-el-Ahmar. Bords de l'Oued Medzesar et de l'Oued Taferma entre Aïn-Magroun et Fratis. Ras-ed-Djerf, vis-à-vis de Djerba; Zarzis, etc. (Let.). — En Algérie: Ouled Naïl près de Biskraoù, à Aïn-Gussera, à Bou-Ghezoul sur les hauts plateaux, entre Boghar et Laghouat et entre cette ville et Djelfa].

Type specimens. Brandt (1959: 113) considered four taxa of hygromiid species described by Letourneux and Bourguignat, 1887 to constitute the species *H. latastei*. Our investigation of the type specimens of these taxa revealed that the species *Helix fratisiana*

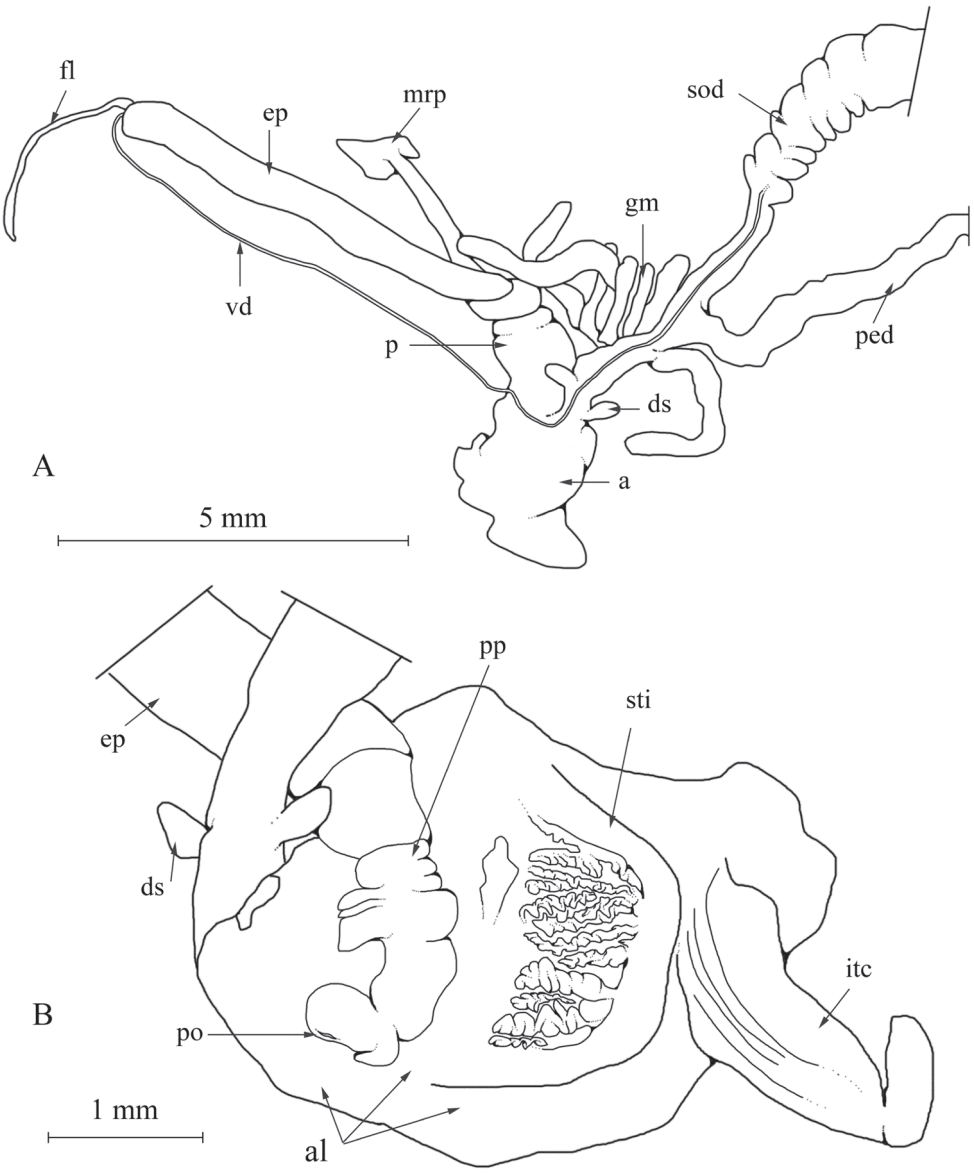


Figure 1. Anatomy of genital organs of *Xerocrassa latastei*; Jorf, 6.12.2015, leg. Ezzine, NMBE 549907/1; **A** situs **B** atrium. Abbreviations: a = atrium; al = atrial lumen; ds = dart sac(s); ep = epiphallus; fl = flagellum; gm = glandulae mucosae; itc = internal tissue cone; mrp = penial retractor muscle; p = penis; ped = pedunculus; po = pore of penial papilla; pp = penial papilla; sod = spermoviduct; sti = stimulator (?); vd = vas deferens.

and *Helix tafermica*, which had been listed by him in the synonymy of *H. latastei*, belong to species of the Hygromiidae living in Tunisia. In order to stabilize nomenclature, we herewith select MHNG-MOLL 115121 as lectotype for *Helix latastei* [hic!]. Thus,

the type locality of this species is herewith restricted to Ketenna [= Kettana]: mouth of Oued El Ferd, Gouv. Gabés, at 33.7575 10.2047; paralectotypes MHNG-MOLL 115121b/4, MHNG-MOLL 115128/2.

Additional specimens examined. Bou Hedma, 29.3.1997, leg. J. Gugel, 34.4958°N 9.488°E, NMBE 516753/1; Boughrara, Medenine, 6.12.2015, leg. Ezzine, NMBE 541952/3, ditto, NMBE 547176/3; ditto, NMBE 541955/7 (preserved); Jorf (El Djorf), Mednine, 6.12.2015, leg. Ezzine, NMBE 541956/1 (preserved), NMBE 549907/1 (anat.); "plaine entre Ras-el-Aïn et Sidi-Salem-Bouguerara", MHNG-MOLL 115118/3, MHNG-MOLL 115126/6, MHNG-MOLL 115127/6, MHNG-MOLL 115129/4; Bir-el-Ahmar MHNG-MOLL 115119/1; Zarzis MHNG-MOLL 115120/2; "Oued el Ftour près de Gabès" MHNG-MOLL 115124/6; "Ras-ed-Djerf, vis-à-vis de Djerba" MHNG-MOLL 115125/1. — Specimens recorded from literature: ruins of Ghighti close Djorf (Djerba) (Jaeckel 1963).

Diagnosis. Shell small to medium sized, thick, basic colour white; protoconch brownish to blackish; three first whorls with granulations; whorls ribbed; suture moderately deep; umbilicus very small, conical.

Description. Shell small to medium sized, depressed globular, thick, basic colour creamy white; protoconch very small, brownish to blackish, smooth, consisting of 1½ whorls; teleoconch consisting of 5½ slightly flattened whorls, sculptured by moderately sized axial ribs; three first whorls brown with whitish granules; lower teleoconch whorls with up to 5 brown spherical bands; suture moderately deep; underside often white; aperture sub-spherical, slightly descending; columellar peristome thick; umbilicus moderately small, conical.

Genital anatomy. The description of the genital organs is taken from an adult and mature specimen collected in El Djorf. Figure 1B shows the lumen of the atrium with its internal structures.

Male part. Penis club-shaped, thick; epiphallus longer than penis; penial retractor muscle inserting at the boundary between penis and epiphallus, with a strong fascia enveloping the genitals; flagellum short; penial papilla subdivided in a simple basal shaft and a subsequent part characterised by deep perpendicular grooves, terminal part of the penial papilla strongly kinked, with central pore at its tip.

Genital atrium. Considerably thickened, lumen filled by two structures: 1) a strong crest of fleshy tissue (here called stimulator), auricle-shaped, the interior side (i.e. opposite to the penial papilla) with zigzag-shaped longitudinal pilasters becoming smooth when entering the interior wall of the atrium, and 2) a longitudinal spoon- or tongue-shaped tissue plate (here called internal tissue cone), with the outer rims bent upwards forming a hollow structure.

Female part. Two very small, almost spherical dart sacs in opposite position; glandulae mucosae simple, tubes randomly attached on the vaginal wall between dart sacs and pedunculus; vagina moderately long, pedunculus formed by a quite strong tube.

Measurements. Lectotype *latastei*: D: 15.9 mm; H: 12.39 mm; PD: 8.58 mm; PH: 6.72; W: 6.25.

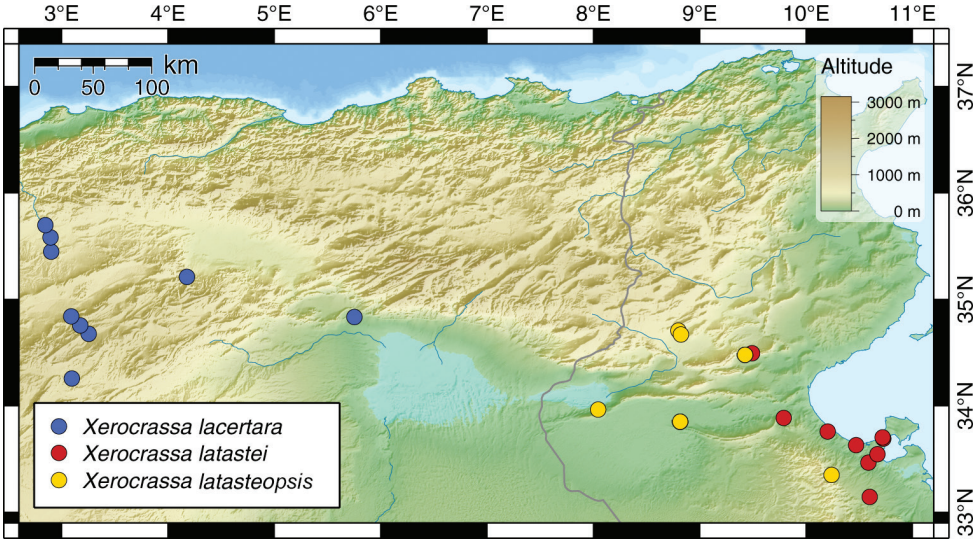


Figure 2. Distribution of *Xerocrassa latastei*, *Xerocrassa latasteopsis* and *Xerocrassa lacertara*.

Distribution (Fig. 2). This species is currently known from the coastal and neighbouring inland areas of central and southern Tunisia. It occurs almost in sympatry with *H. latasteopsis* in some areas of the province Medenine and Sidi Bouzid.

The Senckenberg Museum houses a considerable number of dry shells under the name *H. latatstei* from Libya, based on the collections of Brandt (1959: 112 ff.). They were examined by Neubert during the last years, and they in fact are very similar to *X. latastei* from Tunisia. However, all these shells were collected in the Cyrenaica and its hinterland with the westernmost locality being Marsa Brega (ca. 200 km SSW of Bengasi). So far we have not seen any shells from the Sirte nor the Tripolitanian area towards Tunisia, which embraces almost half of the coastal stripe of Libya. The gap to the Tunisian populations is more than 800 km as the crow flies. This area was visited several times by Kaltenbach (Kaltenbach 1950a; 1950b), but there are no records for *X. latastei* from this area in his rich collection, which is also housed in SMF. As long as no preserved specimens from the Cyrenaica are available, we consider these populations as not conspecific.

Remark. Specimens of this species are characterized by a globose shell with a quite small umbilicus if compared to the large *Cernuella* species, which live sympatrically in southern Tunisia.

The internal structures in the genital atrium are poorly understood. However, when dissecting the atrium, the internal tissue cone is always found to almost completely envelop the penial papilla; the situation shown in Fig. 1B is the result of pulling the penial papilla out of the internal tissue cone. Spreading the opened atrium then leads to a position of this organ on the right side.

***Xerocrassa latasteopsis* (Letourneux & Bourguignat, 1887)**
Figs 2, 3, 5–6

1887 *Helix latasteopsis* Letourneux & Bourguignat, Prodrome de la malacologie terrestre et fluviatile de la Tunisie: 63 [Foum-Hallouf et à Ras-ed-Djerf, vis-a-vis de Djerba].

Type specimens. *latasteopsis*: Foum Hallouf MHNG-MOLL 115131/1 here selected as lectotype [hic!]. paralectotype: Ras-ed-Djerf MHNG-MOLL 115130/1.

Additional specimens. Oasis NE of Tozeur, 10.12.2015, leg. Ezzine, 33.9672°N 8.0421°E, NMBE 541953/1; Bou Hedma, 3.3.2006, leg. I. Abbes, NMBE 551321/X; Oued Medzesar MHNG-MOLL 115122/1; Ksar Sidi Aich 1, Gafsa, 29.4.2014, leg. Ezzine, NMBE 549849/1, 549848/1, 549847/1; Ksar Sidi Aich 2, Gafsa, 34.7061°N 8.7972°E, 9.12.2015, leg. Ezzine, NMBE 549906/1, 549846/1, 547177/1, 541954/1; (Ksar Sidi Aich 1 is located ca. 200 m east of Ksar Sidi Aich 2); Henchir el Zitouna, Medenine, 10.2016, leg. Ezzine, NMBE 551301/9, 551293/6, 551291/1, 551290/1, 551289/1, 551288/1, 549854/1. — Additional specimens in coll. Ezzine/Monastir.

Diagnosis. Shell creamy white throughout, upper teleoconch whorls with fine axial riblets, last whorl almost smooth, umbilicus open, narrow.

Description. Shell medium sized, depressed, creamy white with irregularly dispersed opaque spots, shell walls thick; protoconch very small, brownish to blackish, smooth, consisting of 1½ whorls; teleoconch consisting of up to 6 whorls, upper teleoconch whorls with fine axial riblets and a regular pattern of brownish axial flames fading out as subsutural dots; riblets becoming obsolete on the median teleoconch whorls, last whorl almost smooth with irregular rugosities; suture deep; aperture subspherical, slightly descending; umbilicus open, narrow, conical.

Genital anatomy. The genital anatomy of two adults specimens collected in Henchir el Zitouna and Sidi Aich 2 are illustrated.

Male part. penis club-shaped, thick, with a solid ring-like structure formed by the basis of the penial papilla; epiphallus longer than penis; penial retractor muscle inserting somewhat distal to the boundary between penis and epiphallus, muscle fascia weak; flagellum very short; penial papilla cone shaped, simple, with 2-3 small folds with a central pore at its tip.

Genital atrium. Expanded sac-like structure, with a strongly developed stimulator tissue. The stimulator consists of a thick and tightly upfolded part, connected to the internal tissue cone. The internal tissue cone is fleshy, solid, formed like a stick, and not fully separated from the stimulator.

Female part. Dart sacs in opposite position, very small; glandulae mucosae simple, tubes randomly attached on the vaginal wall between dart sacs and pedunculus; vagina long, pedunculus not strongly developed.

Measurements. Lectotype: H = 14.5 mm; D = 18.34 mm; PH = 9.93 mm; PD = 9.4 mm; W = 6.

Distribution (Fig. 2). This species is known from southeastern Tunisia in the areas north and south of the Chott el Jerid. It also occurs in the Bou Hedma National Park

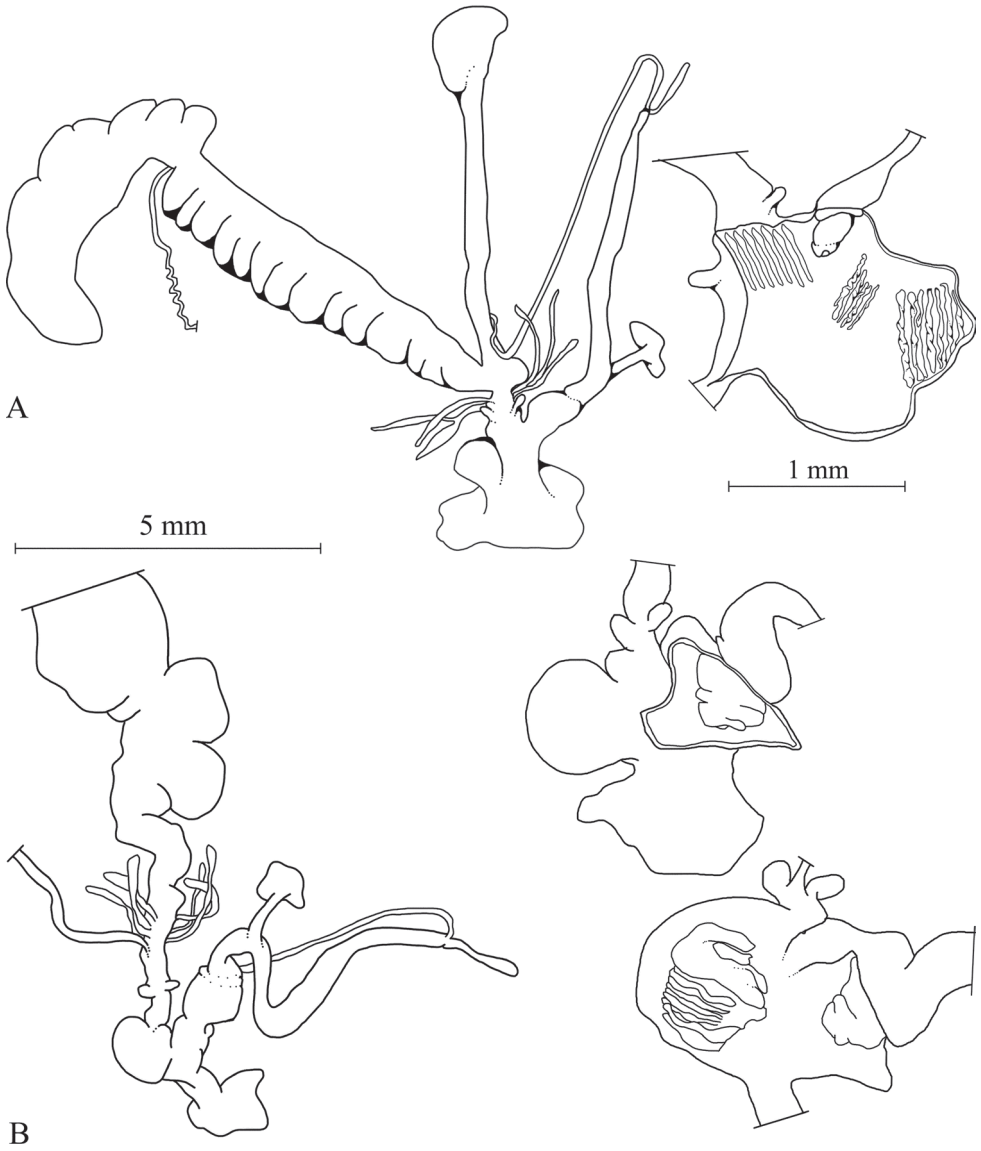


Figure 3. Anatomy of genital organs of *Xerocrassa latasteopsis*; **A** *X. latasteopsis*, NMBE 551301, Henchir el Zitouna: situs, penial lumen and atrial lumen **B** *X. latasteopsis* NMBE 549906, Sidi Aich 2, situs, penial lumen; and atrial lumen.

in central Tunisia, where it obviously comes close to *H. latastei*. Our records from Bou Hedma National Park originate from two different sources, and the exact collecting sites are not known. A sympatric occurrence cannot be excluded. The type locality Foum Hallouf as given by Letourneux and Bourguignat is also imprecise, this term is used for a larger area east of the small hill chain between Dkhilet Toujane and Beharya;

the locality Henchir el Zitouna is situated in the centre of this area, so these specimens can be considered as topotypes (Fig. 2).

Remarks. Besides the genetic difference observed (see Figs 8, 9), there are also slight differences found in the morphology of both, shells, and genital organs. The shell of *X. latasteopsis* is always white (with up to five brown spiral bands in *X. latastei*), the riblets are fine (much stronger in *X. latastei*), the lower whorls are smooth and a bit wrinkled (ribbed throughout in *X. latastei*), and the umbilicus is narrow (somewhat larger in *X. latastei*). The penial papilla is short conical in *X. latasteopsis* (elongate in *X. latastei*), and the flagellum is short if compared to the epiphallus (longer in *X. latastei*).

When describing their *Helix latastei*, Letourneux and Bourguignat mentioned several localities for this species from Algeria. However, it turned out that these localities had been mentioned earlier by Bourguignat in his description of *Helix lacertarum* in 1863. Obviously, Letourneux and Bourguignat in 1887 considered both nominal species to be conspecific without clearly stating this opinion. After examination of all specimens in the collection of Bourguignat we come to the conclusion that, for the time being, the Algerian shells have to be considered as a separate species.

Xerocrassa (*Xerocrassa*) *lacertara* (Bourguignat, 1863)

Figs 2, 7

1863 *Helix lacertarum* Bourguignat Malacologie de l'Algérie, I: 209 [Plaines entre Djelfa et El-Aghouat (de la Péraudière)].

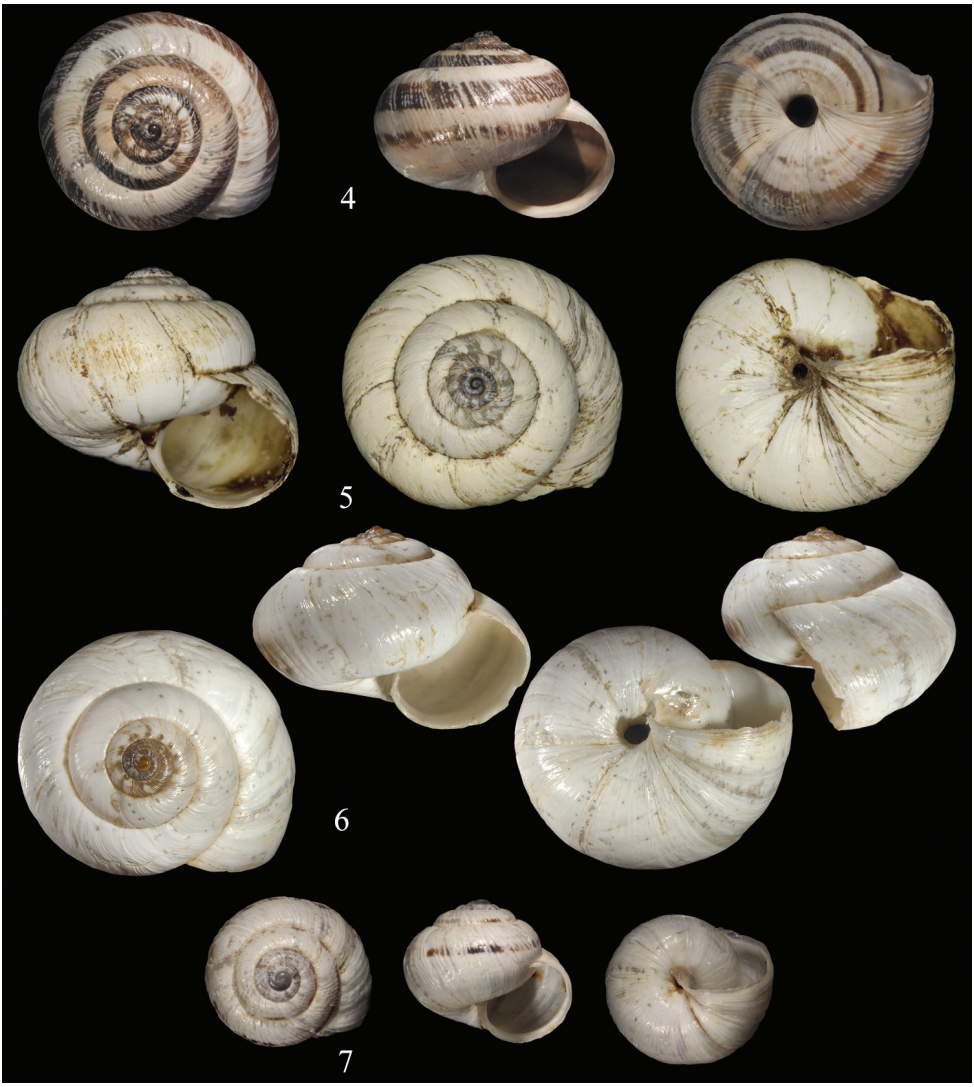
1863 *Helix lacertarum* var. *minor* Bourguignat Malacologie de l'Algérie, I: 209 [collines d'Ogla-Zemera, à 10 lieues nord-ouest de Bou-Saâda (Marès)].

1898 *Helix lacertarum*, Pallary, Comptes rendu de l'association française pour l'avancement des Sciences [Saint-Etienne], 26 (2) [1897]: 557.

Type specimens. *lacertarum*: Syntypes MHNG-MOLL 114001/5; minor: syntype MHNG-MOLL 114006/1.

Additional specimens. "Djebel Sahari près de Djelfa (34.6743°N 3.2552°E) MHNG-MOLL 114003/10; "entre le rocher du Sel et Mesram" (34.8375°N 3.0921°E) MHNG-MOLL 114004/8; "entre Aïn Ouessera et Bou Ghezoul" (35.5819°N 2.8992°E) MHNG-MOLL 114005/11; "Aïn-Seba, près de Bousaada" (35.2118°N 4.1763°E) MHNG-MOLL 114007/1. — Localities mentioned in the synonymy of *X. latastei*, but not represented in Bourguignat's collection: "Ouled Naïl près de Biskraoù" (34.8370°N 5.75104°E); "à Aïn-Gussera" (= Ain Oussera 35.4495°N 2.9045°E); "à Bou Ghezoul sur les hauts plateaux" (= Boughezoul 35.6992°N 2.8482°E); "entre Boghar et Laghouat" (34.7554°N 3.1747°E) "et entre cette ville et Djelfa" (34.2577°N 3.0998°E). — unclear: MHNG-MOLL 114002/1, Saïda (pr. Oran); MHNG-MOLL 114008/1 Sebdou (pr. Oran).

Description. Shell small, globular, basic colour creamy-whitish; protoconch very small, brownish, consisting of two whorls; teleoconch with many axial riblets, surface



Figures 4–7. *Xerocrassa* species. **4** *Xerocrassa latastei,* lectotype MHNG-MOLL 115121, Ketenna [= Kettana], D = 15.9 mm **5** *Xerocrassa latasteopsis*, lectotype MHNG-MOLL 115131, Foum Hallouf, D = 18.34 mm **6** *Xerocrassa latasteopsis,* NMBE 549906, Sidi Aich 2, D = 18.2 mm **7** *Xerocrassa lacertara*, syntype MHNG-MOLL 114001, "Plaines entre Djelfa et El-Aghouat", D = 11.8 mm.

submalleate; whorls well rounded, with a moderately deep suture; last whorl with a single brown band at the periphery, often dissolved to a string of brown stripes; dark spots may occur usually irregularly spread all over the teleoconch, sometimes arranged in axial stripes; aperture semioval, with a small white lip; peristome small, sharp; umbilicus narrow, nearly completely obscured by a reflection of the columellar callus.

Measurements (syntype). D: 11.8 mm; H: 10.1 mm; PD: 6.7 mm; PH: 5.63 mm; W: 5.75.

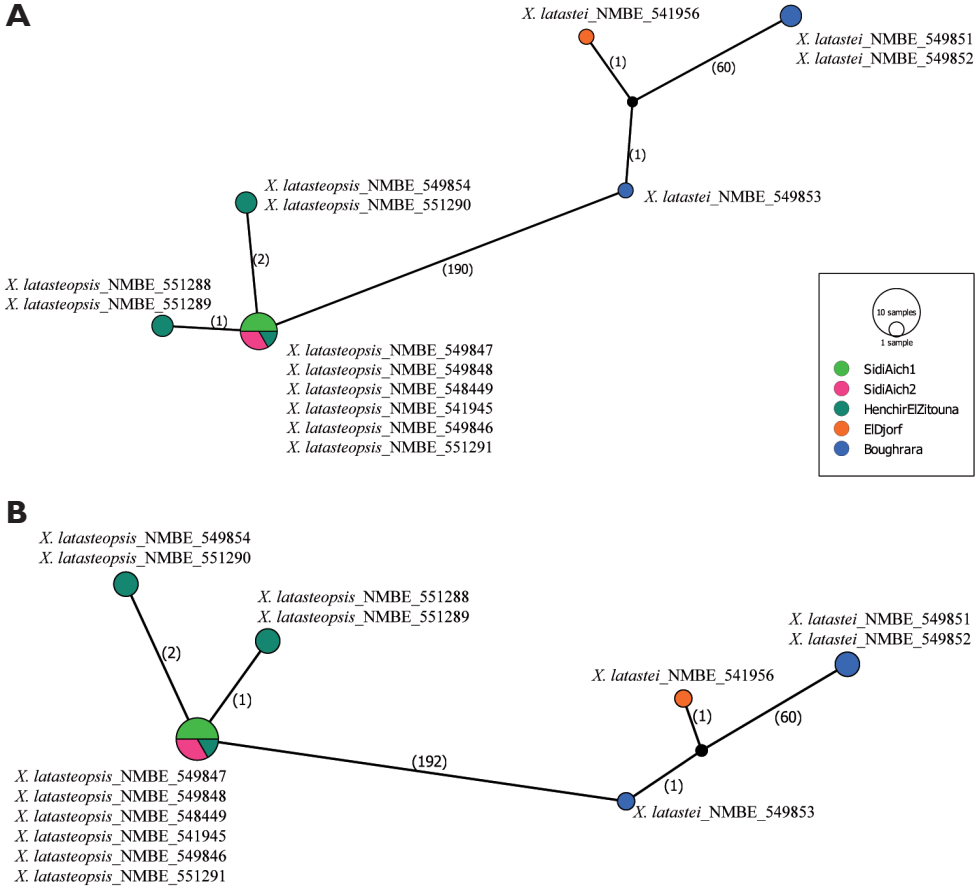


Figure 8. Haplotype Network showed the number of variable sites. **A** Haplotypes and numbers of variables sites based on mitochondrial markers **B** Haplotypes numbers of variables sites based on concatenated mitochondrial and nuclear data.

Distribution (Fig. 2): *Xerocrassa lacertara* is hitherto only known from the collection of Bourguignat, and seems to live restricted to the arid areas in eastern Algeria.

Remark. Deduced from its shell morphology, this species is close to *X. latastei*. Both species share the globular shell form, the glossy shell surface, the ribbing mode, and the colouration pattern. In the absence of preserved specimens, we used these criteria to classify this species within the genus *Xerocrassa*. It differs from *X. latastei* in size (smaller in *X. lacertara*), in the umbilicus, which is more strongly covered in *X. lacertara* than in *X. latastei*, in the more pronounced ribbing pattern of the teleoconch whorls, and the missing granulation of the upper teleoconch (in *X. latastei*), which is malleate in *X. lacertara*.

When describing *X. latastei*, Bourguignat mentioned some of the localities, where he recorded *X. lacertara* 34 years before. This proves that he had no clear concept of

these two species. Looking to the distribution patterns, both species are separated by a large area (ca. 300 km as the crow flies) without any record of the one or the other species. This is not simply an artefact due to undersampling, because the southern part of the province of Constantine is relatively well represented in his collection. For this reason and the pronounced differences in shell morphology we keep these two taxa as separate species until preserved animals from Algeria can be studied.

There are two records for this species from western Algeria south of Oran in MHNG, but their presences in the area needs reconfirmation in order to avoid any mis-labelling in the museum. Pallary (1898) records the species from "sur les berges de l'O. Souag (= O. el Hammam), à 12 kilomètres S.-O. d'Aïn Fekan". These specimens were not seen by the authors, and thus their identity remains uncertain.

Molecular analysis

This dataset consists of two mitochondrial markers (COI and 16S) and one nuclear cluster (5.8S-ITS2-28S). The mitochondrial data was analysed first and afterwards the nuclear marker was added to confirm the results.

Haplotype network and genetic diversity

The results of the anatomical and morphological studies of the Tunisian samples show that there are two *Xerocrassa* species existing: *X. latasteopsis* and *X. latastei*. The nucleotide divergence of these two morphological groups is studied, and a haplotype network is produced. Among fourteen sequences of 1090 bp (655 bp of COI and 435 bp of 16S) of Tunisian *Xerocrassa*, six haplotypes were identified using both markers, suggesting a high haplotype diversity (Hd=0.8022). The haplotypes obtained cluster in two divergent haplo-groups: the first is formed by samples collected from SidiAich1, SidiAich2, and Henchir El Zitouna, and the second was formed by samples collected from El Djorf and Boughrara (Fig. 8A). A high number of variable sites could be found in-between the groups (190 sites: COI: 118 and 16S: 72), but only a low number within the groups (maximum of 60 sites within the group of Boughrara_El Djorf). Thus, the nucleotide divergence was of 17% between haplo-groups, and 5.5% within the haplo-group of Boughrara-El Djorf and a low divergence within Gafsa-Henchir El Zitouna (<1%).

The analyses of each mitochondrial separately showed some differences between COI and 16S. The nucleotide divergence of COI sequences reached 18% between groups, and varied between 0.4% and 6% within haplo-groups. Additionally, the amino acid composition of the partial COI sequence (218 amino acids) displayed eight different amino acids between haplo-groups of which two are of different polarity.

The ribosomal gene 16S showed a high nucleotide divergence between haplo-groups (16%) and a low divergence with a maximum of 2% of nucleotide divergence

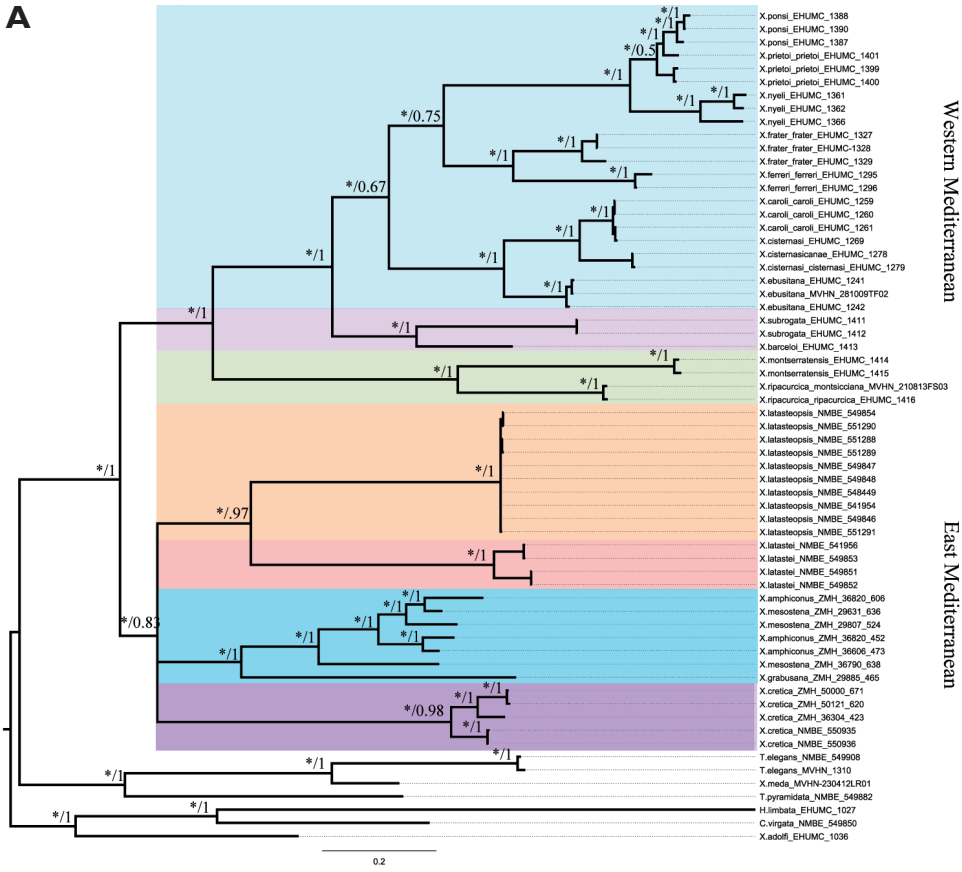


Figure 9. Phylogenetic trees obtained by Bayesian inference (BI) and Maximum Likelihood (ML) methods. **A** Tree inferred based on partial mitochondrial sequences of COI and 16S **B** Tree inferred based on mitochondrial data, partial sequences of 5.8S, complete sequence of ITS2 and partial sequence of 28S rRNA. Posterior probability (PB) obtained from Bayesian analysis and bootstrap values obtained from Maximum likelihood (ML) were presented on each node (*: BS= 100).

within the haplo-group of Boughrara-El Djorf and a monomorphic haplo-group formed by the sequences of Gafsa and Henchir el Zitouna.

The assessment of the nuclear ribosomal cluster 58S-ITS2-28S (1320 bp) showed that all 5.8S and 28S sequences used were identical. The sequences of ITS2 displayed only a single insertion/deletion mutation and one substitution between *X. latasteopsis* and *X. latastei* suggesting an extreme conservation of nuclear sequences in Tunisian *Xerocrassa* species. Adding the ribosomal cluster did not affect the haplotype diversity obtained using the mitochondrial data. In fact, we observed six haplotypes grouped in two haplo-groups with a haplotype diversity of 0.8022, a nucleotide divergence of 8% between *X. latasteopsis* and *X. latastei* (Fig. 8B).

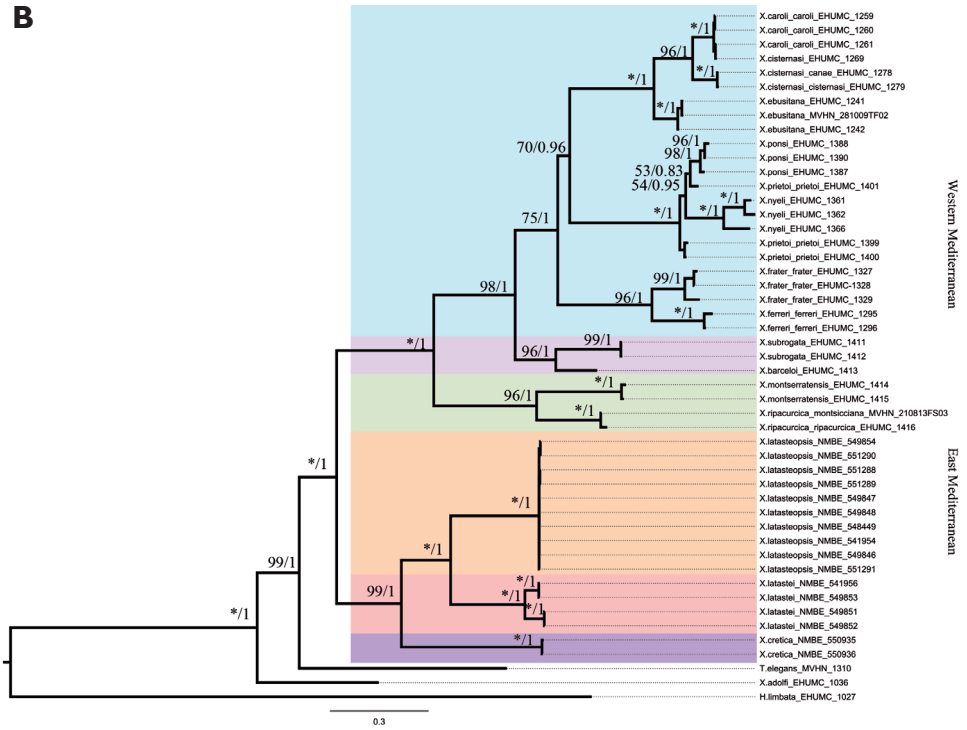


Figure 9. Continued.

Phylogeny

Both topologies of the mitochondrial (mt) data from the ML and BI analyses are identical. The tree obtained is rooted by two *Trochoidea* species, *C. virgata*, *Xerosecta adolfi* and *H. limbata*. Mediterranean *Xerocrassa* species were divided in two groups following the geographical distribution pattern (Fig. 9A): 1) An East-Mediterranean group formed by the Tunisian and the Cretan *Xerocrassa* species. 2) A West-Mediterranean group composed by three clades: one clade comprising the Balearic radiation, and two continental Spanish clades. Both groups are supported by high bootstrap values (BS=100%) and posterior probability (PP=0.83). The East-Mediterranean group shows three highly supported clades: one formed by Tunisian species, one composed by the Cretan *X. cretica* and one formed by the rest of the Cretan *Xerocrassa* radiation. In Tunisia, the *Xerocrassa* species split into two well supported (BS=100%, PP=1) monophyletic clades. In *X. latasteopsis* samples, which were collected from two distinct areas, were grouped in rake proving a low mitochondrial diversity within species.

The position of "*Xerocrassa meda*" close to *Trochoidea* is quite unexpected. In case it is not a mix-up with a specimen from the highly polymorphic *Trochoidea spratti*-group, then the mitochondrial sequences are not informative. Inclusion of nuclear markers in the analysis will probably yield a better result.

For the concatenated tree, all Cretan *Xerocrassa* species except two samples of *X. cretica* had to be excluded because of nuclear data deficiency. Both trees based on Ml and BI analyses show identical topologies; they are rooted by *T. elegans, Xerosecta adolfi* and *H. limbata*. Again, two main Mediterranean clades appear, their node is well supported (Fig. 9B): all Spanish *Xerocrassa* species cluster together forming a single clade (BS=100%, PP=1), which in itself is divided in the three groups, one insular and two continental. Here, the Cretan *X. cretica* clusters with Tunisian *Xerocrassa* species composing a well-supported group (BS=100%, PP=1). The two Tunisian species are well separated and strongly supported (BS=100%, PP=1).

Discussion

The research approach followed here is according to DeSalle (2005) and Hirano et al. (2014), who argue that a biological classification is only valid when using the efforts of a combined study of morphological, anatomical, and molecular characters. Thus, the type areas of *Helix latastei* and *Helix latasteopsis* in Tunisia were visited and living animals and dry shells from the respective localities listed by Bourguignat were collected in order to work with topotypic specimens.

Taxonomic considerations

The type species of *Xerocrassa* Monterosato, 1892 is the east Mediterranean species *Helix seetzeni* L. Pfeiffer, 1847 (by monotypy). *Xerocrassa* is currently characterized by a symmetrical dart apparatus consisting of two small accessory sacs (= appendicula sensu auct.) and usually four branched glandulae mucosae around the vagina, irregular folds at the inner side of the wall of the vagina and the lack of a well-developed appendix at the atrium; the penis is innervated from the right cerebral ganglion (Hausdorf and Sauer 2009: 375). The absence of the atrial appendix is basically the only character state that separates *Xerocrassa* from *Trochoidea* Brown, 1827. Hausdorf and Sauer (2009) report the presence of an atrial "bulge-like stimulatory structure" in some *Xerocrassa* species such as *X. cretica*, *X. franciscoi* and *X. heraclea*, which can be seen as a small protuberance at the side of the atrium in the Tunisian *X. latastei* and *X. latasteopsis* (see Figs 1, 3), the atrium is much wider, and bulge considerably more pronounced. The homology of this organ with the atrial appendix seen in *Trochoidea* is not clear, and there is no other evidence than the similar position at the atrium. The internal tissue crest is here called a stimulator referring to the similarly shaped stimulator found in the atrium of many helicid genera. It seems to consist of two parts, a massive block of tissue, and a separate tongue- or cone-shaped stylus. A similar structure is illustrated by Giusti et al. (1995) for *Trochoidea* species (CAA in their nomenclature). Thus, a morphological separation of the two genera remains difficult, and our results show that the two genera are closely related.

Pallary (1919) based his monotypic genus *Ereminella* on *Helix latastei* Letourneux in Letourneux & Bourguignat, 1887 without delivering any discriminating characters. Brandt (1959) recognized that the species recorded by Bisacchi (1932: 361, Figs 2–4) under *Helicella* (*Xerocrassa*) *pseudosimulata* (Germain 1921) from El Agheila and Soluch-Agedabia (Libya) was a misinterpretation, and identified it with *H. latastei*, which he subsequently affiliated to *Trochoidea* (*Ereminella*). In the same publication, Brandt introduced the monotypic subgenus *Trochoidea* (*Xerobarcana*) (based on *Xerobarcana huggani* Brandt, 1959), and *Trochoidea* (*Xeroregima*) (based on *Trochoidea* (*Xeroregima*) *regimaensis* Brandt, 1959). Both subgenera show the same principal construction of their genital organs and are congruent with what is considered *Xerocrassa* today. *Xerobarcana* is defined as "differing from all other subgenera of this genus [*Trochoidea*] by the rudimentary wart-like flagellum and the conspicuously strong vas deferens" (translated from the original German text). Today, the relative length of the flagellum is considered a character state that encodes on species level, and is widely used in hygromiid and geomitrid taxonomy (Hausdorf 2000: 62); thus, the reduced flagellum reported by Brandt simply constitutes a character state of *Xerocrassa huggani*. The definition of *Xeroregima* is as follows: "Anatomically differentiated from *Trochoidea* (*Trochoidea*) s. str. by the lack of the vaginal appendix [sic!] and the penis, which is club-like swollen at the transition between penis and epiphallus" translated from the original German text). Apparently, Brandt confused the terms vaginal with atrial, and thus exactly described the situation as known in *Xerocrassa*! Even the club-shaped transition between penis and epiphallus is perfectly seen in the majority of Cretan *Xerocrassa* species as well as in *X. latastei*. Consequently, we consider *Xeroregima* as a junior synonym of *Xerocrassa*.

Molecular analysis

As with the morphological and anatomical investigation, the results of our molecular approach show that, independently which maker is considered, Tunisian samples divided into two species and cluster together with the selected *Xerocrassa* species from Crete, the Balearic Islands and Spain, and thus our generic affiliation of the species is correct. There are several remarkable findings, which require deeper examination.

Haplotype network and genetic diversity

The divergence of the COI sequences between Tunisian species (18%) widely exceeded the threshold of 3% as suggested by Hebert et al. (2003) to characterize animal species in general and the threshold of 4% to identify land snails (Davison et al. 2009). In Tunisia, Chott el Jerid is widely described as a geographical barrier for many taxa (Millington et al. 1989; Ben Othmen et al. 2009; Abdallah et al. 2012; Farjallah et al. 2012). Such a barrier may restrict the gene flow between geographically isolated

populations resulting in independent evolution and increase the genetic divergence within species (Funk 2003). In this case, *X. latasteopsis* shows a low divergence between the northern (Sidi Aich 1, Sidi Aich 2) and the southern (Henchir el Zitouna) populations, which share one haplotype. This result disproves this hypothesis for the snail species concerned, suggesting that Chott el Jerid does not restrict the gene flow. It cannot be considered as a barrier for this species. In contrast, *X. latastei* shows a quite high divergence within the population of Boughrara (6%) which could be interpreted as individual diversity.

This high divergence between the two Tunisian *Xerocrassa* species (16%) was also demonstrated by analysis of the 16S marker. High values of genetic divergence were reported for the land slug *Phyllocaulis* (13.1%) (Gomes et al. 2010) and between congeneric species of Ariophantidae and Dyakiidae (4.3 to 10.1%) (Abu-Bakar et al. 2014). Moreover, Liew et al. (2009) reported divergence values of 5% to 25% within *Everettia* spp. (Dyakiidae). The divergence of the 16S between Tunisian *Xerocrassa* species is higher than the divergence of 11.8% between Cretan *Xerocrassa* species as shown by Sauer and Hausdorf (2009). The divergence seen here is quite remarkable but not completely outstanding.

The nuclear cluster 5.8S-ITS2-28S widely confirms the results obtained from the mitochondrial markers. Both, the 5.8S and 28S sequences seem to be conserved within the Tunisian species, and the ITS2 shows only one nucleotide substitution and one insertion/deletion mutation. Thus, the genetic variability is focused on the mitochondrial markers, while the nuclear markers investigated seem to be highly conserved.

Phylogeny

This is the first time that a combined phylogeny for this widespread genus has been shown. As could be expected, the clades follow the distribution pattern of a west and an east Mediterranean group. Each cluster includes an island radiation and a continental radiation. The latter fall in two groups for Europe, and one of them, which includes *Helix montserratensis* Hidalgo, 1870 as its type species [by monotypy] may bear the subgeneric name *Amandana* Fagot, 1891. The results of these combined markers proved the results obtained by mitochondrial markers and confirmed the split between geographical *Xerocrassa* groups. Our results suggest that Tunisian *Xerocrassa* species are more closely related to the Cretan species than to the Spanish and Balearic species. However, within the east Mediterranean clade, the relationship between *X. cretica* and the rest of the Cretan radiation is not that close with a low support of 0.75 in the mitochondrial tree. A direct comparison with the Tunisian species is problematic. The eastern Mediterranean area, especially Libya, and Egypt, is heavily undersampled, and including more species from this area and the Middle East will certainly change the relative position of Tunisian species to the Cretan species as well as the position of *X. cretica* on the tree.

The shell morphology of land snails is extremely affected by environmental conditions (Alonso et al. 1985; Chiba 1999; Pfenninger et al. 2006). The use of these characters in the taxonomic analysis of land snail species were severely criticized (Giusti and Manganelli 1992; Schilthuizen and Gittenberger 1996; Uit de Weerd et al. 2004; Holland and Hadfield 2007). As shown by (Elejalde et al. 2008), a comparison between shell morphological and molecular characters result in incongruent data. However, the integrative approach as used here results in a distinct network of character states enabling to interpret the morphology even of the shells, and to formulate distinctive shell traits. Here, the morphological and anatomical disparity between *X. latasteopsis* and *X. latastei* has been confirmed by phylogenetic analysis. In the reverse conclusion we now can pinpoint the significance of relative flagellum length and form of penial papilla as well as the ribbing mode of the shell, its colouration and other structural details as relevant and useful for species identification within Tunisian *Xerocrassa* species (Hausdorf and Sauer 2009).

Conclusions

This study, based on morphological, anatomical and molecular characters allows the placement of the Tunisian species *Helix latastei* Letourneux, 1887, and *Helix latasteopsis* Letourneux & Bourguignat, 1887 to *Xerocrassa*. This investigation of relationships among species within the genus demonstrates that Tunisian *Xerocrassa* species are more closely related to the Cretan radiation than to the Balearic and Spanish radiation.

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New Philippine species of *Spilosmylus* Kolbe (Neuroptera, Osmylidae)

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Abstract

New species of lance lacewings, *Spilosmylus spilopteryx* **sp. n.** and *Spilosmylus tephrodestigma* **sp. n.**, are described from the Philippines and compared with congeners. Both species are characterised by a distinctive wing pattern, which in the case of *Spilosmylus spilopteryx* **sp. n.** is relatively spectacular among lacewings. An identification key to the species of *Spilosmylus* Kolbe known from the Philippines is also provided.

Keywords

Osmylidae, Spilosmylinae, lance lacewings, Oriental region, Malesia, taxonomy

Introduction

Osmylidae, or lance lacewings, are a small (ca. 225 extant species) family of Neuroptera whose oldest fossil crown group members are known from various Jurassic to Tertiary deposits (Khramov 2014a, b; Oswald 2017; Winterton et al. 2017), and with stem group fossil species known from the Late Permian and throughout the Triassic (Khramov 2014a; Makarkin et al. 2014). Osmylids are present in most biogeographical regions but are notably absent from the Nearctic region, although tertiary-aged fossils are described from the Eocene of the Green River Formation (Makarkin 2017).

They are medium- to large sized neuropterans, usually characterized by strongly patterned wings, sometimes of unusual shape. Their larvae are unmistakable due to their elongate, lance-like mandibles and paired hooked apparatus (i.e., pseudopods) at the apex of the abdomen (Matsuno and Yoshitomi 2016). The larval biology remains one of the most obscure aspects of this lacewing family, since it remains very poorly known with a few exceptions (Brauer 1851; Hölzel and Weissmair 2002; Matsuno and Yoshitomi 2016; Winterton et al. 2017). Osmylidae are also unusual among Neuroptera, with larvae in both riparian (e.g., wet soil adjacent to lotic water bodies) and terrestrial (e.g. leaf litter and subcortical) habitats (New 2003; Winterton et al. 2017). The phylogenetic position and internal relationships of Osmylidae have been long disputed, despite most authors agreeing that Osmylidae represent a relatively early branching lineage of Neuroptera (Withycombe 1925; Haring and Aspöck 2004; Aspöck and Aspöck 2008; Beutel et al. 2010; Randolf et al. 2014; Winterton et al. 2010). This phylogenetic position has been recently confirmed by mitogenomic studies, which recovered the family near the base of lacewing tree, more derived than Coniopterygidae and as a subsequent clade including Sisyridae + Nevrorthidae, sister to all the remaining families of Neuroptera (Wang et al. 2016). In their study of Osmylidae phylogeny, Winterton et al. (2017) found evidence of a sister relationship with Nevrorthidae and support for eight monophyletic osmylid subfamilies. These subfamilies were grouped into two main clades, the first including Gumillinae, Protosmylinae, Spilosmylinae and the second including Osmylinae, Porisminae, Eidoporisminae, Kempyninae and Stenosmylinae. The monophyly of the first lineage is supported by the unbranched hind wing vein CuP (in contrast with a strongly pectinate hind wing CuP vein of other subfamilies). A close relationship between Protosmylinae and Spilosmylinae is supported by molecular data and by the presence of unique features in male and female genitalia, in particular the presence of a narrowly arching gonarcus (Winterton and Wang 2016; Winterton et al. 2017). Spilosmylinae are recognizable due to the presence in the hind wing of a spur vein originating basal to the MP vein and of a basal sclerotised process on the mediuncus (Wang et al. 2011; Winterton et al. 2017). This group is by far the largest subfamily of osmylids, with at least 113 described species, although placed in only three genera: *Thaumatosmylus* Krüger (8 species), *Thyridosmylus* Krüger (20 species) and the most diverse genus of the family, *Spilosmylus* Kolbe (85 species). *Thaumatosmylus* is limited to the Oriental region (New 1991; Wang et al. 2011), while *Thyridosmylus* and *Spilosmylus* have a wider distribution, being also present in the Afrotropical and (in the case of *Spilosmylus*) Australasian regions, although they are most diverse in South-East Asia (Tjeder 1957, New 1986a, 1986b, 1988, 1991, 2003; Winterton et al. 2017). Divergence time estimates support a mid-Jurassic origin for *Thyridosmylus* and *Spilosmylus*, also explaining their unusual biogeographic pattern (Wang et al. 2011; Winterton et al 2017). Despite their ancient origin, the genera of Spilosmylinae are notoriously difficult to delimit using morphology alone and some species are of problematic allocation (New 2003). In particular, *Spilosmylus* is morphologically diverse, including both small and delicate (often yellow-green) species to large robust ones (New 2003). The genus *Thyridos-*

mylus and most species of *Spilosmylus* are best distinguished from *Thaumatosmylus* in the absence of crossveins between M and CuA after the basal crossvein, making a long undivided cell (New 1991, 2003). *Thyridosmylus* itself is mostly recognizable due to the presence of fenestrate markings on the forewing, although this distinction is unclear in some species as they lack the markings. Most species of *Spilosmylus* have intermittent dark dash-like markings between forewing veins Sc and R and/or the presence of an embossed spot (rarely two spots) near the hind margin of the forewing, although these characters are also highly variable (New 1986a, 2003) and are lacking in multiple species. The biology of Spilosmylinae is poorly known. The larvae of a Japanese species were reportedly found near streams (Kawashima 1957).

Malesia is a centre for diversification for *Spilosmylus*, with at least 54 species known from this region (New 2003). Various authors have described species from Malesia (McLachlan 1870; Gerstaecker 1893; Krüger 1913, 1914, Navás 1926), although their descriptions are often inadequate to provide useful comparisons. Banks (1924, 1931, 1937) described several species of *Spilosmylus*, particularly focussing on the Philippines and published the first identification key for species known from this archipelago (Banks 1937). Later, New (1986a, 1986b, 1988, 1991) revised the Oriental and Australasian Osmylidae, describing many new species of Spilosmylinae and provided identification keys to most of the known species. The works of New represent a significant contribution to the characterization of problematic and poorly known species described by earlier authors, and documents the exceptional diversity of *Spilosmylus* in the region. Despite these efforts, the lance lacewings of the Philippines remain poorly known and they received no further attention since then.

Herein, we describe two new species of *Spilosmylus, S. spilopteryx* sp. n. and *S. tephrodestigma* sp. n., from Luzon and compare them with the other species of *Spilosmylus* known from the Philippines. Both species are easily recognizable due to the distinctive wing pattern, easily setting apart them from all other congeners.

Materials and methods

During the last few decades, two different terminology systems were applied to the genital sclerites of Osmylidae. Wang et al. (2011), Winterton and Wang (2016) and Winterton et al. (2017) used an updated version of the classical terminology of Tjeder (1957) and Adams (1969), based on comparisons and homology assessments across the whole family and with other Neuroptera. On the other hand, Aspöck and Aspöck (2008) proposed a different terminology, which was recently extensively applied to Osmylidae by Martins et al. (2016). However, as discussed in length by Winterton et al. (2017), the lack of adequate comparisons among the numerous subfamilies of Osmylidae hampered the recognition of genital sclerites in this family. In some subfamilies of Osmylidae the parameres (*sensu* Tjeder 1957) are absent, and the mediuncus has the role of main intromittent organ (Winterton et al. 2017). Therefore, Aspöck and Aspöck (2008) and Martins et al. (2016) considered the dorso-caudal

sclerite as homologous with the gonocoxites 9 (i.e., parameres of Tjeder 1957), and the gonocoxites 11 (i.e., gonarcus of Tjeder 1957) as absent. However, in Protosmylinae, Spilosmylinae and Osmylinae the parameres are indeed present, and the gonarcus has an inverted "U"-shape typical of many neuropterans (Wang and Winterton 2016) (Fig. 4). Consequently, Osmylidae are in fact no exception with respect to other lacewing families in the overall structure of the genitalic sclerites, although the parameres have been lost in some subfamilies (e.g., Stenosmylinae, Kempyninae, Porisminae). To promote an interchangeability between the two terminologies used in Neuroptera, we consider the gonocoxites 11 *sensu* Aspöck and Aspöck (2008) as present in Osmylidae and homologous with the gonarcus of Tjeder (1957). Here we follow the terminology of Tjeder (1957) as implemented by Winterton et al. (2017).

Wing terminology follows Winterton et al. (2017) and does not assume that MA is fused basally with R to thus represent the posterior most vein of the R field.

Specimens were studied with a Leica MZ 9.5 stereomicroscope and measured with an optical micrometre. Photographs were taken with a Canon EOS 600D digital camera equipped with Canon lens MP-E 65 mm. The obtained images were stacked with the software Zerene Stacker and later post-processed with Adobe Photoshop. Specimens were measured using the following protocol: body length was taken from vertex to tip of the abdomen; wing length was measured longitudinally from base to apex, and wing width was taken as the maximum width perpendicular to the length measurement line. Genitalia were macerated in 10% KOH (potassium hydroxide) at room temperature, later rinsed in acetic acid and water and finally stained in Chlorazol Black. The genitalia were preserved in glycerol in a small vial put beneath the specimen.

Taxonomy

***Spilosmylus spilopteryx* sp. n.**
http://zoobank.org/4FCE4CE0-E7E1-4A4A-A80E-C822F20513CF
Figs 1A, 2A, 3, 4

Material examined. Holotype. Pinned, genitalia in glycerol, preserved beneath the specimen. **PHILIPPINES**, South Luzon, Tigaon, Camerines sur, February 2015, 1 ♂, local collector, (Naturhistorisches Museum Wien).

Diagnosis. Medium sized osmylid with uniformly brown body; both wings with intermittent dark dashes on Sc and R; forewing membrane with a distinct pattern composed by three large light brown markings; hind wing membrane hyaline (Fig. 1A).

Description. *Dimensions.* Body length: 10.48 mm; forewing length 17.46 mm, width 6.03 mm; hind wing length: 16.35 mm, width: 5.08 mm.

Head. Mostly brown. Vertex light brown. Frons and clypeus reddish brown with a central rounded darker marking. Labrum and gena light reddish brown. Maxillary and labial palpi pale. Scape reddish brown, flagellomeres yellowish, slightly darker apically.

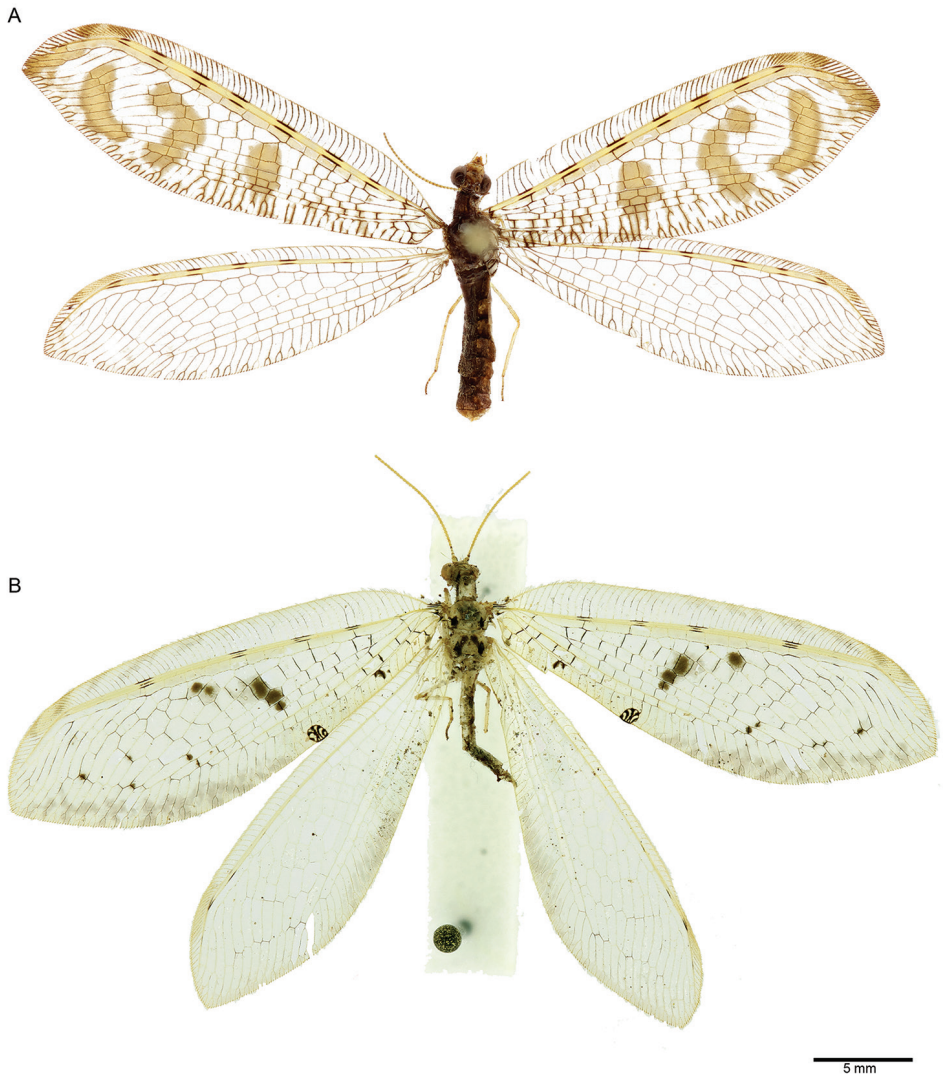

Figure 1. *Spilosmylus* spp., habitus: **A** *Spilosmylus spilopteryx* sp. n. **B** *Spilosmylus tephrodestigma* sp. n.

Thorax. Predominantly brown. Pronotum distinctly longer than wide, with undefined paler stripes running longitudinally to it (Fig. 3); mesonotum and metanotum uniformly brown; thorax covered with dark setae. Legs. Pale brown.

Wings. Forewing relatively broad with a slightly pointed apex, membrane hyaline with conspicuous markings and shades (Fig. 2A). Venation brown. Costal area progressively narrowing toward the apex. Pterostigma brown, lighter medially. Sc and R yellowish, with intermittent, parallel, black dashes. Subcostal area uniformly yellowish. Area between R and Rs uniformly light brown from the first crossvein until the pterostigmal area. Apex of forewing with a brown marking. Forewing medial fork

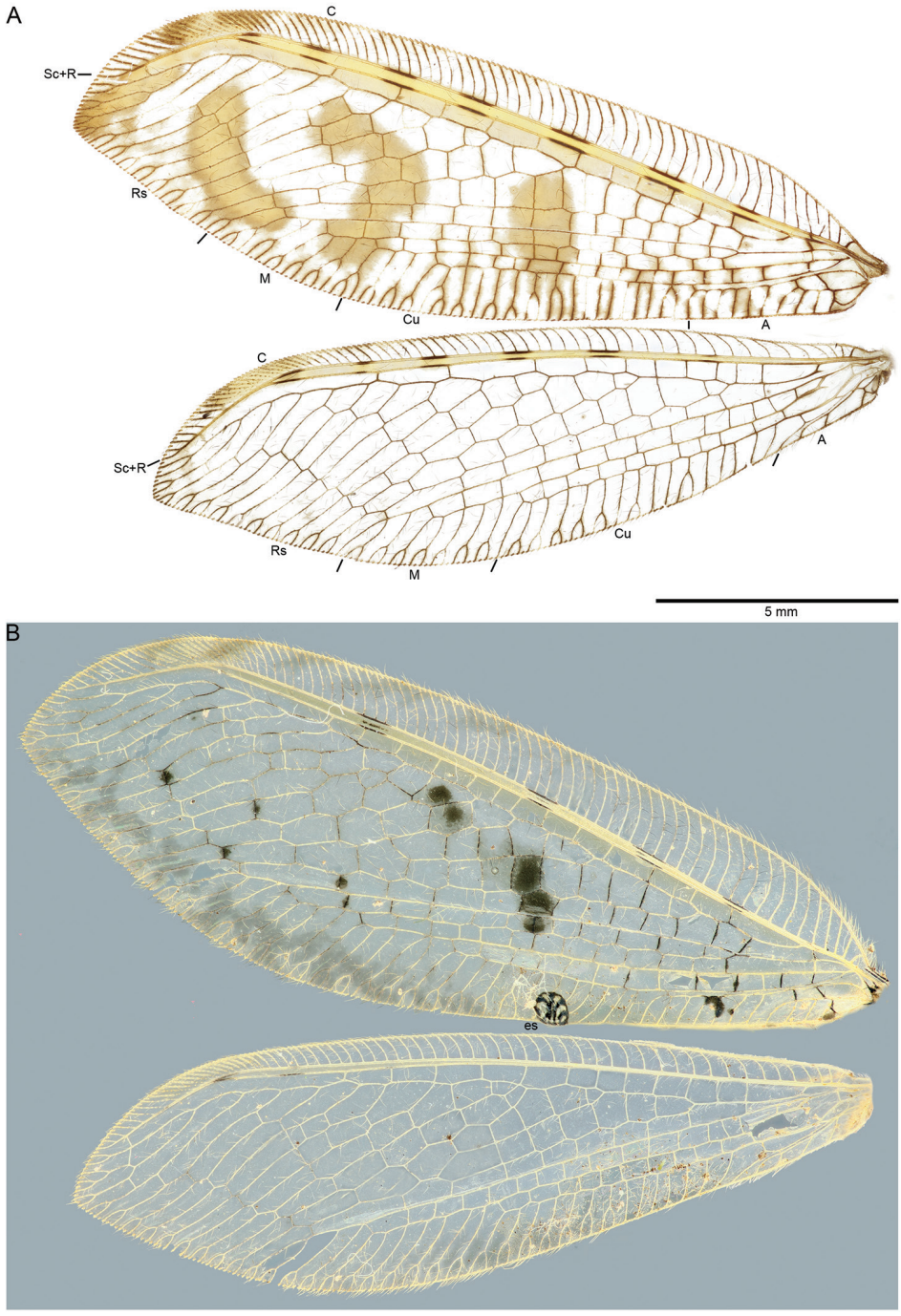

Figure 2. Wings of *Spilosmylus* spp. with main fields labeled: **A** *Spilosmylus spilopteryx* sp. n. **B** *Spilosmylus tephrodestigma* sp. n. Abbreviations: C, Costa; Sc, Subcosta; R, radius; Rs, Radius sector; M, Media; Cu, Cubitus; A, Anal field; es, embossed spot.

Figure 3. *Spilosmylus spilopteryx* sp. n. detail of the head and prothorax.

clearly basal to the first branch of Rs. Forewing membrane with a diagnostic pattern composed by: a basal large, oval brown marking present at forewing middle length, a median elongated marking curved outward in proximity of the internal gradates and an apical elongated marking curved inward covering the external gradates (Fig. 2A). The latter marking is slightly more contrasted than the other two marks. MP, CuA, CuP and anal veins shaded with dark brown and with darkened crossveins. The apex of most veins reaching the hind margin is darkened. Embossed spot absent. Hind wing relatively broad. Sc and R yellowish, with intermittent, parallel, black dashes. Subcostal area yellowish like in the forewing but the rest of the membrane is unmarked with the exception of a few slightly shaded veins along the hind margin (Fig. 2A).

Abdomen. Tergites and sternites uniformly brown. Apex of the abdomen slightly lighter.

Male genitalia. Tergite 9 relatively narrow, extending slightly beyond the ectoproct. Sternite 9 subrectangular. Ectoproct rounded, with a prominent and relatively large callus cercus. Between the two halves of the ectoproct there is a narrow dorsal sclerotization curved downward (Fig. 4A, B). Parameres fused dorsally in an arch-shaped sclerite, rod-like in lateral view (Fig. 4A, B). Mediuncus relatively large, characterized by conspicuous distal paired flanges, connected to the gonarcus by membranes (Fig. 4A, B). Gonarcus narrow, arch-shaped, extending ventro-proximally as a flattened rod; distal section of

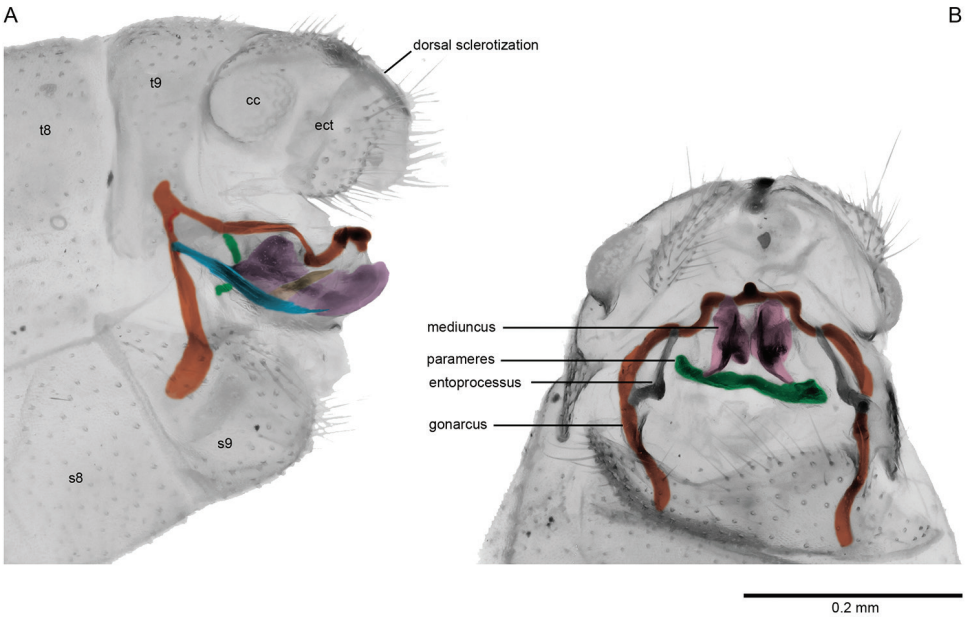

Figure 4. Male genitalia of *Spilosmylus spilopteryx* sp. n.: **A** lateral view **B** ventral view. Abbreviations: t8, tergite 8; s8, sternite 8; t9, tergite 9; st9, sternite 9; ect, ectoproct; cc, callus cercus.

the gonarcus in lateral view distinctly curved downward then bending up again in an almost straight apex; apical section of the gonarcus with a strongly sclerotized median thickening (Fig. 4A, B). Gonarcus equipped with a posterior entoprocessus extending posteriorly, bordering the mediuncus and narrowing apically (Fig. 4A, B).

Etymology. The specific name is a Latinized composite noun of Greek derivation, from *σπίλος*, *spilos*, meaning "marking" and the noun *πτέρυξ*, *pteryx*, meaning "wing", thus *spilopteryx*, "marked wing", in reference to the large cloud-like markings on the forewing.

Comments. *Spilosmylus spilopteryx* sp. n. is a highly distinctive species that cannot be easily confused with any other lance lacewing. This new species of *Spilosmylus* is characterized by a strongly marked wing and the absence of embossed spot on the hind margin of forewing, resembling the condition observed in the closely related genus *Thyridosmylus*. Nevertheless, overall wing shape and venation, the intermittently dashed markings along the Sc–R space, and male genitalic morphology allows us to confidently allocate this species to *Spilosmylus*. The presence of a narrow, dorsal sclerotization between the two halves of the ectoproct is characteristic of many species of *Spilosmylus*, and it might be of systematic relevance within this large genus. Despite several *Spilosmylus* species being characterized by pigmented wings with markings, bands and suffusions (e.g., *S. monticolus* (Banks, 1937), *S. formosus* Banks, 1924, *S. inquinatus* (McLachlan, 1870)), none of them display the extensive and conspicuous markings of this new taxon. Following New (1986, 1991), *Spilosmylus spilopteryx* sp. n.

Figure 5. *Spilosmylus ocellatus* (Krüger, 1914), holotype, habitus and labels (Natural History Museum, Vienna).

appears similar to *S. ocellatus* (Krüger, 1914) but strongly differing in the shape, extent and contrast of forewing markings. In particular, New (1986a) considered *S. ocellatus* as an easily recognizable species thanks to its wing pattern, which vaguely resembles the new species in his drawings, although composed by lighter shading and poorly contrasted markings (New 1986a: figs 115–116, New 1991). Nevertheless, the type specimen of *S. ocellatus*, preserved in the Naturhistoriches Museum Wien (Austria) bears no trace of such intense shading and its wing membrane appears mostly hyaline (Fig. 5). Noteworthy, a hand label of Navás suggest that the latter author also mistook this specimen for the inconspicuously marked *S. modestus* (Gerstaecker, 1893) (as also noted by Krüger 1914) (Fig. 5). Based on the examination of the type material of Krüger, we consider *S. ocellatus* and *S. spilopteryx* sp. n. as two very different taxa only sharing the lack of embossed spot. Further specimens are necessary to assess the identity of the morphospecies attributed by New (1986a) to *S. ocellatus*.

***Spilosmylus tephrodestigma* sp. n.**
http://zoobank.org/7F6F7D1B-38FA-42F3-8215-06BD9F174523
Figs 1B, 2B

Material examined. Holotype. Pinned, abdomen damaged by booklice, genitalia missing. **PHILIPPINES**, North Luzon, Barlig, Mountain Province, July 2014, 1 ex, local collector, [gender indeterminate] (Naturhistorisches Museum Wien).

Diagnosis. Medium sized osmylid with pale body; meso- and metathorax with large brown markings; both wings with small intermittent dark dashes on Sc and R; forewing membrane with well contrasted dark grey spots in the radial and medial area; base of the anal area with a well distinct dark marking; embossed spot present; hind wing membrane hyaline (Fig. 1B).

Description. Dimensions. Forewing length: 21.43 mm, width: 7.14; hind wing length: 19.05 mm, width: 5.87 mm.

Head. Uniformly pale ochre. Vertex, frons and clypeus pale. Labrum, gena and palpi pale. Antenna uniformly pale ochre (Fig. 1B).

Thorax. Predominantly pale ochre. Pronotum distinctly longer than wide, with brown lateral margins; mesonotum with dark brown dots on the posterior portion of the mesoscutum; metanotum with dark brown markings on the metascutum converging apically on the prescutum (Fig. 1B). Legs. Pale.

Wings. Forewing relatively broad with a slightly pointed apex, membrane hyaline with isolated markings and shades (Fig. 2B). Venation of the costal area mostly pale, longitudinal veins predominantly yellowish, crossveins mostly brown. Costal area progressively narrowing toward the apex, with brownish shades toward the pterostigma. Pterostigma light brown, lighter medially. Sc and R yellowish, with 4 parallel black dashes. Subcostal area yellowish with dark streaks paralleling the dark dashes on Sc and R. Forewing medial fork originating basally to the first branch of Rs. Forewing membrane with a diagnostic pattern composed by: a dark grey marking between the origin of the third and fourth branches of Rs, and a series of three dark grey markings forming a stripe extending between the second branch of Rs and MP (Fig. 2B). Gradates with isolated dark spots. Basal cubital and anal crossveins blackish. Anal area with a characteristic curved dark marking at middle length between the wing base and the embossed spot (Fig. 2B). Posterior margin of the wing shaded. Hind wing relatively broad, with hyaline membrane. Venation predominantly yellowish. Posterior margin shaded.

Abdomen. Tergites and sternites uniformly pale ochre. Tip of the abdomen not preserved.

Etymology. The specific epithet is a compound Latinized noun of Greek derivation from *τεφρῶδες*, *tephrodes*, meaning "coal" and *στίγμα*, *stigma*, meaning "spot", thus "ashy spot" referring to the grey spots on the forewing.

Comments. *Spilosmylus tephrodestigma* sp. n. is a more typical species of *Spilosmylus*, displaying a conspicuous embossed spot on the posterior margin of the forewing, which is an autapomorphic character of many species in the genus (Wang et al. 2011). *Spilosmylus tephrodestigma* sp. n. is also easily recognizable from other congeners thanks to the highly characteristic wing pattern composed by a series of dark grey spots forming a linear pattern in the radial area of forewing. *Spilosmylus tephrodestigma* sp. n. is similar to *S. inquinatus* and it might be closely related to the latter, but it is easily set apart thanks to the wing pattern and the presence of dark brown markings on the meso- and metathorax. *Spilosmylus tephrodestigma* sp. n. also lacks the amber shadings typical of *S. inquinatus* and *S. formosus*. The discovery of the genitalia of the new species is necessary to clarify its affinities within the genus.

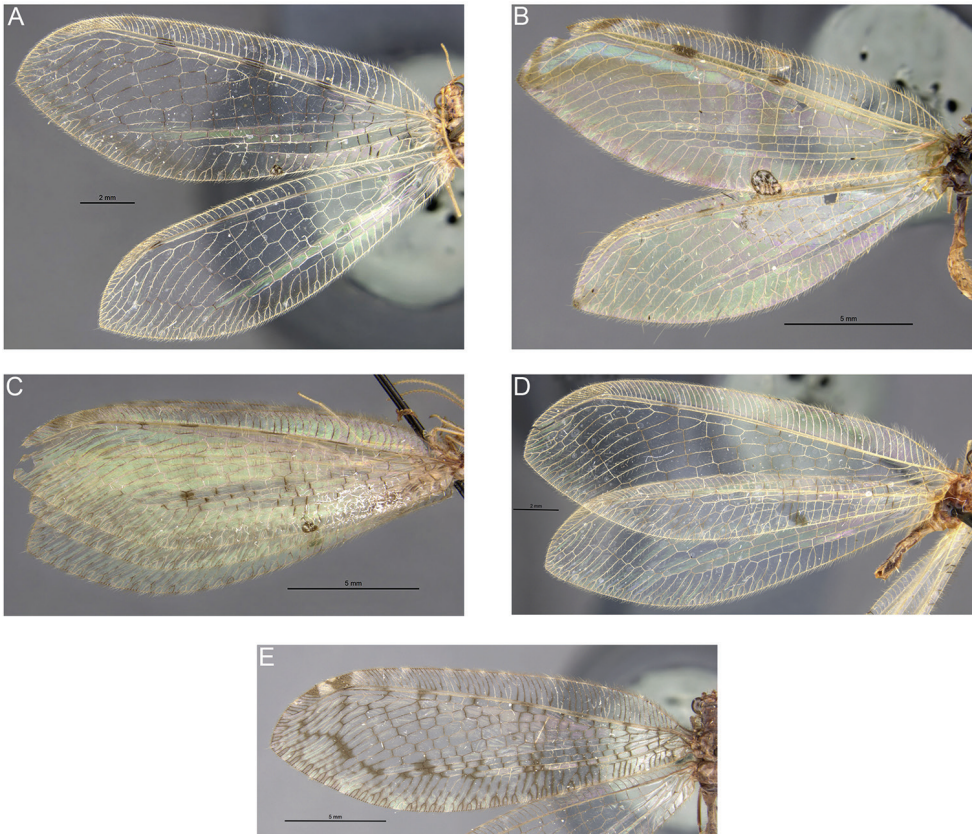

Figure 6. Detail of wings of the holotypes of *Spilosmylus* species described by Nathan Banks from the Philippines. Photographs by Philip D. Perkins, Museum of Comparative Zoology, Harvard University; original photography © President and Fellows of Harvard College. **A** *Spilosmylus alticolus* Banks, 1937 **B** *Spilosmylus formosus* Banks, 1924 **C** *Spilosmylus apoanus* Banks, 1937 **D** *Spilosmylus proximus* Banks, 1937 **E** *Spilosmylus monticolus* (Banks, 1937).

Key to the species of *Spilosmylus* known from the Philippines

1 Forewing with embossed spot (Fig. 2B) **2**
– Forewing without embossed spot (Fig. 2A) **8**
2 Forewing radial and medial area with dark grey spots in the medial area (Fig. 2B) ..... ***S. tephrodestigma* sp. n.**
Forewing radial and medial area without such markings **3**
3 Forewing with diffuse amber shadings (Fig. 6B)..... **4**
– Forewing without amber shadings..... **5**
4 Forewing veins Sc and R with 5 dark dashes, subcostal area unmarked .... ***S. inquinatus* (McLachlan)**
– Forewing veins Sc and R with 2 dark dashes, subcostal area with two distinct, large dark brown markings covering the dark dashes; embossed spot very large (Fig. 6B) ..... ***S. formosus* Banks**

5 Forewing subcostal area with several markings paralleling the dark dashes on Sc and R (Fig. 6A) **6**
– Forewing subcostal area mostly unmarked (Fig. 6D) **7**
6 Forewing with an isolated dark spot in the medial area (Fig. 6C) .... ***S. apoanus*** **Banks**
– Forewing without such a spot (Fig. 6A) ..... ***S. alticolus*** **Banks**
7 Forewing veins Sc and R with 2 dark dashes; medial fork basal to the origin of the first branch of Rs (Fig. 6D) ..... ***S. proximus*** **Banks**
– Forewing veins Sc and R with 5 dark dashes; medial fork in proximity or slightly distal to the origin of the first branch of Rs..... ***S. modestus*** **(Gerstaecker)**
8 Forewing veins Sc and R with 7 dark dashes, subcostal area yellow and unmarked; forewing membrane with 3 large and well distinct light brown markings (Fig. 2A) ..... ***S. spilopteryx*** **sp. n.**
– Forewing subcostal area with dark streaks also covering Sc and R; forewing membrane shaded with dark brown along the outer gradates, rhegma and in proximity of the crossveins of the cubital and medial area but without distinct markings (Fig. 6E) ..... ***S. monticolus*** **(Banks)**

Note: Navás (1926) described a further species of *Spilosmylus* from the Philippines: *S. nephelius* Navás, 1926. The holotype of this species, which was deposited in the private collection of the author, was likely destroyed (c.f. Monserrat 1985). Banks (1937) considered it a probable synonym of *S. inquinatus*.

Acknowledgments

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CHECKLIST

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Review of the tribe Chilocorini Mulsant from Iran (Coleoptera, Coccinellidae)

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Abstract

The Iranian checklist of the tribe Chilocorini Mulsant, 1846 (Coleoptera: Coccinellidae) is updated. In total, 13 species belonging to four genera (*Brumoides* Chapin, 1965, *Chilocorus* Leach, 1815, *Exochomus* Redtenbacher, 1843, and *Parexochomus* Barovsky, 1922) are listed from Iran. An identification key to all genera and species currently known from Iran is presented along with illustrations of adult specimens and male genitalia.

Keywords

checklist, Chilocorini, Coccinelloidea, Iran, review

Introduction

The family Coccinellidae, with nearly 6000 species and 360 genera, belongs currently to the superfamily Coccinelloidea (Coleoptera: Polyphaga) (Robertson et al. 2015, Tomaszewska and Szawaryn 2016). It is divided into two subfamilies: Microweiseinae and Coccinellinae. The subfamily Microweiseinae includes three tribes: Carinodulini, Microweiseini (including Sukunahikonini) and Serangiini (Escalona and Ślipiński 2012); the remaining taxa belong to the subfamily Coccinellinae (Seago et al. 2011, Robertson et al. 2015, Szawaryn et al. 2015, Escalona et al. 2017).

The tribe Chilocorini Mulsant, 1846 contains approximately 250 species belonging to 27 genera (Łączyński and Tomaszewska 2012, Li et al. 2017), of which nine genera have hitherto been recorded from Palaearctic region including: *Brumoides* Chapin, 1965, *Chilocorus* Leach, 1815, *Chujochilus* Sasaji, 2005, *Exochomus* Redtenbacher, 1843, *Parexochomus* Barovsky, 1922, *Phaenochilus* Weise, 1895, *Priscibrumus* Kovár, 1995, *Simmondsius* Ahmad & Ghani, 1966 and *Xanthocorus* Miyatake, 1970) (Kovář 2007).

Although most members of Chilocorini are coccidophagous (Giorgi et al. 2009, Escalona et al. 2017), aphidophagy is also present in some species (Ślipiński and Giorgi 2006); so, the members of this tribe have the potential to be effective biological control agents of coccids and aphids (Drea and Gordon 1990, Ponsonby and Copland 1997).

In the last classification of the former subfamily Chilocorinae by Kovář (2007), the species of the genus *Brumus* Mulsant, 1850 were transferred to *Exochomus* Redtenbacher and the subgenus *Parexochomus* of *Exochomus* was considered as a valid genus, under the name of *Parexochomus* Barovsky, 1922. This classification was followed by Nedvěd and Kovář (2012). Moreover, according to Ślipiński (2007), the subfamily Chilocorinae Mulsant was dissolved and all tribes were lumped into the subfamily Coccinellinae. This classification was confirmed by subsequent morphological and molecular studies (Seago et al. 2011, Robertson et al. 2015). The number of genera and species of this tribe is continuously increasing (Ślipiński and Giorgi 2006, Łączyński and Tomaszewska 2009, Wang and Ren 2010, Łączyński and Tomaszewska 2012, Li et al. 2015, Li et al. 2017) and it is expected that this trend will be continuing.

Although a large number of species of this tribe have hitherto been reported from Iran (Duverger 1983, Kovář 2007, Moddarres-Awal 2012), there is no complete and comprehensive information on the Iranian Chilocorini. The checklist by Abdolahi Mesbah et al. (2016) differs from our view and does not include identification key, diagnosis, and synonymy. Our paper corrects the previous studies on the species of this tribe in Iran, in order to update the information about Iranian Chilocorini.

Materials and methods

This study was mainly based on review of the literature along with the samples collected by the first author. The samples were collected by hand, aspirator, or sweep net in the fields, orchards, and pastures of various provinces of Iran. The specimens were

examined under Olympus stereomicroscope (SZ-ST). The specimens were first boiled in 10% KOH for a maximum of 20 min depending on the darkness of the body color/ sclerotization in order to dissect the genitalia. The dissected genitalia were then transferred into distilled water for a maximum of 10 min to rinse off the KOH. Finally, the slides were prepared using Canada balsam. The slides were examined under a microscope (Olympus CX21) and images were taken using a digital camera and edited in Photoshop software (Adobe Photoshop CS5.1). The specimens were identified to species using available keys and resources (Mader 1955, Fürsch 1961, Bielawski 1984, Kovář 1995, Raimundo and van Harten 2000, Raimundo et al. 2008).

Although the higher classification of Seago et al. (2011) was followed in this study, taxonomy at the species level is based on Kovář (2007). Morphological terminology follows that of Ślipiński (2007). All of the specimens collected and examined during this study are deposited in Plant Protection Department, Lorestan University, Agricultural Faculty, Khorramabad, Iran.

Results and discussion

The Iranian coccinellid species list of the tribe Chilocorini is updated, which includes 13 species belonging to four genera (*Brumoides*, *Chilocorus*, *Exochomus*, and *Parexochomus*).

Although there are some records of *Exochomus flavipes* Thunberg, 1781 from Iran (Ansari pour and Shakarami 2011, Tavakol et al. 2014), re-examination of the voucher specimens of this species showed that these reports are misidentifications and these samples are actually *Parexochomus nigromaculatus* (Goeze, 1777). *Parexochomus flavipes* is morphologically similar to *P. nigromaculatus* but is distinguished from it by the male genitalia, and *P. flavipes* has not hitherto been reported from Palaearctic region (Kovář 2007). It is distributed in the northern states of USA (Gordon 1985) and south and west of Africa (Fürsch 1961).

Mahghari and Ostovan (2006) reported two ladybird species, *Brumus undecempunctata* L. and *Chilocorus stigma* (Say, 1835), from the northern provinces of Iran (Gilan and Mazandaran province) as natural enemies of whiteflies. In coccinellid taxonomy, there is no known species under the name of *Brumus undecempunctata*, while *Chilocorus stigma* has not been reported so far from Palaearctic region (Kovář 2007). According to our knowledge, the presence of these species in Iran is doubtful and not confirmed.

Barovsky (1922) reported *Exochomus kiritshenkoi* Barovsky, 1922 from Iran (Shahrood, H. Christoph leg.). There are also specimens in Zoologichesky Institut (Akademii Nauk SSSR) in St. Petersburg, labeled as *E. kiritshenkoi* which had been collected from Iran (Shahrood, H. Christoph leg). Kovář (1995) however identified these specimens as *E. gebleri* Weise.

Data on the presence of *E. bifasciatus* in Iran are based on Kovář (2007). Since we do not have any information (particularly morphological) about this species, it is excluded from the identification key of Iranian species of Chilocorini.

Subfamily Coccinellinae Latreille, 1807

Tribe Chilocorini Mulsant, 1846

Diagnosis. Body size small to medium (2.0–8.0 mm), with downward directed head inserted into prothorax to some extent; dorsum usually without obvious pubescence. Head wider than long, flattened ventrally; clypeus variously expanded laterally and wholly concealing antennal insertions. Mandibles triangular, strong with an apical tooth and heavily developed molar teeth; maxillary palps relatively long, terminal palpomere parallel sided to weakly enlarged apically; labial palp clearly separated basally, inserted on ventral side of prementum. Antenna composed of 7–10 antennomeres, markedly short with a fusiform club composed of three terminal antennomeres. Prosternum fairly elongate in front of coxae; prosternal process narrow, parallel sided without carinae. Hind wings with large anal lobe. Elytra irregularly punctate, with epipleuron wide and complete to apex, frequently with foveae for receiving apices of femora. Abdomen with five or six ventrites; postcoxal lines at abdominal ventrite 1 variable, without associated pits and pores. Male genitalia with symmetrical tegmen, penis guide sometimes asymmetrical; parameres well developed, apically setose; penis a simple, single sclerite with sizeable basal capsule. Coxites triangular and faintly sclerotized, usually without styli; bursa copulatrix with infundibulum or fleshy lobe, with sperm duct composed of two parts of different diameter; spermatheca bean-shaped, sclerotised without well differentiated nodulus or ramus, with large accessory gland (after Ślipiński 2007).

Key to the Iranian species and genera of Chilocorini

1 Fronto-clypeal plate emarginate anteriorly (Fig. 14). Postcoxal line on abdominal ventrite 1 merging with posterior margin of ventrite or running very close to it (Fig. 15). All tibiae with tooth at outer side; tibial spurs absent (Fig. 16). Elytron brown or reddish brown with 3 small orange discal spots in transverse row, usually partially fused (Fig. 2). Male genitalia with penis guide as long as parameres (Figs 17, 18), penis as in Figs 19, 20. (Body circular, strongly convex, 3.5–4.5 mm long) ..... ***Chilocorus bipustulatus*** **Linnaeus**

– Fronto-clypeal plate not emarginate. Postcoxal line on abdominal ventrite 1 distant from posterior margin of ventrite (Figs 21, 22). Mid-and hind tibiae smoothly arcuate; with 2 apical spurs (Fig. 23) **2**

2 Antenna composed of 8 antennomeres (Fig. 24). Body yellow with two small black spots on each elytron, one behind the other (Fig. 1). Male genitalia with parameres slightly longer than penis guide (Fig. 25); penis as in Fig. 26. (Body broadly oval, 2.0–2.5 mm long) ..... ***Brumoides adenensis*** **Fürsch**

– Antenna composed of 10 antennomeres (Figs 27, 28) **3**

3 Elytra black with red spots or red-brown with or without black spots. Body size 2.8–5.0 mm ..... ***Exochomus*** **Redtenbacher 4**

– Elytra completely black. Body size 2.2–4.5 mm ..... ***Parexochomus*** **Barovsky 10**

4 Elytra black; each elytron with two small or medium sized, separated red spots..... **5**

– Elytra orange to red-brown, with or without black spots, or elytra black with large pale maculae of irregular shape **6**

5 Each elytron with two similar and equally-sized rounded spots (Fig. 6). Male genitalia with penis guide approximately as long as parameres (Figs 29, 31); penis as in Fig. 30. Body oval, 3.5–4.5 mm long..... ***E. quadriguttatus*** **Fleischer**

– Each elytron with two differently sized and shaped spots (Figs 7, 8). Male genitalia with penis guide clearly shorter than parameres (Figs 32, 33); penis as in Fig. 34. Body subcircular, 3.5–4.0 mm long..... ***E. quadripustulatus*** **Linnaeus**

6 Background of elytra black; elytral maculae large and of irregular shape, brown or orange **7**

– Background of elytra orange to red-brown; with or without contrasting markings..... **8**

7 Humeral part with brown macula (Fig. 9); male genitalia with penis guide longer than parameres (Fig. 35); penis as in Fig. 36. Form oblong, body length 4.3–5.0 mm..... ***E. undulatus*** **Weise**

– Humeral part with orange macula surrounding a black round spot (Fig. 3). Body form oblong, 3.0–5.0 mm long) ..... ***E. ericae*** **Crotch**

8 Elytra brown without markings; (Body subcircular, 3.5–4.0 mm long)..... ..... ***E. quadripustulatus*** **Linnaeus**

– Each elytron with 4 nearly equally sized, small, black spots similarly distributed **9**

9 Pronotum reddish orange, with a medio basal ungulate black spot (Fig. 5). Tarsal claw simple (Fig. 37). Male genitalia with penis guide as long as parameres (Fig. 38); penis as in Fig. 39. Body nearly of spindle form, 2.8–4.5 mm long ..... ***E. octosignatus*** **Gebler**

– Pronotum entirely black except for dark bordering oflateral and anterior margins (Fig. 4).Tarsal claw with small basal tooth (Fig. 40). Male genitalia with penis guide distinctly shorter than parameres (Figs 41, 42); penis as in Fig. 43. Body subcircular, 4.0–5.0 mm long..... ***E. gebleri*** **Weise**

10 Body pubescent **11**

– Body glabrous..... **12**

11 Body covered with dense, moderately long setae (Fig. 13). Male genitalia with penis guide shorter than parameres (Figs 44, 45); penis as in Figs 46, 47. Body short oval to nearly circular, 2.8–2.9 mm long..... ***P. pubescens*** **Küster**

– Body apparently glabrous, but actually with minute sparse setae particularly at pronotum (Fig. 10). Form oblong, 2.2–2.7 mm long..... ..... ***P. melanocephalus*** **Zubkov**

12 Pronotum yellow (Fig. 11). Male genitalia as in Figs 48, 49, 50. Body oval and highly convex, 3.8–4.2 mm long..... ***P. nigripennis*** **Erichson**

– Pronotum black with yellow lateral margins (Fig. 12). Male genitalia as in Figs 51–55. Body broadly oval, moderately convex, 3.1–4.5 mm long..... ..... ***P. nigromaculatus*** **Goeze**

Updated checklist of the Iranian species of Chilocorini

Brumoides Chapin, 1965

Brumoides Chapin, 1965: 237. Type species: *Coccinella suturalis* Fabricius, 1798, by original designation.

Diagnosis. Body length 2.0–3.5 mm. Dorsum glabrous; yellowish or brown, elytra with dark markings. Eye distinctly emarginate. Antenna composed of 8 antennomeres; terminal antennomere small, partly embedded in penultimate one. Clypeus short; labrum exposed. Pronotal base bordered; prosternal process extremely narrow, without carinae; without hypomeral fovea. Fore tibia narrow, simple, middle and hind tibiae with two apical spurs; tarsal claws appendiculate or weakly thickened basally. Abdominal ventrite 6 visible in males; abdominal postcoxal lines separated medially, each arcuately recurving apically and reaching or nearly reaching midpoint of lateral line (after Ślipiński 2007).

Ecology. Various species of *Brumoides* have been associated with mealybugs (Ślipiński 2007), namely *Coccidohystrix insolita* (Hemiptera: Pseudoccocidae), *Dactylopius confusus* (Hemiptera: Dactylopiidae), *Ferrisia virgata* (Hemiptera: Pseudococcidae), and *Phenacoccus solenopsis* (Hemiptera: Pseudoccocidae) (Gordon 1985, Gautam 1990, Hodek and Honěk 2009, Arif et al. 2012, Giorgi et al. 2014). Some species of this genus, such as *Brumoides suturalis* (F.) feed on some whitefly species, such as *Aleurolobus barodensis* (Maskell) (Inayatullah 1984, Hodek and Honěk 2009) in addition to feeding on some coccids, such as *F. virgata* (better for development) and *Planococcus pacificus* (better for oviposition) (Gautam 1990).

Brumoides adenensis Fürsch, 1987

Figs 1, 21, 24–26

Brumoides adenensis Fürsch, 1987: 44.

General distribution. Middle East (that includes Iran, Saudi Arabia, United Arab Emirates, Yemen) (Kovář 2007), Southern Africa (Łączyński and Tomaszewska 2012).

Distribution in Iran. Iran (Kovář 2007) – no specific distribution known.

Remarks. The species descriptions and photographs by Fürsch (1987) and Raimundo et al. (2008) were used with some modifications.

Chilocorus Leach, 1815

Chilocorus Leach, 1815: 116. Type species: *Coccinella cacti* Linnaeus, 1767, by monotypy.

Diagnosis. Body length 2.5–4.8 mm. Dorsal body glabrous; elytra black or brown with white or orange markings; eye clearly emarginate. Antennae short, composed of

Figures 1–9. Dorsal habitus of Chilocorini species. **1** *Brumoides adenensis* Fürsch **2** *Chilocorus bipustulatus* Linnaeus **3** *E. ericae* Crotch **4** *E. gebleri* Weise **5** *E. octosignatus* Gebler **6** *E. quadriguttatus* Fleischer **7, 8** *E. quadripustulatus* Linnaeus **9** *E. undulatus* Weise.

8 antennomeres; with scape symmetrical; 8th antennomere either as long as or markedly longer than antennomere 7. Clypeus long; labrum partly exposed. Pronotal base unbordered; prosternal process narrow without carinae; hypomeral fovea absent. All tibiae flattened and angulate externally, without apical spurs; tarsal claws strongly appendiculate. Elytral margin not reflexed with indistinct bead; epipleural foveae weak. Abdominal ventrite 6 visible in males; abdominal postcoxal lines separated medially, each running parallel to hind margin of ventrite (after Ślipiński 2007).

Ecology. Although various scale insects are primary hosts of *Chilocorus* (Escalona et al. 2017), some species at least accept aphids as prey (Gordon 1985, Drea and Gordon 1990, Ślipiński 2007, Hodek and Honěk 2009). Nonetheless, there are some reports about some species of this genus, such as *Chilocorus stigma* (Say) which feed on some whitefly species, such as *Aleurocanthus woglumi* Ashby (Dowell and Cherry 1981, Hodek and Honěk 2009).

***Chilocorus bipustulatus* (Linnaeus, 1758)**
Figs 2, 16, 15–20

Coccinella bipustulata Linnaeus, 1758: 367.
Coccinella fasciata Müller, 1776: 68.
Coccinella transversoguttata Börner, 1776: 250.
Coccinella frontalis Thunberg, 1792: 105. [Homonym]
Coccinella testudo Florencourt Chassot, 1796: 214.
Coccinella strigata Fabricius, 1798: 79. [Homonym]
Chilocorus olivetorum Costa, 1839: 104.
Chilocorus minor Sahlberg, 1903: 86.

Material examined. 8♂, 3♀: Iran, Lorestan province, V.2013, lgt. Amir Biranvand, det. Biranvand. 2♂, 1♀: Iran, Semnan province, V.2015, lgt. Mino Toozandejani, det. Biranvand.

General distribution. Afrotropical region, Nearctic region, Palaearctic region (Mader 1955, Gordon 1985, Kovář 2007, Canepari 2011) and Oriental region (Poorani 2002).

Distribution in Iran. Widely distributed (Duverger 1983, Moddarres-Awal 2012).

Ecology. This species feeds on a wide range of Hemiptera species: *Agonoscena pistaciae* (Psyllidae), *Aonidiella orientalis* (Diaspididae), *Bemisia tabaci* (Aleyrodidae), *Chrysomphalus dictyospermi* (Diaspididae), *Eulecanium prunastri* (Coccidae), *Euphyllura olivina* (Psyllidae), *Salicola kermanensis* (Diaspididae), *Lepidosaphes malicola* (Diaspididae), *Leucaspis pusilla* (Diaspididae), *Maconellicoccus hirsutus* (Pseudococcidae), *Ommatissus binotatus lybicus* (Tropiduchidae), *Parlatoria blanchardi* (Diaspididae), *Parlatoria oleae* (Diaspididae), *Phloeomyzus passerinii* (Aphididae), *Planococcus citri* (Pseudococcidae), *Pseudaulacaspis pentagona* (Diaspididae), *Psylla pyricola* (Psyllidae) (Moddarres-Awal 2012) and other coccids, particularly armoured scales (Hodek 1973, Stansly 1984).

***Exochomus* Redtenbacher, 1843**

Exochomus Redtenbacher, 1843:11. Type species: *Coccinella quadripustulata* Linnaeus, 1758, by subsequent designation of Thomson, 1859.

Diagnosis. Body length 2.8–5.5 mm. Dorsal body glabrous; elytra black, brown, or yellow, often with contrasting red or yellow markings; sometimes (in lighter coloured species) with black stripes along lateral margins of elytra. Antenna composed of 10 antennomeres, minute terminal antennomere embedded in penultimate one; pronotal basal margin completely bordered with submarginal line; prosternal process narrow, truncate apically, without carinae; elytral epipleura clearly narrowing, without foveae; abdominal postcoxal lines complete or nearly so, semicircular, reaching to inner end of lateral line; meso- and metatibiae each with two apical spurs; tarsal claws with or without basal tooth (after Li et al. 2015).

Figures 10–13. Dorsal habitus of Chilocorini species. **10** *Parexochomus melanocephalus* Zubkov **11** *P. nigripennis* Erichson **12** *P. nigromaculatus* Goeze **13** *P. pubescens* Küster.

Ecology. Most species of this genus are aphidophagous and coccidophagous (Gordon 1985, Kovář 1995, Magro et al. 2010). Nontheless, there are some reports about some species of the genus feeding on aleyrodids e.g., *Exochomus bimaculosus* Mulsant which feeds on *Bemisia tabaci* (Gennadius) (Yigit 1992, Leite et al. 2003, Hodek and Honěk 2009).

Exochomus bifasciatus Barovsky, 1927

Exochomus bifasciatus Barovsky, 1927: 200.

General distribution. China, Iran, Kazakhstan (Kovář 2007).

Distribution in Iran. Iran (Kovář 2007) – no specific distribution provided**.**

***Exochomus ericae* Crotch, 1874**
Fig. 3

Exochomus ericae Crotch, 1874: 193.
Chilocorus nigropictus Fairmaire, 1876: 94.
Chilocorus picturatus Fairmaire, 1876: 94.
Exochomus anchorifer Allard, 1870: 9.

General distribution. Algeria, Iran, Morocco, Tunisia (Mader 1955, Duverger 1983, Kovář 2007).

Distribution in Iran. Dasht Arzhanregion, Kerman, Nowshahr region (Duverger 1983).

Remarks. We used the species descriptions and photographs of Mader (1955) with some modifications.

***Exochomus gebleri* Weise, 1885**
Figs 4, 40–43

Exochomus gebleri Weise, 1885: 55.

Material examined. 5♂, 2♀: Iran, Yazd province, spring and summer 2013, lgt. Mehdi Zare Khormizi, det. Biranvand.

General distribution. Afghanistan, Iran, Turkey (Kovář 2007).

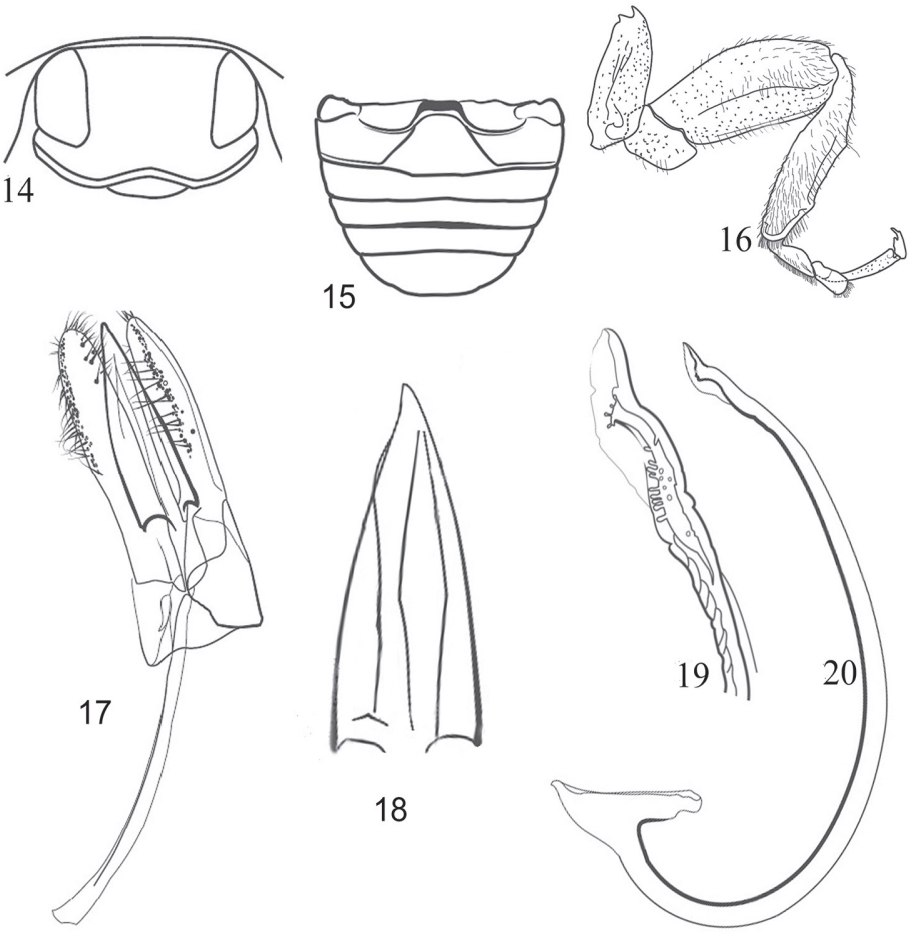
Distribution in Iran. Golestan, Semnan (Kovář 1995), Lorestan (Jafari and Kamali 2007), Fars (Moddarres-Awal 2012), Yazd (current study).

***Exochomus octosignatus* (Gebler, 1830)**
Figs 5, 37–39

Coccinella octosignata Gebler, 1830: 225.
Coccinella deserta Motschulsky, 1840: 175.
Coccinella desertorum Gebler, 1841: 376.
Brumus lasioides Weise, 1879: 135.
Brumus conjunctus Fleischer, 1900: 118.

General distribution. Afghanistan, Armenia, Azerbaijan, France, Iran, Iraq, Italy, Kazakhstan, Kyrgyzstan, Mongolia, Russia, Tajikistan, Turkmenistan, Turkey, Uzbekistan (Kovář 2007).

Distribution in Iran. Khameshorkn region (Duverger 1983), Khorasan (Moodi and Mossadegh 1995, Yaghmaei and Kharrazi Pakdel 1995), Chaharmahal and Bakhtiari (Bagheri and Mossadegh 1996), East Azerbaijan, Gilan, Isfahan, Kerman, Qom, Tehran, Sistan and Baluchestan (Moddarres-Awal 2012).



Figures 14–20. Morphological details and male genitalia of Chilocorini species. **14–20** *Chilocorus bipustulatus*: **14** Head **15** Abdominal postcoxal lines **16** Leg **17** Tegmen **18** Penis guide of tegmen **19** Penis apex **20** Penis.

Ecology. This species feeds on the mealybugs *Phenacoccus aceris* and *Planococcus citri* (Pseudococcidae) (Moddarres-Awal 2012).

***Exochomus quadriguttatus* Fleischer, 1900**
Figs 6, 29–31

Exochomus quadriguttatus Fleischer, 1900: 118.
Exochomus cordiformis Roubal, 1926: 245.
Exochomus illaesicollis Roubal, 1927: 135.

Material examined. 3♂, 8♀: Iran, Semnan province, VII.2015, lgt. Mino Toozandejani, det. Biranvand.

General distribution. Caucasus, Iran, Lebanon, Syria (Duverger 1983), Armenia, Turkey (Kovář 2007).

Distribution in Iran. Sagdar region (Duverger 1983), Kerman (Moddarres-Awal 2012), Semnan (current study).

***Exochomus quadripustulatus* (Linnaeus, 1758)**
Figs 7–8, 32–34

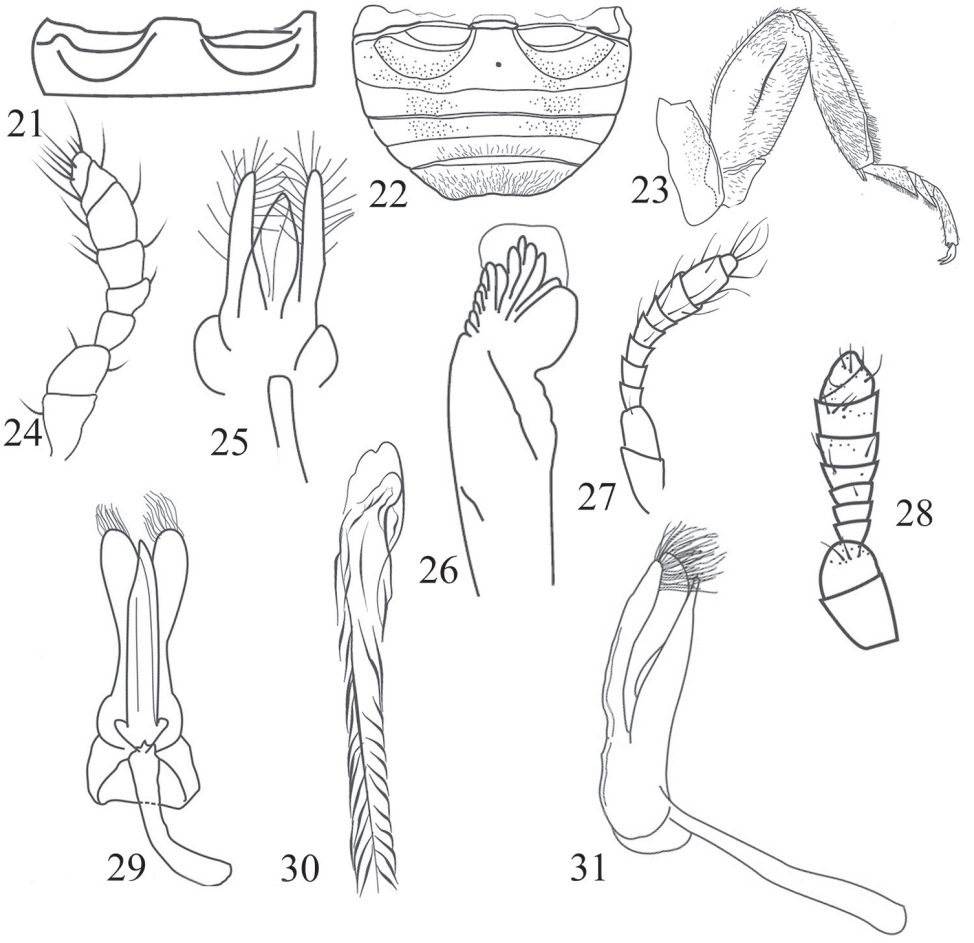
Coccinella quadripustulata Linnaeus, 1758: 367.
Coccinella lunulata Gmelin, 1790: 1662.
Coccinella quadriverrucata Fabricius, 1792: 288.
Coccinella cassidoides Donovan, 1798: 74.
Coccinella varia Schrank, 1798: 444.
Coccinella distincta Brullé, 1832: 273
Coccinella iberica Motschulsky, 1837: 422.
Coccinella floralis Motschulsky, 1837: 423.
Exochomus haematideus Costa, 1849: 62.
Exochomus unicolor Schaufuss, 1862: 50
Exochomus sexpustulatus Kraatz, 1873:192
Exochomus bilunulatus Weise, 1879: 133.
Exochomus koltzei Weise, 1879: 134.
Exochomus reitteri Schneider, 1881: 16
Exochomus vittatus Fuente, 1910: 444

Material examined. 60♂, 75♀: Iran, Lorestan province, in all seasons, 2013, 2014, 2015, 2016, 2017, lgt. Amir Biranvand, det. Biranvand. 3♂, 3♀: Iran, Semnan province, V.2015, lgt. Mino Toozandejani, det. Biranvand.

General distribution. Palaearctic Region, Oriental region, Australian region, Nearctic region (USA: California) (Canepari 2011, Li et al. 2015).

Distribution in Iran. Widely distributed (Duverger 1983, Moddarres-Awal 2012).

Ecology. This species feeds on various species of Hemiptera, namely: *Aonidiella orientalis* (Diaspididae), *Aphis fabae* (Aphididae), *Callaphis juglandis* (Aphididae), *Chromaphis juglandicola* (Aphididae), *Eriosoma lanigerum* (Aphididae), *Eulecanium prunastri* (Coccidae), *Euphyllura olivina* (Psyllidae), *Maconellicoccus hirsutus* (Pseudococcidae), *Parlatoria oleae* (Diaspididae), *Psylla pyricola* (Psyllidae), *Saissetia oleae* (Coccidae) (Moddarres-Awal 2012), and other aphids and Coccidae (Uygun 1981, Ülgentürk and Toros 2001, Kaydan et al. 2006, Kaydan et al. 2012).



Figures 21–31. Morphological details and male genitalia of Chilocorini species. **21**, **24–26** *Brumoides adenensis*: **21** Abdominal postcoxal lines **24** Antenna **25** Tegmen **26** Penis apex **22** *Parexochomus pubescens*: Abdominal postcoxal lines **23, 28** *P. nigripennis*: **23** Hind leg **28** Antenna **27** *Exochomus undulatus*: Antenna **29–31** *E. quadriguttatus*: **29** Tegmen, ventral view **31** Tegmen, lateral view **30** Penis apex.

Exochomus undulatus Weise, 1878

Figs 9, 27, 35–36

Exochomus undulatus Weise, 1878: 93

Material examined. 10♂, 16♀: Iran, Lorestan province, in all seasons, 2013, 2015, 2016, lgt. Amir Biranvand, det. Biranvand.

General distribution. Palestine (Mader 1955), Caucasus (Duverger 1983), Afghanistan, Azerbaijan, Egypt, Georgia, Iraq, Iran, Lebanon, Syria, Tajikistan (Kovář 2007).

Distribution in Iran. Lorestan (Jafari and Kamali 2007), Chaharmahal and Bakhtiari, Fars, Isfahan, Kerman, Khorasan, Kohgiluyeh and Boyer-Ahmad, Qazvin (Moddarres-Awal 2012), Tehran (Ghanbari et al. 2012), Markazi (Ahmadi et al. 2012), Yazd (Zare Khormizi et al. 2016).

Ecology. This species feeds usually on *Euphyllura olivina* (Hemiptera: Psyllidae) (Moddarres-Awal 2012).

Parexochomus Barovsky, 1922

Exochomus (*Parexochomus*) Barovsky, 1922: 293. Type species: *Exochomus pubescens* Küster, 1848, by subsequent designation of Chapin 1965.
Parexochomus: Kovář 2007: 595.

Diagnosis. Body length 3.0–3.5 mm. Dorsal body glabrous or pubescent, dark brown or black with lateral margins of pronotum or at least anterior angles yellow or red. Antenna composed of 10 antennomeres, minute terminal antennomere embedded in penultimate one; terminal maxillary palpomeres stout, nearly parallel-sided; pronotal basal margin entirely bordered with submarginal line; prosternal process narrow, rounded apically, without carinae; elytral epipleura clearly narrowing towards apex, without foveae; abdominal postcoxal lines complete and semicircular, reaching to middle of lateral line; meso- and metatibiae each with two apical spurs; tarsal claws with basal tooth (after Li et al. 2015).

Ecology. The species of *Parexochomus* are aphidophagous or coccidophagous (Moddarres-Awal 2012).

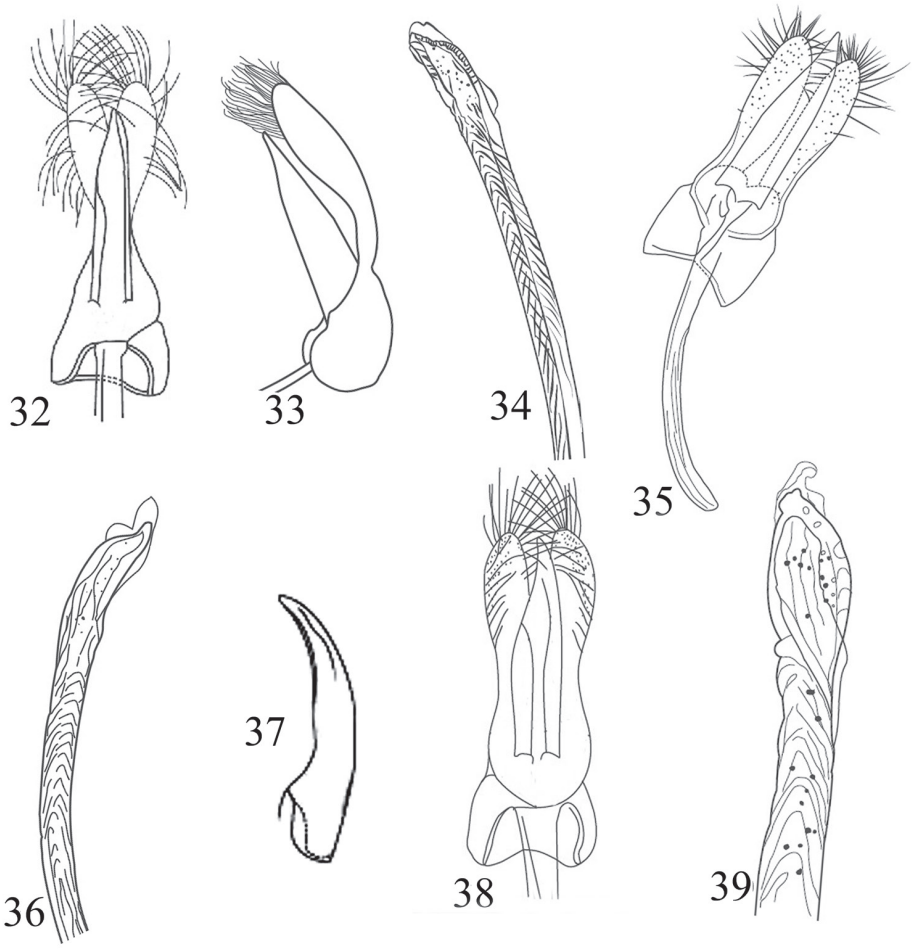
Parexochomus melanocephalus (Zubkov, 1833)

Fig. 10

Coccinella melanocephala Zubkov, 1833: 339.
Exochomus russicollis Mulsant, 1850: 1033.

General distribution. Southern Russia, Caucasus (Mader 1955), Azerbaijan, Armenia, Bulgaria, Georgia, Iran, Kazakhstan, Tajikistan, Turkmenistan, Turkey, Uzbekistan (Kovář 2007).

Distribution in Iran. Razavi Khorasan (Yaghmaei and Kharrazi Pakdel 1995), Lorestan (Jafari and Kamali 2007), Chaharmahal and Bakhtiari, Khorasan (Moddarres-Awal 2012), Kerman (Salehi et al. 2011), Hormozgan (Fallahzadeh et al. 2013).



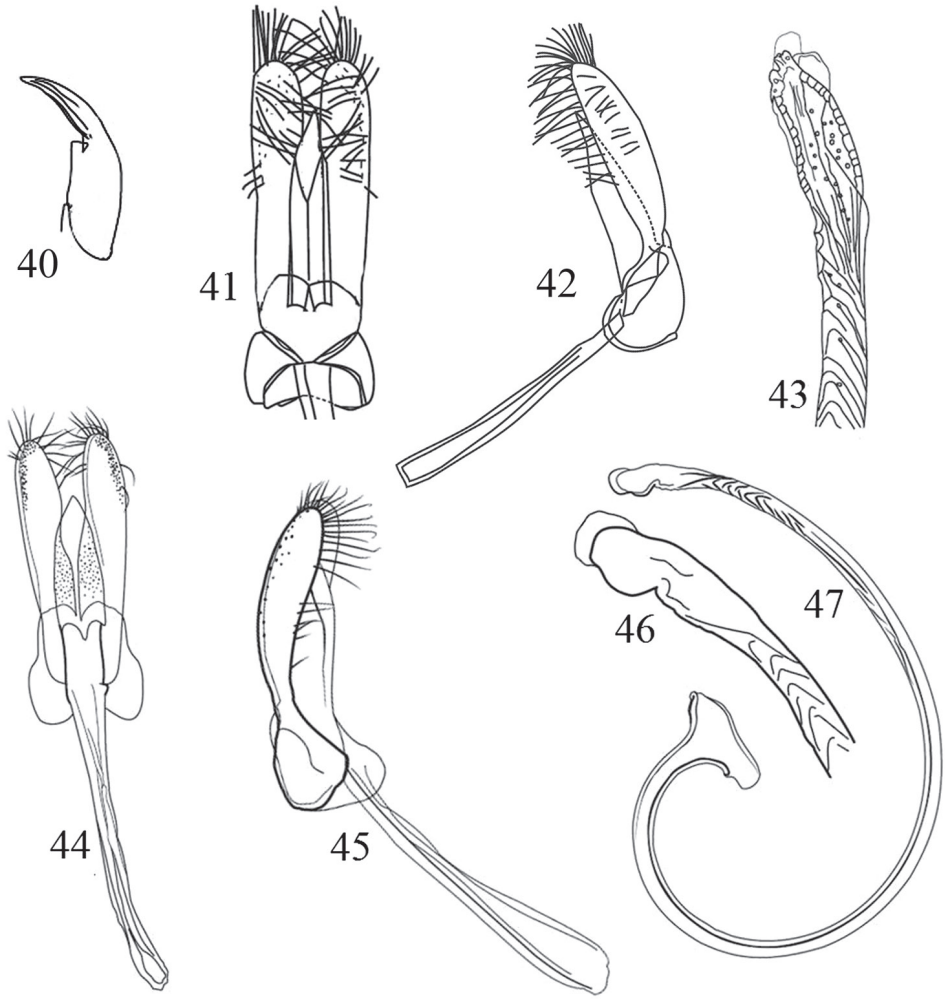
Figures 32–39. Morphological details and male genitalia of Chilocorini species **32–34** *E. quadripustulatus*: **32, 33** Tegmen in ventral and lateral view **34** Penis apex **35–36** *E. undulatus*: **35** Tegmen, ventral view **36** Penis apex **37–39** *E. octosignatus*: **37** Tarsal claw **38** Tegmen, ventral view **39** Penis apex.

***Parexochomus nigripennis* (Erichson, 1843)**
Figs 11, 23, 28, 48–50

Chilocorus nigripennis Erichson, 1843: 267.
Exochomus xanthoderus Fairmaire, 1864: 648.

Material examined. 10♂, 16♀: Iran, Lorestan province, VII.2014, lgt. Amir Biranvand, det. Biranvand.

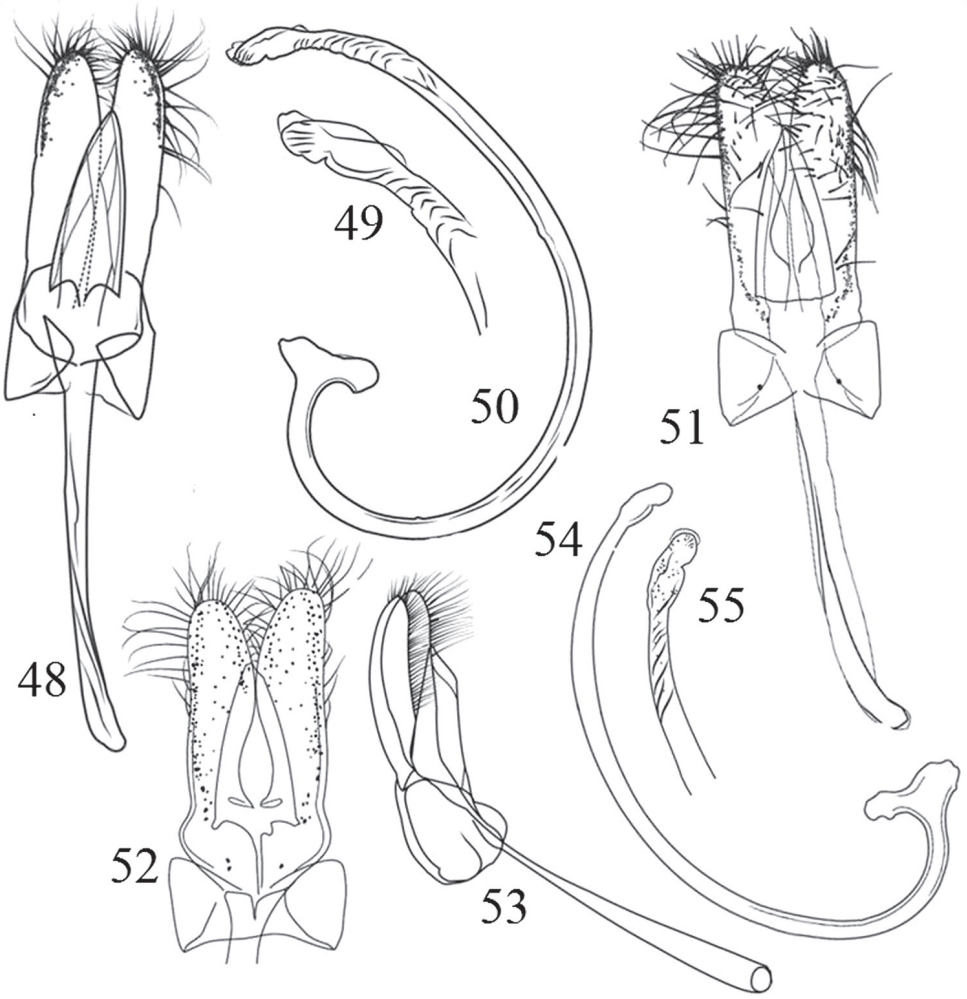
General distribution. Oriental region (Poorani 2002), Afrotropical region, Mediterranean region, Middle East (Kovář 2007).



Figures 40–47. Morphological details and male genitalia of Chilocorini species. **40–43** *E. gebleri*: **40** Tarsal claw **41, 42** Tegmen in ventral and lateral view **43** Penis apex **44–47** *P. pubescens*: **44–45** Tegmen in ventral and lateral view **46** Tip of penis **47** Penis.

Distribution in Iran. Golestan (Montazeri and Mossadegh1995), Lorestan (Jafari and Kamali 2007), Gilan (Hajizadeh et al. 2003), Fars, Kerman, Khorasan, Khuzestan, Sistan, and Baluchestan (Moddarres-Awal 2012), Lorestan (current study).

Ecology. This species feeds usually on the following hemipterans: *Acanthococcus abaii* (Eriococcidae), *Agonoscena pistaciae* (Psyllidae), *Bemisia tabaci* (Aleyrodidae) (Moddarres-Awal 2012).



Figures 48–55. Morphological details and male genitalia of Chilocorini species. **48–50** *P. nigripennis*: **48** Tegmen, ventral view **49** Penis apex **50** Penis **51–55** *P. nigromaculatus*: **51–53** Tegmen, ventral and lateral view **54** Penis **55** Penis apex.

***Parexochomus nigromaculatus* (Goeze, 1777)**
Figs 12, 51–55

Coccinella nigromaculata Goeze, 1777: 248. *Coccinella testudinare* Geoffroy in Fourcroy, 1785: 151. *Coccinella aurita* Scriba, 1791: 101. *Coccinella humerale* Townson, 1800: 167.

Chilocorus rufipes Stephens, 1832: 375. *Exochomus collaris* Küster, 1849: 100. *Exochomus pyrenaeus* Kraatz, 1873: 194.

Material examined. 75♂, 90♀: Iran, Lorestan province, spring and summer 2013, 2014, 2015, 2016, 2017, lgt. Amir Biranvand, det. Biranvand. 3♂, 1♀: Iran, Semnan province, VI.2015, lgt. Mino Toozandejani, det. Biranvand.

General distribution. Palaearctic region (Duverger 1983, Kovář 2007).

Distribution in Iran. Widely distributed (Duverger 1983, Moddarres-Awal 2012).

Ecology. This species feeds usually on the following species of Hemiptera: *Agonoscena pistaciae* (Psyllidae), *Aonidiella orientalis* (Diaspididae), *Bemisia tabaci* (Aleyrodidae), *Diuraphis noxia* (Aphididae), *Eulecanium prunastri* (Coccidae), *Euphyllura olivina* (Psyllidae), *Maconellicoccus hirsutus* (Pseudococcidae), *Therioaphis maculata* (Aphididae) (Moddarres-Awal 2012) and other aphids and Coccidae (Uygun 1981, Atlıhan and Özgökçe 2002, Kaydan et al. 2012).

***Parexochomus pubescens* (Küster, 1848)**
Figs 13, 22, 44–47

Exochomus pubescens Küster, 1848: 94
Exochomus apicatus Fairmaire, 1884: 59.
Exochomus circumcinctus Sahlberg, 1903: 36.
Platynaspis flavilabris Motschulsky, 1849: 155.
Platynaspis flavilabris Mulsant, 1850b: 947. [Homonym]
Exochomus gestroi Fairmaire, 1875: 540.
Exochomus lugubrivestis Mulsant, 1853: 194.
Exochomus saharae Sicard, 1929: 60

Material examined. 3♂, 5♀: Iran, Lorestan province, VII.2014, lgt. Amir Biranvand, det. Biranvand.

General distribution. Oriental region, Palestine, Syria (Poorani 2002), Afghanistan, Algeria, Egypt, France, Greece, Iran, Israel, Italy, Libya, Morocco, Saudi Arabia, Spain, Tunisia (Kovář 2007).

Distribution in Iran. Angohran region, Hormozgan, Tehran (Karaj), Khuzestan (Susangerd), Ramine region, Daran region, Sagdan region (Duverger 1983), Lorestan (Jafari and Kamali 2007), Fars, Kerman, Khorasan, Khuzestan, Sistan, and Baluchestan (Moddarres-Awal 2012).

Ecology. This species feeds on *Bemisia tabaci* (Hemiptera: Aleyrodidae) and *Tetranychus turkestani* (Acari) (Moddarres-Awal 2012).

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Phylogenetic study of the genus *Sternolophus* Solier (Coleoptera, Hydrophilidae) based on adult morphology

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Abstract

The phylogeny of the hydrophilid genus *Sternolophus* Solier, 1834 was examined in this study using 60 morphological adult characters, eight of them continuous and 52 discrete. The cladistic analysis resulted in a single most parsimonious tree with two major subclades corresponding, respectively, to species previously assigned to the subgenera *Sternolophus* s. str. Solier and *Neosternolophus* Zaitzev, although they are not re-instated. The species groups *S. angolensis* (Erichson, 1843) and *S. solieri* Castelnau, 1840 are recovered as monophyletic. The biogeography and diversification of the species of *Sternolophus* are briefly discussed.

Keywords

biogeography, cladistic analysis, diversification, species groups, water scavenger beetles

Introduction

The genus *Sternolophus* Solier, 1834 is widely distributed in the tropics of the Old World, with only few species occurring in the temperate zones. In a recent taxonomic revision of the genus by Nasserzadeh and Komarek (2017), the number of species was increased from nine (Hansen 1999) to 17.

The phylogeny of *Sternolophus* has been poorly studied. Zaitzev (1909) split the genus into two subgenera, *Sternolophus* s. str. Solier, 1834 and *Neosternolophus* Zaitzev, 1909. His classification was based on the absence or presence of an emargination on the anterior clypeal margin. Although this subdivision was accepted by Orchymont (1919), this author considered the length of the spine on the metaventrite a more significant character. Smetana (1980) elevated *Neosternolophus* to generic rank based on the emargination of the anterior clypeal margin, but this change was later opposed by Hansen (1991). This subgeneric division was also rejected by Watts (1989) based on the wide inter- and intraspecific variation of the mentioned character within the Australian species. The phylogenetic relationships of *Sternolophus* species were also studied by Hansen (1991), Short (2010), Short and Fikáček (2013) and Toussaint et al. (2017), although these studies (with the exception of Short 2010) are mainly focused either on family- and tribe-level relationships (Hansen 1991; Short and Fikáček 2013) or had a biogeographic focus (Toussaint et al. 2017). Short (2010) included seven species of *Sternolophus* in his analysis of the subtribe Hydrophilina which resulted in the monophyly of the subgenus *Sternolophus* s. str. and the lack of resolution for species of *Neosternolophus*.

Nasserzadeh and Komarek (2017) suggested changes to the subgeneric classification, and proposed two new species groups (the groups *S. angolensis* (Erichson, 1843) and *S. solieri* Castelnau, 1840) based on highest morphological similarity and without including a phylogenetic approach. These authors considered *S. angolensis*, *S. inconspicuus* (Nietner, 1856), *S. mundus* (Boheman, 1851) and *S. solitarius* Nasserzadeh and Komarek, 2017 as members of the *angolensis* group, and placed *S. angustatus* (Boheman, 1851), *S. elongatus* Schaufuss, 1883, *S. mandelai* Nasserzadeh and Komarek, 2017, *S. rufipes* (Fabricius, 1792), and *S. solieri* in the *solieri* group. They left the remaining species (*S. australis* Watts, 1989, *S. decens* Zaitzev, 1909, *S. immarginatus* Orchymont, 1911, *S. insulanus* Nasserzadeh and Komarek, 2017, *S. jaechi* Nasserzadeh and Komarek, 2017, *S. marginicollis* (Hope, 1841), and *S. prominolobus* Nasserzadeh and Komarek, 2017) ungrouped.

Here the first comprehensive phylogenetic analysis of the genus *Sternolophus* is provided, based on a cladistics analysis of adult morphological characters. Considering the phylogenetic results, the biogeography and diversification of the species are briefly discussed.

Materials and methods

Taxon sampling. More than 4000 specimens in all the 17 species of *Sternolophus* were studied as ingroup, and *Hydrochara flavipes*, belonging to the tribe Hydrophilini, was included as outgroup. A total of 271 specimens were measured. The specimens were obtained on loan from the following institutions and collections:

AEZS	coll. A. Short, University of Kansas, Lawrence, KS, USA
CBSU	Collection of Department of Biology, Shiraz University, Iran
HMIM	Hayek Mirzayans Insect Museum, Tehran, Iran

ISNB	Institut Royal des Sciences Naturelles de Belgique, Bruxelles, Belgique
MNHN	Muséum National d'Histoire Naturelle, Paris, France
MNHUB	Museum der Alexander Humboldt Universität, Berlin, Germany
NHML	Natural History Museum, London, UK
NMB	Naturhistorisches Museum Basel, Basel, Switzerland
NMW	Naturhistorisches Museum Wien, Vienna, Austria
NRM	Swedish Museum of Natural History, Stockholm, Sweden
OUMNH	Oxford University Museum of Natural History, UK
SAMA	South Australian Museum, Adelaide, Australia
SMTD	Staatliches Museum für Tierkunde, Dresden, Germany
ZMUC	Zoological Museum University of Copenhagen, Denmark

The examined specimens are listed in Appendix 1. The specimens were selected according to: 1) geographical distribution, 2) morphological variation, and 3) status as type specimens.

Preparation for morphological studies. To study the male genitalia, the aedeagus was extracted and macerated in lactic acid for at least four days to become hydrated and cleared before examination. Bursa copulatrix, spermatheca, and spermathecal gland were also dissected (for details see Nasserzadeh et al. 2005) and mounted in DMHF or Euparal on transparent cards and pinned below the associated specimens. Morphological data for each species were obtained using a stereomicroscope (Zeiss Stemi SV11). Measurements were made through a micrometric eyepiece and presented in figures 1, 8, 14–15, 20–21. Line drawings of characters were adapted from Nasserzadeh and Komarek (2017). Photographs were taken using a 650D Canon digital camera.

Character selection and coding. Character selection and character state definition follow Smetana (1980), Nasserzadeh et al. (2005) and Nasserzadeh and Komarek (2017). A total of 60 characters (eight continuous and 52 discrete) was selected and scored from zero to 59 (see Table 1). Eight continuous characters involving ranges and ratios were treated as such, avoiding the use of *ad hoc* methods to establish ranges (Goloboff et al. 2008). Discrete characters contained 45 binary and seven multistate. Characters 0, 2–6, and 8–45 correspond to the external morphology, characters 1, 7 and 46–55 were derived from the aedeagus, and characters 56–59 were coded from the female genital membranous tube. Characters and character state compositions approach the logic of neomorphic and transformational pattern as indicated by Sereno (2007). There are no missing characters in the data matrix, and the inapplicable characters were coded as '?' (Appendix 2).

Phylogenetic analysis. Cladistic analyses were performed on all characters in 'Tree Analysis using New Technologies' (TNT) (Goloboff et al. 2008) with 'traditional' search based on 5000 replicates, through 'tree bisection reconnection' (TBR) branch swapping holding 100 trees by collapsing rule 'min. length=0'. Discrete characters were treated as unordered, and multistate characters were treated as polymorphic (e.g. [0 1]). The same analysis was performed only on the discrete characters and the consensus tree was obtained using strict and majority-rule methods. An analysis including

Table 1. List of morphological characters, character states, and codes.

Codes	List of characters and character states
Continuous characters	
0	Average length of body in millimeters.
1	Average length of aedeagus in millimeters (Fig. 15a).
2	Ratio width of head (from outer lateral margin of eyes) / width of clypeus in anterior margin (connecting with labrum) in males.
3	Ratio width of head in outer margin of eyes / length of clypeus (from the centre of frontoclypeal suture (Fig. 3a) to anterior margin of clypeus).
4	Ratio average length of body / average length of aedeagus.
5	Length of hind femur (Fig. 13a) / widest part (Fig. 13b).
6	Ratio distance of bare area between the apical angle of the pubescent part of submentum to the base of mentum (Figs 5c, 6c) / width of anterior margin of submentum (connecting to the mentum) (Figs 5d, 6d).
7	Ratio length of aedeagus (Fig. 15a)/width (widest part of the parameres) (Fig. 15b).
Discrete characters	
External body morphology	
8	Lateral sides of body: (0) rather parallel; (1) rather rounded.
9	Body in lateral view: (0) distinctly convex; (1) moderately convex.
10	Femora with basal hydrofuge pubescent: (0) absent; (1) present.
11	If femora pubescent basally, pubescence distribution on hind femur: (0) very narrow, in anterior part of femur connecting with coxa, sometimes slightly extended marginally to the connecting border with trochanter (Fig. 14b); (1) more expanded, covering a wider area from attachment part of femur to coxa posteriorly toward trochanter (Fig. 13).
12	Coloration of legs in comparison with ventrites: (0) unicolored; (1) not unicolored.
13	Coloration of femur: (0) uniformly black to rufous; (1) not uniformly colored, femur distinctly darker proximally and lighter distally, rufo-testaceous to rufous.
14	Irregular transversal row of 11–13 deep punctures on medial part of the labrum: (0) absent; (1) present.
15	Few deeper punctures near the basal margin of labrum (Fig. 4a): (0) absent; (1) present.
16	Length of the rufous to testaceous coloration on the anterior part of labrum /length of labrum: (0) ¼ to ⅙; (1) ½ to ⅓.
17	Paired and irregularly distributed antero-lateral groups of punctures on the clypeus (Fig. 4b): (0) semicircular (Figs 1–3); (1) arc-shaped (Fig. 4).
18	The paired antero-lateral groups of punctures on the clypeus separated: (0) narrowly (narrower than 1/6 width of clypeus at anterior margin of eyes); (1) widely (wider than 1/5 width of clypeus at anterior margin of eyes).
19	Anterior margin of clypeus: (0) entire (Fig. 4); (1) sinuated/emarginated medially (Figs 1–3).
20	If anterior margin of clypeus emarginated or sinuated medially: (0) sinuated smoothly (Fig. 2); (1) weakly emarginated; (2) distinctly emarginated (Fig. 3); (3) strongly and widely emarginated (Fig. 1).
21	Apex of fourth maxillary palpomere: (0) without infuscation; (1) distinctly darkend.
22	Length of maxillary palpus (Fig. 7) /width of clypeus in anterior margin of eye: (0) short (0.8); (1) almost equal (1.0); (2) moderately long (1.2–1.3); (3) long (1.4).
23	Mentum with anteromedial impression: (0) absent; (1) present (Figs 5–7).
24	If mentum with anteromedial impression, the pubescent area of submentum: (0) triangular-shape, lateral sides more straight (Fig. 6); (1) semicircular-shape, lateral sides more rounded (Fig. 7); (2) belly-shape/domical-shape, rounded lateral sides (Fig. 5)

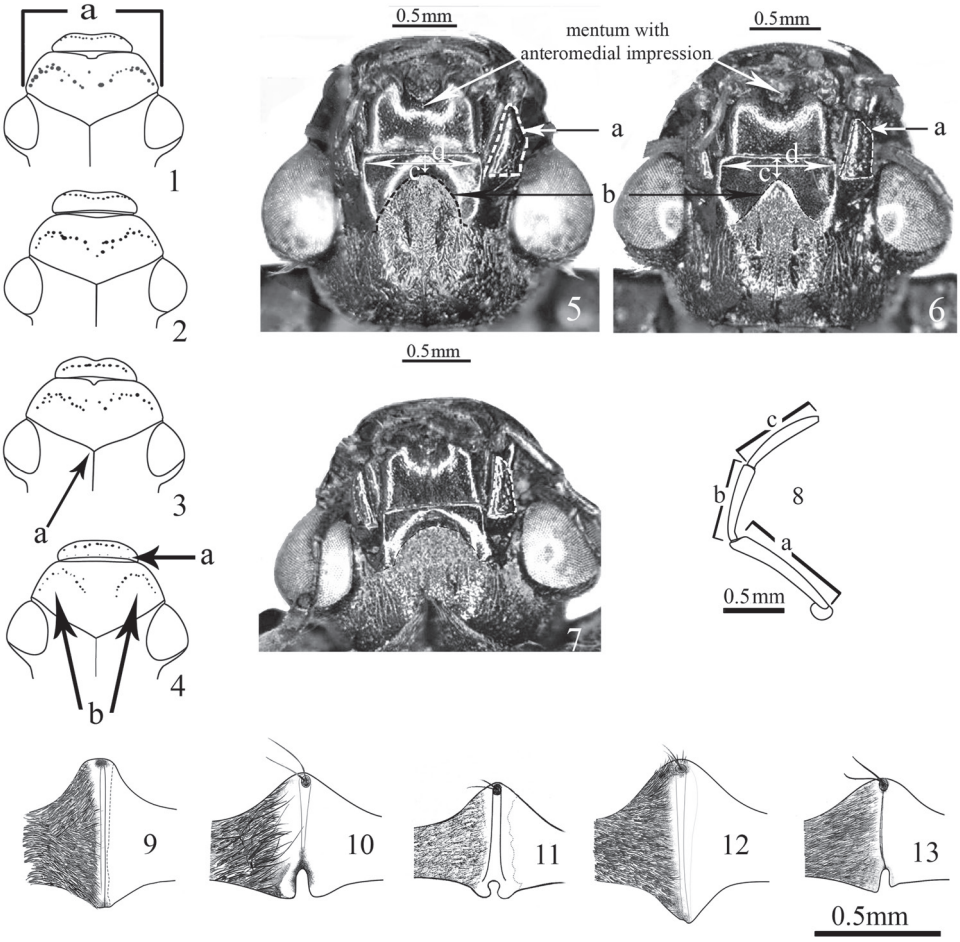
Codes	List of characters and character states
25	Outer lateral margin of maxilla: (0) rounded, without projection; (1) not rounded, more or less straight, with or without a projection (Figs 5–7).
26	If lateral margin of maxilla is straight: (0) no projection on lateral margin is recognizable (Fig. 7); (1) a distinct projection is recognizable (Figs 5–6).
27	If lateral margin of maxilla bears a distinct projection: (0) it is located approximately on anterior third (Fig. 6); (1) it is located approximately on medial portion (Fig. 5).
28	Scattered deep punctures on pronotum: (0) absent; (1) present.
29	Mesal edge of prosternal carina: (0) sharp (Figs 9, 10, 12, 13); (1) blunt (Fig. 11).
30	Deep or weak division on posterior end of mesal edge of prosternal carina: (0) absent (Figs 8, 11); (1) present (Figs 10, 11, 13).
31	If mesal edge of carina not divided and knob-like, posterior protrusion between procoxae: (0) absent (Fig. 7); (1) present (Fig. 10).
32	If mesal edge of carina divided on posterior end, the division is: (0) deep with a notch (Fig. 9); (1) more or less weak and without a deep notch (Fig. 12).
33	Number of longitudinal series of punctures on the elytra: (0) four; (1) five.
34	If the number of longitudinal series of punctures on the elytra is four, irregular punctures between last lateral series 4 and elytral margin: (0) absent; (1) present.
35	If number of longitudinal series of punctures on the elytra is four and irregular punctures between last lateral series and elytral margin present, the width of punctures in interspace of lateral margin of elytra (between lateral series and elytral margin): (0) about ¾ or more; (1) about ½; (2) about ⅓ or less.
36	If irregular punctures between lateral series 4 and elytral margin reaching 1/2 width of interspace, irregular punctures distributed: (0) densely; (1) loosely.
37	Length of spine on metaventrite: (0) short, never reaching anterior margin of first ventrite (Fig. 14); (1) long, exceeding anterior margin of first ventrite (Fig. 15).
38	If length of spine on metaventrite long, spine: (0) straightly elongated almost in parallel to the ventral side; (1) slightly and gradually bend upward distally toward posterior end.
39	If the spine of metaventrite short, spine at posterior end (or apex): (0) not sharp/pointed, not bent ventrally; (1) sharp and slightly bent ventrally.
40	If the spine of metaventrite short, spine: (0) reaching mid-length of 1st ventite or shorter (Fig. 12); (1) exceeding mid-length of 1st ventrite (Fig. 13).
41	If the spine of metaventrite long, spine: (0) not reaching mid-length of 2nd ventrite (1) hardly reaching mid-length of 2nd ventrite; (2) exceeding mid-length of 2nd ventrite and extending to 3/4 length of ventrite 2; (3) reaching anterior margin of 3rd ventrite.
42	Sternal keel of metaventrite: (0) slim, almost as wide as the spine of metaventrite at mid-length (Fig. 14); (1) wide, distinctly wider than the spine on metaventrite at mid-length (Fig. 15).
43	Abdominal ventrite 5 hydrofuge pubescence: (0) uniform; (1) with a glabrous posteromedian area.
44	Apical margin of ventrite 5: (0) entire; (1) emarginated.
45	Male claw of fore leg: (0) weakly curved and short; (1) strongly curved and distally elongated.
Aedeagus morphology	
46	Inner and outer lateral margins of paramere on anterior half: (0) without distinct curvature and straight (Fig. 17); (1) with curvature, i.e. width of paramere changes from mid-length toward the apex. (Figs 18–21).
47	If paramere with curvature in lateral margins on anterior half: (0) outer lateral margin concave at about mid-length (Fig. 20); (1) outer lateral margin concave at about apical third (Figs 16, 18, 19, 21).

Codes	List of characters and character states
48	If paramere with outer lateral margin concave at about apical third: (0) the posterior ⅔ smoothly and widely convex with no impression (Fig. 19); (1) a weak curvature projected lateromedially (just before the apical third) (Figs 16, 18, 21).
49	If outer lateral margin of paramere concave at about apical third without a smooth convex curve, the apex of paramere: (0) clavate (Figs 18, 21); (1) not clavate (Fig. 16).
50	Sclerotized dorsal shield of median lobe of aedeagus: (0) without sharp anterior carina; (1) with sharp anterior carina (Fig. 16).
51	Sclerotized dorsal shield of median lobe of aedeagus: (0) flat to subcylindrical (Figs 17, 18, 21); (1) tectiform (Figs 16, 19, 20).
52	Lateral lobules of median lobe of aedeagus: (0) absent (Fig. 16); (1) present.
53	If lateral lobules of median lobe of aedeagus present, lateral lobule at widest part (Fig. 20a) / total width of the median lobe on apical portion of the sclerotized dorsal shield (Fig. 20b): (0) less than 2/10 (lobules with small size) (Fig. 18); (1) almost 3/10 (lobules with moderate size) (Figs 17, 21); (2) almost 4/10 (lobules with large size) (Figs 19, 20).
54	If lateral lobules of median lobe of aedeagus present, the sclerotized dorsal shield: (0) without snout-shaped process apically that protrudes between the lateral lobules (Figs 18, 20); (1) with a weak snout-shaped process apically that protrudes between the lateral lobules (Figs 17, 19, 21).
55	If lateral lobules of median lobe of aedeagus present these lobules: (0) not inflated; (1) inflated (Fig. 19).
Female genital tube morphology	
56	Connection between bursa copulatrix and ejaculatory duct: (0) lateral; (1) anterior.
57	Connection of spermathecal duct and spermathecal gland to spermathecal bulb: (0) separate; (1) via one joined duct.
58	Length of spermathecal duct/bursa (from apex to common oviduct): (0) less than 1/2; (1) 1/2 to equal; (2) two times longer.
59	Longitudinal rows of small tooth-like spines on the membranous wall of the bursa: (0) absent; (1) present.

all continuous and discrete characters was also conducted by retaining suboptimal trees 0.5 steps longer than the most parsimonious tree; the resulting trees were summarized by strict and majority-rule consensus methods.

The synapomorphic characters and character states are mapped on the single most parsimonious cladogram (analysis A). Branch support was calculated by bootstrap (Felsenstein 1985), jack-knife (Farris et al. 1996), and symmetric resampling (Goloboff et al. 2003), with 2000 replicates. Different numbers of replicates (up to 5000) did not affect the results. In resampling analysis, the results of the absolute frequency summarize method was used, which were slightly higher than the analysis using frequency difference.

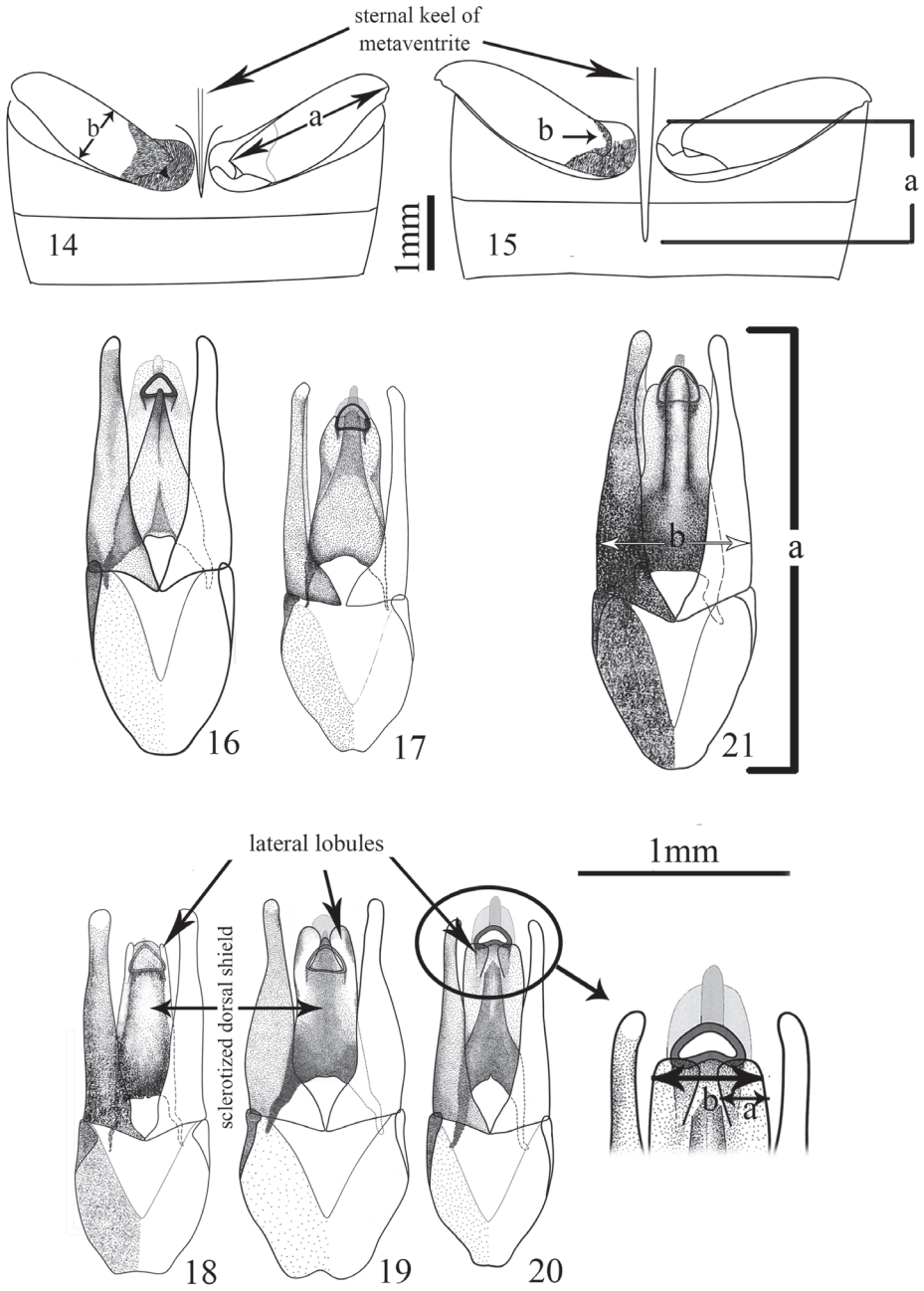
The consistency and retention indices (Kluge and Farris 1969; Farris 1989) of discrete characters were calculated using PAUP version 4.0b10 (Swofford 2002) (analysis D). All 52 discrete characters were equally weighted, and multistate characters were treated as unordered. Heuristic searches were selected with 20000 random additions followed by branch swapping using TBR and holding a single tree (NCHUCK = 1, CHUCKSCORE = 1) (Alipanah et al. 2010).



Figures 1–13. 1–4 Dorsal view of head **1** *Sternolophus acutipenis* **a** width of clypeus at anterior margin of eyes **2** *S. jaechi* **3** *S. marginicollis* **a** centre of frontoclypeal suture **4** *S. solieri* **a** deeper punctures near the basal margin of labrum **b** paired antero-lateral groups of punctures on the clypeus (Nasserzadeh and Komarek 2017) **5–7** Ventral view of head **5** *Sternolophus acutipenis* **6** *S. angustatus* **7** *S. decens* **a** maxilla **b** pubescent area on submentum **c** bare area of submentum **d** base of mentum **8** Maxillary palpus of *Sternolophus acutipenis* **a–c** length of palpus segments (Nasserzadeh and Komarek 2017) **9–13** Prosternal carina **9** *Sternolophus acutipenis* **10** *S. angustatus* **11** *S. decens* **12** *S. jaechi* **13** *S. solieri* (Nasserzadeh and Komarek 2017).

Results

The parsimony analysis of all characters (analysis A) resulted in a single most parsimonious tree of 146.130 steps (Fig. 22). When suboptimal trees 0.5 steps longer than the most parsimonious tree were retained (analysis C), six most parsimonious trees were obtained. The consensus of these trees, either using strict or majority-rule methods, was congruent with the single most parsimonious tree from analysis A, except for slight differences in the position of the species within clades C and M (Fig. 23a, b). The analysis



Figures 14–21. 14–15 Hind femur with the spine on metaventrite **14** *Sternolophus acutipenis* **a** length of femur **b** widest part of hind femur **15** *S. mandelai* **a** length of spine **b** basal pubescent area (modified from Nasserzadeh and Komarek 2017) **16–21** Dorsal view of aedeagus **16** *Sternolophus acutipenis* **17** *S. angolensis* **18** *S. angustatus* **19** *S. immarginatus* **20** *S. marginicollis* **a** lateral lobules at widest part of median lobe **b** total width of median lobe on apical portion of the sclerotized dorsal shield **21** *S. solitarius* **a** length **b** widest part of the parameres (modified from Nasserzadeh and Komarek 2017).

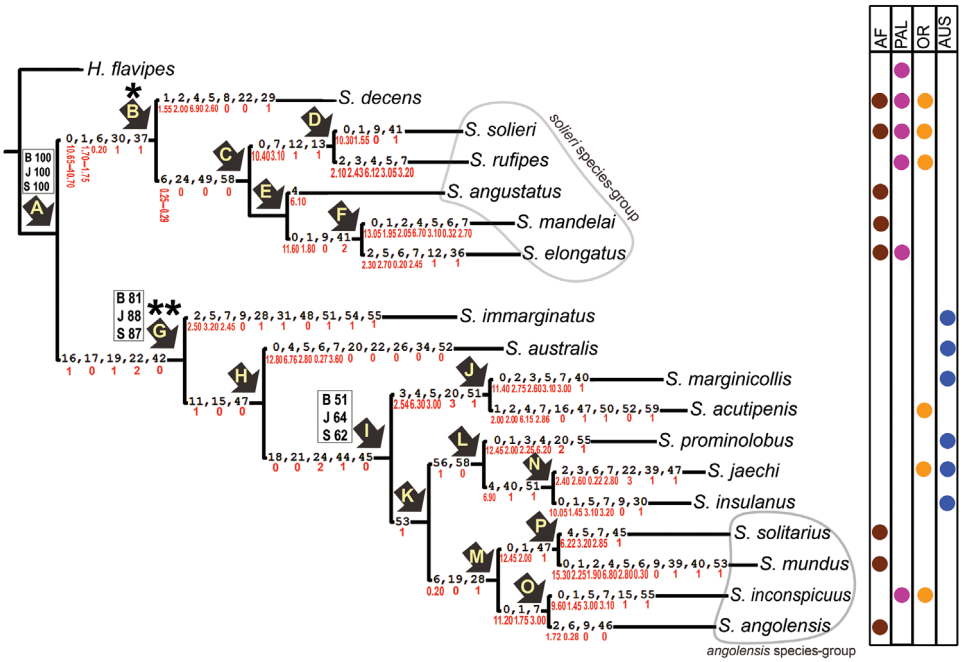


Figure 22. Single most parsimonious tree (146.130 steps) based on 60 morphological characters (52 discrete and 8 continuous). Bootstrap (B), Jackknife (J) and Symmetric (S) support values over 50% are mentioned above the corresponding branches, respectively. The arrows with capital letters indicate the clades. Synapomorphies are shown on the branches, and character states in red. Table on the right shows distribution of the species by region (AF = Afrotropical, PAL = Palaearctic, OR = Oriental, AUS = Australian). The two major clades are marked as (*) and (**) indicating *Sternolophus* s. str. and *Neosternolophus* respectively. Species groups *angolensis* and *solieri* (see Nasserzadeh and Komarek 2017) are shown in closed irregular ovals.

of discrete characters only (analysis B) resulted in 36 most parsimonious trees of 110 steps. The consensus trees using both strict and majority-rule methods were different from previous trees in the position of the species in clade B (Fig. 24a, b). Analysis using PAUP on the 52 discrete characters (analysis D) estimated 38 parsimony informative characters, with consistency index (CI) = 0.56 and retention index (RI) = 0.72.

As shown in the single most parsimonious tree obtained with analysis A (Fig. 22), the examined *Sternolophus* species are divided into two major monophyletic clades, B and G, with 6 and 11 species respectively. Clade B contains *S. decens* as sister to clade C that is composed of five species, *S. solieri*, *S. rufipes*, *S. angustatus*, *S. mandelai*, and *S. elongatus*. Clade B is supported by five characters (0: 10.65–10.70, 1: 1.70–1.75, 6: 0.20, 30: 1, 37: 1), although it is weakly supported statistically. Except for the elongated spine on the metaventrite (37: 1), the characters sustaining this clade were homoplastic. The topology of clade B was slightly different in analysis C (Fig. 23), and the clade was not maintained in analysis B, with the six species unresolved in the strict consensus (Fig. 24a), whereas in the majority-rule consensus tree (Fig. 24b) *S. decens* was resolved as sister to clade G in 64% of the cases (24 out of 36 trees).

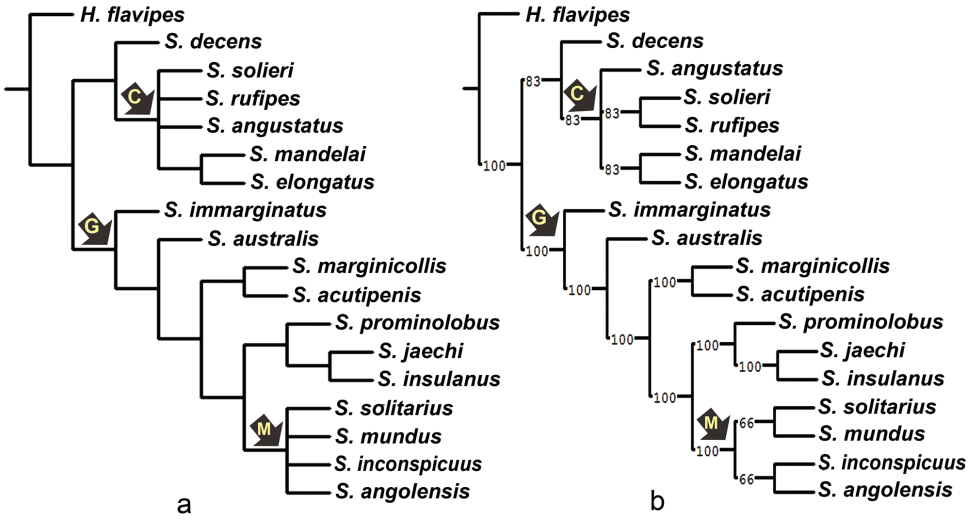


Figure 23. Results of the phylogenetic analysis based on 60 (continuous and discrete) morphological characters, with a suboptimum value of 0.5 step longer **a** strict consensus tree **b** majority-rule consensus tree of six most parsimonious trees (length 146.130), numbers on the branches indicate majority rule support for node. The arrows with capital letters indicate selected clades.

The monophyly of clade G was well supported in all analyses (Figs 22–24). Monophyly of this clade is supported by the following five synapomorphies: the rufous to testaceous coloration of the labrum exceeding one third of its length (16: 1); the semicircular arrangement of the paired antero-lateral group of punctures on clypeus (17: 0); the presence of an emargination on the anterior margin of clypeus (19: 1); the moderately long maxillary palpus (22: 2); and the slim sternal keel of metaventrite (42: 0). All analyses also agreed in the monophyly of clade I, although with weaker support (Figs 22–24). Five synapomorphies sustain this clade: the narrow distance between paired antero-lateral groups of punctures on the clypeus (narrower than one-sixth of the width of clypeus at anterior margin of eyes) (18: 0); the absence of infuscation on the apex of fourth maxillary palpomere (21: 0); the belly shape of the pubescent area of submentum (24: 2); the presence of an emargination on the apical margin of ventrite 5 (44: 1); and the weakly curved and short male claw on fore leg (45: 0). Based on the results of analysis A (Fig. 22), *S. australis* is sister to clade I, whereas *S. immarginatus* is sister to the clade formed by *S. australis* and clade I. In all analyses, clades K, L, M, and N were found to be monophyletic with the same configuration. These clades are supported by one, two, three, and three synapomorphies, respectively (Fig. 22); however, the position of the four species within clade M was unstable in all analyses.

The comparison of the trees obtained using all characters (Figs 22, 23) with those obtained using only discrete characters (Fig. 24) reveals the influence of continuous characters in the formation of clade B. The exclusion of continuous characters from the analysis causes the species within this clade to collapse in a polytomy (Fig. 24). Clade B is supported by three continuous and two discrete synapomorphies. Similarly, continu-

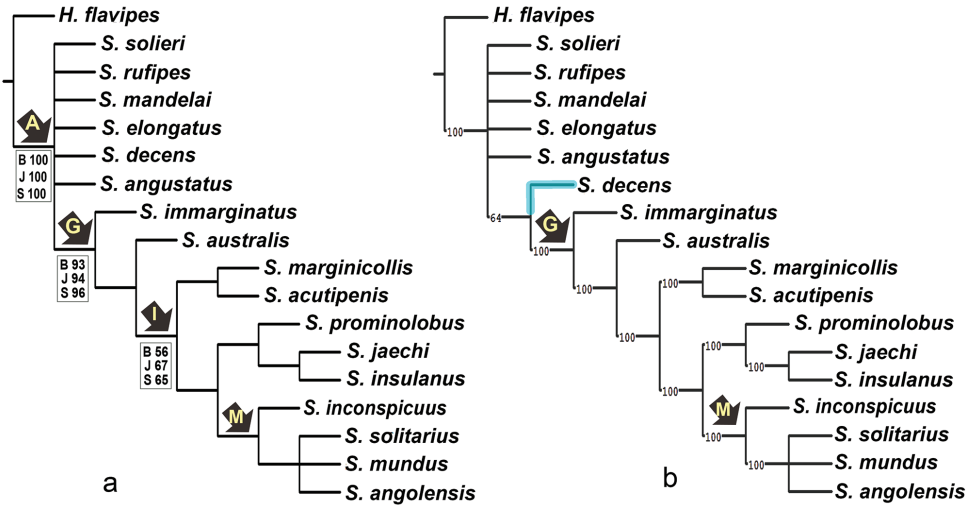


Figure 24. Results of the phylogenetic analysis based on 52 discrete morphological characters. **a** strict consensus tree. Bootstrap (B), Jackknife (J) and Symmetric (S) support values over 50% are mentioned above the corresponding branches **b** majority-rule consensus tree of 36 most parsimonious trees (length 110). Numbers on the branches indicate majority rule support for nodes. Arrows with capital letters indicate selected clades.

ous synapomorphies outnumber discrete synapomorphies within clade B, except for clade C with one continuous and three discrete synapomorphies (Fig. 22). The importance of continuous characters in shaping clade B can be explained by the fact that this character set (0 to 7) provides diagnostic features for separating the morphologically very similar species of the *solieri* species group (clade C) (Nasserzadeh & Komarek 2017). In all analyses, the topology of clade G remained consistent except for slight changes in clade M and variable support for clades G and I (Figs 22–24). On the other hand, *Sternolophus decens* was recovered in clade B in five of the six most parsimonious trees obtained using both continuous and discrete characters combined (Fig. 23b), whereas it was sister to clade G in more than 60% of the 36 most parsimonious trees obtained using discrete characters only (e.g., Fig. 24b), showing that the position of this taxon is also highly influenced of continuous characters.

Discussion

Taxonomy. The species formerly included in the subgenera *Sternolophus* s. str. and *Neosternolophus* were recovered into two major subclades, B and G, respectively. However, due to the following considerations, subgeneric status was not re-instated: i) Unreliable topology of clade B in different analyses and absence of support for its monophyly as well as monophyly of the subclades. ii) Questionable position of *S. decens* within clade B. *Sternolophus decens* was included in the subgenus *Sternolophus* s. str. by Zaitzev (1909), and was found to be closely related to *S. rufipes* and *S. solieri* by Short (2010).

However, it was recovered in a monophyletic clade together with *S. marginicollis* (and some unidentified *Sternolophus* species) by Toussaint et al. (2017), which was included in the subgenus *Neosternolophus* by Zaitzev (1909). In the trees obtained in analyses A and C (Figs 22–23), *S. decens* was recovered as sister to clade C. The species of this clade (*S. solieri*, *S. rufipes*, *S. angustatus*, *S. mandelai* and *S. elongatus*) (Fig. 22) were grouped in the *solieri* species group by Nasserzadeh and Komarek (2017) based on highest morphological similarity. iii) A nearly similar topology was obtained for clade G in the different analyses, all of them including *S. marginicollis*, with strong support. Based on the topology obtained here and those of Short (2010) and Toussaint et al. (2017), we believe that reinstating subgenera within *Sternolophus* is premature and would not reflect the evolutionary history of the genus. Further investigations including larval and molecular characters of as many species of the genus as possible, as well as other techniques such as scanning electron microscopy, are required to resolve its phylogenetic relationships.

Short (2010), in his phylogenetic analysis of the subtribe Hydrophilina based on adult-morphological characters, found evidence for monophyly of the subgenus *Sternolophus* s. str., but the species formerly grouped in the subgenus *Neosternolophus* were unresolved and formed a basal polytomy within the genus. In our analysis, on the contrary, strong evidence was found for monophyly of *Neosternolophus*, whereas monophyly of *Sternolophus* s. str. is more questionable for the reasons mentioned above.

Finally, the four species (*S. solitarius*, *S. mundus*, *S. inconspicuus* and *S. angolensis*) grouped by Nasserzadeh and Komarek (2017) as the *angolensis* species group based on morphological similarities, are resolved here as clade M confirming their close relationship, although weakly supported (Fig. 22).

Biogeography and diversification. In Figure 22 (right table), clade C consists of the *solieri* species group distributed in the Afrotropical, Palaearctic and Oriental regions. Distribution of *S. decens* overlaps with those of clade D. On the other hand, most members of clade G have an Oriental-Australasian distribution. The exceptions are representatives of the *angolensis* species group, with *S. solitarius*, *S. mundus,* and *S. angolensis* restricted to the Afrotropical Region whereas *S. inconspicuus* is widely distributed in the Oriental Region to the eastern boarder of the Palaearctic Region. *Sternolophus insulanus* and *S. jaechi* are two sister species with insular distribution in the Malay Archipelago (see Appendix 1).

Toussaint et al. (2017) postulated an Afrotropical origin for *Sternolophus*, dispersing toward Australia in the Oligocene/Miocene. There are many New Cenozoic fossil findings of taxa closely related to *Sternolophus* in Europe and North America (e.g. Fikáček et al. 2008, 2010a, 2010b), whereas the only record of this genus is a dubious fossil likely belonging to *S. rufipes* from the Early Pliocene of the Tsubusagawa Formation in Japan (Hayashi et al. 2003). The current distribution of *Sternolophus* in the Old World, i.e. without protruding into northern Asia, Europe, Tasmania and New Zealand (Nasserzadeh and Komarek 2017), which were largely covered by ice, and its absence in the fossil records from Europe and America, suggest a sensitivity of this group to climate change and glacial periods as inhibitor factors for its distribution, and also highlight the effect of eustatic changes in accelerating its dispersal in the Old World towards Australia.

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Appendix 1

App1. List of the specimens examined.

Species	Number of examined specimens	Collections	Total number of studied specimens	Geographical diversity of the examined specimens	Distribution of the species
S. acutipenis	10 (5 ♂♂, 5 ♀♀)	NMW	124	India, Thailand, Vietnam	Oriental Region
S. angolensis	20 (9 ♂♂, 11 ♀♀)	MNHUB, NMW, ZMUC	270	Burkina Faso, Comoros, Egypt, Guinea, Namibia, Tanzania, Togo, Zimbabwe	Afrotropical Region
S. angustatus	7 (5 ♂♂, 2 ♀♀)	NMW, NRM, ZMUC	50	Botswana, Namibia, South Africa, Tanzania, Zimbabwe	Eastern Afrotropical Region
S. australis	6 (4 ♂♂, 2 ♀♀)	FMNH, SAMA	37	Australia	Australian Region (only Australia)
S. decens	17 (7 ♂♂, 8 ♀♀)	NMB, NMW, HMIM	202	Iran, Oman, Pakistan, Saudi Arabia	Palaearctic and Oriental Regions (from East Africa to India)
S. elongatus	28 (19 ♂♂, 9 ♀♀)	ISNB, NMB, NMW, SMTD	301	Angola, Cameroon, Congo, Egypt, Eritrea, Ethiopia, Guinea, Madagascar, Saudi Arabia, Socotra Island (Yemen),	Afrotropical and Palaearctic Regions (Africa and Arabian Peninsula)
S. immarginatus	5 (3 ♂♂, 2 ♀♀)	SAMA, SMTD	30	Australia	Australian (only Australia)
S. inconspicuus	18 (11 ♂♂, 7 ♀♀)	MNHN, NNW, SMTD	234	China, India, Indonesia, Japan, Nepal, Sri Lanka, Thailand, Vietnam	Oriental Region, including southern China and Japan
S. insulanus	9 (6 ♂♂, 3 ♀♀)	NMW, ZMUC	37	Indonesia (Sulawesi & Papua)	Sulawesi to New Guinea
S. jaechi	6 (4 ♂♂, 2 ♀♀)	FMNH, NMW	13	Indonesia & Malaysia (Borneo Island)	Malay Peninsula, Borneo
S. mandelai	14 (7 ♂♂, 7 ♀♀)	NMW, SMTD	130	Gabon , Guinea, Namibia	Afrotropical Region
S. marginicollis	22 (13 ♂♂, 9 ♀♀)	NMW, SAMA, ZMUC	330	Australia, Indonesia, New Caledonia, Papua New Guinea, Philippines	Philippines and Sulawesi to New Guinea, Australia, New Caledonia and Fiji
S. mundus	20 (11 ♂♂, 9 ♀♀)	ISNB, MNHN, NNW, SMTD, ZMUC	416	Gabon, Kenya, Sudan, Tanzania, Uganda	Afrotropical Region

Species	Number of examined specimens	Collections	Total number of studied specimens	Geographical diversity of the examined specimens	Distribution of the species
S. prominolobus	10 (4 ♂♂, 6 ♀♀)	HMIM, NMW, SAMA	10	Australia	Eastern Australia
S. rufipes	30 (17 ♂♂, 13 ♀♀)	ISNB, NNW, SMTD, AEZS	1302	China, India, Indonesia, Japan, Nepal, Philippines, Thailand, Singapore, Vietnam	Eastern Palaearctic Region, Oriental Region
S. solieri	33 (17 ♂♂, 13 ♀♀)	HMIM, ISNB, NMW, SMTD	635	Afghanistan, Burkina Faso, Cape Verde, Egypt, Guinea, Iran, Mali, Pakistan, Saudi Arabia, Senegal, South Sudan	Northern half of Afrotropical Region to northwestern India
S. solitarius	7(6 ♂♂, 1 ♀)	NMW, HMIM	12	Mauritius (Rodrigues Island), Madagascar	Madagascar, Mascarene Islands
Hydrobius fuscicps	4 (2 ♂♂, 2 ♀♀)	HMIM, CBSU		Iran	Holarctic
Hydrochara flavipes	5 (3 ♂♂, 2 ♀♀)	HMIM, CBSU		Iran	Western Palaearctic Region

Appendix 2

App 2. Data matrix for cladistic analysis of *Sternolophus* species based on adult morphological characters. Inapplicable data are represented by '?'.

	0 0	0 1	0 2	0 3	0 4	0 5	0 6	0 7	0 0 1 8 9 0	1 1 1 1 1 1 2 3 4 5	1 1 1 1 6 7 8 9	2 2 2 2 2 2 0 1 2 3 4 5	2 2 2 2 6 7 8 9	3 3 3 3 3 3 0 1 2 3 4 5	3 3 3 3 6 7 8 9	4 4 4 4 4 4 0 1 2 3 4 5	4 4 4 4 6 7 8 9	5 5 5 5 5 5 0 1 2 3 4 5	5 5 5 5 6 7 8 9
Sternolophus acutipenis	12.30	2.00	2.00	2.54	6.15	3.00	0.19	2.85	1 1 1	1 0 0 1 0	0 0 0 1	3 0 2 0 2 1	1 1 0 0	0 0 ? 0 12	? 0 ? 1	0 ? 0 0 1 0	1 1 1 1	1 1 0 ? ? ?	0 0 1 1
S. angolensis	11.20	1.75	1.72	2.50	6.40	2.90	0.28	3.00	1 0 1	1 0 0 1 0	1 0 0 0	? 0 3 0 2 1	1 1 1 0	0 0 ? 0 1 0	? 0 ? [01]	0 ? 0 0 1 0	0 ? ? ?	0 0 1 1 1 0	0 0 1 0
S. angustatus	10.70	1.75	2.20	2.30	6.10	3.00	0.30	2.80	1 1 1	0 0 0 1 1	0 0 1 0	? 1 1 0 0 1	1 0 0 0	1 ? 0 0 1 1	0 1 1 ?	? 3 1 0 0 1	1 1 1 0	0 0 1 0 0 0	1 0 0 0
S. australis	12.80	1.90	2.20	2.30	6.76	2.80	0.27	3.60	1 1 1	1 0 0 1 0	1 0 1 1	0 1 0 0 1 1	0 ? 0 0	0 0 ? 0 0 ?	? 0 ? 0	0 ? 0 0 01	1 0 ? ?	0 0 0 ? ? ?	0 0 1 0
S. decens	10.65	1.55	2.00	2.36	6.90	2.60	0.20	2.90	0 1 1	0 0 01 [01]	0 1 1 0	? 1 0 0 1 1	1 0 0 1	1 ? 1 0 1 1	0 1 0 ?	? 0 1 0 0 1	1 1 1 1	0 0 1 0 0 0	0 0 1 0
S. elongatus	11.60	1.80	2.30	2.30	6.40	2.70	0.20	3.00	1 0 1	0 1 0 1 1	0 0 1 0	? 1 1 0 0 1	1 0 0 0	1? 0 0 1 1	1 1 1 ?	? 2 1 0 0 1	1 1 1 0	0 0 1 0 0 0	1 0 0 0
S. immarginatus	12.05	1.80	2.50	2.50	6.70	3.20	0.19	2.45	1 0 1	0 0 0 1 1	1 0 1 1	1 1 2 0 1 1	1 1 1 0	0 1 ? 0 1 2	? 0 ? 0	0 ? 0 0 0 1	1 1 0 ?	0 1 1 2 1 1	0 0 1 0
S. inconspicuus	9.60	1.45	2.10	2.50	6.60	3.00	0.20	3.10	1 1 1	1 0 0 1 1	1 0 0 0	? 0 3 0 2 1	1 1 1 0	0 0 ? 0 1 0	? 0 ? [01]	0 ? 0 0 1 0	1 0 ? ?	0 0 1 1 0 1	0 0 1 0
S. insulanus	10.05	1.45	2.20	2.45	6.90	3.10	0.15	3.20	1 0 1	1 0 0 1 0	1 0 0 1	1 0 2 0 2 1	1 1 0 0	1 ? 1 0 1 0	? 0 ? 0	1 ? 0 0 1 0	1 0 ? ?	0 1 1 1 0 0	1 0 0 0
S. jaechi	12.40	1.80	2.40	2.60	6.90	2.90	0.22	2.80	0 1 1	1 0 0 1 0	1 0 0 1	[01] 0 3 0 2 1	1 1 0 0	0 1 ? 0 12	? 0 ? 1	1 ? 0 0 1 0	1 1 1 1	0 1 1 1 0 0	1 0 0 0
S. mandelai	13.05	1.95	2.05	2.30	6.70	3.10	0.32	2.70	1 0 1	0 0 0 1 1	0 1 1 0	? 1 1 0 0 1	1 0 0 0	1 ? 1 0 1 1	0 1 1 ?	? 2 1 0 0 1	1 1 1 0	0 0 1 0 0 0	1 0 0 0
S. marginicollis	11.40	1.80	2.75	2.60	6.30	3.10	0.19	3.00	1 1 1	1 0 0 1 0	1 0 0 1	[23] 0 2 0 2 1	1 1 0 0	0 0 ? 0 1 1	0 0 ? [01]	1 ? 0 0 1 0	1 0 ? ?	0 1 1 2 0 0	0 0 1 0
S. mundus	15.30	2.25	1.90	2.50	6.80	2.80	0.30	2.90	1 0 1	1 0 0 1 0	1 0 0 0	? 0 3 0 2 1	1 1 1 0	0 1 ? 0 1 0	? 0 ? 1	1 ? 0 0 1 0	1 1 1 0	0 0 1 2 1 0	0 0 1 0
S. prominolobus	12.45	2.00	2.05	2.25	6.20	2.90	0.15	2.90	0 1 1	1 0 01 0	1 0 0 1	2 0 2 0 21	1 1 0 0	0 1 ? 0 1 1	1 0 ? 0	0 ? 0 0 1 0	1 0 ? ?	0 0 1 1 0 1	1 0 0 0
S. rufipes	10.40	1.70	2.10	2.43	6.12	3.05	0.25	3.20	1 1 1	0 1 1 1 1	0 1 1 0	? 1 1 0 0 1	1 0 0 0	1 ? 1 0 1 1	0 1 1 ?	? 3 1 0 0 1	1 1 1 0	0 0 1 0 0 0	1 0 0 0
S. solieri	10.30	1.55	2.17	2.30	6.64	2.90	0.29	3.10	1 0 1	0 1 1 1 1	0 1 1 0	? 1 1 0 0 1	1 0 0 0	1 ? 1 0 1 1	0 1 0 ?	? 1 1 0 0 1	1 1 1 0	0 0 1 0 0 0	1 0 0 0
S. solitarius	12.45	2.00	2.00	2.50	6.22	3.20	0.20	2.85	1 1 1	1 0 0 1 0	1 0 0 0	? 0 2 0 2 1	1 1 1 0	0 1 ? 0 1 0	? 0 ? 0	0 ? 0 0 1 1	1 1 1 0	0 0 1 1 1 0	0 0 1 0
Hydrochara flavipes	15.50	2.70	2.20	2.30	5.70	2.90	0.19	3.40	1 1 0	? 1 1 0 1	0 1 1 0	? 1 1 1 ? 0	? ? 0 0	0 0 ? 1 ? ?	? 0 ? 0	0 ? 1 1 0 1	1 1 1 1	0 0 0 ? ? ?	1 1 2 0

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Review of *Perdita* subgenus *Procockerellia* Timberlake (Hymenoptera, Andrenidae) and the first *Perdita* gynandromorph

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Abstract

A systematic study of *Perdita* subgenus *Procockerellia* Timberlake and the related subgenus *Allomacrotera* Timberlake results in the synonymy of the latter with the former, and two specific synonymies: *Perdita* (*Hexaperdita*) *glamis* Timberlake is a junior synonym of *Perdita* (*Procockerellia*) *stephanomeriae* Timberlake, while *Perdita* (*Procockerellia*) *brachyglossa* Timberlake is a junior synonym of *Perdita* (*Cockerellia*) *imbellis* Timberlake. *Perdita* (*Procockerellia*) *moldenkei* Timberlake is moved to subgenus *Cockerellia* Ashmead. A revised subgeneric diagnosis and key to the three included species are provided. Diagnoses of species are updated with novel characters; distributions and biological data are expanded. A gynandromorph of *P.* (*Procockerellia*) *moabensis* Timberlake, the first known in the genus *Perdita*, is reported.

Keywords

Apoidea, *Allomacrotera*, *Stephanomeria*, scopal hairs, distribution

Introduction

The panurgine genus *Perdita* Smith, 1853 (Hymenoptera: Andrenidae: Panurginae) is the most speciose bee genus in North America with 636 currently recognized species and 127 additional subspecies (Portman et al. 2016b). It is also diverse at the subgeneric

level with 17 subgenera currently recognized (Michener 2007). Among these, the subgenera *Procockerellia* Timberlake, 1954 and *Allomacrotera* Timberlake, 1960 have had a complicated and intertwined taxonomic history.

Procockerellia was originally described by Timberlake (1954) to include two species: *Perdita* (*Procockerellia*) *albonotata* Timberlake, 1954 (type species) and *P.* (*P.*) *stephanomeriae* Timberlake, 1954. *Perdita* (*P.*) *excellens* Timberlake, 1958 was subsequently described (Timberlake 1958). Upon the discovery of the male of *P.* (*P.*) *stephanomeriae*, Timberlake (1960) split *Procockerellia*, moving *P.* (*P.*) *stephanomeriae* into the new subgenus *Allomacrotera*. Later, Timberlake (1971) described *P.* (*P.*) *brachyglossa* Timberlake, 1971 and *P.* (*P.*) *moabensis* Timberlake, 1971. Then, Timberlake (1980) described *P.* (*P.*) *moldenkei* Timberlake, 1980 and moved *P.* (*P.*) *moabensis* into *Allomacrotera* due to the discovery of the male. More recently, *P.* (*P.*) *excellens* was synonymized with *P.* (*Xeromacrotera*) *cephalotes* (Cresson, 1878) by Portman et al. (2016a).

To summarize the taxonomic status, three species currently constitute *Procockerellia*: *P.* (*P.*) *albonotata*, *P.* (*P.*) *brachyglossa*, and *P.* (*P.*) *moldenkei*, while two species constitute *Allomacrotera*: *P.* (*A.*) *moabensis* and *P.* (*A.*) *stephanomeriae*.

Here, we assess the taxonomic status of these subgenera and revise the included species. Synonymies and changes presented here result in a single subgenus, *Procockerellia*, containing three species: *P.* (*P.*) *albonotata*, *P.* (*A.*) *moabensis*, and *P.* (*A.*) *stephanomeriae*. These changes reduce the total number of *Perdita* subgenera to 16 and the number of species to 634. A revised key to species of *Procockerellia,* and updated species accounts are presented. Lastly, during the course of this study, a gynandromorph of *P. moabensis* was discovered; its aberrant morphology is described. This specimen is the first described gynandromorph in the genus *Perdita*.

Methods

Morphological terms follow Michener (2007). The metasomal terga and sterna are abbreviated to T and S, respectively. Specimens were examined using a Leica MZ12 microscope and images and measurements were taken with Keyence VHX-500 and VHX-5000 Digital Imaging Systems. Scanning electron microscope images were taken with a Quanta FEG 650 Scanning Electron Microscope. Images were compiled into plates using Adobe Photoshop CS5 and maps were made using ArcGIS 10.2. The following acronyms are used for institutions housing the type material in the current study:

BBSL USDA ARS Pollinating Insects Research Unit, Logan, Utah.
CAS California Academy of Sciences, San Francisco, California. Robert Zuparko.
SEMC Snow Entomological Museum Collection, Lawrence, Kansas. Michael Engel and Jennifer Thomas.

All specimens are deposited in the BBSL collection unless otherwise noted.

Systematics

Subgenus *Procockerellia* Timberlake

Perdita (*Procockerellia*) Timberlake, 1954: 402. Type species: *Perdita* (*Procockerellia*) *albonotata* Timberlake, 1954, by original designation.

Perdita (*Allomacrotera*) Timberlake, 1960: 131. Type species: *Perdita* (*Procockerellia*) *stephanomeriae* Timberlake, 1954, by original designation and monotypy. **Syn. n.**

Subgeneric diagnosis. *Procockerellia* can be recognized by two characters. First, the unique scopal hairs of the female are long, dense, and tightly corkscrew-shaped, appearing kinky or crimped (Fig. 1). Second, the male S8 is apically narrowed into a carinate median keel (Fig. 2), rather than having a club-shaped apical process as found in related and similar subgenera *Cockerellia* Ashmead, 1898, *Hexaperdita* Timberlake, 1954, and *Pentaperdita* Cockerell and Porter, 1899. *Callomacrotera* Timberlake, 1954 also has a median carina on S8, but the apical process is short and spade-shaped (rather than long and narrow), and the subgenus can be separated by numerous other morphological characters (Timberlake 1954). *Procockerellia* can be further recognized by having the maxillary palpi 3- or 5-jointed in both sexes, mandibles expanded medially in the females and female pygidial plate truncate, lacking a median emargination. The male hind tarsal claws can be either simple or bidentate.

Biology. Although specimens of *Procockerellia* have been collected on many plant families (see below), our results support the idea that all the species are specialists on the plant genus *Stephanomeria* Nutt. (Asteraceae), since only *Stephanomeria* pollen has been found in the scopae of all three species. Bees are active in the early morning before the flowers close, and may also be active in the evening. The nesting biology is

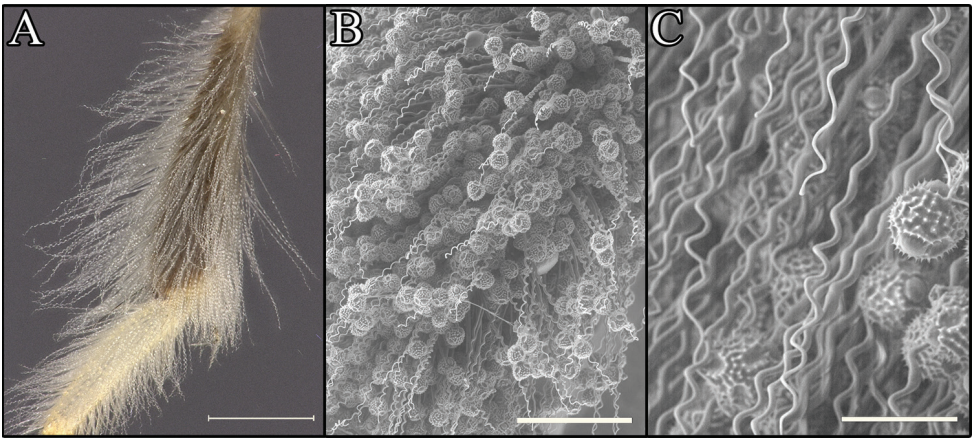

Figure 1. Tibial scopal hairs of *Procockerellia*. **A** *Perdita albonotata* (BBSL972407) **B** *P. moabensis* (36765 (BBSL)) **C** *P. moabensis* (36765 (BBSL)). Scale bars: 500 μm (**A**), 200 μm (**B**), 50 μm (**C**).

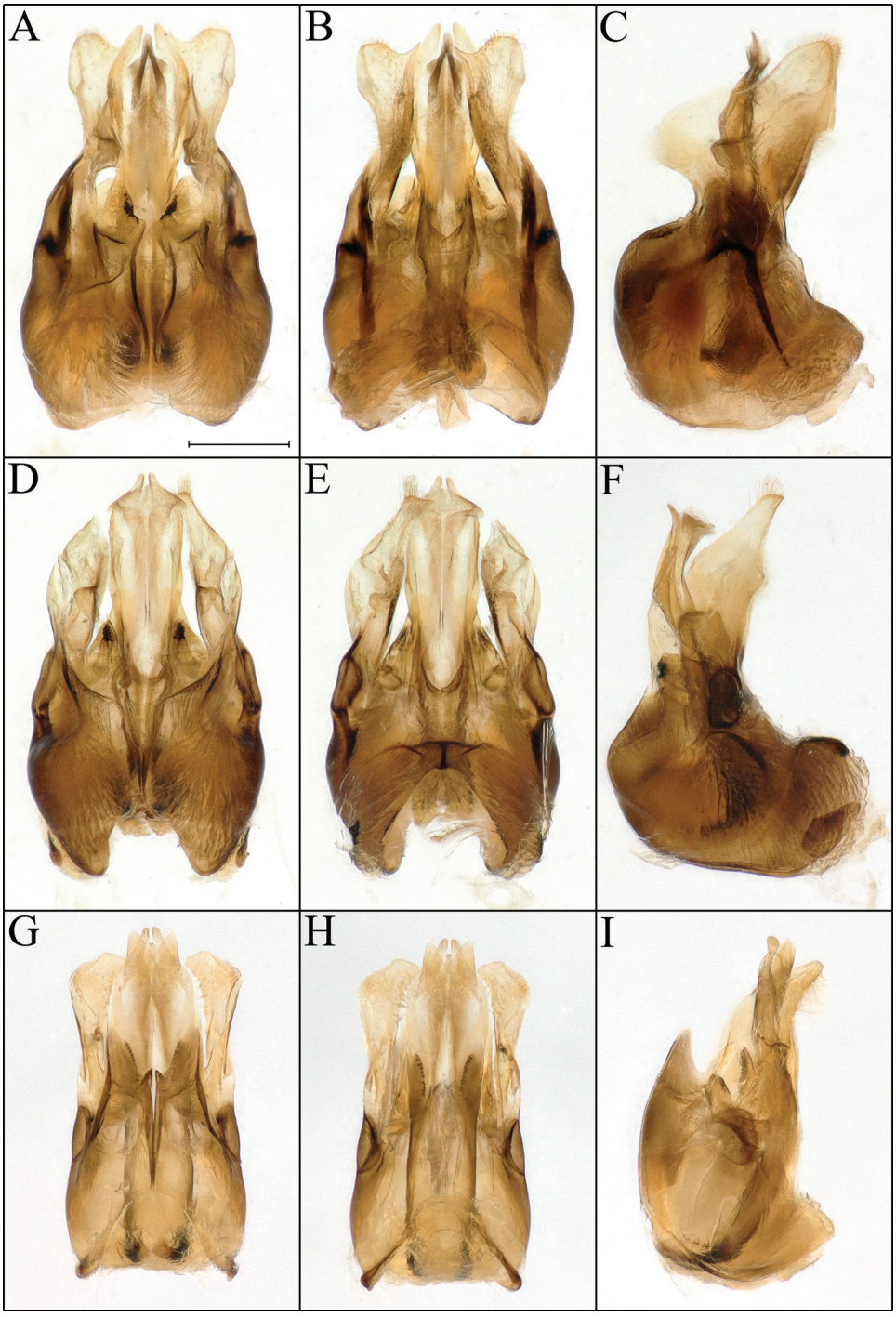

Figure 2. *Procockerellia* male genitalia. *Perdita albonotata* (BBSL529462) **A** dorsal view **B** ventral view **C** lateral view. *Perdita moabensis* (BBSL779598) **D** dorsal view **E** ventral view **F** lateral view. *Perdita stephanomeriae* (BBSL317528) **G** dorsal view **H** ventral view **I** lateral view. Scale bar: 250 μm, all images are the same scale.

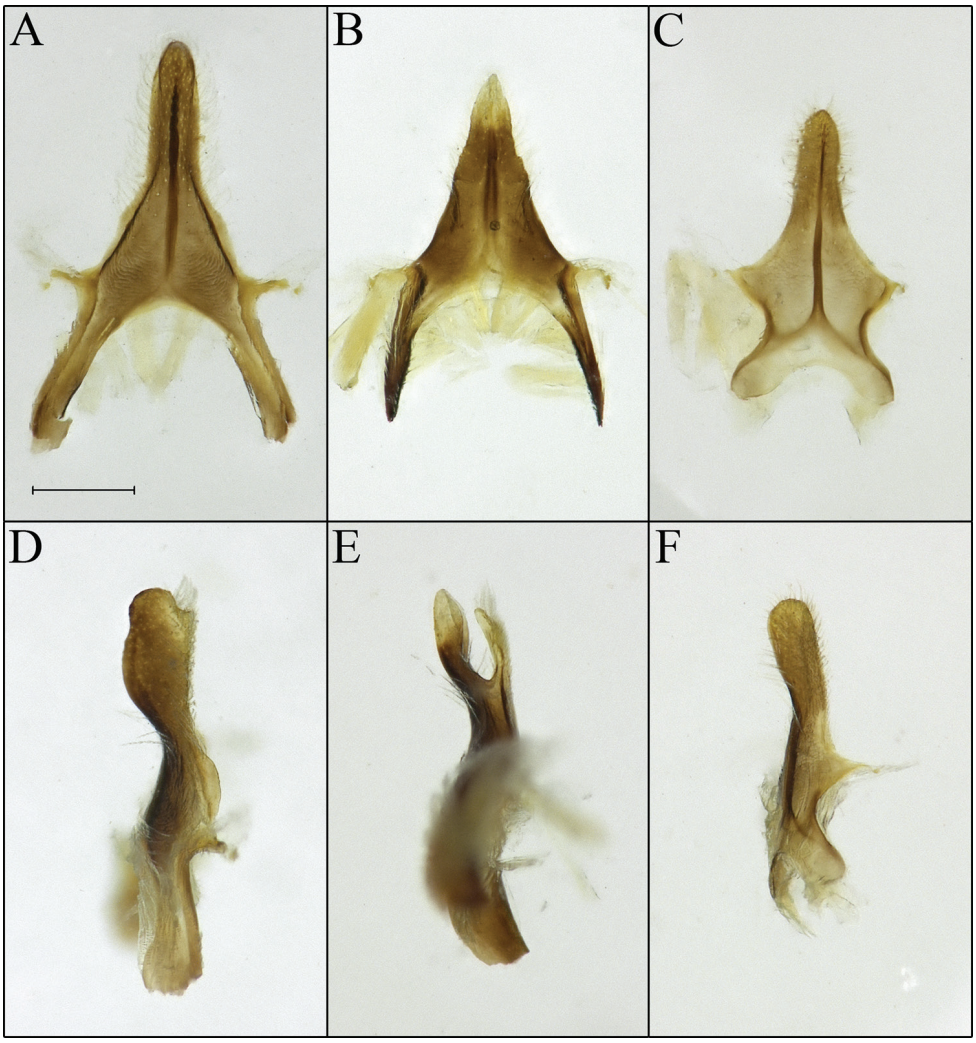

Figure 3. *Procockerellia* male S8. **A** *Perdita albonotata* (BBSL529469) ventral view **B** *P. moabensis* (BBSL779598) ventral view **C** *P. stephanomeriae* (BBSL317528) ventral view **D** *P. albonotata* lateral view **E** *P. moabensis* lateral view **F** *P. stephanomeriae* lateral view. Scale bar: 250 μm, all images are the same scale.

unknown, but they are presumably ground nesting bees like other species of *Perdita*. Both *P. albonotata* and *P. moabensis* are found throughout the flowering season from spring to fall, suggesting they are multivoltine. Thus, the flight period of *Procockerellia* matches the bloom period of the genus *Stephanomeria*, which contains species that collectively bloom from spring to fall (Gottlieb 1972). The paucity of collection events renders the phenology of *P. stephanomeriae* unclear.

Remarks. The relationship between *Procockerellia, Allomacrotera* and closely-related subgenera is ambiguous, though *Prockerellia* is clearly a member of the monophyletic group made up of the subgenera *Callomacrotera*, *Cockerellia*, *Hexaperdita*, *Penta-*

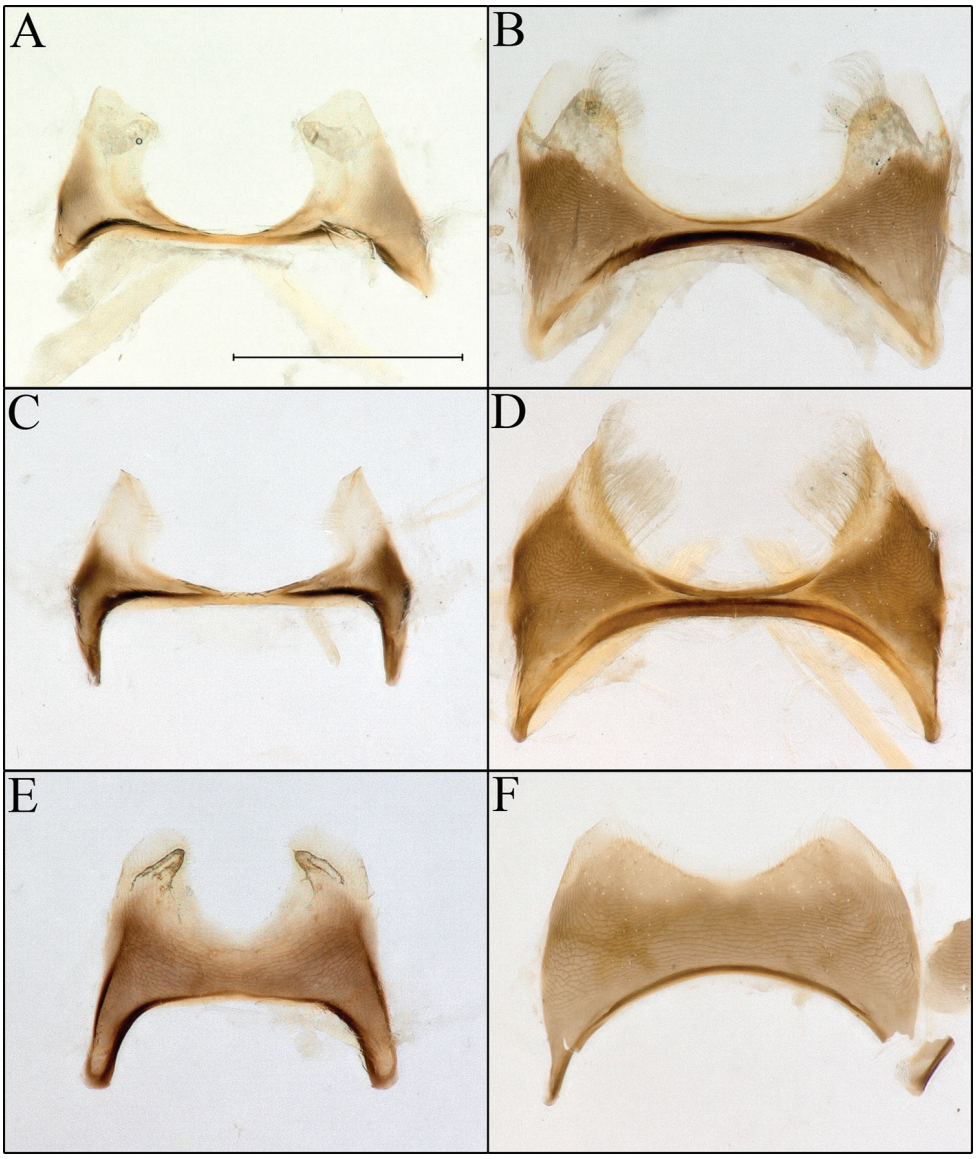

Figure 4. *Procockerellia* male S7 and S6. *Perdita albonotata* (BBSL529462) **A** S7 **B** S6. *Perdita moabensis* (BBSL779598) **C** S7 **D** S6. *Perdita stephanomeriae* (BBSL317528) **E** S7 **F** S6. Scale bar: 500 μm, all images are the same scale.

perdita and *Xeromacrotera* Timberlake, 1954 (Danforth 1996). The reduced number of maxillary palpi suggests an affinity to the subgenus *Pentaperdita*, which has the maxillary palpi 5-jointed (Timberlake 1954). Danforth (1996) suggested a close relationship to *Cockerellia*, though the species of *Procockerellia* also bear a general resemblance to the monotypic subgenus *Xeromacrotera*, which also has an uncertain phylogenetic relationship (Portman et al. 2016a).

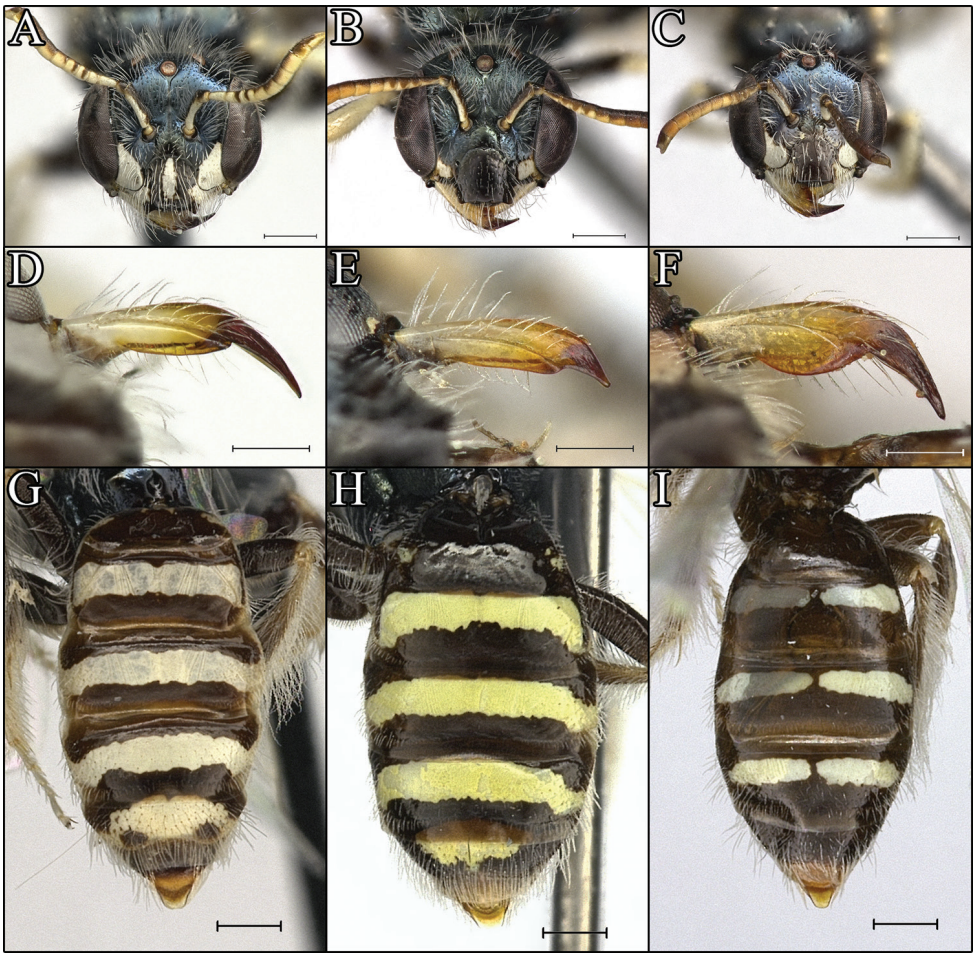

Figure 5. *Procockerellia* females. Faces: **A** *Perdita albonotata* (BBSL668188) **B** *P. moabensis* (BBSL23824) **C** *P. stephanomeriae* (BBSL531898). Scale bars = 500 μm. Mandibles: **D** *P. albonotata* (BBSL640552) **E** *P. moabensis* (BBSL515687) **F** *P. stephanomeriae* (BBSL317523). Scale bars = 250 μm. Metasomas: **G** *P. albonotata* (BBSL311790) **H** *P. moabensis* (BBSL471657) **I** *P. stephanomeriae* (BBSL531898). Scale bars: 500 μm.

The many similarities in scopal hairs (Fig. 1), morphology, genitalia (Fig. 2), apical sterna (Figs 3, 4), coloration and general gestalt (Figs 5, 6) all support a close relationship between *Procockerellia* and *Allomacrotera* that does not justify different subgenera. The structural similarities between S6, S7, and S8 in the males of *P. albonotata* and *P. moabensis* suggest that these are sister species, which would render *Allomacrotera* paraphyletic. In particular, *P. albonotata* and *P. moabensis* have S6 and S7 deeply divided and emarginate and S6 with pronounced lateral hair tufts; these characters are lacking in *P. stephanomeriae* (Figs 3, 4). Timberlake (1980) and Michener (2007) also reported that *Allomacrotera* lacked lateral furrows in the flanks of the pronotum, but all three species contained in the two subgenera have the flanks of the pronotum moderately impressed.

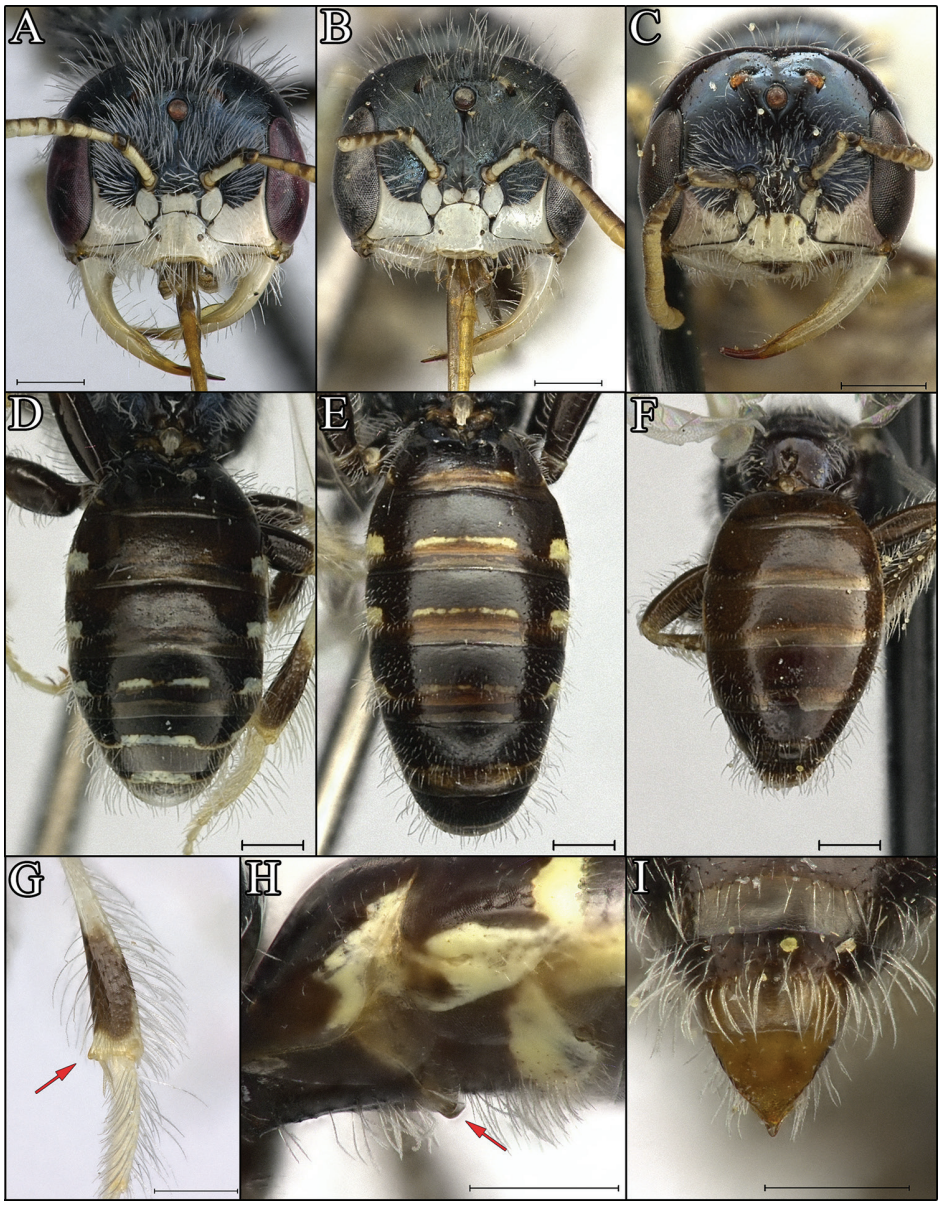

Figure 6. *Procockerellia* males. Faces (of large males): **A** *Perdita albonotata* (BBSL529482) **B** *P. moabensis* (36868 (BBSL)) **C** *P. stephanomeriae* (BBSL317506). Metasomas: **D** *P. albonotata* (BBSL529482) **E** *P. moabensis* (BBSL311790) **F** *P. stephanomeriae* (BBSL317506). Identifying characters: **G** *P. albonotata* tibial nub (BBSL669043) **H** *P. moabensis* S1 flange (BBSL238283) **I** *P. stephanomeriae* pointed pygidial plate (BBSL317506). Scale bars: 500 μm.

The close relationship between *P. albonotata* and *P. moabensis* suggests two possible solutions to fix the classification of *Procockerellia* and *Allomacrotera*. Either (1) *P. moabensis* should be moved from *Allomacrotera* to *Procockerellia*, or (2)

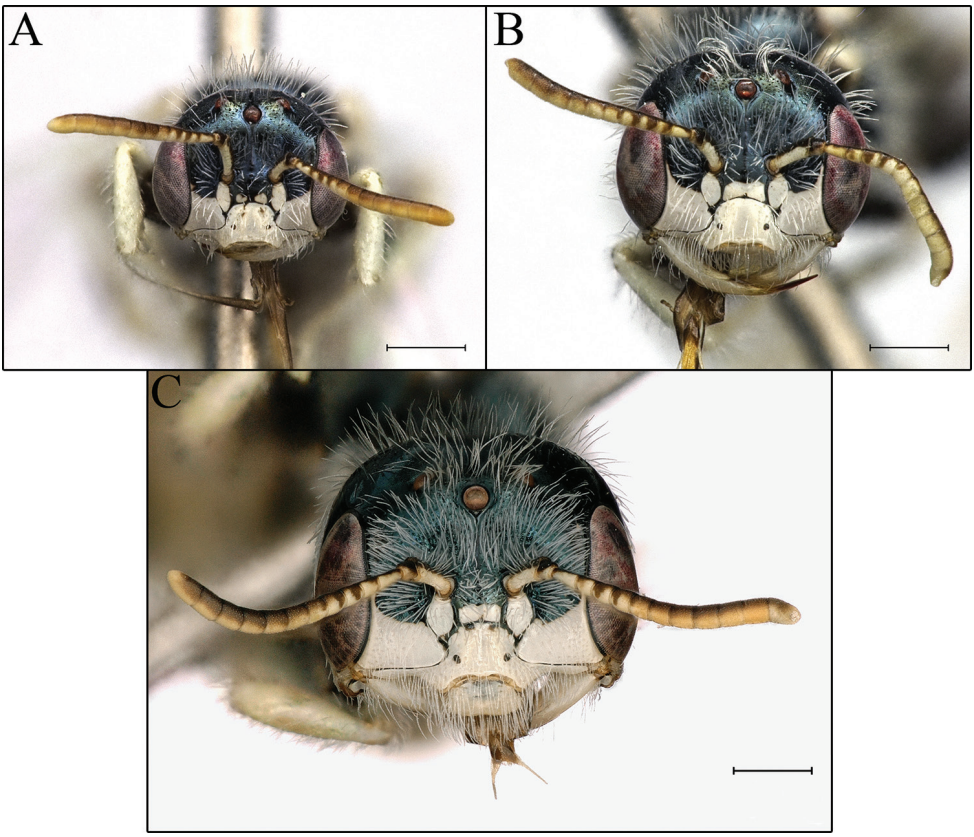

Figure 7. Male head variance in *Perdita albonotata*. **A** small male (BBSL529648) **B** medium male (BBSL532560) **C** large male (BBSL532480). Scale bars: 500 μm, all images are the same scale.

Allomacrotera and *Procockerellia* should by merged. If *P. moabensis* were to be moved to *Procockerellia*, it would eliminate the sole defining character of *Allomactera* (bifurcate hind tarsal claws in the male) because both *P. moabensis* and *P. stephanomeriae* share this character, while *P. albonotata* lacks it. In addition, the only remaining character unique to *Allomacrotera* would be the 3-jointed maxillary palpi. However, we agree with Timberlake (1954) that the reduction of maxillary palpi is more important for classification than the specific number of palpi. Indeed, a similar pattern can be seen in the Halictoides group of subgenus *Perdita sensu stricto*, which includes incredibly similar species in which the maxillary palpi collectively range in number from one to five (Timberlake 1958). Therefore, we have chosen to synonymize *Allomacrotera* with *Procockerellia* due to the shared characters of the corkscrew-shaped scopal hairs (Fig. 1) and keel-shaped apical process of S8 (Fig. 3).

The species of *Procockerellia* are distinctive in *Perdita* due to their unique scopal hairs, which are especially long, dense, and tightly corkscrew-shaped, appearing crimped under all but the highest magnification (Fig. 1). This type of scopal hair morphology is rare, and to our knowledge only occurs in one other group, the panurgine

genus *Panurgus* Panzer, 1806 (Pasteels et al. 1983). The corkscrew hairs of *Procockerellia* encircle the hind tibia and basitarsus, and the tips are slightly clavate (Fig. 1C). Scopal hairs are also present on the hind femur and trochanter, though these are minutely branched rather than corkscrew-shaped. Similar to the related subgenera *Callomacrotera*, *Cockerellia*, *Hexaperdita*, *Pentaperdita*, *Xeromacrotera* (Danforth 1996), the species of *Procockerellia* initially pack dry pollen into the scopa and then cap it with pollen that has been moistened with nectar (Timberlake 1954, Norden et al. 1992, Portman and Tepedino 2017). Pollen loads on museum specimens indicate that *P. albonotata* and *P. moabensis* cap approximately the last 20% of the pollen load on the anterior face of the hind tibia with moistened pollen. The pollen on the trochanter, femur, basitarsus, and posterior face of the hind tibia are not moistened. The proportion of moist and dry pollen carried by *P. stephanomeriae* is unknown due to a lack of specimens with full pollen loads.

The males of *Procockerellia* vary greatly in size. Similar to many other *Perdita*, the male head size increases and becomes more quadrate with larger body size (fig. 7, Norden et al. 1992, Portman et al. 2016a). However, the distribution of head sizes is continuous, and there are not discrete classes as seen in the Perditini species *Macrotera portalis* (Danforth 1991). The function of the large, quadrate heads is unknown, though it could be used for inter-male aggression and/or grasping females during mating (Norden et al. 1992, Danforth and Neff 1992).

Key to species. Females:

1 Vertex and frons strongly shining, lacking tessellation (Fig. 5C); metasoma with narrowly-interrupted pale bands extending straight to lateral margins (Fig. 5I); pronotal collar with slight carina dorso-laterally; inner margin of mandible broadly expanded (Fig. 5F); maxillary palpi 3-segmented..... ..... ***P. stephanomeriae*** **Timberlake**

– Vertex and frons (at least laterally and ventrally) with medium or dense tessellation (Fig. 5A–B); pronotal collar with prominent rounded nub apicolaterally; metasoma with pale bands complete and curving apically on lateral margins (Fig. 5G–H); inner margin of mandible not broadly expanded (Fig. 5D–E); maxillary palpi 5-segmented..... **2**

2 Frons and vertex heavily tesselate and dullish; face with light marks limited to small, transverse lateral marks, or even absent (Fig. 5B); metasomal bands generally yellowish; pygidial plate broadly truncate apically (Fig. 5H)..... ..... ***P. moabensis*** **Timberlake**

– Frons and vertex slightly tessellate and shining; face with pale, triangular lateral marks reaching level of antennae, clypeus with lateral margins white and often with a median white band (Fig. 5A); pygidial plate slightly narrower and more rounded apically (Fig. 5G); metasomal bands generally white (Fig. 5G), but sometimes yellowish..... ***P. albonotata*** Timberlake

Males:

1 Frons and vertex strongly tessellate and dull (Fig. 6B); S1 medially with small, outflexed apical margin (Fig. 6H) .....***P. moabensis*** **Timberlake**

– Frons and vertex weakly tessellate and shining; S1 unmodified **2**

2 Apex of hind tibia with small nub above tibial spurs (Fig. 6G); T7 broadly rounded, without a point; hind tarsal claws simple; pronotal collar with prominent rounded nub laterally; metasoma generally with white or yellowish markings (Fig. 6D) .....***P. albonotata*** **Timberlake**

– Apex of hind tibia lacking a nub; T7 ending in a small triangular point (Fig. 6I); hind tarsal claws bidentate; pronotal collar with sharp transverse carina dorso-laterally; metasoma generally lacking markings (Fig. 6F)..... .....***P. stephanomeriae*** **Timberlake**

***Perdita* (*Prockerellia*) *albonotata* Timberlake**

Figures 1A, 2A–C, 3A, D, 4A–B, 5A, D, G, 6A, D, G, 7, 8A

Perdita (*Procockerellia*) *albonotata* Timberlake, 1954: 403, ♂♀. Holotype female: USA, California, San Bernardino Co., Morongo Valley, 29 September 1944, at flowers of *Stephanomeria exigua* Nutt. [CAS, type no. 14414]. Examined.

Measurements. Female (n=10): head width 1.5 mm (1.3–1.8 mm), body length 5.8 mm (5.1–6.8 mm). Male (n=11): head width 1.4 mm (1.2–1.7 mm), body length 5.1 mm (4.0–6.2 mm).

Diagnosis. Both sexes of *P. albonotata* have five maxillary palpi and the frons and vertex are weakly tessellate and slightly shining. The female generally has the most extensive facial markings in the subgenus, but the extent is highly variable. In the typical form, the face has the following markings: the clypeus is marked with white on the lateral margins and has a medial white band, the white paraocular marks are broadly triangular and reach the level of the antennal sockets, and the supraclypeal area can be dark or with a pair of white spots (Fig. 5A). The mandible is only slightly expanded on the inner margin and has a long apical tooth (Fig. 5D). The pronotal lobe can be white or dark. The white (sometimes yellowish) abdominal bands on T2–T5 are entire and curve towards the apical margins laterally (Fig. 5G).

The male is unique in *Procockerellia* due to the simple hind tarsal claws and the presence of an apical nub on the anterior face of the hind tibia, just above the tibial spurs (Fig. 6G). In addition, the male has the pygidial plate broadly and evenly rounded, and the discs of terga have small white markings, with the markings generally more pronounced on the apical terga (Fig. 6D).

Distribution. Arid regions of the West in Arizona, California, Idaho, Nevada and Utah: Great Basin, southern Colorado Plateau, Mojave Desert (Fig. 8A). There is a single record from the Sonoran Desert. Frequently found in sandy areas.

Phenology.

Month:	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
# of records	11	2575	529	313	655	243	142	0

Floral records. Asclepiadaceae (1 ♂): *Asclepias* sp. 1 ♂, **Asteraceae** (61 ♂ 26 ♀): *Asteraceae* sp. 1 ♂ 1 ♀, *Baileya pleniradiata* 2 ♂, *Grindelia squarrosa* 1 ♂, *Helianthus* sp. 1 ♀, *Isocoma acradenia* 1 ♂, *Malacothrix sonchoides* 3 ♂ 1 ♀, *Pectis papposa* 2 ♂, *Rafinesquia neomexicana* 1 ♂, *Senecio spartioides* 1 ♂, *Stephanomeria exigua* 3 ♂ 3 ♀, *S. pauciflora* 2 ♂ 1 ♀, *S.* sp. 44 ♂ 19 ♀, **Brassicaceae** (6 ♂ 15 ♀): *Streptanthus* sp. 6 ♂ 15 ♀, **Euphorbiaceae** (4 ♂): *Croton* sp. 1 ♂, *C. wigginsii* 3 ♂, **Fabaceae** (1 ♂): *Acacia greggii* 1 ♂, **Loasaceae** (1 ♂): *Mentzelia multiflora* 1 ♂, **Malvaceae** (1 ♂): *Sphaeralcea* sp. 1 ♂, **Polemoniaceae** (1 ♂): *Eriastrum wilcoxii* 1 ♂, **Polygonaceae** (3 ♂): *Eriogonum cernuum* 1 ♂, *E. deflexum* 1 ♂, *E. nummulare* 1 ♂.

Additional material examined. Total specimens: 1403 ♂ 3060 ♀. **USA: ARIZONA: Coconino County:** Colorado River, Fossil Rapids (36.27333 -112.525): 1 ♂, 28 Sep 2001, T.L. Griswold, J. Sterling, *Isocoma acradenia*; **Mohave County:** Littlefield, N (36.88 -113.92): 1 ♂, 8 May 1998, M. Andres, K. Keen, K. Receveur, C. Schultz; Littlefield (36.88 -113.92): 1 ♂, 15 Jun 1983, W.J. Hanson; Mesquite, 8 mi E (36.81 -113.98): 3 ♂, 26 Apr 1973, F.D. Parker, P.F. Torchio. **CALIFORNIA: Riverside County:** Palm Springs (33.832 -116.5453): 1 ♂, 2 May 1953, R.M. Bohart; **San Bernardino County:** (34.868 -115.7731): 2 ♀, 28–29 Apr 2012, collector unknown; 5 ♀, 24–25 May 2012, collector unknown; (34.8794 -115.7808): 3 ♀, 16–17 Aug 2011, collector unknown; 10 ♀, 5–6 Oct 2011, collector unknown; 1 ♀, 23–24 Oct 2011, collector unknown; 1 ♀, 28–29 Apr 2012, collector unknown; 11 ♂ 104 ♀, 24–25 May 2012, collector unknown; Black Canyon (35.1223 -115.394): 2 ♀, 30 May 1998, F.D. Parker; Cedar Canyon (35.16192 -115.44098): 1 ♀, 30 May 1998, F.D. Parker; Hole-in-the-Wall, 2 mi S (35.01783 -115.3843): 7 ♂ 13 ♀, 30 May 1998, F.D. Parker; Kelso, 1 km NE (35.0197 -115.6394): 2 ♂ 3 ♀, 31 May 1998, F.D. Parker. **IDAHO: Franklin County:** Preston (42.09 -111.87): 1 ♂ 1 ♀, 8 Aug 1972, G.E. Bohart. **NEVADA: Churchill County:** Sand Mountain, 25 mi SE Fallon (39.3163 -118.4128): 1 ♀, 13 Jul 1980, L. Hanks; **Clark County:** 0.42 mi E McClanahan Spr. (35.6949 -115.1788): 3 ♂ 1 ♀, 12 May 2004, T.L. Griswold, E. Ahlstrom; 3 ♂ 1 ♀, 25 May 2004, E. Ahlstrom, L. Saul; 1 mi SW Little Virgin Peak (36.5927 -114.21): 3 ♀, 8 Jun 2004, E. Ahlstrom, D. Skandilis; 1.3 mi ESE Mule Spr. (36.0261 -115.5594): 1 ♂, 9 Jun 2004, L. Saul, D. Skandilis; 2.3 mi E Sheep Mtn. (35.7473 -115.2407): 6 ♂ 9 ♀, 12 May 2004, T.L. Griswold, E. Ahlstrom; 3.0 mi SE Rainbow Mtn. (36.0832 -115.4479): 2 ♂, 23 Aug 2004, E. Ahlstrom, *Pectis papposa*; 3.5 mi SW Cow Spr. (35.5382 -115.0665): 14 ♂ 1 ♀, 25 May 2004, S.M. Higbee, D. Skandilis; 3.9 mi SSW Whitney Pocket (36.4669 -114.1537): 3 ♂ 7 ♀, 26 May 2005, R. Andrus, S.M. Higbee; Black Mesa, W (36.1607 -114.789): 1 ♂, 9 Oct 1998, T.L. Griswold, *Eriogonum deflexum*; Black Ridge, 4.6 mi NW (36.5975 -114.3594): 1 ♂ 1 ♀, 21 Apr 2005, R. Andrus, *Stephanomeria pauciflora*; Black Wash (36.4113 -114.0778): 1 ♂, 12 Aug 1998, T.L. Griswold, *Baileya pleniradiata*; Bow-

man Reservoir, E (36.6222 -114.467): 4 ♂ 7 ♀, 5 Aug 1998, M. Andres, C. Schultz; Christmas Tree Pass, W (35.2708 -114.823): 1 ♂, 6 Jun 1998, F.D. Parker; Corn Creek Springs (36.4407 -115.363): 1 ♀, 28 May 1998, F.D. Parker; Eldorado Valley (35.5697 -114.878): 2 ♂, 10 Jun 1998, M. Andres, K. Keen, K. Receveur, C. Schultz; Fire Canyon Wash (36.4548 -114.5088): 18 ♂ 51 ♀, 5 Aug 1998, M. Andres, C. Schultz; Halfway Wash, 1.8 mi NW Virgin River (36.6859 -114.3322): 1 ♂, 6 May 2004, E. Ahlstrom; Highland Range, NW (35.6703 -115.0715): 1 ♂ 1 ♀, 9 Jun 1998, M. Andres, K. Keen; Hot Creek Valley (36.27417 -115.07056): 2 ♂ 2 ♀, 20 Aug 1998, F.D. Parker; Jean Lake, 2.24 mi ENE (35.814 -115.2051): 1 ♂, 10 May 2005, S.M. Higbee, *Rafinesquia neomexicana*; Jean Lake, NE (35.8067 -115.2233): 1 ♂ 3 ♀, 8 Oct 1998, T.L. Griswold; Jean, N (35.8102 -115.2998): 1 ♂, 8 Oct 1998, T.L. Griswold; Juanita Springs Ranch, S Riverside (36.6383 -114.2478): 1 ♂, 15 May 1983, F.D. & J.H. Parker; Kyle Canyon (36.3268 -115.3418): 1 ♀, 28 May 1998, F.D. Parker; Las Vegas Dunes (36.286 -114.9667): 1 ♂ 28 ♀, 22 May 1998, M. Andres, K. Keen, K. Receveur, C. Schultz; Las Vegas, NE (36.2798 -115.0355): 1 ♂, 7 Oct 1998, T.L. Griswold; Mesquite (36.8055 -114.0664): 1 ♂, 4 Oct 1988, P.F. Torchio, R.W. Rust, *Croton* sp.; 14 ♂ 6 ♀, 8 May 1994, P.F. Torchio, D.F. Veirs, *S.* sp.; Mesquite (36.8144 -114.0703): 3 ♀, 19 Sep 1997, F.D. Parker; Mica Peak (36.3348 -114.1445): 2 ♂ 1 ♀, 7 Jun 1998, T.L. Griswold, F.D. Parker; Mormon Mesa (36.5702 -114.4197): 1 ♂ 1 ♀, 5 Aug 1998, M. Andres, C. Schultz; Mormon Mesa (36.7437 -114.375): 10 ♀, 5 Aug 1998, M. Andres, C. Schultz; Mormon Mesa (36.7447 -114.3778): 36 ♂ 180 ♀, 20 May 1998, M. Andres, K. Keen, K. Receveur, C. Schultz; Mormon Well Rd (36.4355 -115.351): 1 ♀, 15 Sep 1998, W.R. Bowlin; Mormon Well Rd (36.4361 -115.3519): 1 ♂, 15 Sep 1998, W.R. Bowlin; Mormon Well Road (36.534 -115.1067): 6 ♂ 8 ♀, 1 Jul 1998, M. Andres, C. Schultz; Mormon Well Road (36.5492 -115.0995): 6 ♂, 16 Jul 1998, M. Andres, C. Schultz; Mud Wash (36.43 -114.1527): 1 ♀, 19 Sep 1998, W.R. Bowlin; Overton, NE (36.5693 -114.4193): 7 ♂ 5 ♀, 21 May 1998, M. Andres, K. Keen, K. Receveur, C. Schultz; Peek a Boo Canyon (36.5032 -115.1577): 2 ♂ 17 ♀, 16 Jul 1998, M. Andres, C. Schultz; Peek a Boo Canyon (36.5065 -115.1477): 15 ♂ 44 ♀, 16 Jul 1998, M. Andres, C. Schultz; Peek a Boo Canyon (36.515 -115.1327): 8 ♂ 13 ♀, 16 Jul 1998, M. Andres, C. Schultz; Peek a Boo Canyon (36.5325 -115.1128): 12 ♂ 42 ♀, 16 Jul 1998, M. Andres, C. Schultz; Peek-A-Boo Cyn. (36.5522 -115.0991): 1 ♂, 8 Sep 2004, T.L. Griswold, *E. cernuum*; Piute Valley (35.46483 -115.052): 8 ♂ 25 ♀, 10 Jun 1998, M. Andres, K. Keen, K. Receveur, C. Schultz;Piute Valley (35.47266 -115.04816): 4 ♂ 1 ♀, 11 Jun 1998, M. Andres, K. Receveur, *S.* sp.; Pulsipher Wash (36.807 -114.1095): 1 ♂ 2 ♀, 16 Sep 1998, W.R. Bowlin; Pulsipher Wash (36.8073 -114.1143): 4 ♂ 19 ♀, 16 Sep 1998, W.R. Bowlin; Pulsipher Wash (36.8083 -114.1138): 6 ♀, 16 Sep 1998, W.R. Bowlin; Riverside, 4.5 mi SW (36.69137 -114.26056): 1 ♂ 2 ♀, 19 Sep 1997, F.D. Parker; Sandstone Bluffs, E (36.0895 -115.4523): 9 ♂ 24 ♀, 25 Jun 1998, T.L. Griswold; St. Thomas Gap, 0.4 mi E(36.4084 -114.0937): 1 ♂, 20 May 2004, E. Ahlstrom; 109 ♂ 118 ♀, 20 May 2004, S.M. Higbee, E. Ahlstrom; 72 ♂ 100 ♀, 20 May 2004, S.M. Higbee, E. Ahl-

strom, D. Skandilis, L. Saul; 1 ♂, 28 Jun 2004, E.D. Rentz, *Eriastrum wilcoxii*; 2 ♀, 27 Apr 2005, S.M. Higbee; 109 ♂ 387 ♀, 12 May 2005, D. Allen, E. Ahlstrom, R. Andrus, S.M. Higbee; 59 ♂ 193 ♀, 25 May 2005, S.M. Higbee, E. Ahlstrom; 1 ♂, 26 May 2005, S.M. Higbee, E. Ahlstrom; 1 ♂, 8 Jun 2005, D. Allen, *S. exigua*; 1 ♂, 8 Jun 2005, S.M. Higbee; 1 ♂, 8 Jun 2005, S.M. Higbee, *Acacia greggii*; 2 ♂, 7 Sep 2005, A. Portoluri, *C. wigginsii*; 1 ♂, 7 Sep 2005, E. North; 1 ♂, 13 Oct 2005, T.L. Griswold, *C. wigginsii*; St. Thomas Gap (36.4023 -114.093): 1 ♂ 4 ♀, 27 May 1998, F.D. Parker; 57 ♂ 59 ♀, 7 Jun 1998, F.D. Parker; 8 ♂ 37 ♀, 12 Aug 1998, M. Andres, T.L. Griswold, C. Schultz; 15 ♂ 46 ♀, 12 Aug 1998, T.L. Griswold, C. Schultz; St. Thomas Gap (36.4041 -114.0933): 1 ♂, 25 May 1998, M. Andres, K. Receveur, C. Schultz, *B. pleniradiata*; 68 ♂ 158 ♀, 26 May 1998, M. Andres, K. Keen, K. Receveur, C. Schultz; 1 ♂, 7 Jun 1998, F.D. Parker; 6 ♂ 15 ♀, 8 Jun 1998, T.L. Griswold, *Streptanthus* sp.; 13 ♂ 54 ♀, 27 Aug 1998, O.J. Messinger, S. Messinger, C. Schultz; 21 ♂ 34 ♀, 6 Oct 1998, T.L. Griswold; 4 ♂ 1 ♀, 6 Oct 1998, T.L. Griswold, *S.* sp.; St. Thomas Gap (36.4058 -114.0937): 19 ♂, 29 Jun 1998, M. Andres, C. Schultz; 4 ♂, 29 Jun 1998, M. Andres, K. Keen, K. Receveur, C. Schultz; St. Thomas Gap (36.4075 -114.0937): 2 ♀, 4 Aug 1998, M. Andres, C. Schultz; 8 ♂ 5 ♀, 18 Sep 1998, W.R. Bowlin; 6 ♂ 30 ♀, 6 Oct 1998, T.L. Griswold; St. Thomas Gap (36.4083 -114.125): 71 ♂ 63 ♀, 11 May–12 Jun 1984, R.C. Bechtel, J.B. Knight; Stewarts Point, NW, 9R (36.3853 -114.4123): 1 ♂, 6 May 1998, M. Andres, K. Keen, K. Receveur, C. Schultz; Tramp Ridge, E (36.3905 -114.1287): 2 ♂ 5 ♀, 4 Aug 1998, M. Andres, C. Schultz; Virgin Mountains, W (36.5768 -114.2042): 1 ♂, 6 Oct 1998, T.L. Griswold; Virgin Valley (36.6868 -114.2643): 1 ♂ 10 ♀, 12 Aug 1998, T.L. Griswold, C. Schultz; 7 ♂ 18 ♀, 6 Oct 1998, T.L. Griswold; Whitney Pocket, NW (36.5448 -114.1765): 1 ♀, 26 May 1998, T.L. Griswold; Whitney Pocket (36.5288 -114.1557): 2 ♂ 7 ♀, 27 May 1998, F.D. Parker; **Lincoln County:** Tule Desert (37.1703 -114.2829): 1 ♂, 17 Aug–30 Sep 1983, R.C. Bechtel, J.B. Knight; 1 ♀, 15 Sep 1983, R.C. Bechtel, J.B. Knight; **Mineral County:** Marrietta, 3 mi S (38.2 -118.3): 1 ♀, 16 Aug 1998, F.D. Parker; **Nye County:** 16 mi E Gabbs (38.8605 -117.623): 26 ♂ 38 ♀, 22 Aug 1998, F.D. Parker; Hot Crk Vly (38.16666 -116.20222): 18 ♂ 19 ♀, 20 Aug 1998, F.D. Parker. **UTAH: Cache County:** Cornish (41.9756 -111.9525): 2 ♂, 4 Aug 1959, G.E. Bohart, *S.* sp.; 1 ♀, 4 Aug 1959, G.E. Bohart, R.A. Nielsen, *S.* sp.; **Garfield County:** Calf Creek (37.7645 -111.4046): 1 ♂, 18 Jun 2003, S.M. Higbee, *S. exigua*; Hole in the Rock Road, Halfway Hollow (37.6338 -111.4449): 7 ♂ 8 ♀, 5–19 Jul 2003, H. Ikerd; 1 ♂, 19–29 Jul 2003, H. Ikerd; Twentyfive Mile Wash (37.5596 -111.3048): 1 ♂ 1 ♀, 18 Sep 2003, A. Johansen, *Asteraceae* sp.; **Kane County:** 2.38 mi SE Stave Spr. (37.2344 -112.8769): 5 ♂ 3 ♀, 14–15 Jun 2007, H. Ikerd, K. Davidson; Dry Fork, N (37.441 -111.2307): 1 ♂, 1 Jul 2003, C. Boyers, *Mentzelia multiflora*; 1 ♂, 18 Sep 2003, J. Tolliver, *S. exigua*; Kitchen Corral Spr., 1.0 mi N (37.2298 -112.1165): 1 ♀, 6 Aug 2003, S.M. Higbee; 1 ♂, 6 Aug 2003, S.M. Higbee, *Senecio spartioides*; Paria River W, on HWY 89 (37.12655 -111.95152): 2 ♀, 21 Aug 2008, T.L. Griswold; Sooner Rocks, 0.6 mi WNW (37.333 -111.0713): 3 ♀, 4 Jun 2003, H. Ikerd; **Millard County:** Oak City

(39.37 -112.33): 1 ♀, 24 Jun 1949, G.E. Bohart, *Helianthus* sp.; **Tooele County:** 0.5 mi E Wig Mountain (40.3183 -113.0553): 1 ♀, 6 Jul 2005, E. Jarrell, J.S. Wilson; 1 ♀, 6 Jul 2005, J. Wilson; 1 ♂, 15 Aug 2005, J. Wilson, *Grindelia squarrosa*; 0.6 mi NW Little Granite Mt. (40.2038 -112.845): 1 ♂, 10 Jun 2003, R. Andrus, *S. pauciflora*; 0.7 mi NW Little Granite Mt. (40.2057 -112.8456): 2 ♂ 1 ♀, 10 Jun 2003, O.J. Messinger, *S.* sp.; 5 ♂ 4 ♀, 10 Jun 2003, O.J. Messinger, R. Andrus; 2 ♂ 2 ♀, 20 Jun 2003, O.J. Messinger, C. Boyers; 3 ♀, 18 Jul 2003, R. Andrus, C. Boyers; 1 ♂, 9 Sep 2003, O.J. Messinger, H. Ikerd; 1 ♂ 4 ♀, 4 Aug 2005, J.S. Wilson, L. Wilson; 17 ♂ 60 ♀, 22 Aug 2005, J.S. Wilson; 17 ♂ 26 ♀, 14 Sep 2005, O.J. Messinger, K.T. Huntzinger; 7 ♀, 29 Sep 2005, K.T. Huntzinger, T.L. Griswold; 1.3 mi W Little Granite Mt. (40.1977 -112.8612): 4 ♂ 3 ♀, 10 Jun 2003, O.J. Messinger, R. Andrus; 1 ♀, 10 Jun 2003, R. Andrus, *Malacothrix sonchoides*; 2 ♂ 3 ♀, 20 Jun 2003, O.J. Messinger, C. Boyers; 1 ♀, 1 Jul 2003, J.S. Wilson, O.J. Messinger; 4 ♂, 18 Jul 2003, R. Andrus, C. Boyers; 17 ♂ 33 ♀, 22 Aug 2005, J.S. Wilson; 1 ♂, 14 Sep 2005, K.T. Huntzinger, O.J. Messinger; 10 ♂ 20 ♀, 14 Sep 2005, O.J. Messinger, K.T. Huntzinger; 2 ♂ 5 ♀, 29 Sep 2005, K.T. Huntzinger, T.L. Griswold; 1.8 mi WNW Simpson Butte (40.082 -112.9347): 1 ♂ 6 ♀, 23 Jun 2005, J.S. Wilson, E. Jarrell; 2 mi N Little Granite Mt. (40.225 -112.833): 2 ♂ 3 ♀, 10 Jun 2003, O.J. Messinger, R. Andrus; 1 ♂, 1 Jul 2003, J.S. Wilson, O.J. Messinger; 6 ♂ 24 ♀, 22 Aug 2005, J.S. Wilson; 1 ♂, 14 Sep 2005, O.J. Messinger, K.T. Huntzinger; 2.8 mi NNW Little Granite Mtn. (40.238 -112.8495): 2 ♂, 5 Jul 2005, J.S. Wilson, *S.* sp.; 1 ♂, 5 Jul 2005, J.S. Wilson, E. Jarrell; 2.8 mi W Simpson Buttes (40.0698 -112.9366): 18 ♂ 5 ♀, 17 Jul 2003, R. Andrus, C. Boyers; 26 ♂ 5 ♀, 30 Jul 2003, J.S. Wilson, C.M. Davidson; 3 ♀, 13 Jun 2005, J.S. Wilson, *S. exigua*; 2 ♂ 12 ♀, 13 Jun 2005, J.S. Wilson, E. Jarrell; 4 ♂ 2 ♀, 23 Jun 2005, E. Jarrell,*S.* sp.; 2 ♂, 23 Jun 2005, J.S. Wilson, *M. sonchoides*; 10 ♂ 4 ♀, 23 Jun 2005, J.S. Wilson, *S.* sp.; 15 ♂ 21 ♀, 23 Jun 2005, J.S. Wilson, E. Jarrell; 1 ♂, 8 Jul 2005, E. Jarrell, *S.* sp.; 1 ♂, 8 Jul 2005, J. Wilson, E. Jarrell; 6 ♂, 20 Jul 2005, J.S. Wilson, E. Jarrell; 24 ♂ 21 ♀, 22 Aug 2005, J.S. Wilson; 4 ♂ 3 ♀, 30 Aug 2005, O.J. Messinger; 1 ♂ 2 ♀, 15 Sep 2005, K.T. Huntzinger, *S.* sp.; 1 ♂, 15 Sep 2005, O.J. Messinger, *E. nummulare*; 5 ♂ 21 ♀, 15 Sep 2005, O.J. Messinger, K.T. Huntzinger; 1 ♀, 28 Sep 2005, T.L. Griswold, *S.* sp.; 11 ♂ 33 ♀, 28 Sep 2005, T.L. Griswold, K.T. Huntzinger; 3.5 mi N Wig Mt. (40.3648 -113.088): 5 ♂ 11 ♀, 19 Jun 2003, O.J. Messinger, C. Boyers; 2 ♂ 1 ♀, 17 Jul 2003, R. Andrus, C. Boyers; 4.17 mi SE Wig Mt.(40.2779 -113.0068): 1 ♂, 6 Jul 2005, E. Jarrell; 1 ♂ 1 ♀, 6 Jul 2005, J. Wilson, E. Jarrell; 7 ♂ 3 ♀, 15 Aug 2005, J.S. Wilson, E. Jarrell; 1 ♂, 13 Sep 2005, O.J. Messinger, K.T. Huntzinger; 1 ♂, 26 Sep 2005, T.L. Griswold, K.T. Huntzinger; 4.5 mi SSW White Rock (40.2691 -112.9495): 2 ♀, 1 Aug 2003, J.S. Wilson, C.M. Davidson; 2 ♂, 5 Jul 2005, E. Jarrell, J.S. Wilson; 2 ♂ 1 ♀, 5 Jul 2005, J. Wilson, E. Jarrell; 1 ♂, 5 Jul 2005, J.S. Wilson, *Sphaeralcea* sp.; 2 ♂ 1 ♀, 20 Jul 2005, J.S. Wilson, E. Jarrell; 4.6 mi WSW Little Granite Mtn. (40.1774 -112.9218): 7 ♂ 12 ♀, 23 Jun 2005, J.S. Wilson, E. Jarrell; 1 ♂, 8 Jul 2005, J. Wilson, E. Jarrell; 1 ♂ 3 ♀, 20 Jul 2005, J.S. Wilson, E. Jarrell; 1 ♂ 14 ♀, 22 Aug 2005, J.S. Wilson; 2 ♀, 30 Aug 2005, O.J.

Messinger; 2 ♂ 6 ♀, 15 Sep 2005, O.J. Messinger, K.T. Huntzinger; 2 ♂ 2 ♀, 28 Sep 2005, T.L. Griswold, K.T. Huntzinger; 6.3 mi N Wig Mt. (40.4007 -113.0909): 1 ♂, 21 Jun 2005, J.S. Wilson, E. Jarrell; 1 ♂ 1 ♀, 18 Jul 2005, J.S. Wilson, E. Jarrell; 3 ♂, 15 Aug 2005, J.S. Wilson, E. Jarrell; Camels Back Ridge, 3 mi NNE (40.1705 -112.9314): 1 ♂ 1 ♀, 18 Jul 2003, J.S. Wilson, C.M. Davidson; Camels Back Ridge, 3 mi NNE (40.1706 -112.9297): 1 ♂, 12 Jun 2003, R. Andrus, *M. sonchoides*; 13 ♂, 17 Jun 2003, J.S. Wilson; 1 ♂ 5 ♀, 30 Jul 2003, J.S. Wilson, C.M. Davidson; Dugway Proving Grounds, Cedar Ridge, S (site 7) (40.2519 -112.821): 1 ♂, 24 Jun 1997, T. Toler; Dugway Proving Grounds, Dugway, 7 km W (site 6)(40.2333 -112.8313): 4 ♂ 18 ♀, 24 Jun 1997, T. Toler; Dugway Proving Grounds, East Dugway Dunes (40.22111 -112.74361): 1 ♀, 26 Jun 2003, R.L. Johnson; Dugway Proving Grounds, N; Tabbys Peak, 9 km SW (site 13) (40.4296 -113.0946): 10 ♂ 9 ♀, 24 Jun 1997, T. Toler; 3 ♂ 3 ♀, 1 Jul 1997, T. Toler; Dugway Proving Grounds, North Wig Dunes (site 12) (40.22111 -112.74361): 1 ♀, 28 Jul 1997, T. Toler; Dugway Proving Grounds, Wig Mtn., 4.5 km NE (site 8) (40.355 -113.0484): 1 ♂ 2 ♀, 3 Jun 1997, T. Toler; 1 ♂, 24 Jun 1997, T. Toler; Dugway Proving Grounds, Wig Mtn., 8 km E (site 9) (40.2975 -112.9694): 1 ♀, 10 Jun 1997, T. Toler; 1 ♂ 3 ♀, 24 Jun 1997, T. Toler; 2 ♂, 1 Jul 1997, T. Toler; Dugway Proving Grounds, dunes N Wig Mtn. (40.3615 -113.0856): 1 ♀, 18 Jun 2003, R.L. Johnson; 2 ♀, 26 Jun 2003, R.L. Johnson; Dugway Proving Grounds; Dog Area (Ditto), 1 km W (site 19B) (40.1741 -112.9191): 1 ♂ 1 ♀, 3 Jun 1997, T. Toler; Dugway Proving Grounds; Dog Area (Ditto), 8 km N (site 19B) (40.2551 -112.9022): 4 ♂ 10 ♀, 24 Jun 1997, T. Toler; 1 ♂, 1 Jul 1997, T. Toler; Dugway Proving Grounds; Dog Area (Ditto), 8.5 km NE (site 1) (40.2373 -113.8452): 1 ♂ 3 ♀, 24 Jun 1997, T. Toler; Dugway Proving Grounds; Dog Area (Ditto), 9 km N (site 21B) (40.2668 -112.9478): 3 ♂ 3 ♀, 3 Jun 1997, T. Toler; 1 ♀, 24 Jun 1997, T. Toler; Dugway Proving Grounds; Wig Flats, S (site 18) (40.17113 -112.94218): 1 ♀, 3 Jun 1997, T. Toler; Dugway Proving Grounds; Wig Mtn., 10 km WNW (site 12) (40.4 -113.0894): 3 ♂ 6 ♀, 24 Jun 1997, T. Toler; 2 ♂ 1 ♀, 1 Jul 1997, T. Toler; 1 ♂, 24 Jul 1997, T. Toler; **Washington County:** 1.12 mi WSW Stave Spr. (37.2558 -112.9235): 1 ♀, 7 Jul 2006, B. Hays, F. Nicklen; Firepit Kn., 0.72 mi E (37.3494 -113.0912): 1 ♀, 24 Apr 2007, H. Ikerd, K. Davidson; Firepit Kn., 0.73 mi SE (37.3459 -113.0922): 1 ♀, 26 Jun 2007, K. Davidson; Kolob Terrace Road, .63mi NW, Tabernacle Dome (37.305 -113.1016): 1 ♀, 31 May 2007, H. Ikerd; Oak Creek Cyn. (37.2131 -112.9961): 1 ♂, 8 Jun 2006, F. Nicklen, *Asclepias* sp.; Paradise Canyon (37.1442 -113.613): 1 ♀, 19 Jun 1983, D. Beck; 1 ♀, 11 Jul 1983, D. Beck; Spendlove Kn., 0.28 mi NNE (37.3404 -113.1059): 1 ♀, 13 Jun 2006, B. Hays, F. Nicklen; 1 ♀, 22 Jun 2006, B. Hays, F. Nicklen; 1 ♀, 13 Jun 2007, H. Ikerd, K. Davidson; St. George, 5 mi N (37.1738 -113.6186): 1 ♀, 22 Jun 1972, F.D. Parker, D. Vincent; Warner Valley; Warner Valley Rd. (37.02541 -113.43376): 25 ♂ 90 ♀, 14–15 May 2012, K. Williams, E. Sadler, D. Denlinger, A. Kelley; 7 ♂ 11 ♀, 15–16 May 2012, K. Williams, E. Sadler, D. Denlinger, A. Kelley; 61 ♂ 372 ♀, 16–17 May 2012, K. Williams, E. Sadler, D. Denlinger, A. Kelley.

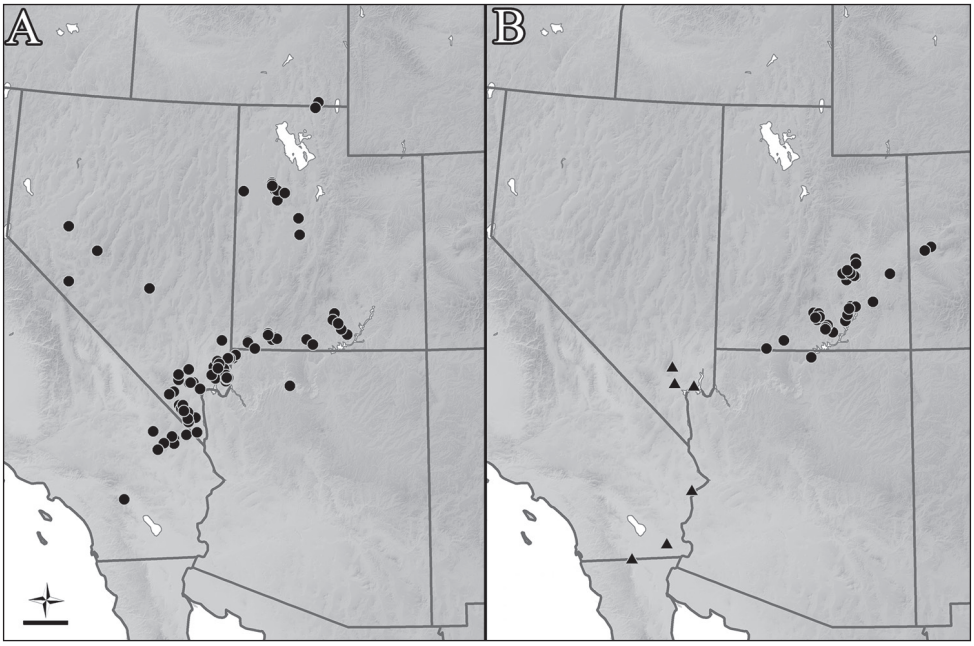

Figure 8. Occurrence maps. **A** *Perdita albonotata* **B** *P. moabensis* (circles) and *P. stephanomeriae* (triangles). Scale bar: 100 km.

***Perdita* (*Procockerellia*) *moabensis* Timblerlake**
Figures 1B–C, 2B, E, 4D–E, 5B, E, H, 6B, E, H, 8B, 9

Perdita (*Procockerellia*) *moabensis* Timberlake, 1971: 7, ♀; Timberlake, 1980: 15, ♂. Holotype female: USA, Utah, Grand Co., Moab, 8 August 1963, G.F. Knowlton [SEMC]. Examined.
Perdita (*Allomacrotera*) *moabensis*; Timberlake, 1980: 15 (change of subgenus).

Measurements. Female (n=10): head width 1.6 mm (1.5–1.7 mm), body length 6.0 mm (5.3–6.4 mm). Male (n=10): head width 1.7 mm (1.2–1.9 mm), body length 5.8 mm (4.6–6.4 mm).

Diagnosis. Both sexes of *P. moabensis* have the maxillary palpi 5-segmented and the frons and vertex are strongly tessellate and dull. Additionally, the head and mesosoma often have a more greenish (rather than bluish) cast. The female can be recognized by the relatively reduced facial markings which range from transverse lateral marks (Fig. 5B) to entirely absent. In addition, the mandibles are only slightly expanded medially (Fig. 5E) and the metasomal bands are curved towards the apical margins (Fig. 5H).

The male of *P. moabensis* is unique in having the apical margin of S1 slightly flexed out medially (Fig. 6H) as well as S8 bifurcate apically (Fig. 3E). Both characters are subtle but diagnostic in the male, as this is the only *Perdita* species known to have either of these characters. The male can be further distinguished by the strongly tessellate

and dull frons and vertex, the evenly rounded pygidial plate, and the bidentate hind tarsal claws (though this may be hard to see).

Distribution. Colorado, Utah and Arizona: Colorado Plateau (Fig. 8B).

Phenology.

Month:	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
# of records	0	9	16	51	376	70	0	0

Floral records. Apocynaceae (2 ♂ 1 ♀): *Cycladenia humilis* 2 ♂ 1 ♀, **Asteraceae** (11 ♂ 19 ♀): *Helianthus annuus* 1 ♂, *Lygodesmia* sp. 1 ♀, *Stephanomeria exigua* 8 ♂ 8 ♀, *S. pauciflora* 2 ♀, *S.* sp. 1 ♂ 3 ♀, *Vanclevea stylosa* 1 ♂ 5 ♀, **Commelinaceae** (1 ♂): *Tradescantia occidentalis* 1 ♂, **Fabaceae** (1 ♂): *Psoralea* sp. 1 ♂, **Lamiaceae** (1 ♂): *Poliomintha incana* 1 ♂, **Malvaceae** (1 ♂): *Sphaeralcea coccinea* 1 ♂, **Polemoniaceae** (3 ♂): *Gilia inconspicua* 2 ♂, *G.* sp. 1 ♂.

Additional material examined. Total specimens: 192 ♂ 327 ♀ 1 gynandromorph. **USA: ARIZONA: Coconino County:** Colorado River, Lee's Ferry (36.86583 -111.58783): 2 ♀, 9 Jun 2001, L.E. Stevens, *Stephanomeria pauciflora*. **COLORADO: Mesa County:** (39.0295 -108.6276): 3 ♀, 15–16 Jun 2011, collector unknown. **UTAH: Emery County:** Buckskin Springs (38.62 -110.6733): 14 ♂ 26 ♀, 5 Aug 1997, F.D. Parker; Flat Top Pass (38.5417 -110.4906): 1 ♂, 16 Jun 2000, F.D. Parker; Gilson Butte Well (38.5876 -110.583): 27 ♂ 64 ♀, 20 Aug 2001, F.D. Parker; 2 ♀, 22–26 Aug 2001, F.D. Parker, C. Lambkin, M. Metz, M. Hauser; 2 ♂ 2 ♀, 27 Aug 2001, M.E. Irwin, F.D. Parker, M. Metz, M. Hauser, C. Lambkin; Gilson Butte, 4 air mi N (38.64 -110.63): 6 ♂ 22 ♀, 20–23 Jul 1981, D.F. Veirs, T.L. Griswold, F.D. Parker; 1 ♀, 21 Jul 1981, D.F. Veirs, T.L. Griswold, F.D. Parker; 11 ♂ 21 ♀, 5 Aug 1997, F.D. Parker; Gilson Butte, 7 km NW; 30 km N Hanksville (38.6333 -110.6333): 1 ♂ 1 ♀, 21 Aug 2001, F.D. Parker, M.E. Irwin, C. Lambkin, M. Metz, M. Hauser; Goblin Valley turnoff, 25 mi N Hanksville (38.6299 -110.568): 4 ♂ 20 ♀, 16 Sep 1979, F.D. Parker, D.F. Veirs; Goblin Valley, sand dunes (38.64 -110.63): 1 ♂ 3 ♀, 16 Sep 1979, C.L. Hatley, G. Briggs; 12 ♀, 16 Sep 1979, F.D. Parker, D.F. Veirs; Goblin Valley, wash (38.5961 -110.7028): 14 ♀, 16 Sep 1979, F.D. Parker, D.F. Veirs; Iron Wash, 32 km SW Green River (38.7833 -110.4333): 1 ♂, 21 Aug 2001, M.E. Irwin, C. Lambkin, M. Metz, M. Hauser; Little Flat Top, 4 km E (38.5333 -110.45): 2 ♂ 2 ♀, 22 Aug 2001, M.E. Irwin, F.D. Parker, M. Metz, M. Hauser, C. Lambkin; 1 ♂ 9 ♀, 27 Aug 2001, F.D. Parker, C. Lambkin, M. Metz, M. Hauser, M.E. Irwin; 4 ♀, 27 Aug 2001, M.E. Irwin, F.D. Parker, M. Metz, M. Hauser, C. Lambkin; Little Flat Top, 8 km E (38.5167 -110.4333): 1 ♂, 20 Aug 2001, M.E. Irwin, F.D. Parker, M. Metz, M. Hauser, C. Lambkin; 2 ♂ 1 ♀, 22–26 Aug 2001, M.E. Irwin, F.D. Parker, M. Metz, M. Hauser, C. Lambkin; 1 ♂, 27 Aug 2001, M.E. Irwin, F.D. Parker, M. Metz, M. Hauser, C. Lambkin; Little Flat Top (38.5333 -110.4833): 2 ♂ 2 ♀, 20 Aug 2001, F.D. Parker, M.E. Irwin; 1 ♀, 27 Aug 2001, F.D. Parker, C. Lambkin, M. Metz, M. Hauser, M.E. Irwin; 5 ♂ 6 ♀, 27 Aug 2001, F.D. Parker, M.E. Irwin; Little Gilson Butte, 0.5 air mi E (38.5924 -110.594): 9 ♂ 5 ♀, 5 Aug 1997, F.D. Parker; Little Gilson Butte, 0.5 mi E (38.58 -110.59): 1 ♀, 27 Aug 1985, T.L. Griswold,

Vanclevea stylosa; Little Gilson Butte, 2 air mi E(38.5917 -110.5737): 2 ♂ 6 ♀, 24–26 Aug 1981, D.F. Veirs, T.L. Griswold, F.D. Parker, *S. exigua*; 1 ♂, 25 Aug 1981, D.F. Veirs, T.L. Griswold, F.D. Parker; 2 ♀, 5 Aug 1997, F.D. Parker; Little Gilson Butte, 2 mi E (38.5917 -110.5737): 26 ♂ 24 ♀, 5 Aug 1997, F.D. Parker; Little Gilson Butte, near (38.58 -110.59): 2 ♂, 21 Aug 1980, A.S. Menke, F.D. Parker; Mollys Castle turnoff (38.5492 -110.6184): 1 ♂ 4 ♀, 28 Aug 1985, T.L. Griswold, *V. stylosa*; San Rafael Desert, Butte (38.0 -110.0): 1 ♂, 31 Jul 2000, F.D. Parker; San Rafael Reef, E edge, 2.8 mi S I-70 (38.88694 -110.44416): 1 ♂, 15 Jun 1991, S. Sipes, W.R. Bowlin, *Cycladenia humilis*; 1 ♀, 16 Jun 1991, S. Sipes, W.R. Bowlin, *C. humilis*; 1 ♂, 17 Jun 1991, S. Sipes, W.R. Bowlin, *C. humilis*; South Temple Wash (38.65 -110.66): 1 ♀, 22 Aug 1989, J. Burner, *Lygodesmia* sp.; South Temple Wash (38.65 -110.6667): 5 ♂ 4 ♀, 21 Aug 2001, M.E. Irwin, C. Lambkin, M. Metz, M. Hauser, F.D. Parker; 4 ♂ 1 ♀, 22–26 Aug 2001, F.D. Parker, C. Lambkin, M. Metz, M. Hauser, M.E. Irwin; 1 ♂, 22–26 Aug 2001, M.E. Irwin, C. Lambkin, M. Metz, M. Hauser; Temple Mountain, 3.5 mi SSE San Rafael Desert (38.638 -110.6636): 2 ♂ 19 ♀, 5 Aug 1997, F.D. Parker; Wild Horse Creek, N Goblin Valley(38.5961 -110.7028): 1 ♂ 4 ♀, 21–23 Jul 1981, D.F. Veirs, T.L. Griswold, F.D. Parker; 1 ♀, 13 Sep 1982, F.D. & J.H. Parker; 1 ♀, 13 Sep 1982, F.D. & J.H. Parker, *S.* sp.; 3 ♀ 1 ♀, 13 Sep 1983, F.D. & J.H. Parker; 1 ♀, 13 Sep 1983, F.D. & J.H. Parker, *S.* sp.; 12 ♂ 4 ♀, 5 Aug 1997, F.D. Parker; Wildhorse Creek, 2 mi E (38.5819 -110.7833): 6 ♂, 21 Aug 2001, collector unknown; Wildhorse Creek, 2 mi N (38.618 -110.675): 3 ♂, 21 Aug 2001, F.D. Parker; Wildhorse Creek (38.5852 -110.7008): 4 ♂ 2 ♀ 1 gynandromorph, 21 Aug 2001, F.D. Parker; **Garfield County:** Alvey Wash, 12 km S Escalante (37.6813 -111.4858): 1 ♂, 24 May 2002, M.E. Irwin, F.D. Parker; Calf Creek (37.7645 -111.4046): 1 ♂, 4 Jul 2001, O.J. Messinger, *Sphaeralcea coccinea*; 1 ♂ 1 ♀, 11 Aug 2003, S.M. Higbee, *S. exigua*; 1 ♂, 23 Sep 2003, S.M. Higbee, *S. exigua*; Duffy Mesa, 2 mi NNW (39.10327 -108.45874): 1 ♂, 17 Jun 2003, H. Ikerd, *S. exigua*; 1 ♂ 1 ♀, 15 Sep 2003, A. Johansen; 1 ♂ 1 ♀, 29 Sep 2003, S.M. Higbee, *S.* sp.; Escalante Riv., jct Death Hollow, 1.3 mi W (37.7808 -111.5353): 1 ♂, 5 Sep 2001, B. Morgan; 1 ♂, 21 Aug 2002, C.M. Davidson, *S. exigua*; 1 ♂ 1 ♀, 13 Aug 2003, O.J. Messinger; Hole in the Rock Road, Halfway Hollow (37.6338 -111.4449): 1 ♂ 8 ♀, 5–19 Jul 2003, H. Ikerd; 1 ♂ 4 ♀, 19–29 Jul 2003, H. Ikerd; Hwy 95, jct Hwy 276, 12 mi S (37.9046 -110.452): 1 ♂, 24 May 2000, F.D. Parker; Hwy 95, jct Hwy 276, 9 mi S (37.9107 -110.577): 2 ♂, 24 May 2000, F.D. Parker; 1 ♂, 24 May 2000, F.D. Parker, *Psoralea* sp.; Red Breaks, 2.3 mi NW (37.6924 -111.379): 1 ♂, 25 Jun 2003, H. Ikerd, *Helianthus annuus*; Ticaboo, 4 mi S (37.6142 -110.7168): 1 ♂, 25 May 2000, F.D. Parker; Ticaboo, 5 mi N (37.7368 -110.6635): 1 ♂, 25 May 2000, F.D. Parker; Woodruff Springs, 54 km S Hanksville (37.8661 -110.6178): 2 ♀, 22–27 May 2002, M.E. Irwin, F.D. Parker; **Kane County:** Billy Pasture, 0.4 mi N (37.2059 -112.2912): 1 ♂, 5 Sep 2003, T.L. Griswold, *Tradescantia occidentalis*; Coral Pink Sand Dunes State Park, all Swales (37.03521 -112.73325): 1 ♂ 1 ♀, 30 Jul 1994, C.B. Knisley, J.M. Hill; Dry Fork, N (37.441 -111.2307): 1 ♂, 18 Sep 2003, J. Tolliver, *S. exigua*; Dry Fork (37.4817 -111.2205): 1 ♂, 5 Jun 2000, J. Grixti, *Poliomintha incana*; 1 ♀, 18 Sep 2003, J. Tolliver, *S. exigua*; Sunset Natural Arch,

Figure 9. *Perdita moabensis* gynandromorph (BBSL468079). Scale bar: 500 μm.

0.72 mi NE (37.3822 -111.0374): 1 ♂, 5 Jun 2001, L. Topham, *Gilia inconspicua*; 1 ♂, 5 Jun 2001, R. Andrus, *G. inconspicua*; Twentyfive Mile Wash, 1.5 mi S (37.5322 -111.1788): 1 ♂, 4 Jun 2001, S. Messinger, *S. exigua*; Twentyfive Mile Wash, 1.6 mi S (37.5308 -111.1773): 1 ♂, 4 Jun 2001, S. Messinger, *G.* sp.; **Wayne County:** The Notch, 6 mi N Hanksville (38.45107 -110.68065): 1 ♂, 16 Aug 1992, T.L. Griswold.

Gynandromorph. A single gynandromorph of *P. moabensis* was discovered during the course of this study. It was collected in a pan trap on the 21st of August 2001 at Wildhorse Creek in Emery County, UT by Frank Parker (accession number BBSL468079). The gynandromorph is almost entirely female, except the left side of the head (Fig. 9), the left foreleg, and the left midleg are male. The face is split cleanly down the middle; the female half has an antenna with 12 antennal segments, whereas the male half has 13 antennal segments. The pronotal collar on the male side is slightly more protuberant than the female side, but not as much as in a typical male. The rest of the thorax, including the wings and hind legs, are entirely female. This unique specimen is the first known gynandromorph in *Perdita*. Further, this is the first recorded gynandromorph in Panurginae; all previously known gynandromorphs in the family Andrenidae have been restricted to the genus *Andrena* (Wcislo et al. 2004, Hinojosa-Díaz et al. 2012), although an intersex (blended male and female characters) *Acamptopoeum submetallicum* (Spinola) has been previously documented (Ramos and Ruz 2013).

Remarks. Timberlake (1980) reported that the male of this species had only four maxillary palpi. Examination of many specimens (including the female holotype) reveals that the species always has five maxillary palpi.

***Perdita* (*Procockerellia*) *stephanomeriae* Timberlake**
Figures 2G–I, 3C, F, 4E–F, 5C, F, I, 6C, F, I, 8B

Perdita (*Procockerellia*) *stephanomeriae* Timberlake, 1954: 404, ♀; Timberlake 1960: 132, ♂. Holotype female: USA, California, San Diego Co., 12 miles south of Ocotillo, 12 November 1939, P.H. Timberlake, at flowers of *Stephanomeria pauciflora* [CAS type no. 14720]. Examined.

Perdita (*Allomacrotera*) *stephanomeriae*; Timberlake 1960: 131 (change of subgenus).

Perdita (*Hexaperdita*) *glamis* Timberlake, 1980: 16, ♂. Holotype male: USA: California: Imperial Co., Glamis, 13 June 1965, G.E. Wallace [CAS type no. 14544]. Examined. **Syn. n.**

Measurements. Female (n=10): head width 1.5 mm (1.4–1.6 mm), body length 5.6 mm (5.2–6.3 mm). Male (n=4): head width 1.5 mm (1.4–1.6 mm), body length 4.9 mm (4.6–5.2 mm).

Diagnosis. Both sexes have the maxillary palpi 3-jointed (whereas the other two species of *Prockerellia* have 5-jointed maxillary palpi) and the frons and vertex are barely tessellate and strongly shining (e.g. Fig. 6C). The transverse dorso-lateral carina on the pronotal collar found in both sexes is distinctive; other *Procockerellia* have a rounded nub laterally. The female has the face marked with white laterally on the clypeus and a triangular mark on the lateral area (Fig. 5C), similar to lighter females of *P. albonotata*. The female can be further recognized by the broad median expansion of the mandibles (Fig. 5F) and narrowly interrupted metasomal bands that don't curve to the apical margin laterally (Fig. 5I). The male is unique in having a small point apically on the pygidial plate (Fig. 6I). It can be further distinguished by the bidentate tarsal claws and the lack of light bands on the metasoma (Fig. 6F).

Distribution. Nevada and California: Mojave and Sonoran Deserts (Fig. 8B).

Phenology.

Month:	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**
# of records	0	0	2	0	0	0	15	1

Floral records. Asteraceae: *Stephanomeria* sp. 1 ♂ 6 ♀.

Additional material examined. Total specimens: 4 ♂ 12 ♀. **USA: CALIFORNIA: San Bernardino County:** Vidal, 1 mi S (34.1062 -114.50738): 1 ♂ 6 ♀, 6 Oct 1988, T.L. Griswold, *Stephanomeria* sp. **NEVADA: Clark County:** 2.2 mi SSW Mormon Well (36.6165 -115.1111): 1 ♀, 14 Jun 2004, E. Ahlstrom, D. Skandilis;

Las Vegas, NE (36.2798 -115.0355): 1 ♂, 7 Oct 1998, T.L. Griswold; Pinto Ridge (36.2422 -114.5493): 2 ♂ 5 ♀, 9 Oct 1998, T.L. Griswold.

Remarks. As a result of this study, *P. stephanomeriae* is hereby returned to its original subgeneric assignment, *Procockerellia*. This species appears rare, especially compared to *P. albonotata* and *P. moabensis*, which can be common and locally abundant. Extensive all season sampling conducted in 1998, 2004, 2005 in Clark County, Nevada in the eastern Mojave Desert yielded large numbers of *Procockerellia*. It is therefore interesting that while *P. albonotata* was widely distributed and abundant, *P. stephanomeriae* was rarely detected.

The holotype of *P. glamis* was examined and found to clearly match *P. stephanomeriae*. The mouthparts of the holotype of *P. glamis* are not exposed, which likely led Timberlake (1980) to incorrectly place and describe the species in subgenus *Hexaperdita* since he could not see the reduced number of palpi.

Subgenus *Cockerellia* Ashmead

Cockerellia Ashmead, 1898: 284. Type species: *Perdita hyalina* Cresson, 1878, by original designation.

Philoxanthus Ashmead, 1898: 285. Type species: *Perdita beata* Cockerell, 1895, by original designation.

Perdita (*Cockerellia*) *imbellis* Timberlake

Perdita (*Cockerellia*) *imbellis* Timberlake, 1968: 21, ♂. Holotype male: USA, Arizona, Coconino Co., 28 May 1954, F. Werner [CAS type no. 13525]. Examined.

Perdita (*Cockerellia*) *hilaris* Timberlake, 1968: 2, ♂ (syn. Timberlake 1980). Holotype male: USA, Utah, Dixie State Park, 13 June 1961, G.E. Bohart [CAS type no. 14554]. Not examined.

Perdita (*Procockerellia*) *brachyglossa* Timberlake, 1971: 6, ♀. Holotype female: USA, Arizona, Coconino Co., Four and one-half miles southwest of Marble Canyon, 30 August 1967, P.H. Timberlake, on *Thelesperma* [CAS type no. 14448]. Examined. **Syn. n.**

Remarks. *Perdita* (*Procockerellia*) *brachyglossa* was described from a single female specimen. Timberlake (1971) reported that the female had four maxillary palpi. Examination of the holotype revealed four maxillary palpi on one side and one palp on the other. In every morphological character except the palpi, the holotype of *P. brachyglossa* clearly matches *P. imbellis*. It seems likely that the mouthparts of the holotype of *P. brachyglossa* are either damaged or aberrant.

Due to a *lapsus calami* in Timberlake (1980), *Perdita albomaculata* Timberlake, 1980, *Perdita imbellis* Timberlake, 1968, and *Perdita luculenta* Timberlake, 1968 were all referred to as being in subgenus *Cockerellula* Strand, 1932. They all belong in subgenus *Cockerellia*.

Perdita (*Cockerellia*) *moldenkei* Timberlake, 1980

Perdita (*Procockerellia*) *moldenkei* Timberlake, 1980: 14, ♂. Holotype male: USA, California, San Diego Co., Ocotillo-Borrego area, 27 March 1972, J.L. Neff, at nectaries of *Encelia farinosa* [CAS type no. 14614]. Examined.

Remarks. Timberlake's description of *P. moldenkei* reports the lone type specimen as having five maxillary palpi. However, examination of the type reveals that the specimen clearly has six maxillary palpi on both sides. Timberlake must have miscounted the number of maxillary palpi and described *P. moldenkei* in the incorrect subgenus as a result.

Perdita moldenkei is clearly a member of subgenus *Cockerellia*, and is hereby moved to that subgenus. Based on the examination of the type of *P. moldenkei*, we believe that it is a likely synonym of *P. verbesinae* Cockerell, 1896. However, we have not examined the various syntypes and varieties of *P. verbesinae* in order to confirm this; examination of the syntypes of *P. verbesinae* should be addressed in a broader revision of *Cockerellia*.

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Seahorses of the *Hippocampus coronatus* complex: taxonomic revision, and description of *Hippocampus haema*, a new species from Korea and Japan (Teleostei, Syngnathidae)

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Abstract

Morphological and molecular analyses were conducted on 182 specimens belonging to the *Hippocampus coronatus* complex (*H. coronatus* sensu lato), collected in Korea and Japan 1933–2015, in order to clarify the taxonomic status of the species within this complex. Three species are recognized based on the shape of the coronet, the number of trunk rings (TrR) and tail rings (TaR), and presence or absence of a wing-tip spine (WS) at the dorsal fin base. *Hippocampus coronatus* Temminck & Schlegel, 1850 (*H. coronatus* sensu stricto), is diagnosed by 10 TrR, 37–40 TaR, an extremely high coronet (55.7–79.0 % head length) with four tips on the corona flat (CoT), and one WS. *Hippocampus sindonis* Jordan & Snyder, 1901 is diagnosed by 10 TrR, 35–38 TaR, a moderately high coronet (36.3–55.4 % HL) with five CoT, and no WS. A new species, *H. haema* is described on the basis of 140 specimens, characterized by 10 TrR, 35–38 TaR, a moderately high coronet (34.1–54.9 % head length) with four CoT, and two WS. *Hippocampus haema* is only known from the Korea Strait, western Kyushu, and East/Japan Sea. Recognition of the three species is supported by differences in mitochondrial DNA fragments (cytochrome *b*, 16S rRNA, and 12S rRNA).

Keywords

Genetic distance, morphology, molecular systematics, Pacific Ocean, taxonomy

Introduction

The seahorse genus *Hippocampus* (Teleostei: Syngnathidae) exhibits a wide range of inter- and intra-specific variation, for example in skin filaments, color, and body proportions. Therefore, taxonomic relationships within *Hippocampus* have been controversial (Lourie et al. 1999, 2016), and more than 140 species have been named within this genus (Lourie et al. 2016; Eschmeyer et al. 2017). For example, Lourie et al. (2016) reviewed the genus and considered 41 species as valid, while Kuiter (2009) recognized *ca*. 79 valid species. Six species of *Hippocampus* have been recorded from Korea and Japan, viz., *H. coronatus* Temminck & Schlegel, 1850, *H. mohnikei* Bleeker, 1853, *H. histrix* Kaup, 1856, *H. kuda* Bleeker, 1852, *H. trimaculatus* Leach, 1814, and *H. sindonis* Jordan & Snyder, 1901. Another two species, *H. kelloggi* Jordan & Snyder, 1901 and *H. bargibanti* Whitley, 1970, were only recorded from Japan (Choi et al. 2002; Lourie et al. 2004; Kim et al. 2005; Senou et al. 2006; Kim et al. 2013; Senou 2013; Lourie et al. 2016).

The species (or species group) *H. coronatus* sensu lato has been defined by possessing ten trunk rings, 34–40 tail rings, a bony armor, double gill openings (Lourie et al. 1999, 2004; Kim et al. 2005; Kuiter 2009; Foster and Gomon 2010; Senou 2013; Lourie 2016), and a tall coronet on the head, which exhibits a wide range of height variation (Jordan and Snyder 1901; Mitani 1956; Lourie et al. 1999, 2004). Some authors have stated that this group includes two species, *H. coronatus* (sensu stricto), which has an extremely high coronet and a snout length ~2.33 times the head length, and *H. sindonis*, which has a moderately high coronet and a snout length ~3 times the head length (Jordan and Snyder 1901; Okada and Matsubara 1938; Matsubara 1955; Lourie et al. 1999; Senou 2002; Lourie et al. 2004; Senou 2013), while others considered the variation in coronet height only as intraspecific variation (Mitani 1956; Araga 1984; Senou 1993). Based on variation in mitochondrial DNA (partial 12S rRNA), Mukai et al. (2000) suggested that the *H. coronatus* complex (*H. coronatus* sensu lato) consists of two genetically diverged groups.

Although the Korean seahorse (Korean name: *Haema*) has been identified as *H. coronatus* (Mori 1928; Chyung 1977; Kim et al. 2001; Kim et al. 2005), the height of its coronet and the number of tail rings appear to agree better with that described for *H. sindonis* (Jordan and Snyder 1901; Lourie et al. 1999, 2004; Kim et al. 2013; Senou 2013; Han et al. 2014). In fact, *H. sindonis* has often been confused with *H. coronatus* (Lourie et al. 1999, 2016), and the height of the coronet in the type series of *H. coronatus* varies (Boeseman, 1947). These controversies have contributed to the uncertainty about the distribution of *H. coronatus* in both Korea and Japan, and led to its classification in the Data Deficient (DD) category of the International Union for Conservation of Nature and Natural Resources (IUCN) Red List, as there is a lack of information on population trends (Zhang and Pollom 2016). The present study aims to clarify the taxonomic status of Korean seahorses, redescribing *H. coronatus* and *H. sindonis* and describing a new species, all belonging to the *H. coronatus* complex.

Materials and methods

Material examined

A total of 182 specimens of *H. coronatus* sensu lato collected from Korean and Japanese waters (Fig. 1) were subjected to morphological analyses. Voucher specimens were deposited in Korea [Department of Marine Biology, Pukyong National University (**PKU**); National Institute of Biological Resources (**NIBR**)], Japan [Maizuru Fisheries Research Station, Field Science Education and Research Center, Kyoto University (**FAKU**); Kagoshima University Museum (**KAUM**); Kanagawa Prefectural Museum of Natural History (**KPM**)], Europe [Naturalis Biodiversity Center (**RMNH**), The Netherlands], and the United States [Smithsonian National Museum of Natural History (**USNM**)].

Morphological analysis

Procedures used for counts and measurements follow Lourie (2003) and are presented in Fig. 2.

Morphological terms are abbreviated as:

TrR	trunk rings	**FTrDS**	first TrR dorsal spine
TaR	tail rings	**LTrDS**	last TrR dorsal spine
DsR	TrR and TaR supporting the dorsal fin	**WS**	wing-tip spine: a thick-recurved spine on dorsal fin base as in *H. coronatus* and *H. haema*
D	dorsal fin rays		
A	anal fin rays	**ACS**	anterior coronet spine
P	pectoral fin rays	**PCS**	posterior coronet spine: 5th tip on corona flat
CS	cheek spine below the operculum	**Coa**	corona: posterior crest of coronet
ES	eye spine above the eye	**CoT**	number of tips on corona flat

Measurements are abbreviated as:

SL	standard length	**CHMC**	coronet height from mid-point of cleithral ring to the median groove on corona
HL	head length		
CHGO	coronet height from gill opening to the median groove on corona (along central depression between 1st and 2nd tip on it)	**SnL**	snout length
		ED	eye diameter
		TrL	trunk length
		TaL	tail length

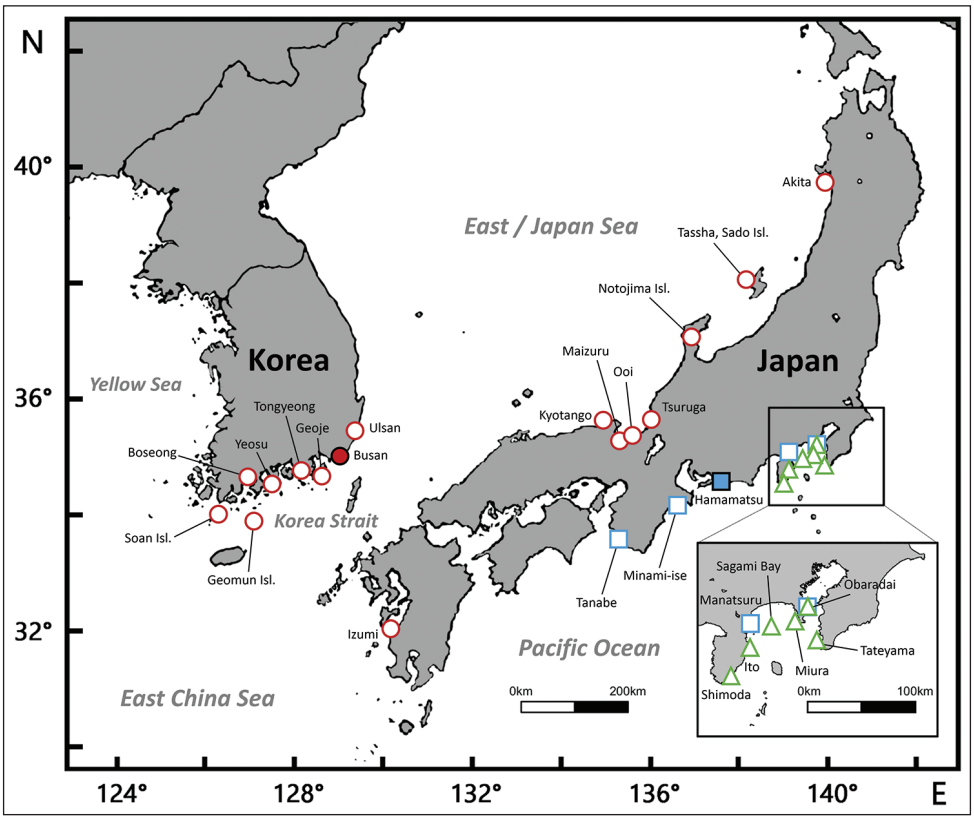

Figure 1. Distribution of the species within the *Hippocampus coronatus* complex: *H. haema* (red circles; the filled red circle indicates the holotype), *H. coronatus* (green triangles), and *H. sindonis* (blue squares; the filled blue square indicates the holotype).

Meristic data were obtained from soft X-rays of the 182 *H. coronatus* sensu lato specimens. Measurements were obtained using the microscope-integrated Active Measure software (Shinhanoptics, Seoul, Korea). The coronet height was measured as CHMC (Lourie 2003) and CHGO (Temminck and Schlegel 1850; Jordan and Snyder 1901) (Fig. 2) so that our results could be compared to those reported in previous studies (Temminck and Schlegel 1850; Jordan and Snyder 1901; Lourie et al. 1999). Sexual dimorphism analysis was conducted on the 152 adults (80 females and 72 males). These are all the specimens over 53.9 mm, which is the minimum SL at maturation defined for *H. coronatus* sensu lato (Choi et al. 2006).

Molecular analysis

Tissue from the right eye ball or from the right-side of the tail was used to isolate genomic DNA from 22 specimens with moderately high coronets, collected in Busan,

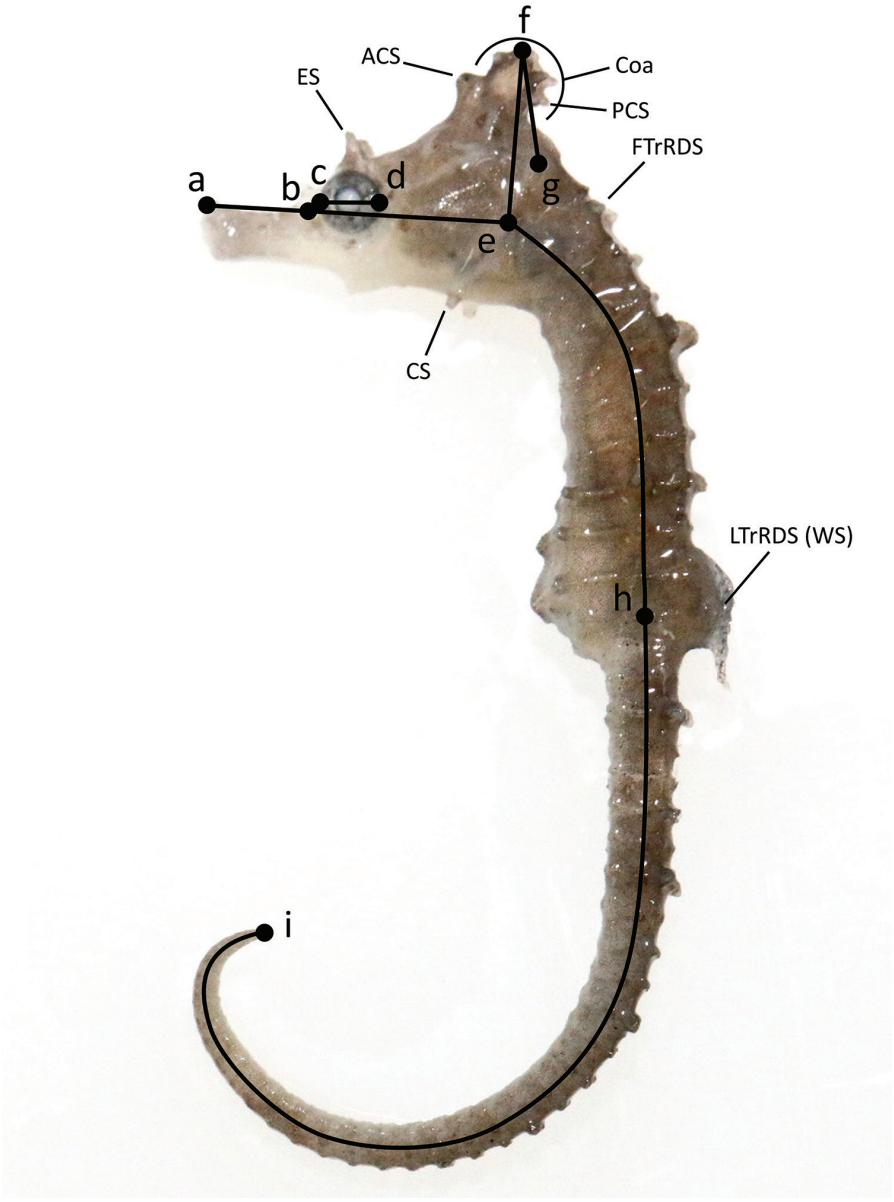

Figure 2. Meristic and morphometric characters used in *Hippocampus* analyses following Lourie (2003). Abbreviations: eye spine (ES), cheek spine (CS), anterior coronet spine (ACS), posterior coronet spine (PCS), corona (Coa), dorsal spine of the first trunk ring (FTrRDS), dorsal spine of the last trunk ring (LTrRDS; Wing-tip spine [WS] as in *H. coronatus* and *H. haema*). Points used for measurements: **a** tip of snout (upper jaw) **b** anterior side of tubercle/spine **c** anterior edge of orbit **d** posterior edge of orbit **e** mid-point of cleithral ring **f** median groove (central depression) of coronet **g** gill opening **h** mid-point of lateral ridge of the last trunk ring **i** tail tip. Measurements: **a–b** snout length (SnL) **c–d** eye diameter (ED), **a–e** head length (HL) **e–f** coronet height from mid-point of cleithral ring (CHMC) **f–g** coronet height from gill opening (CHGO) **e–h** trunk length (TrL) **h–i** tail length (TaL) **a–e–h–i** standard length (SL). Photographed specimen *H. haema* PKU 10129 (paratype).

Tongyeong, Boseong, Soan Island, Maizuru, and Minami-ise, and from four specimens with extremely high coronets collected in Miura. Isolation was performed using an AccuPrep® Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea), according to the manufacturer's instructions.

Three partial mitochondrial DNA loci (cytochrome *b* [cyt *b*], 16S rRNA, and 12S rRNA) were amplified via polymerase chain reaction (PCR), which was conducted on an S1000™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). The PCR solutions consisted of 3 µl 10× Ex Taq buffer (20 mM Mg^{2+} plus), 2.4 µl 2.5 mM dNTPs, 1 µl each primer, 0.1 µl TaKaRa Ex Taq DNA polymerase (Takara Bio, Kusatsu, Shiga, Japan), 3 µl genomic DNA, and distilled water to bring the total volume to 30 µl. The PCR amplification of cyt *b* was conducted using primers Shf2 (5'-TTGCAACCGCATTTTCTTCAG-3') and Shr2 (5'-CGGAAGGTGAGTCCTCGTTG-3') under the following conditions: initial denaturation at 94°C for 2:30 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1:15 min; final extension at 72°C for 5 min (Lourie and Vincent 2004). Using the universal primers 16Sal-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16Sbr-H (5'-CCGGTCTGAACTCAGATCACGT-3'), 16S rRNA was amplified as follows: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 1 min, and extension at 72°C for 1 min; final extension at 72°C for 10 min (Palumbi 1996). The amplification of 12S rRNA was conducted using primers OMT16SF (5'-TGCCAGCCACCGCGGTTATACCT-3') and tRNA02 (5'-GGATGTCTTCTCGGTGTAAG-3') (both from Mukai et al. 2000), under the following conditions, which were modified from Mukai et al. (2000): initial denaturation at 95°C for 2:30 min; 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 70°C for 2 min; final extension at 70°C for 5 min. Amplified PCR samples were purified using a Davinch™ PCR Purification Kit (Davinch-K, Seoul, Korea), according to the manufacturer's instructions. Sequencing reactions were performed in a DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad) using an ABI BigDye(R) Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA).

Sequences of the three gene regions belonging to members of the *H. coronatus* complex (*H. coronatus* and *H. sindonis*), its sister species (*H. mohnikei*), some members of the *H. kuda* complex (*H. kuda*, *H. reidi*, and *H. ingens*) (Lourie et al. 1999, 2004), and one outgroup (*Syngnathus schlegeli*) were retrieved from the GenBank database (www.ncbi.nlm.nih.gov) (Table 1). Sequences obtained for each species were concatenated and each gene region was treated as a partition. To compare our results with that of Mukai et al. (2000), an additional analysis focusing on 12 rRNA sequence variation was performed. GenBank sequences were aligned with those obtained in the present study using BioEdit7 (Hall 1999), and pairwise genetic distances were calculated using the Kimura 2-parameter model (Kimura 1980) on MEGA6 (Tamura et al. 2013). Neighbor-joining (NJ) trees were constructed in MEGA6, and confidence levels were assessed using 1000 bootstrap replications.

Table 1. GenBank accession numbers and sources of the mitochondrial gene sequences used in the evaluation of the phylogenetic relationships among species belonging to the *Hippocampus coronatus* complex.

Species	Locus	Accession No.	Source
Hippocampus haema sp. n.	cyt *b*	KP744863–KP744882	Present study
	16S rRNA	KP744883–KP744902	
	12S rRNA	KP744903–KP744922	
H. coronatus	cyt *b*	KT167545–KP167548	Present study
	16S rRNA	KT167549–KP167552	
	12S rRNA	KT167553–KP167556	
	12S rRNA	AB032030	Mukai et al. (2000)
H. sindonis	cyt *b*	KT167539–KP167540	Present study
	16S rRNA	KT167541–KP167542	
	12S rRNA	KT167543–KP167544	
	12S rRNA	AB032029	Mukai et al. (2000)
H. mohnikei	complete mitogenome	KT780446	Zhang et al. (2017)
	12S rRNA	AB032028	Mukai et al. (2000)
H. kuda	complete mitogenome	AP005985	Kawahara et al. (2008)
H. reidi	complete mitogenome	KJ123692	Wang et al. (2016)
H. ingens	complete mitogenome	KF680453	Zhang et al. (2015)
Syngnathus schlegeli	complete mitogenome	AP012318	Song et al. (2014)

Systematics

***Hippocampus coronatus* Temminck & Schlegel, 1850**

Figs 3F–G, 4B, 5C, 6B, 6E, Tables 2–3

English name: Crowned seahorse, New Korean name: *Wanggwan-haema*, Japanese name: *Tatsu-no-otoshigo*

Hippocampus coronatus Temminck and Schlegel 1850: 274, pl. 120 (fig. VII) (Lectotype: RMNH.PISC.D 1543; Paralectotype: RMNH.PISC.D 1544; type locality: Japan; Boeseman 1947: 196); Kaup 1853: 229; Jordan and Snyder 1901: 18; Matsubara 1955: 431; Jordan et al. 1913: 100; Boeseman 1947: 195; Burgess and Axelrod 1972: 212; Araga 1984: 89; Senou 1993: 489, 1294; Lourie et al. 1999: 88; Mukai et al. 2000: 139; Senou 2000: 536; Senou 2002: 536, 1508; Lourie et al. 2004: 42; Yoshino and Senou 2008: 76; Kuiter 2009: 129; Kohno et al. 2011: 127; Senou 2013: 635, 1911; Lourie 2016: 106; Lourie et al. 2016: 21.

Material examined. Japan. RMNH.PISC.D 1543 (lectotype of *H. coronatus*, photograph from RMNH), female, 103.3 mm SL, von Siebold collection. RMNH.PISC.D 1544 (paralectotype of *H. coronatus*, photograph from RMNH), female, 100.2 mm SL, von Siebold collection. FAKU 137348–137351, 4, 96.4–112.6 mm SL, Miura, Kanagawa, Nov 2014, H. Sugawara. KAUM-I 20721, 1, 73.7 mm SL, Takane, Hamasa, Tateyama, Chiba, 34°58'38"N; 139°47'19"E, depth 20 m, 2 Dec 2008, M. Aizawa. KPM-NI

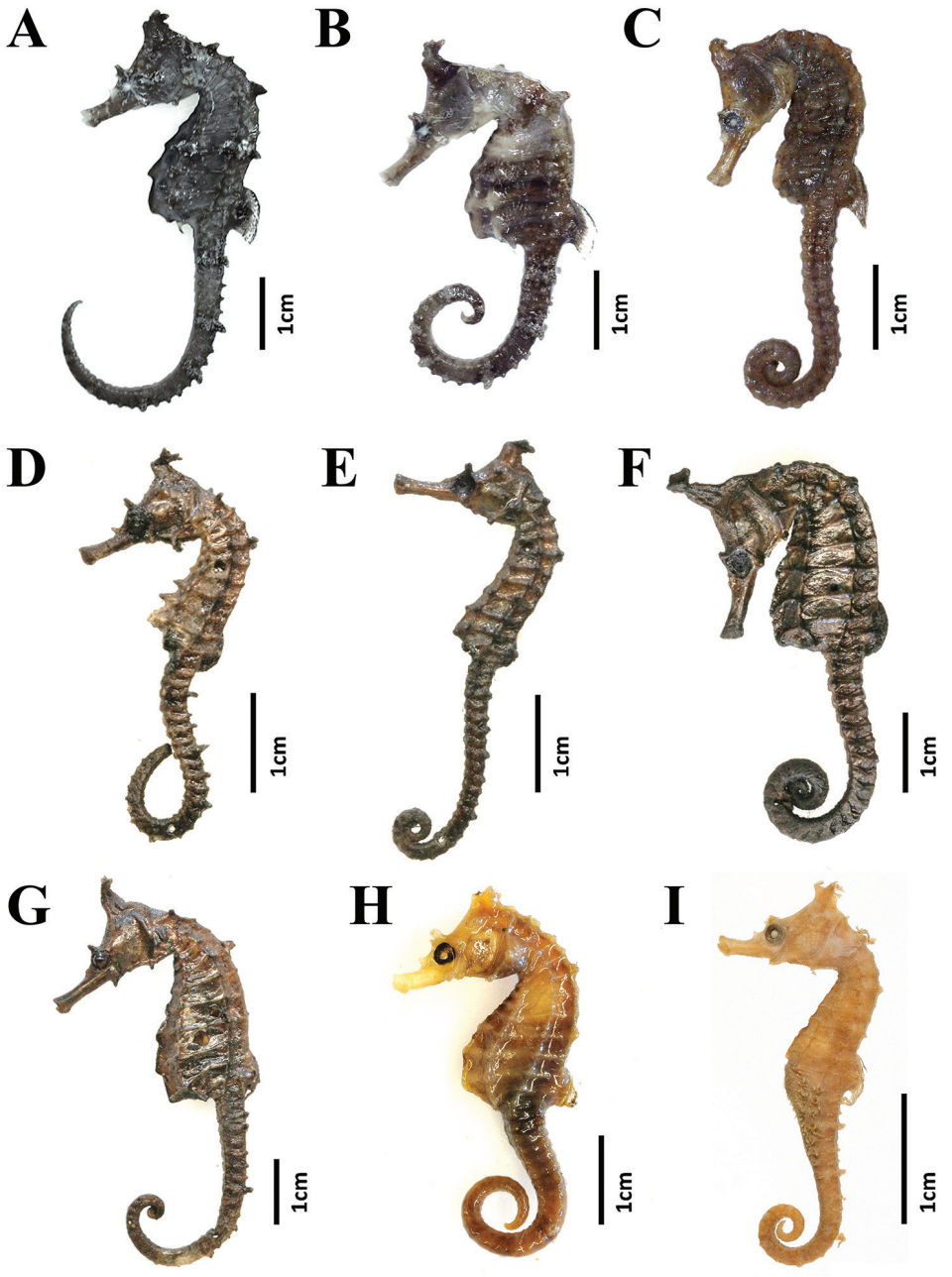

Figure 3. Specimens within the *Hippocampus coronatus* complex examined in the present study. **A–E** *H. haema* **A** PKU 9641 (holotype, Busan, Korea) **B** FAKU 135644 (paratype, Maizuru, Japan) **C** KPM-NI 24769 (paratype, Akita, Japan) **D** RMNH.PISC.D 1541 (paratype, Japan) **E** RMNH.PISC.D 1542 (paratype, Japan) **F–G** *H. coronatus* **F** RMNH.PISC.D 1543 (lectotype, Japan) **G** RMNH.PISC.D 1544 (paralectotype, Japan) **H–I** *H. sindonis* **H** RMNH.PISC 3924 (Japan) **I** USNM 49730 (holotype, Hamamatsu, Japan).

Table 2. Meristic and morphometric characters assessed in the species comprising the *Hippocampus coronatus* complex.

	***H. haema* sp. n.**	***H. coronatus***			***H. sindonis***			
	Present study	**Present study**	**Temminck and Schlegel (1850)**	**Jordan and Snyder (1901)**	**Lourie et al. (1999)**	**Present study**	**Jordan and Snyder (1901)**	**Lourie et al. (1999)**
N	140	28	5	–	7	14	1	6
SL (mm)	15.9–113.9	24.1–133.0	?–127.0	90.0–115.0	–	30.9–108.3	38.0	–
Counts								
TrR	10	10	–	10	10	10	10	10
TaR	35–38 (36)	37–40 (39)	–	38–40	38–40 (39)	35–38 (36)	37	36–38 (37)
DsR	2 + 0, 2 + 1	2 + 0, 2 + 1	–	2 + 1	2 + 0	2 + 0, 2 + 1	2 + 0	2 + 1
D	11–14 (13)	12–15 (14)	–	13–14	14	11–15 (12)	15	11–15 (12)
A	4	4	–	–	–	4	–	–
P	10–13 (12)	10–13 (12)	–	11	12	11–14 (11)	14	12–14
CS	1	1	–	–	1	1	–	1
ES	1–2 (1)	1	–	–	1	2	2	2
WS	1	1	1	–	1	0	–	0
CoT	4	4	4	–	–	5	–	–
Measurements								
% HL								
CHGO	22.7–41.6 (32.2)	43.0–60.1 (51.6)	44.4	42.9	–	26.8–41.0 (33.9)	35.7	–
CHMC	34.1–54.9 (44.5)	55.7–79.0 (67.4)	–	–	–	36.3–55.4 (45.9)	–	–
SnL	28.8–49.0 (38.9)	35.6–44.2 (39.9)	44.4	42.9	40.0–43.4 (41.7)	28.7–37.2 (33.0)	35.7	30.3–35.7 (33.0)
% SnL								
ED	27.1–68.9 (48.0)	32.3–62.9 (47.6)	–	33.3	–	41.5–69.0 (55.3)	57.1	–
% TrL								
HL	57.3–88.7 (73.0)	56.6–71.3 (64.0)	–	60.0–66.7 (63.4)	–	57.2–80.1 (68.7)	75.0	–
% TaL								
TrL	37.4–57.2 (47.3)	42.6–64.5 (53.6)	–	50.0–71.4 (60.7)	–	38.3–52.1 (45.2)	50.0	–

N (number of samples), SL (standard length), TrR (trunk rings), TaR (tail rings), DsR (rings supporting dorsal fin), D (dorsal fin rays), A (anal fin rays), P (pectoral fin rays), CS (cheek spine), ES (eye spine), WS (wing-tip spine on dorsal fin base), CoT (tips on corona flat), HL (head length), CHGO (coronet height from gill opening), CHMC (coronet height from mid-point of cleithral ring), SnL (snout length), ED (eye diameter), TrL (trunk length), TaL (tail length). Bracket represents mode in counts and median in measurements

1375, 1, 82.0 mm SL, 6 Sep 1964. KPM-NI 7301–7302, 2, 110.5–117.9 mm SL, depth 4 m, 12 Jul 2000; KPM-NI 7535, 1, 124.1 mm SL, 19 Dec 2000, S. Gosho; KPM-NI 7718–7720, 3, 113.1–115.6 mm SL, depth 1–12 m, 18 Jan 2001, S. Gosho; KPM-NI 8075, 1, 115.3 mm SL, depth 3–4 m, 26 Jul 2001, K. Uchino & D. Kanbayashi; in front

Table 3. Frequency distribution of meristic counts among species within the *Hippocampus coronatus* complex. Holotypes and lectotypes are marked by an asterisk.

	Tail rings						
	35	**36**	**37**	**38**	**39**	**40**	***N***
Hippocampus haema sp. n.	17	53*	50	18			138
H. coronatus			1	9*	15	3	28
H. sindonis	4	4	4*	2			14
	Dorsal fin rays						
	11	**12**	**13**	**14**	**15**		***N***
H. haema	1	22	89	28*			140
H. coronatus		1	6	18*	3		28
H. sindonis	1	8	1	3	1*		14
	Pectoral fin rays						
	10	**11**	**12**	**13**	**14**		***N***
H. haema	6	45	65	24*			140
H. coronatus	2	4	18*	4			28
H. sindonis		7	5	1	1*		14

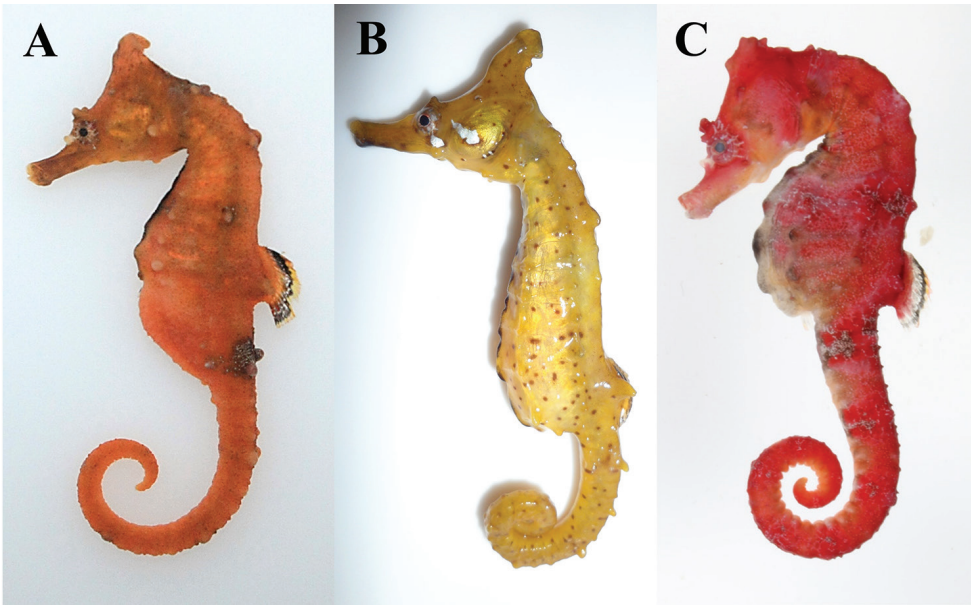

Figure 4. Coloration of fresh specimens. **A** *Hippocampus haema* (paratype, PKU 9424) **B** *H. coronatus* (FAKU 137351) **C** *H. sindonis* (FAKU 137339).

of Misaki Marine Biological Station, The University of Tokyo, Aburatsubo Bay, Koajiro, Miura, Kanagawa. KPM-NI 14854, 1, 24.1 mm SL, in front of Keikyu Aburatsubo Marine Park, Koajiro, Miura, Kanagawa, T. Mukai. KPM-NI 19270, 1, 113.7 mm SL, Cape of Manazuru, Obaradai, Yokosuka, Kanagawa, 1 Jul 2000, T. Yokoo. KPM-NI 19272, 1, 108.5 mm SL, Kannonzaki, Tatara-hama, Obaradai, Yokosuka, Kanagawa, 12

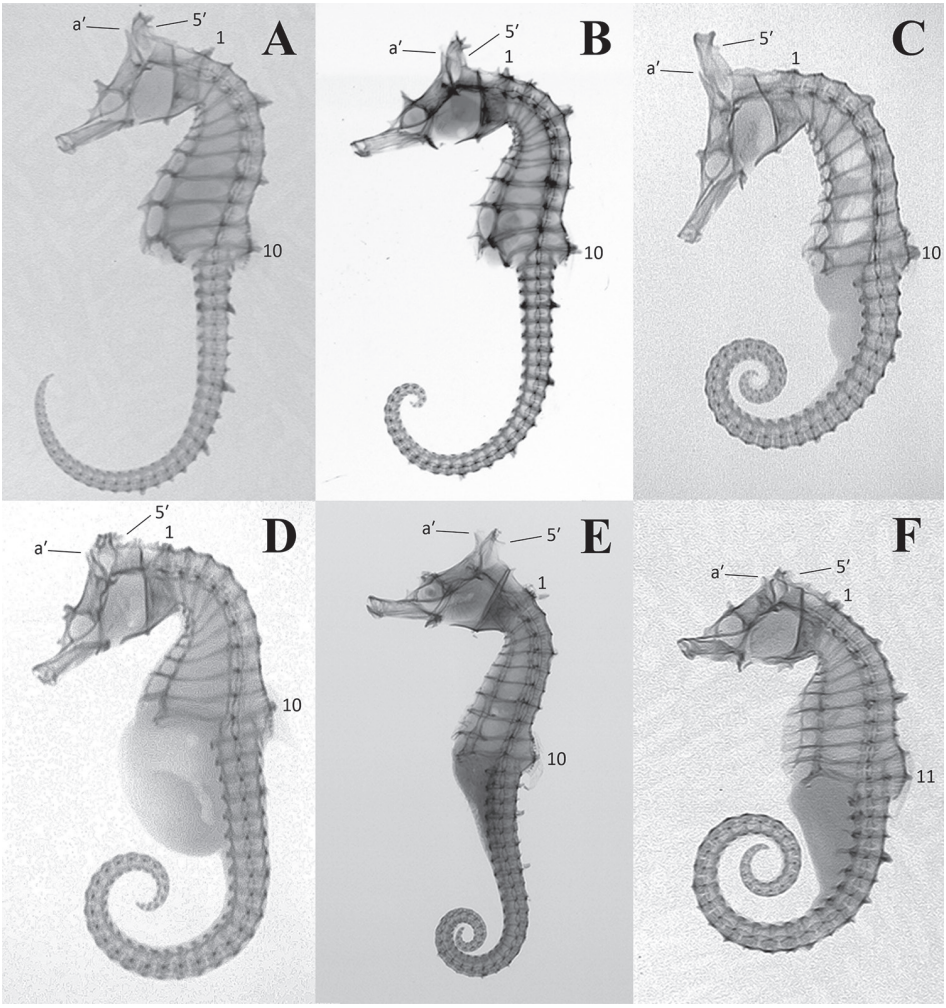

Figure 5. X-radiographs of *Hippocampus* specimens. **A** *H. haema* PKU 9641 (holotype) **B** *H. haema* NI-BR-P 5412 (paratype) **C** *H. coronatus* FAKU 137348 **D** *H. sindonis* FAKU 137340 **E** *H. sindonis* USNM 49730 (holotype) **F** *H. mohnikei* FAKU 135643. a' indicates the anterior coronet spine; 5' indicates the posterior coronet spine (the 5th tip on the corona); the first (1) and last (10 or 11) trunk rings are marked.

Dec 1998, T. Yokoo. KPM-NI 18765, 18772, 2, 27.8–28.4 mm SL, 14 Jun 2006, Y. Miyazaki; KPM-NI 21540, 1, 39 mm SL, 6 Jul 2003; KPM-NI 21541, 1, 53.4 mm SL; 19 Jun 2004; KPM-NI 25371, 1, 103.5 mm SL, depth 7 m, 27 Jun 2009, S. Shimizu; in front of Tateyama Station of Field Science Center, Tokyo University of Marine Science and Technology, Banda, Tateyama, Chiba. KPM-NI 27901–27903, 3, 51.4–67.5 mm SL, 2–6m depth, 5 Oct 2010, N. Takeuchi; KPM-NI 29380, 1, 47.8 mm SL, depth 2–6 m, 3 Jun 2011, N. Takeuchi; Gouchome, Shimoda, Shizuoka. KPM-NI 30596, 1, 133.0 mm SL, Sagami bay, Kanagawa Hadano High School, Kanagawa.

Diagnosis. A species of *Hippocampus* having a bony body; double gill openings; ring (R: TrR + TaR) 10 + 37–40, mode 10 + 39 (lectotype: 10 + 38); extremely high

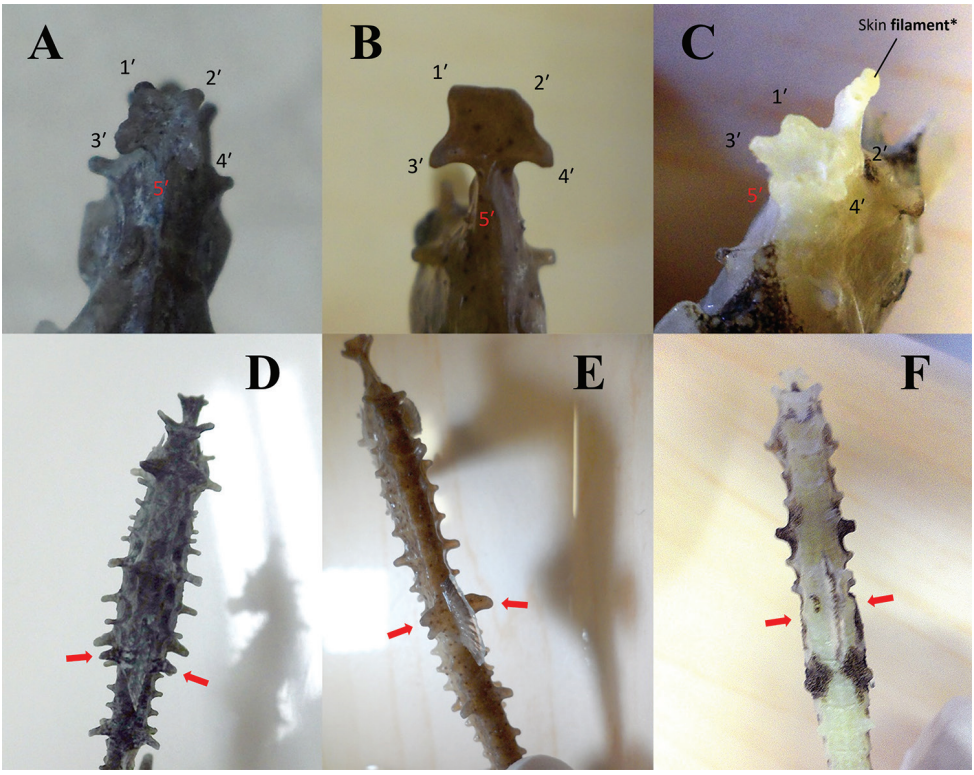

Figure 6. Distinctive morphological characters among species within the *Hippocampus coronatus* complex. **A–C** Tips on the corona flat **A** *H. haema* (PKU 9641, holotype) **B** *H. coronatus* (KPM-NI 7720) **C** *H. sindonis* (KPM-NI 19797). Numbers indicate coronet tips; the 5th coronet tip (posterior coronet spine) is indicated in red. The * indicates the appendage growing on the anterior coronet spine, which is a skin filament **D–F** Dorsal fin base spines (red arrows; wing-tip spines in **D** and **E**) **D** *H. haema* (PKU 9641, holotype) **E** *H. coronatus* (KPM-NI 7720) **F** *H. sindonis* (KPM-NI 19797).

coronet, straight or inclined backwards; CoT 4; CHGO 43.0–60.1 % HL; CHMC 55.7–79.0 % HL; WS thick and recurved.

Description. Head and trunk folded at approximately right angle; snout elongated and fused; pelvic and caudal fins absent; prehensile tail; D 12–15, mode 14 (lectotype: 14); A 4; P 10–13, mode 12 (lectotype: 12); D always greater than or equal to P; CS 1; ES 1; SnL 35.6–44.2 % HL; ED 32.3–62.9 % SnL; HL 56.6–71.3 % TrL; TrL 42.6–64.5 % TaL; flat and smooth skin generally covering armor-plated body; ACS degenerative; Coa expanded; CoT 4 arising from degenerative PCS; WS two fused LTrRDS (lower more developed than upper and recurved; upper LTrRDS occasionally standing out [Fig. 6E]); dorsal and lateral spines more prominent on 1st, 4th, 7th, and 10th TrR than on other TrRs, except occasionally for lateral spines on 10th TrR, occasionally; usually no skin filaments on body, but, occasionally, a strand was observed on ACS or on the forward part of Coa; blunt (or absent) body spine; often whitish radial blotches from iris to surrounding eye and striped-pattern body; occasionally semicircular band present on dorsal fin; variable color, light to dark red-brown or yellow, sometimes showing numerous thin whitish striations and/or dark small dots

along body; male brood pouch sometimes speckled with fine white and dark spots (Kuiter 2009); no particular sexual dimorphism, apart from male brood pouch.

Distribution. Southeastern coast of Honshu (Japan), from Izu Peninsula (Shizuoka Prefecture) to Boso Peninsula (Chiba Prefecture) (Fig. 1). *Hippocampus coronatus* lives in weed habitats, especially in floating *Sargassum* (Kuiter 2009; Senou 2013), within shallow areas (0–20 m depth).

Etymology. The Latin word *coronatus* means crowned. The new Korean name, *Wanggwan-haema* means 'crowned seahorse', in agreement with the English and scientific names. In fact, *Haema*, which has the connotation 'common' and 'fish species belonging to the genus *Hippocampus*' in Korean, has been used to name seahorses commonly found in Korea, whereas *Wanggwan-haema* has been informally used to refer to *H. coronatus* in Korean. In addition, the word *wanggwan* [crown] is more suited for *H. coronatus*, whose coronet is considerably higher than that of *H. haema*. The Japanese name *Tatsu-no-otoshigo* literally means 'dragon's bastard child'.

Remarks. Temminck and Schlegel (1850) described *H. coronatus* based on five specimens. Boeseman (1947) designated one of these specimens RMNH.PISC.D 1543 as the lectotype. As a consequence the other three specimens RMNH.PISC.D 1541, RMNH.PISC.D 1542, and RMNH.PISC.D 1544 became paralectotypes, except that RMNH.PISC 3924 was reidentified as *H. mohnikei* (see remarks of *H. sindonis* below). However, two of the specimens described in Boeseman (1947), RMNH.PISC.D 1541 and 1542, have a moderately high coronet, not agreeing with the *H. coronatus* described in the present study and being more similar to *H. haema* (see species description below). The lectotype RMNH.PISC.D 1543 and the paralectotype RMNH.PISC.D 1544 have an extremely high coronet, which agrees with the present description of *H. coronatus*. Our 28 specimens have an extremely high coronet, a wing-tip spine on the dorsal fin base, and CoT 4, as described and illustrated in Temminck and Schlegel (1850). The phylogenetic trees obtained in the present study also support the differentiation of these 28 specimens from *H. sindonis* and *H. haema* (Fig. 7).

The type series does not match Temminck and Schlegel (1850)'s description on the basis of five dried specimens and an illustration which was based on a small male seahorse (Temminck and Schlegel 1850; Kaup 1853). The lectotype (RMNH.PISC.D 1543) and the paralectotype (RMNH.PISC.D 1544) are large female seahorses (100.2–103.3 mm SL), and RMNH.PISC.D 1541, 1542, and RMNH.PISC 3924 are small female seahorses (67.5–74.0 mm SL). RMNH.PISC 3924 is preserved in spirits unlike the other specimens, therefore Boeseman's inclusion of this sample is questionable. The original illustration of *H. coronatus* from Temminck and Schlegel (1850) might be the missing fifth dry specimen (personal communication, M. van Oijen).

The type locality of *H. coronatus* has not been established. Although it is thought to be Nagasaki (Eschmeyer et al. 2017), no specific locality information is provided for the type series or in previous studies (Temminck and Schlegel 1850; Boeseman 1947; Lourie et al. 1999). Seahorses are used historically as charm for safe-birth in East Asia (Korea, Japan, and China) and as a trinket in western culture (Lourie et al. 1999; Scales 2009). Thus, we cannot exclude the possibility that dried specimens might be from someone's folkloric collection (MacLean, 1973). This historical element might support

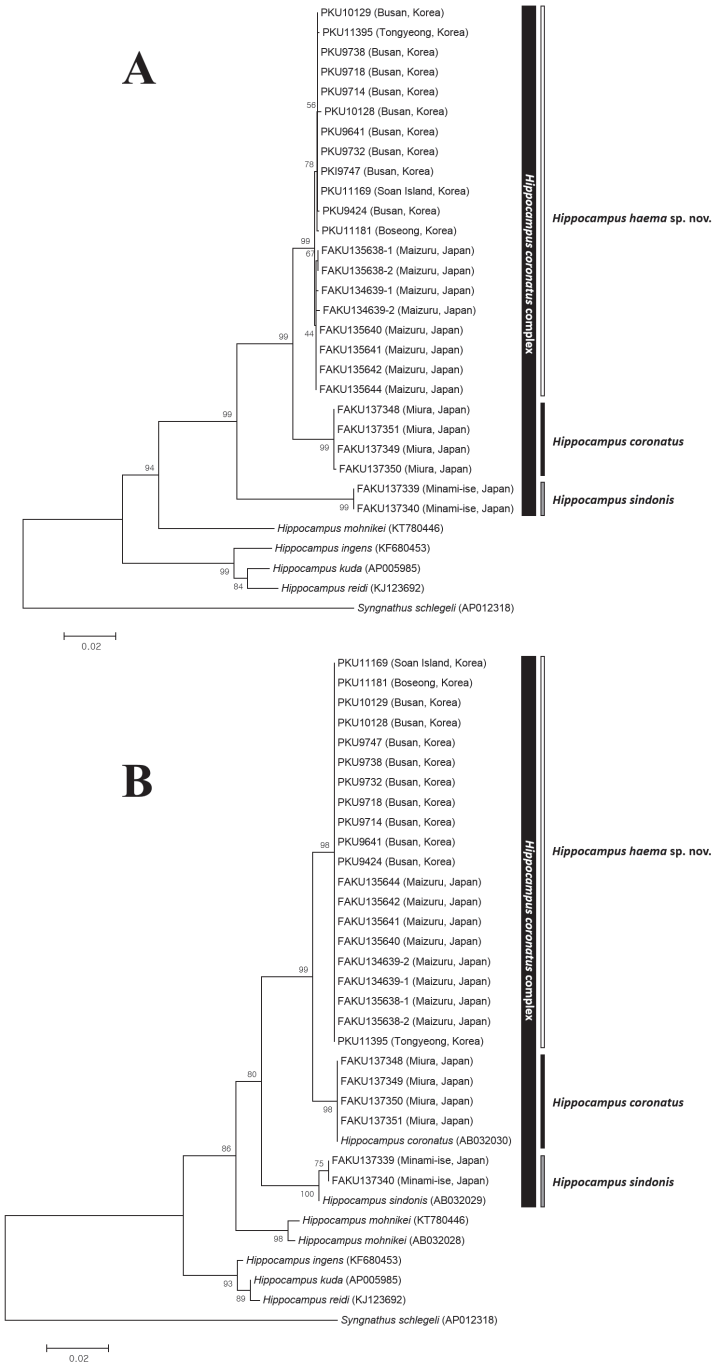

Figure 7. Neighbor-joining tree showing the relationships among species of *Hippocampus* based on mtDNA sequences. **A** tree produced using multiple loci (cytochrome *b*, 16S rRNA, and 12S rRNA) as partitions **B** tree produced using 12S rRNA, only. Numbers in branches indicate bootstrap probabilities obtained from 1000 bootstrap replications. Scale bar = genetic distance of 0.02.

that the type series was not caught in the Nagasaki area. Therefore, it is possible that collectors not only gathered specimens from Nagasaki, but Edo (present-day Tokyo) as well, which is the habitat of *H. coronatus* in this study (see Fig. 1; personal communication, M. van Oijen; MacLean 1973; Compton and Thujsse 2013; Nofuji et al. 2013).

Although *H. coronatus* sensu stricto was considered to be distributed along the coast of Japan and southern coast of Korea, we only found records from the Pacific Ocean. Mori (1928) reported *H. coronatus* off Korea for the first time, but the original data consisted only of checklists, not providing descriptions; thus, Mori (1928) might be reporting the occurrence of *H. haema* or *H. coronatus*. Therefore, the distribution of *H. coronatus* needs to be reviewed. In Korea and Japan, seahorse identification has been generally treated as a laborious task, leading to taxonomic controversy and misidentifications; thus, we recommend a careful revision of *H. coronatus* recorded from Korea and Japan.

Senou (2002) and (2013) suggested that the publication date for *H. coronatus* was in 1847. However, based on Sherborn and Jentick (1895), Boeseman (1947), Mees (1962), Bauchot et al. (1982), and Eschmeyer et al. (2017), the year should be 1850.

***Hippocampus sindonis* Jordan & Snyder, 1901**

English name: Painted seahorse, Korean name: *Sindo-haema*, Japanese name: *Hanatatsu*
Figs 3H–I, 4C, 5D–E, 6C, 6F, Tables 2–3

Hippocampus sindonis Jordan and Snyder 1901: 17, pl. 11 (Holotype: USNM 49730; type locality: Totomi bay, off Hamamatsu, Totomi Province, Shizuoka, Japan); Jordan et al. 1913: 100; Matsubara 1955: 431; Araga 1984: 89; Lourie et al. 1999: 119; Mukai et al. 2000: 139; Senou 2000: 536; Senou 2002: 536, 1508; Lourie et al. 2004: 74; Yoshino and Senou 2008: 76; Kuiter 2009: 131; Senou 2013: 635, 1911; Lourie 2016: 108; Lourie et al. 2016: 39.

Hippocampus coronatus: Burgess and Axelrod 1972: 211; Araga 1984: 89; Senou 1993: 489 (left fig.), 1294 (non Temminck & Schlegel).

Hippocampus mohnikei: Jordan and Snyder 1901: 18; Jordan et al. 1913: 98; Boeseman 1947: 196; Matsubara 1955: 431; Burgess and Axelrod 1972: 210; Araga 1984: 89 (non Bleeker).

Hippocampus japonicus: Burgess and Axelrod 1972: 211 (non Kaup).

Material examined. Japan. USNM 49730 (holotype of *H. sindonis*, photograph and radiograph from USNM), male, 49.1 mm SL, Totomi bay, off Hamamatsu, Totomi Province, Shizuoka, dredged by the U.S. Fish Commission Steamer *Albatross* (Jordan and Snyder 1901). RMNH.PISC 3924 (photograph from RMNH), 1 female, 74.0 mm SL. FAKU 121388, 1, 69.4 mm, Tanabe, Wakayama, Jan 1969. FAKU 137339, 1 93.0 mm, Hozaura, Minami-ise, Watarai, Mie, depth 20–25 m, Nov 2014, H. Sugawara. FAKU 137340, 1, 95.9 mm, Nayaura, Minami-ise, Watarai, Mie, depth 25–30 m, Mar 2014, H. Sugawara. KPM-NI 19257, 1, 59.4 mm SL, 16 May 1999, D. Sugita; KPM-NI 19258, 1, 44.4 mm SL, 18 Oct 1997, M. Kojima; KPM-NI 19259, 1, 30.1

mm SL, 5 Jul 1998, T. Kamano; KPM-NI 19261, 1, 43.3 mm SL, 7 Aug 1998, N. Ogata; KPM-NI 19262, 1, 32.2 mm SL, 25 Aug 1998, N. Ogata; Kannonzaki, Tatarahama, Obaradai, Yokosuka, Kanagawa. KPM-NI 19475, 1, 82.1 mm SL, 23 Sep 2007 K. Okubo; KPM-NI 19797–19798, 2, 75.1–99.8 mm SL, 18 Oct 2007, K. Okubo; KPM-NI 21947, 1, 75.4 mm SL, K. Okubo; Manatsuru, Ashigarashimo, Kanagawa.

Diagnosis. A species of *Hippocampus* having a bony body; double gill openings; R 10 + 35–38 (holotype: 10 + 37); coronet moderately high; CoT 5; CHGO 26.8–41.0 % HL; CHMC 36.3–55.4 % HL; a very blunt or truncated spine on the dorsal fin base; no WS on dorsal fin base.

Description. Head and trunk folded at approximately right angle; snout elongated and fused; pelvic and caudal fins absent; prehensile tail; D 11–15, mode 12 (holotype: 15); A 4; P 11–14, mode 11 (holotype: 14); D always greater than or equal to P; CS 1; ES 2 (anterior ES smaller than posterior ES); SnL 28.7–37.2 % HL; ED 41.5–69.0 % SnL; HL 57.2–80.1 % TrL; TrL 38.3–52.1 % TaL; coarse skin often covering armor-plated body; moderately high coronet; CoT, 5; body spines blunt, truncated, or absent; spines on 1st, 4th, 7th, and 10th TrR more prominent than on other TrRs, except for the lateral spine on the 10th TrR; several skin filaments on ACS and ES, and prominent TrR and TaR spines, or skin filaments absent on these structures; variable coloration on fresh specimens, including white, red, yellow, brown, and grey; variable patterns on fresh specimens, often presenting white radial blotches on iris and surrounding eye, stripes and/or blotches on body, and, occasionally, a semicircular stripe on dorsal fin; preserved specimens, black, pale white, brown, or grey; no sexual dimorphism apart from male brood pouch.

Distribution. Southeastern coast of Honshu (Japan), from Tanabe (Wakayama Prefecture) to Boso Peninsula (Chiba Prefecture) (Fig. 1). *Hippocampus sindonis* lives in a wide range of habitats, from shallow high-energy algae reefs to soft bottom habitats (Kuiter 2009), at 2–30 m depth (Senou 2013).

Etymology. The specific name *sindonis* was derived from the name of M. Sindo, an assistant curator of fishes at Stanford University (Jordan and Snyder 1901; Lourie 2016). The English name was coined by Kuiter (2009). The Japanese name *Hanatatsu* literally means *'hana* (flower or blossom, which indicates gorgeous) + *tatsu* (dragon, or the abbreviation of the word "*Tatsu-no-otoshigo*: seahorse")', and refers to the beautiful color and skin filaments of the species.

Remarks. The 14 Japanese specimens of *H. sindonis* have a moderately high coronet with five CoT, and a couple of prominently blunted or truncated spines on the dorsal fin base, therefore corresponding to the description and holotype of *H. sindonis* provided by Jordan and Snyder (1901). In the 12S rRNA tree, our *H. coronatus* specimens (voucher number: FAKU 137348–137351) appeared in the same clade as Mukai et al.'s (2000) high coronet specimen (GenBank accession number AB032030) whereas our *H. sindonis* specimens (voucher numbers FAKU 137339–137340) formed a clade with Mukai et al.'s (2000) low coronet specimen (accession number AB032029) (Fig. 7B). *Hippocampus sindonis* is considered the most external group within the *H. coronatus* complex because of its homogenous CoT (= 5) and no WS, as found in *H. coronatus* complex outgroups (e.g., *H. mohnikei* and *H. trimaculatus*).

RMNH.PISC 3924 was labeled 'Hippocampus fasciatus Kaup 1853' (Boeseman 1947), which is a nomen nudum in *Hippocampus*. Boeseman (1947) noted that RMNH. PISC 3924 was related to *H. coronatus* and *H. mohnikei*, and that its morphology agreed with Jordan and Snyder's (1901) description as well as with Bleeker's (1853) *H. mohnikei* specimens. However, we found that Bleeker's *H. mohnikei* (RMNH.PISC 7259, 3 specimens) differ from RMNH.PISC 3924 in their TrR number (11 in Bleeker's specimens vs. 10 in RMNH.PISC 3924). Thus, RMNH.PISC 3924 belongs to the *H. coronatus* complex, and its ES 2 and coronet features (moderately high coronet with 5 CoT) allow identifying it as *H. sindonis*. Jordan and Snyder (1901) stated that *H. sindonis* was distinguished from *H. mohnikei* by dorsal fin features (D 15 and long dorsal fin base in *H. sindonis* vs. D 11–13 and short dorsal fin base in *H. mohnikei*), but their key did not consider individual variations. Our *H. sindonis* specimens agree with both *H. mohnikei* and *H. sindonis* descriptions, but the paradoxical inconsistency between the original description and type series of *H. mohnikei* requires a further taxonomic review of this species, and, therefore, we compared our specimens with '*H. mohnikei*' holotype and not to the original description of the species (Lourie et al. 1999; Eschmeyer et al. 2017).

Nakamura (1999a) described a single specimen of *H. sindonis* caught off Kumamoto, Japan, which is questionable, as there are no other records of *H. sindonis* from western Kyushu. This record may have been based on *H. haema* because spines were not mentioned in Nakamura's description. Kim et al. (2013) recorded a *H. sindonis* specimen from Korean waters (voucher: NIBR-P 5412; Fig. 5B). However, the morphology of this specimen indicates that it rather belongs to *H. haema* and we include it in the type series of *H. haema*. Thus, there are no reliable records of *H. sindonis* from Korea.

***Hippocampus haema* sp. n.**

http://zoobank.org/13F12FB3-B435-4AD4-B02F-110E20C06C56

New English name: Korean seahorse, Korean name: *Haema*, New Japanese name: *Himetatsu*

Figs 3A–E, 4A, 5A–B, 6A, 6D, Tables 2–3

Hippocampus coronatus: Jordan and Snyder 1901: 19; Mori 1928: 5; Boeseman 1947: 195; Mitani 1956: 30; Chyung 1977: 272; Araga 1984: 89; Senou 1993: 489 (right fig.), 1294; Kim and Lee 1995: 76; Nakamura 1999b: 125; Senou 2000: 536; Choi et al. 2002: 141; Senou 2002: 536, 1508; Kim et al. 2005: 203; Choi et al. 2006; Yoshino and Senou 2008: 76; Kohno et al. 2011: 127; Senou 2013: 635, 1911; Han et al. 2014: 423 (non Temminck & Schlegel).

Hippocampus cf. *coronatus*: Kuiter 2009: 128.

Hippocampus sindonis: Nakamura 1999a: 124; Yoshino and Senou 2008: 76; Kim et al. 2013: 42 (non Jordan & Snyder).

Hippocampus kuda: Kim et al. 2001: 67, Myoung et al. 2002: 74 (non Bleeker).

Hippocampus sp.: Kim and Ryu 2017: 110.

Holotype. PKU 9641, 1, female, 90.3 mm SL, Namcheon Harbor, Namcheon 1-dong, Suyeong-gu, Busan, Korea, 35°08'16"N; 129°06'51"E, 9 Aug 2013, H. J. Kwun, hand net.

Paratypes. 139 specimens: specimens (74.0–99.0 mm SL). **Korea:** NIBR-P 5412, 1, female, 74.0 mm SL, off Geomun Island, Yeosu-si, Jellanam-do, depth 18 m, 17 Apr 2009, T. S. Park, SCUBA Diving & hand net. NIBR-P 1602, 1, 59.4 mm SL, Wonpo, Yeosu-si, Jeollanam-do, 27 Aug 2006, J. H. Ryu. NIBR-P 19724, 3, 58.4–71.8 mm SL, 25 Jan 2012, H. G. Cho & S. H. Lee; NIBR-P 19725–19727, 19729, 7, 33.3–102.2 mm SL, 13 Sep 2012, Y. Eun, S. Lee & S. S. Hong; Jisepo-ri, Irun-myeon, Geoje-si, Gyeongsangnam-do. PKU 6097, 1, 77.5 mm SL, 30 Aug 2011; PKU 9422–9424, 3, 80.6–92.3 mm SL, 12 Jul 2013, hand net; PKU 9704, 1, 82.3 mm SL, 1 May 2013, J. M. Lee; PKU 9705–9712, 8, 65.7–98.1 mm SL, 26 Jul 2012, J. M. Lee; PKU 9713–9717, 9719–9720, 7, 61.6–85.1 mm SL, 9 Dec 2012, J. M. Lee; PKU 9721–9723, 3, 80.8–91.5 mm SL, 20 Aug 2012, J. M. Lee; PKU 9724–9731, 8, 73.2–113.9 mm SL, 17 Jul 2012, J. M. Lee; PKU 9732–9740, 9, 62.4–100.2 mm SL, 17 Aug 2012, J. M. Lee; PKU 9741–9747, 7, 56.4–81.9 mm SL, 21 Jun 2012, J. M. Lee; PKU 9748, 1, 56.8 mm SL, 11 Sep 2012, J. M. Lee; PKU 10128–10129, 2, females, 52.2–62.7 mm SL, 23 Oct 2013, H. J. Kwun; PKU 54069–54074, 6, 32.3–77.5 mm SL, 21 Mar 2015, J. M. Lee; Namcheon Harbor, Namcheon 1-dong, Suyeong-gu, Busan, 35°08'16"N; 129°06'51"E, hand net. PKU 7230–7233, 4, 41.9–83.7 mm SL, Ulsan, 14 Sep 2012, hand net. PKU 10277, 1, 72.7 mm SL, Minrak Harbor, Millak-dong, Suyeong-gu, Busan, 35°09'14"N; 129°07'51"E, 20 Feb 2014, H. J. Yu & W. J. Lee, hand net. PKU 11159, 1, 30.9 mm SL, Hak-ri, Ilgwang-myeon, Gijang-gun, Busan, 22 Jul 2014, J. Y. Bae, hand net. PKU 11170–11180, 11, 74.2–102.4 mm SL, Soan Island, Soan-myeon, Wando-gun, Jeollanam-do, May 2014, S. Rho, bottom trawl. PKU 11181–11182, 2, 71.8–84.2 mm SL, Gunhak village, Jeonil-ri, Hoecheon-myeon, Boseong-gun, Jeollanam-do, 24 Dec 2013, S. Rho, bottom trawl. PKU 11266, 1, 74.1 mm SL, 24 Jul 2014; PKU 11634, 1, 69.9 mm SL, 25 Sep 2014; Hwayang-myeon, Yeosu-si, Jeollanam-do, hand net. PKU 11395–11401, 7, 62.3–98.7 mm SL, Jangu Island, Suwol-ri, Dosan-myeon, Tongyeong-si, Gyeongsangnam-do, Sep 2014, K. S. Han & H. D. Mun, Shrimp beam trawl. PKU 11449, 1, 81.0 mm SL, Jul 2014; PKU 11635–11637, 3, 15.9–84.7 mm SL, 24 Sep 2014; Gijang-gun, Busan, hand net. **Japan:** RMNH.PISC.D 1541–1542 (photograph by RMNH), 2, female, 67.5–68.5? mm SL, von Siebold collection. FAKU 109359, 1, 58.0 mm SL, Tassha, Sado Island, Niigata, 24 Oct 1955. FAKU 135638, 2, 82.7–88.9 mm SL, 22 Sep 2011; FAKU 135639, 2, 53.2–86.0 mm SL, 23 Aug 2010; FAKU 135640, 135644, 2, 76.2–86.8 mm SL, 29 Jul 2011; FAKU 135641, 1, 61.4 mm SL, 20 Aug 2008; FAKU 135642, 1, 57.4 mm SL, 6 Sep 2008; Maizuru Bay, Maizuru, Kyoto, Y. Kai. FAKU 136087, 1, 76.1 mm SL, Tsuruga, Fukui, 28 Jun 2014. FAKU 136119, 1, 59.2 mm SL, Kamai, Kyotango, Kyoto, 19 Jul 2014, F. Tashiro. KPM-NI 1615, 1, 91.6 mm SL, Aug 1933. KPM-NI 6770, 1, 57.9 mm SL, Azo, Tsuruga, Fukui, depth 5 m, 13 Aug 1999, T. Nomura. KPM-NI 24769, 1, female, 83.3 mm SL,

Akita, H. Sugiyama. KPM-NI 31204, 1, 47.5 mm SL, Takahama-cho, Ooi, Fukui, 2 Oct 2012, M. Mune. KPM-NI 31620, 1, 72.7 mm SL, 27 Feb 2013; KPM-NI 31707, 1, 60.6 mm SL, 11 Mar 2013; Ogurui, Takahama-cho, Ooi, Fukui, depth 7 m, M. Mune. KPM-NI 31880–31883, 4, 76.6–85.5 mm SL, depth 0–2 m, 7 May 2013; KPM-NI 36111–36112, 2, 77.1–78.6 mm SL, depth 1–3 m, 28 Apr 2014; Agurizaki Point, Ooshima, Ooi-cho, Ooi-gun, Fukui, M. Mune. KPM-NI 35122–35123, 2, 46.3–46.8 mm SL, Tanoura, Takahama, Ooi-cho, Ooi, Fukui, depth 0–1 m, 3 Jul 2013, M. Mune. KPM-NI 35291–35297, 7, 54.5–74.9 mm SL, Koda Fishing Port, Notojimakouda-machi, Notojima Island, Nanao, Ishikawa, depth 1–3 m, 2013, H. Masaki. KAUM-I 12745, 1, 100.5 mm SL, 12 Oct 2007, depth 5 m, kept in Kagoshima Aquarium and dead on 8 Dec 2008; KAUM-I 12746, 1, 96.9 mm SL, 13 Feb 2008, kept in Kagoshima Aquarium and dead on 4 Aug 2008; off Nagashima Station, Faculty of Fisheries, Kagoshima University, Usui, Azuma, Izumi, Kagoshima, M. Yamada. KAUM-I 19885, 1, male, 99.0 mm SL, off Nagashima Station, Faculty of Fisheries, Kagoshima University, Usui, Azuma, Izumi, Kagoshima, 32°13'22"N; 130°10'31"E, 13 Feb 2008, Kagoshima Aquarium, hand net, kept in Kagoshima Aquarium and dead on 30 Apr 2007.

Diagnosis. A species of *Hippocampus* having a bony body; double gill openings; R 10 + 35–38, mode 10 + 36 (holotype: 10 + 36); coronet moderately high and turned back on top; CoT 4; CHGO 22.7–41.6 % HL; CHMC 34.1–54.9 % HL; a WS on the dorsal fin base.

Description. Head and trunk folded at approximately right angle; snout elongated and fused; pelvic and caudal fins absent; prehensile tail; D 11–14, mode 13 (holotype: 14); A 4; P 10–13, mode 12 (holotype: 13); D always greater than or equal to P; CS 1; ES 1–2 (in ES 2, anterior ES smaller than posterior ES), mode 1 (holotype: 2); SnL 28.8–49.0 % HL; ED 27.1–68.9 % SnL; HL 57.3–88.7 % TrL; TrL 37.4–57.2 % TaL; often flat and smooth skin covering armor-plated body; coronet turned back on top; CoT 4 arising from degenerative PCS (5th coronet tip); WS two fused spines (lower spine more developed than upper spine, recurved; occasionally, upper spine stands out giving appearance of two dorsal fin base spines); dorsal and lateral spines at 1st, 4th, 7th, and 10th TrR more prominent than on other TrRs, except for lateral spines on 10th TrR (occasionally none or degenerative spine); Several skin filaments on body, ACS, and prominent dorsal and lateral spines on 1st, 4th, and 7th TrR; Several colors when fresh: black, white, orange, yellow, magenta, claret, brown, grey with black, red, or white stripe, and frostlike whitish or grey striations along prominent TrR and TaR; whitish radial blotches from iris to surrounding eye often present; semicircular band on dorsal fin occasionally present; when fixed in alcohol, specimens become black, white, brown, and grey; blunt (or absent) body spine; no particular sexual dimorphism except for male brood pouch. Minimum size at sexual maturity, 53.9 mm SL in males.

Distribution. Korea: southern and southeastern coasts of the Korean Peninsula (from Soan Island to Ulsan); Japan: western coast of Kyushu (western Kagoshima Prefecture), northwestern coast of Honshu (from Kyoto Prefecture to Akita Prefecture)

(Fig. 1). Lives in floating *Sargassum* and weeds on shallow soft bottom habitats from 0–18 m depth (e.g. Kim et al. 2016).

Etymology. The Korean word *Haema* means 'seahorse', which connotes 'representative' and 'common'. Thus, the scientific and Korean names *Haema* were chosen to indicate that this seahorse is the one most commonly found in Korea. The Japanese name *Himetatsu* means 'princess seahorse' or 'dwarf seahorse', and refers to its lower coronet and smaller body compared to *H. coronatus*.

Remarks. Temminck and Schlegel (1850) described the extremely high coronet as follows: coronet height (CHGO, based on the inquiry of type specimens and on Jordan and Snyder [1901]'s description) of *H. coronatus* is identical to its SnL, 1/5 shorter than remaining HL (i.e., 4/9 of HL). All *H. haema* specimens present a moderately high coronet (CHGO 22.7–41.6 % HL and CHMC 34.1–54.9 % HL) when compared to *H. coronatus* (extremely high coronet, CHGO 43.0–60.1 % HL and CHMC 55.7–79.0 % HL). Our *H. sindonis* specimens (including the holotype, USNM 49730) differ from *H. haema* in their 5 CoT and blunt or truncated LTrDS (vs. CoT 4 and WS [recurved LTrDS] in *H. haema*) (Fig. 6). The genetic distance between *H. haema* and *H. coronatus* is greater than that between species of the *H. kuda* complex (i.e., *H. kuda*, *H. reidi*, and *H. ingens*), supporting specific distinctness (Fig. 7; Table 4).

Our data also suggest the existence of two subgroups, one from Korea and another from Japan: cyt *b* sequences of *H. haema* collected in these two areas consistently present two base pairs (bp) differences (0.3%–0.8% genetic distance). Based on molecular results, *H. haema* is more closely related to *H. coronatus* than to *H. sindonis* (Fig. 7; Table 4), but based on coronet height and on the number of TaR, except for CoT and WS, it is more similar to *H. sindonis* (Tables 2 and 3).

Hippocampus haema was collected off the southern and southeastern coasts of Korea, but we were not able to collect *H. haema* off the western or northeastern coasts of Korea; only *H. mohnikei* was collected from all Korean waters. A few studies have reported *H. coronatus* from the western coast of Korea (Lee and Seok 1984; Hwang 1998; Hwang et al. 1998; Hwang et al. 2005), but these publications are mostly checklists, similar to that of Mori (1928), and *H. mohnikei* is not referred to in written records. Such inconsistency might be the result of misidentifications. The northern boundaries of *H. coronatus* in Korean waters determined in our study are similar to the distributions found by Choi et al. (2002) and Kim et al. (2005), who stated *H. coronatus* was limited to the southern coast of Korea, similarly to *H. mohnikei*. We found that the habitat of *H. haema* is affected by the Tsushima Warm Current (Briggs 1995; Nakabo 2009; Ishizu et al. 2017) and, therefore, *H. haema* might only rarely be found off the western and northeastern coasts of Korea.

Discussion

The NJ trees based on cyt *b* (670 bp), 16S rRNA (405 bp), and 12S rRNA (344 bp) recovered three monophyletic groups within the *H. coronatus* complex, all supported

Table 4. Pairwise genetic distances between *Hippocampus* species and the outgroup *Syngnathus schlegeli* based on multiple loci (cytochrome *b*, 16S rRNA, and 12S rRNA) and on 12S rRNA only. Asterisks indicate intraspecific pairwise distances calculated from one base pair difference.

Multiple loci	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Hippocampus haema sp. n. (1)	0.000–0.004							
H. coronatus (2)	0.025–0.028	0.000–0.001*						
H. sindonis (3)	0.075–0.079	0.082	0.000					
H. mohnikei (4)	0.104–0.108	0.114–0.115	0.121	–				
H. kuda (5)	0.131–0.135	0.139–0.140	0.148	0.110	–			
H. reidi (6)	0.134–0.138	0.143–0.144	0.153	0.111	0.020	–		
H. ingens (7)	0.131–0.136	0.139–0.140	0.151	0.109	0.031	0.028	–	
Syngnathus schlegeli (8)	0.241–0.244	0.247–0.248	0.251	0.232	0.217	0.219	0.231	–
12S rRNA	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Hippocampus haema sp. n. (1)	0.000							
H. coronatus (2)	0.015	0.000						
H. sindonis (3)	0.042–0.046	0.042–0.045	0.000–0.003*					
H. mohnikei (4)	0.049–0.052	0.058	0.042–0.052	0.006				
H. kuda (5)	0.074	0.074	0.055–0.058	0.039	–			
H. reidi (6)	0.074	0.074	0.074–0.078	0.049–0.052	0.055	–		
H. ingens (7)	0.068	0.068	0.071–0.074	0.046–0.049	0.055	0.009	–	
Syngnathus schlegeli (8)	0.216	0.208	0.204–0.208	0.211–0.213	0.195	0.180	0.191	–

by high bootstrap probabilities (Fig. 7): viz. *Hippocampus coronatus* group, *H. sindonis* group, and *H. haema* group. This evidence strongly supports the existence of three species, *H. coronatus*, *H.* cf. *coronatus,* and *H. sindonis*, as suggested by Kuiter (2009).

Lourie et al. (1999, 2004), based on the rings supporting the dorsal fin base (DsR), stated *H. coronatus* had '2 + 0 (TrR + TaR)' and *H. sindonis* had '2 + 1'. However, Jordan and Snyder (1901) described *H. sindonis* as '2 + 0' and *H. coronatus* as '2 + 1', which is the reverse. Moreover, all species within the *H. coronatus* complex described in the present study include '2 + 0' and '2 + 1' forms (Table 2). Thus, DsR is an inappropriate characteristic to diagnose the species studied here. *Hippocampus coronatus* has only one supraorbital spine whereas *H. sindonis* has two and *H. haema* has either one or two spines. Many ichthyologists have attempted to distinguish *H. coronatus* and *H. sindonis* based on color and skin filaments (especially Jordan and Snyder 1901). However, Curtis (2006) refuted the use of skin filaments on its key to distinguish *H. hippocampus* from *H. guttulatus*, as skin filaments grow irregularly in both species. Lourie et al. (1999) and Szabó et al. (2011) also suggested that color and skin filaments were affected by environment and/or growth, and therefore should be considered of limited diagnostic value. In the present study, several color and skin filament patterns were found in *H. haema*, which is in agreement with Mitani's (1956) data for specimens sampled from Maizuru Bay, Japan. This author interpreted these as intraspecific variations, but, given the results obtained in this study by molecular analyses, we do not agree that *H. coronatus* and *H. sindonis* should be treated as a single species.

Hippocampus coronatus is ranked as DD in the IUCN Red List due to the lack of information on its population trends and to the uncertainty of its distributions, originating from taxonomic controversies (Zhang and Pollom 2016). *Hippocampus sindonis* is ranked as Least Concern (LC) because no major threat has been reported for its distribution (Fritzsche et al. 2010). The distribution of *H. coronatus* is similar to that of *H. sindonis* (i.e., southeastern coast of Honshu, Japan), and there is no data supporting its potential threat with distribution uncertainty. However, *H. coronatus* distribution has a narrower range than that of *H. sindonis* (Fig. 1), so it is more likely to be affected by human pressure. For these reasons, *H. coronatus* will likely be ranked above or equal to *H. sindonis* after further surveys of its population trends. To improve the conservation of these species, a better taxonomic understanding is required to resolve the DD rank of *H. coronatus* regarding the uncertainty of its distribution, as well as more data on its biology, habitat, and abundance. Previous studies considering the biology of *H. coronatus* conducted on local Korean areas (Choi et al. 2006, 2012; Huh et al. 2014; Park and Kwak 2015), might, in fact, indicate the biology of *H. haema*. Overfishing could potentially threat *H. haema* due to by-catch, given the species low density and patchy distribution (Choi et al. 2012; Zhang and Pollom 2016), and its wide distribution requires the study of populations across the entire area.

Key to species of the genus *Hippocampus* in Korea and Japan

1 No lump on bony body; double gill openings; 10–11 trunk rings..... **2**
– Reddish lumps on fleshy body; single gill opening; 12 trunk rings..... ***Hippocampus bargibanti* Whitley, 1970**
2 11 trunk rings..... **3**
– 10 trunk rings..... **6**
3 Blunt spine or no spine on body..... **4**
– Sharp spine on body..... ***Hippocampus histrix* Kaup, 1856**
4 One blunt cheek spine; trapezoid-shape coronet; no dorsal spot..... **5**
– Two blunt cheek spines; moderately high triangle-shape coronet; no dorsal spot..... ***Hippocampus mohnikei* Bleeker, 1853**
– One recurved and sharp cheek spine; very low triangular coronet (degenerative coronet); three dorsal spots (on the 1st, 4th, and 7th trunk rings) but sometimes absent..... ***Hippocampus trimaculatus* Leach, 1814**
5 Wide body; 34–38 (36) tail rings..... ***Hippocampus kuda* Bleeker, 1852**
– Narrow body; 39–41 (40) tail rings..... ***Hippocampus kelloggi* Jordan & Snyder, 1901**
6 Four tips on corona flat (5th tip degenerated, and separated from the other four); wing-tip spine on dorsal fin base..... **7**
– Five tips on corona flat (5th tip developed, and combined with the other four); no wing-tip spines on dorsal fin base..... ***Hippocampus sindonis* Jordan & Snyder, 1901**

7 37–40 (39) tail rings; coronet height from gill opening 43.0–60.1 % head length; coronet height from mid-point of cleithral ring 55.7–79.0 % head length ..... ***Hippocampus coronatus*** **Temminck & Schlegel, 1850**

– 35–38 (36) tail rings; coronet height from gill opening 22.7–41.6 % head length; coronet height from mid-point of cleithral ring 34.1–54.9 % head length ..... ***Hippocampus haema*** **sp. n.**

*This key was compiled from Lourie et al. (1999, 2004), Senou (2013), Lourie (2016), and the current study data.

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SHORT COMMUNICATION

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The use of low cost compact cameras with focus stacking functionality in entomological digitization projects

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Abstract

Digitization of specimen collections has become a key priority of many natural history museums. The camera systems built for this purpose are expensive, providing a barrier in institutes with limited funding, and therefore hampering progress. An assessment is made on whether a low cost compact camera with image stacking functionality can help expedite the digitization process in large museums or provide smaller institutes and amateur entomologists with the means to digitize their collections. Images of a professional setup were compared with the Olympus Stylus TG-4 Tough, a low-cost compact camera with internal focus stacking functions. Parameters considered include image quality, digitization speed, price, and ease-of-use. The compact camera's image quality, although inferior to the professional setup, is exceptional considering its fourfold lower price point. Producing the image slices in the compact camera is a matter of seconds and when optimal image quality is less of a priority, the internal stacking function omits the need for dedicated stacking software altogether, further decreasing the cost and speeding up the process. In general, it is found that, aware of its limitations, this compact camera is capable of digitizing entomological collections with sufficient quality. As technology advances, more institutes and amateur entomologists will be able to easily and affordably catalogue their specimens.

* Shared 1st authorship

Keywords
Focus Stacking, Compact Camera, Canon-Cognysis, Mass Digitization, Entomology, Collections

Introduction

Many museums rely on the help of volunteers for collection work (Flemons and Berents 2012; Holmes 2003). One such effort is the digitization of the vast quantities of specimens in the collections (Mathys et al. 2013; Mathys et al. 2015). Although controversy exists when describing species using photographic material exclusively (e.g., Pape (2016) and Ceriaco et al. (2016) in response), a photographic inventory of collections adds to documenting biodiversity, increases accessibility for other researchers and instances, adds to increased ecological knowledge, and helps experts and students screen specimens in an affordable way (Beaman and Cellinese 2012; Garrouste 2017).

Museums usually own a small number of digital imaging systems, constraining the digitization of collections, and can barely keep up with new additions to the collections. The professional setups typically require some level of training to use and have a high cost (€ 3.000 – € 30.000, Brecko et al. (2014)). Unsurprisingly, the price tag prevents amateur naturalists and smaller museums from acquiring such a system. Consequently, the rate of digitization not only depends on the number of volunteers but also on the infrastructure available in museums or institutes. Time-saving techniques are sometimes used, for instance whole drawer imaging (e.g., Mantle et al. (2012)). These techniques have major limitations and the resulting images often lack the resolution necessary for taxonomic accuracy or it fails to capture all required information (e.g., limited angles in which the specimen was shot or specimens covering the labels, Brecko et al. (2014); Hudson et al. (2015)).

The rapid advancement in imaging technology and software over the past few years has resulted in high-quality, user-friendly and more affordable imaging systems (e.g., the focus stacking method currently used in the Royal Belgian Institute of Natural Sciences (RBINS) and the Royal Museum for Central Africa (RMCA) of which the price is approximately € 3.000 or € 1800 when excluding the pc required for post-processing, Table 1); Brecko et al. (2014)). These systems are primarily intended to digitize type specimens, produce images for publications, retain a digital back-up of specimens prior to loans, or to avoid loans altogether. They typically involve single-lens reflex (SLR) cameras with interchangeable macroscopic (producing images on a 1:1 scale or smaller) lenses which are generally too expensive for the average volunteer to invest in. Cheaper digital cameras usually do not provide the user with the flexibility nor the image quality of an SLR camera, but manufacturers often include extra features to improve their functionality. Among these is the possibility to take macroscopic images, the quality of which has improved substantially the last decade (Pratt 2015).

The applicability of a compact camera was tested in view of a small digitization project of the genus *Calligrapha* (Coleoptera – Chrysomelidae) in the Royal Belgian Institute for Natural Sciences (RBINS) in September - November 2016 (Merckx et al. in prep).

Table 1. Comparison in price (minimum prices) and processing speed of the Canon-Cognisys setup with both TG-4's stacking modes. [1a]Canon EOS 600D with 60mm EF-S f/2.8 macro lens; [1b]Canon EOS 600D with 65mm MP-E f/2.8 macro lens; [2]off-camera flashes and platform; [3]price for lifetime license of Helicon Focus Lite; [4]post-processing time depends on processor type and speed among other factors; [5]data from Brecko et al. (2014), depends on #images in stack (here: 20); [6]already has stacking included in processing time.

	Canon-Cognisys		**TG-4 manual**	**TG-4 internal**
Camera	€ 880[1a]	€ 1500[1b]	€ 350	
Stacking set-up	€ 700		N/A	
Stacking software cost[2]	€ 100		€ 100	€ 0
Lightbox cost	€ 120[3]		€ 25	
Total cost	**€ 1800**	**€ 2420**	**€ 475**	**€ 375**
#images in stack	Unlimited		29	10
Image resolution	4.3 µm/pixel		1.3 µm/pixel	1.9 µm/pixel
Time to produce image	5" per image in stack		3"	13"[6]
Post-processing time[4]	17"[5]		28"	

In this study, we assess whether a compact, (low cost) camera can replace a professional setup when it comes to digitizing entomological collections. Image quality, digitization speed, and ease-of-use were compared with the Canon-Cognisys setup and whether there are limitations to the usability of the camera.

Methods

Camera

The Olympus Stylus TG-4 Tough (TG-4) was used in this test. Several compact cameras focusing on macro functionality are available on the market; however, they either lack internal focus stacking (e.g., for a comparison with the Nikon Coolpix AW130, see Cameradecision (2017)) or are more expensive (e.g., some of the Panasonic Lumix line-up).

The camera is a rugged, dust- (IPX6) and waterproof (IPX8) outdoor camera with an in-camera focus-stacking feature. This camera generally gets good reviews in terms of its macro capabilities (e.g., Keller 2015). It has two stacking methods: internal stacking (in which the camera processes a stack of 10 pictures with a built-in stacking algorithm) and focus bracketing (in which the camera takes up to 30 pictures to form a stack that has to be processed by dedicated software afterwards, from here on referred to as 'manual stacking'). In the latter, the focal step size can be set to three options: narrow, normal and wide. The differences between these settings were tested (Suppl. material 2) but since the effects were rather marginal, the narrow setting was always used. Note that the first of the 30 pictures serves as an overview and should not be included when stacking as this will lead to artefacts in the final image. The internal

stacking function exports an 8MP picture, whereas the manual stacking function results in a 16MP picture. Both methods were compared to the setup currently in use at the RBINS, the Canon-Cognisys setup (for specifications, see Brecko et al. 2014). The tested compact camera is approximately four times less expensive than the Canon-Cognisys setup currently in use by the RBINS (Brecko et al. 2014, Table 1).

The camera's capabilities were tested using five insect specimens varying in size and colour. Its image quality was compared with that of the professional setup, assessing image sharpness and level of detail and presence of stacking artefacts. In addition, distance to lens, zoom level and stacking method were altered.

Specimen choice

Specimens from the genera *Aplagiognathus* (Coleoptera - Cerambycidae) and *Elytrimitatrix* (Coleoptera - Cerambycidae) were selected. The *Aplagiognathus* specimen was chosen for its larger size (length: 4.9 cm, width: 1.8 cm, height: 1.6 cm), uniform colour and microsculpture. The *Elytrimitatrix* specimen (length: 2.5 cm, width: 0.7 cm (2.3 cm including antennae), height: 0.5 cm) was chosen for its hairy abdomen, which often poses a problem when stacking (Brecko et al. 2014). Additionally, picture quality was assessed on images of *Polistes dominula* (Hymenoptera - Vespidae), *Forficula auricularia* (Dermaptera - Forficulidae) and *Archips podana* (Lepidoptera - Tortricidae) to test the applicability of the camera on a range of taxonomic groups.

Lightbox and stacking software

Our own lightbox design was used, specifically made to be used with the compact camera. The body consists of a cylindrical plastic container with a hole on top that fits the lens of the camera. Inside, the top of the cylinder is lined with 59 12V, dimmable, white LED lights, covered by tracing paper to reduce light reflection on the specimens (Suppl. material 1).

Manual stacking was initially performed using the free software package CombineZP (http://alan-hadley.software.informer.com). A recent review showed that this software package underperforms in comparison with commercial packages like Helicon Focus (http://www.heliconsoft.com/heliconsoft-products/helicon-focus/) and Zerene stacker (http://zerenesystems.com/cms/home), mostly when complex structures like hairs are involved (Brecko et al. 2014). Problems with stacking (i.e., artefacts) were also encountered by us, and therefore switched to Helicon Focus as stacking software. The Helicon Focus software has a two-week free trial after which one has to pay for a lifetime license to the 'lite' package or the Pro package respectively, the latter adding more functions including retouching tools and batch mode, which can greatly improve the digitization workflow (e.g., stacking a large batch of images overnight).

Tested settings

To ascertain the compact camera's performance and to find the optimal position of the specimens, firstly the two specimens of longhorn beetles (*Aplagiognathus* and *Elytrimitatrix*) were photographed. The camera's two stacking methods, internal and manual stacking, were visually assessed and compared to macro-photographs of these specimens from the Canon-Cognisys setup. Next, the object-lens distance (11–5 cm, with 2 cm increments) and optical zoom (1–4 times) were altered to find an optimal set of parameters. Finally, pictures of *Archips podana*, *Polistes dominula* and *Forficula auricularia* (shot in manual stacking mode with specimens at an optimal distance from the lens) were visually assessed as well, to explore applicability in a wider taxonomic range.

Results

Manual stacking, internal stacking, and professional setup

A comparison of the two stacking settings (internal and manual stacking) with the professional setup can be seen in Figure 1. The picture of the latter retains its sharpness towards the edges (Figure 1B), whereas images made by both stacking methods of the compact camera (Figure 1C–D) are less sharp there. Despite the softer edges of the camera's images, the fine setae are clearly visible regardless of the stacking method. The image quality is also influenced by the positioning of the insect, the type and intensity of light and, most importantly, the stacking algorithm and software used. All three pictures conserve plenty of detail, generally sufficient for taxonomic screening. All features such as setae, elytral and prothoracic punctures, folds and dimensions can be distinguished properly, regardless of the method used. The compact camera and the professional setup are comparable in terms of usability when used for taxonomic studies.

Assuming the handling time to position the specimen is similar in all situations, the time required to finish one stacked image differs more among methods (Table 1). The Canon-Cognisys setup requires an average of 5 seconds to take one image in the stack. The compact camera we tested is faster, requiring 3 seconds to produce a complete stack of 29 pictures (ISO-100; f/2.3–4.9, depending on optical zoom, focal length: 6–18 mm) in manual mode. In both cases, off-camera image stacking is required to attain the desired result. The speed at which images are stacked strongly depends on the software package and the processing power of the computer; using the Helicon Focus software package and a Dell Latitude E5570 (i7–6820HQ Intel core processor and 8GB RAM) in all comparisons, processing a set of 29 images required on average 28 seconds. In the internal stacking mode, approx. 13 seconds are needed to make and process the final stacked picture.

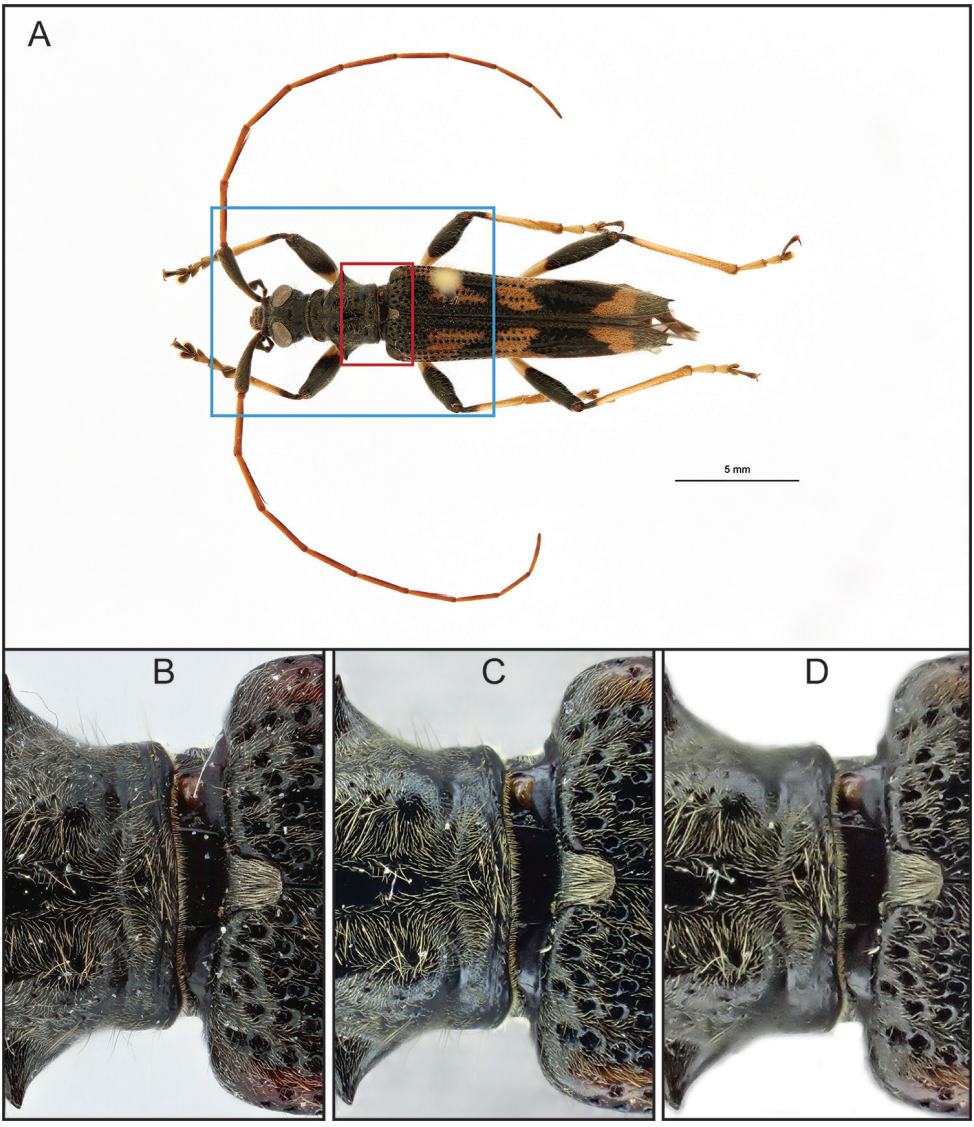

Figure 1. Comparison of the *Elytrimitatrix* digitized with the professional setup (**A** shot with the 60 mm macro lens and **B** with the Canon MP-E 65 mm lens), the compact camera's manual focus stacking mode (**C**) and internal stacking mode (**D**). **A** depicts the whole specimen as would be shot for publication purposes. The red box indicates the section shown in **B, C, D** and the blue box indicates how the specimen was framed in these three images. Note that the stronger reflections in **C, D** are the result of a different lighting setup.

Zoom versus object-lens distance

To assess any noticeable reduction in sharpness when altering the optical zoom, sample pictures at four levels of magnification were taken. No so-called 'sweet spot' (optimal zoom range of a lens) at a certain zoom level could be observed (Figure 2). The in-

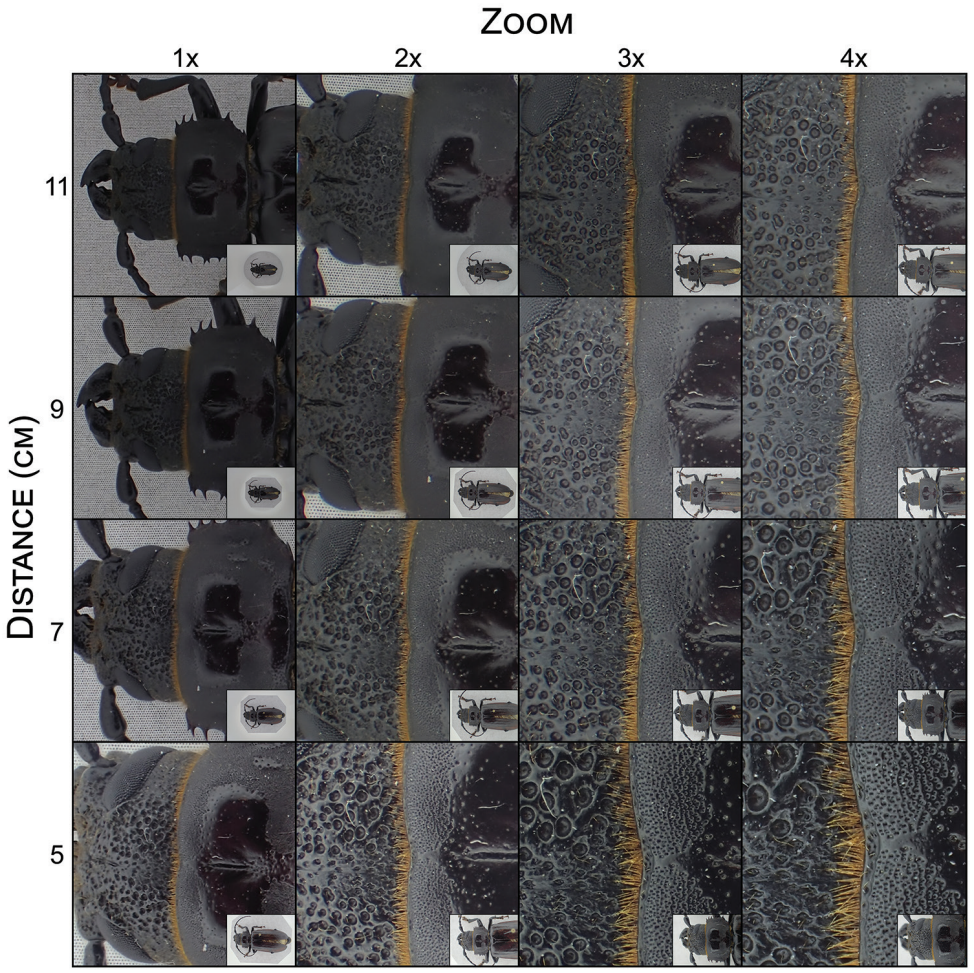

Figure 2. Visualization of the variation in image quality, level of detail and proportion of the specimen fitting the frame (insets) at different levels of optical magnification (1–4 times) and distance from the lens (11–5 cm). Every image, shot with the compact camera, is composed of 29 manually stacked images at the narrow setting and cropped to equal dimensions (approx. 1/24 of the original image). Quality and detail improve as lens distance decreases and/or the zoom increases at the cost of reduced depth of field and a smaller portion of the specimen fitting the image frame.

creased magnification does reduce the focus depth (depth of field) of the stacked image, relative to a fully zoomed-out image. This affects sharpness along the edges of the head and prothorax. However, as the specimen is placed closer to the lens, up to the minimum focus distance of 1 cm (not shown in Figure 2), the effect on the depth of field is less pronounced; the camera focuses on a point closer to the lens, but the individual distance between every image in the stack remains the same. Larger specimens, such as the *Aplagiognathus* species in Figures 2 and 3, do not fit the frame at higher zoom or closer proximity to the lens. This is where the professional setup outperforms

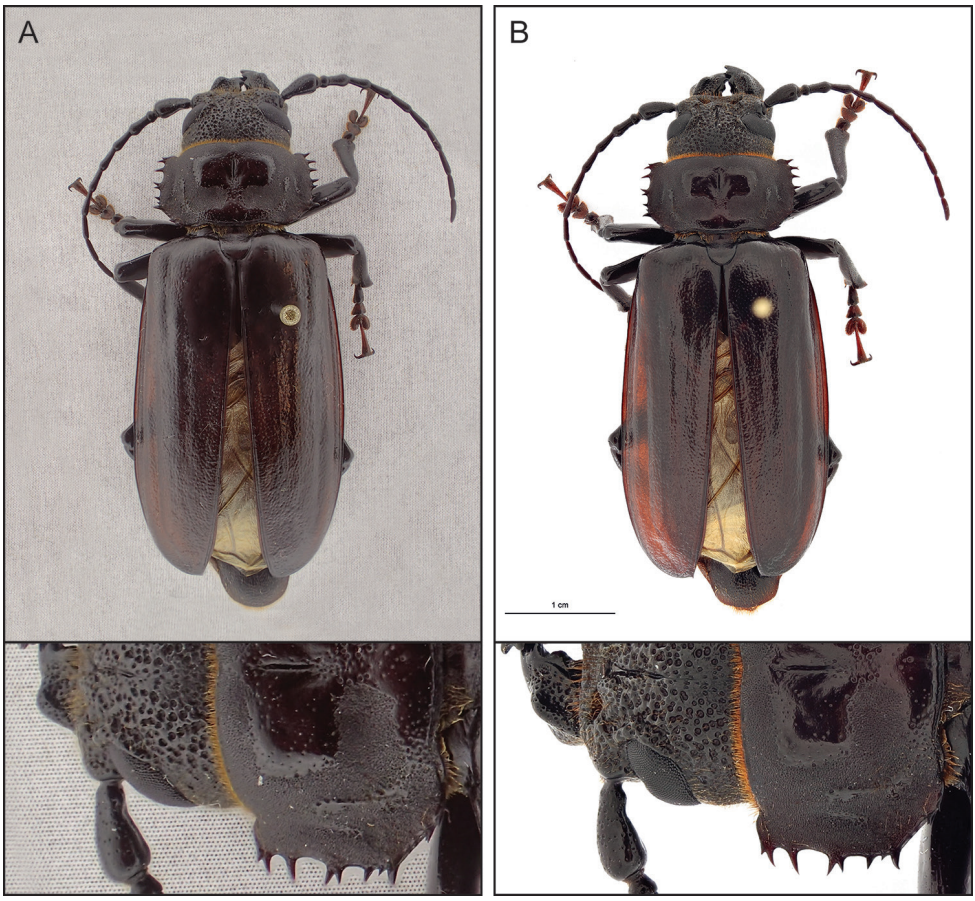

Figure 3. Comparison of image quality between the compact camera (**A**) and the professional setup (**B**) with the specimen occupying the same proportion of the frame. A detail is shown below. The compact camera was set up 5 cm from the specimen with the optical zoom at 1×, 29 images (narrow setting) were manually stacked. The professional setup outperforms the compact camera, producing a sharper image when specimens larger than a few centimetres are set to fill the frame optimally.

the compact camera; producing a sharp image of the specimen as a whole and retaining more detail than a similar image shot with the compact camera (Figure 3). Moreover, the professional setup has the functionality to take images within a specific focus range, alter the step size between every image in the stack, and exchange lenses according to the specimen's size. As a consequence, a larger range of specimen shapes and sizes can be photographed without loss of quality and resolution.

Applicability in a wider taxonomic range

Figure 4 shows manually stacked images of three non-Coleopteran insects, shot by the compact camera (manual mode, narrow setting). In general, picture quality is compa-

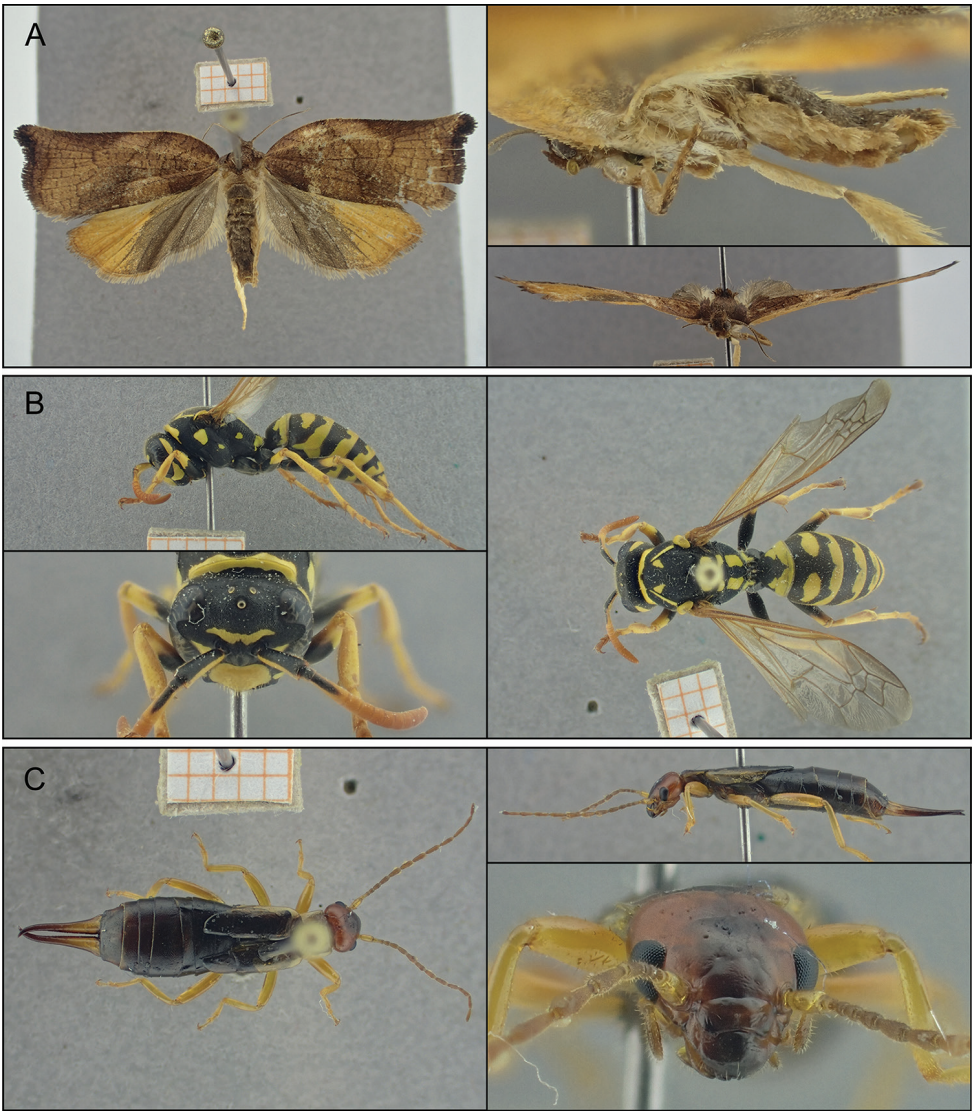

Figure 4. Images of different taxonomic groups, shot by the compact camera in manual mode (narrow setting). **A** large fruit-tree tortrix (*Archips podana* (Lepidoptera - Tortricidae)) **B** European paper wasp (*Polistes dominula* (Hymenoptera - Vespidae)), and **C** common earwig (*Forficula auricularia* (Dermaptera - Forficulidae)).

rable to the results shown above. The images tend to be less sharp further away from the centre, where the camera was focused. This is likely a combination of reduced corner sharpness (an optical limitation present in most lenses) and subsequent imperfect stacking of these less sharp regions of the image. Additionally, some patches of the wings in the micro-moth (Figure 4A) are less sharp than neighbouring areas. These imperfections are likely related to a combination of the relatively large distance between individual images of a stack and the limited number of pictures within a stack.

Discussion

Internal stacking versus manual stacking

The internal stacking and the manual stacking mode of a compact camera were compared with a professional museum imaging setup. We found that in terms of picture detail and centre sharpness, the compact camera's images are often comparable to the professional setup when it comes to image quality. However, pictures shot with the first, likely due to its limiting 10 (internal stacking mode) or 29 (manual stacking mode) images per stack and limited options defining the focal distance between each image ("wide", "normal" and "narrow"), were more prone to local loss of focus (e.g., along the edges). The latter is especially clear when the object of interest spans the whole frame. The narrow setting results in marginally sharper images, barely noticeable in areas with more depth. However, due to the limited focus range, extremities (i.e., legs and antennae), and 'deeper' parts of the body fall out of focus. This can be alleviated by focusing exactly in the middle (i.e., mid-depth) of the specimen, for example more towards the head instead of the highest point of the abdomen. The normal setting usually solves this problem, broadening the focus range sufficiently to include the whole specimen. The professional setup is more versatile as its number of images in a stack can be adjusted, based on a predefined focus range and step size. Decreasing the step size results in a smoother transition from slice to slice and setting the focus range ensures the fore- and background to be out of focus. Therefore, the professional setup can provide a sharp image across the whole specimen, regardless of its shape or size.

The relatively small sensor size of the TG-4 (6.17 mm × 4.55 mm), when compared to any SLR camera (e.g., Canon APS-C: 22.3 mm × 14.9 mm), is unable to capture the amount of detail the professional setup can and, together with the limited number of images in a stack, can result in a less detailed image with parts of the frame being less sharp, especially when framing a large specimen (i.e., fully zoomed out and more distant from the lens). Nevertheless, the images shot by the compact camera retain key taxonomic features such as hairs and punctures. Additionally, the above-mentioned stacking imperfections are often corrigible in the stacking software. This, however, requires the user to select manually which parts of one slice should be used in the final stacked image, increasing the processing time per stacked image.

When comparing the internal and manual stacking, it was found that a sharper image is achieved in manual stacking mode. This result is influenced by several factors, including the higher number of pictures in a manual stack (29 versus 10 in internal mode), the higher image resolution to 16MP (instead of 8MP) and the possibility to adjust focus range from narrow to wide. We should note, however, that the quality of manually stacked pictures also depends on the capabilities and limitations of the stacking software. Results varied when stacking the same batch of images in the freely available CombineZP software after which we opted to use the professional Helicon Focus software. Even though manual stacking is more time consuming (28 seconds per stack versus 13 seconds with internal stacking), most of this work can easily be batched in the stacking software and ran without user interaction using the Helicon

pro license. The time spent transferring, organising, and labelling files onto the computer to prepare for Helicon's stacking depends on the number of images and can easily add several minutes to the process. Another advantage of the stacking software, are the options to fine-tune several stacking algorithm parameters like smoothing and radius (http://www.heliconsoft.com/helicon-focus-main-parameters/) to improve the final image quality.

Apart from technical aspects, internally stacked pictures can easily be checked for incorrect focus on the camera's LCD screen whereas errors in manually stacked pictures due to some parts of the specimen being not in focus are usually only discovered after processing. We would only recommend the internal stacking mode when no workstation and/or sufficient hard drive space are available (i.e., 29 image slices of one specimen can take up to 100Mb unstacked and 5Mb when stacked, whereas an automatically stacked image usually takes up below 2Mb).

Zoom versus object-lens distance

The optical zoom did not substantially affect image quality. The feature that mattered most was the distance to the lens; the smaller the distance between the specimen and the lens, the more details could be discerned (e.g., punctuation, hairs). Nevertheless, there is a subtle functional difference between zoom versus distance to lens. Increasing the optical zoom slightly compresses the image stack, resulting in a smaller focus range. Zooming in is therefore practical when capturing details (e.g., microstructures and small setae) but less so when framing a specimen that requires more focus depth (e.g., a frontal view or legs stretching down far below the specimen's body). Consequently, it is recommended to position such specimens closer to the lens instead of zooming in to profit from the larger focus range, the opposite is true for small specimens. Even though the focus compression effect is small, it is easy to take into account when positioning the specimen and might help retain more details in the stacked image. It could also prove to be helpful to adjust the focal step size, where a narrow step size often generates marginally better results, but could miss some parts of bigger specimens whereas a normal or wide setting wouldn't.

One other drawback of using the compact camera tested in this study on larger specimens is the trade-off between detail and a full view of the specimen (Figure 3). Taking images of large specimens with the highest possible quality (in terms of detail) is impossible unless several pictures, taken by moving the camera above the object, can be 'stitched' together (using so-called micro panorama software). This would require more processing time and could again decrease the overall image quality due to misalignments. In practice, however, we found that this procedure is unfeasible; at such close distances, parallax differences cause large shifts of objects closer to the lens compared to more distant ones, making it impossible for the program to stitch images together. In this respect, we conclude that the setup is perfect for small specimens with a maximum of around 1–2 cm in length, but leads to decreased quality for bigger specimens because of the greater distance from the lens required to fit the specimen in the frame.

Taxonomic range

In light of applicability to a wider taxonomic range than just Coleoptera, we tested the manual stacking mode to three other specimens: a European paper wasp (*Polistes dominula* (Hymenoptera - Vespidae)), a common earwig (*Forficula auricularia* (Dermaptera - Forficulidae)) and a large fruit-tree tortrix (*Archips podana* (Lepidoptera - Tortricidae)). In general, the resulting pictures are of good quality and detail. Some errors can remain, however, due to the limited focal step size adjustability, for example a slight tilt of the wing in Lepidoptera can cause certain parts to be out of focus. For large specimens or large-winged insects, this might pose an inconvenience. Note that these specimens surpassed the 'ideal' range of 1–2 cm. Additionally, the typical reduction of image sharpness towards the corners might influence the optimal positioning of a specific specimen. We recommend to always evaluate this beforehand.

Conclusions

When it comes to digitization of entomological collections, it seems that compact camera models such as the TG-4, used in this study, cannot out-compete professional imaging systems such as the Canon-Cognisys setup. This is in part due to the limited number of images in a stack and lower versatility when it comes to specimen dimensions. In situations where higher quality images are preferred (e.g., type material), specimens should be digitized with a professional, high quality setup. Nevertheless, compact camera models are a valuable addition to the professional setup for rapid specimen digitization. The ease of use and affordability could help reduce the digitization backlog of large museums or be the primary means to digitize specimens of personal collections or smaller institutes. This camera performs best for small specimens (around 1–2 cm) because they can be positioned closer to the lens without falling out of frame or reach the camera's minimum focus distance. The manual stacking function, with 29 images, generates the best results, but has a significantly longer (post-)processing time. The latter can however be avoided by investing in a professional stacking software package with batching functionality. We do not recommend using the automatic stacking mode unless no workstation with stacking software or sufficient hard drive space is available. It generates a lower quality image; however, depending on the taxonomic group, it should still show key taxonomic features with sufficient detail to be useful to experts.

Trade-offs aside, budget compact cameras are constantly improved upon, including their macro capabilities and functions. The emergence of focus stacking features is an important step towards affordable professional-grade macroscopic images. Consequently, digitization of insect specimens has become affordable for most people and institutes. The internal stacking function could eliminate the cost of a dedicated stacking program and further costs (i.e., lightbox) are negligible. Together with a good volunteer program, a combination of a professional setup for type specimen digitization and compact cameras with focus stacking functionality could drastically speed up digitization efforts in an affordable way.

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Supplementary material 1

Figure S1

Authors: Jan E.J. Mertens, Martijn Van Roie, Jonas Merckx, Wouter Dekoninck

Data type: PNG File (.png)

Explanation note: Lightbox setup, the camera rests on top of the cut-off bucket, its lens protruding though the hole in the middle. The specimen is usually shielded from direct light by a free-standing cylinder of tracing paper (not depicted). The LED strips on the inside are powered through a 12V adapter.

Link: https://doi.org/10.3897/zookeys.712.20505.suppl1

Supplementary material 2

Figure S2

Authors: Jan E.J. Mertens, Martijn Van Roie, Jonas Merckx, Wouter Dekoninck

Data type: JPG File (.jpg)

Explanation note: Comparison of the narrow (**C, F**), normal (**A, B, D, G**) and wide (**E, H**) focal step size in two specimens of different 'depth' (**A, C–E**: *Allochroma* sp., 2 mm deep; **B, E–H**: *Doryphora* sp., 12 mm deep, measured from top of elytra to lowest tarsi). The narrow setting is marginally sharper in some areas; however, deeper parts of the specimen are not in focus. The wide setting produces artefacts around some of the edges, sometimes resulting in less sharp regions.

Link: https://doi.org/10.3897/zookeys.712.20505.suppl2