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Three new species of the leafhopper genus *Dayus* Mahmood from China (Hemiptera, Cicadellidae, Typhlocybinae, Empoascini)

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Abstract

Three new species of the Oriental empoascine leafhopper genus *Dayus* Mahmood are described from China: *D. bifurcatus* **sp. n.**, *D. trifurcatus* **sp. n.** and *D. serratus* **sp. n.** A key to distinguish all Chinese species of the genus is provided.

Keywords

Auchenorrhyncha, leafhopper, taxonomy, morphology

Introduction

The Oriental typhlocybine leafhopper genus *Dayus* was established by Mahmood in 1967 with *D. elongatus* Mahmood (Singapore) as its type species. Subsequently, Dworakowska (1971) described *D. takagii* Dworakowska (Japan) and transferred *D. upoluanus* (Osborn, 1934) (Western Samoa) and *D. euryphaessus* (Kirkaldy, 1907) (Fiji) to the geuns, Dworakowska and Viraktamath (1978) added a new species: *D. formosus* from China (Taiwan) and Qin and Zhang (2007) added three new species from China: *D. lii* Qin & Zhang, *D. membranaceus* Qin & Zhang and *D. lamellatus* Qin & Zhang.

Here we describe three new species of *Dayus* from China and provide a key for the separation of all known Chinese species. The specimens examined are deposited in the Institute of Entomology, Guizhou University, Guiyang, Guizhou, China (GUGC) and The Natural History Museum, London (BMNH).

Materials and methods

The methods and terminology follow Zhang (1990) except for the nomenclature of wing, for which we follow Dworakowska (1993). Male specimens were dissected under a MOTIC B1 SMS-168 SERIES microscope. Figures were made using an OLYMPUS CX41 and enhanced using Adobe Illustrator CS4. Pictures were taken with VHX-1000C and dealt with by Adobe Illustrator CS4. The body length is measured from the apex of the head to the apex of the forewing.

Results

Genus *Dayus* Mahmood
http://species-id.net/wiki/Dayus

Dayus Mahmood, 1967: 39.

Type species. *Dayus elongatus* Mahmood, 1967 by original designation.

Diagnosis. Vertex (Fig. 1) slightly longer medially than next to eye. Forewing (Fig. 22) with 3rd apical cell petiolate, cua cell broad distally; veins RP, MP' and MP''+CuA' arise from m cell. Hindwing (Fig. 23) with apically broad m cell. Male pygofer (Figs 3, 13, 24) abruptly and strongly narrowing caudad; dorsal bridge about half length of pygofer (Figs 15, 26); with few rigid microsetae distally; elongate ventral appendage present, extended beyond pygofer. Subgenital plate (Figs 5, 17, 27) with basal group of macrosetae and one or two oblique rows of more distal macrosetae. Connective (Figs 7, 19, 29) completely fused with the base of aedeagus. Aedeagus (Figs 8, 20, 30) with basal apodeme absent; shaft strongly curved posteriorly at base with one or two pairs of processes.

Key to the Chinese species (males)

1 Aedeagus with one pair of processes **2**
– Aedeagus with two pairs of processes 6
2 Aedeagal processes with basal serrated lobes (Figs 29–30) ... ***D. serratus* sp. n.**
– Aedeagal processes not dentate basally **3**
3 Aedeagal processes unbranched **4**
– Aedeagal processes branched **5**

4 Aedeagal processes arising near midlength of shaft .....***D. membranaceus***
– Aedeagal processes arising at apex of shaft..... ***D. formosus***
5 Aedeagal processes trifurcate, subapical on shaft (Figs 19–20).....
.....***D. trifurcatus* sp. n.**
– Aedeagal processes bifurcate, at apex of shaft (Figs 7–8)....***D. bifurcatus* sp. n.**
6 Aedeagal processes bifurcate; apical pygofer ventral appendage branched ....***D. lii***
– Aedeagal processes not bifurcate; apical pygofer ventral appendage unbranched 7
7 Apical aedeagal processes straight, subapical processes leaf-like.....***D. lamellatus***
– Apical aedeagal processes hook-shaped, subapical processes slim ....***D. takagii***

***Dayus bifurcatus* Yu & Yang, sp. n.**
http://zoobank.org/8636DDEB-EDC7-4A8C-B6EA-6D7AEF8756DC
http://species-id.net/wiki/Dayus_bifurcatus
Figures 1–9

Description. Length, male 3.0 mm.

General color reddish to reddish brown. Both sides of coronal suture with a brownish patch.

Male ventral abdominal apodemes reaching segment 5 (Fig. 4). Male pygofer with dorsoposterior margin sinuate; pygofer appendage with dorsal margin sinuate in lateral view, tapering to apex (Fig. 3). Subgenital plate about twice as broad basally than distally, with three lateral macrosetae in basal group, an oblique line of 14 macrosetae and several long fine setae subbasally to apex and ca.12 short microsetae at outer margin (Fig. 5). Paramere as in Fig. 6. Aedeagus shaft long and narrow, slightly depressed dorsoventrally, similar in width throughout length in ventral view, with a pair of short bifurcate apical processes (Figs 7, 8). Anal tube process short (Fig. 9).

Type material. Holotype male. China: Zhejiang Province, Fengyang mountain, 30 July 2009, coll. Junqiang Ni.

Etymology. The new species name alludes to the pair of apical bifurcate aedeagal processes.

Remarks. The new species can be distinguished mainly by the shape of the aedeagal shaft and its process configuration as noted in the description.

***Dayus trifurcatus* Yu & Yang, sp. n.**
http://zoobank.org/1FCC60EB-60FC-490D-8E7B-7E69594D814C
http://species-id.net/wiki/Dayus_trifurcatus
Figures 10–23

Description. Length, male 4.5–4.6 mm, female 4.7–4.8 mm.

General color yellowish.

Male ventral abdominal apodemes reaching segment 4 (Fig. 14). Male pygofer with dorsoposterior margin strongly sinuate (Fig. 13); ventral appendage expanded at distal 2/3, thereafter abruptly tapering to spine-like apex (Figs 13, 16). Subgential plate abruptly expanded laterobasally about twice as broad basally than distally; with 11 lateral macrosetae in basal group, an oblique line of 17 macrosetae and several long fine setae subbasally to apex and ca.35 short microsetae at outer margin (Fig. 17). Paramere as in Fig. 18. Aedeagus shaft very long and narrow, cylindrical, nearly straight in lateral view, with a subapical trifurcate process on each side, branches slender (Figs 19, 20). Anal tube process relatively long (Fig. 21).

Type materials. Holotype male. China: Beipei, Chongqing, 6 May 2008, coll. Zaihua Yang. Paratypes, 13♂♂, 5♀♀, same data as holotype (GUGC and 1♂, 1♀ in BMNH).

Etymology. The new species name alludes to the trifurcate processes of the aedeagus.

Remarks. The new species can be distinguished mainly by the strongly sinuate posterior margin of the pygofer and shape of the aedeagal shaft and configuration of its process as noted in the description.

***Dayus serratus* Yu & Yang, sp. n.**
http://zoobank.org/A163BC19-73A4-459A-BA07-125F00EF9D8A
http://species-id.net/wiki/Dayus_serratus
Figures 24–30

Description. Length, male 3.9mm.

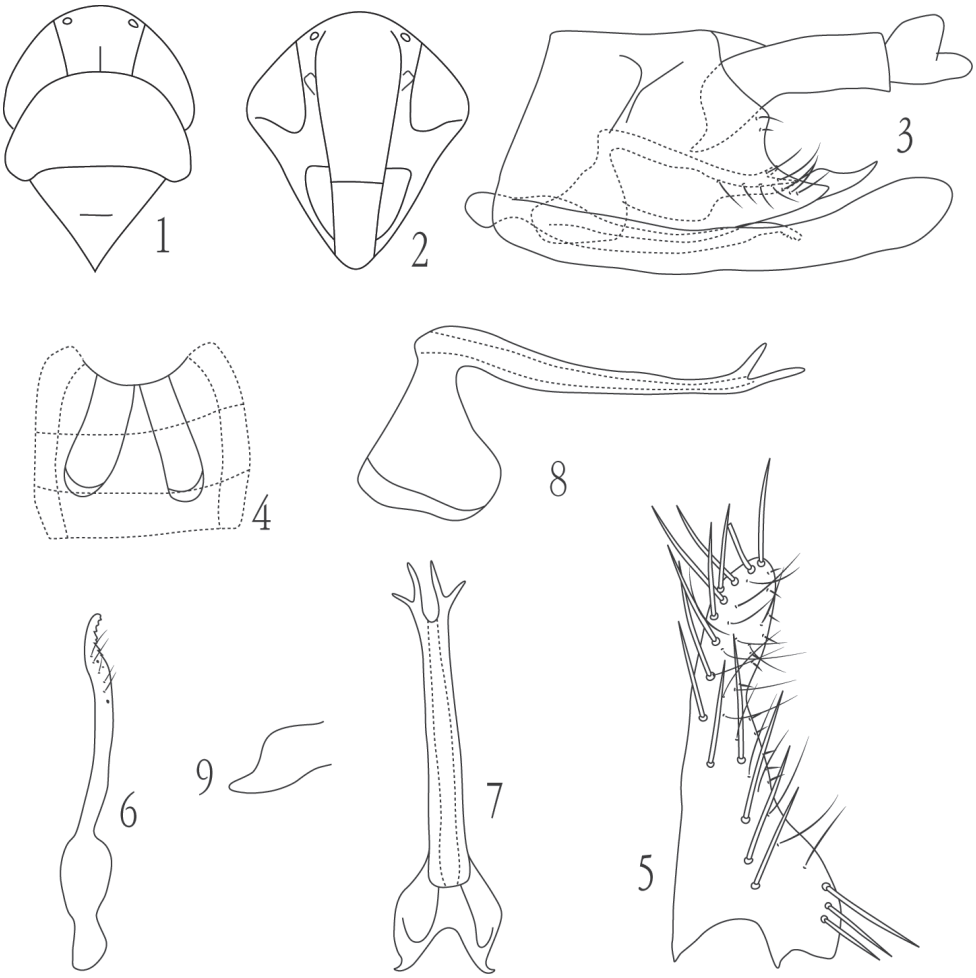
General color yellowish.

Male ventral abdominal apodemes reaching segment 3 (Fig. 25). Male pygofer (Fig. 24) with dorsoposterior margin concave, tapering caudally; ventral appendage with dorsal margin slightly sinuate, tapered to acute apex. Subgenital plate slightly broader basally, with 9 apically rounded lateral macrosetae in basal group, an oblique line of 12 macrosetae and several long fine setae sub-basally to apex and ca.32 short microsetae at outer margin (Fig. 27). Parameres as in Fig. 28. Aedeagus shaft long, basal half strongly dorsoventrally depressed, distal half narrow and cylindrical, serrate laterally at base on dorsal surface; with two long processes arising at midlength on each side of shaft, basally each process with a lateral lamellate serrate lobe (Figs 29, 30). Anal tube process short (Fig. 24).

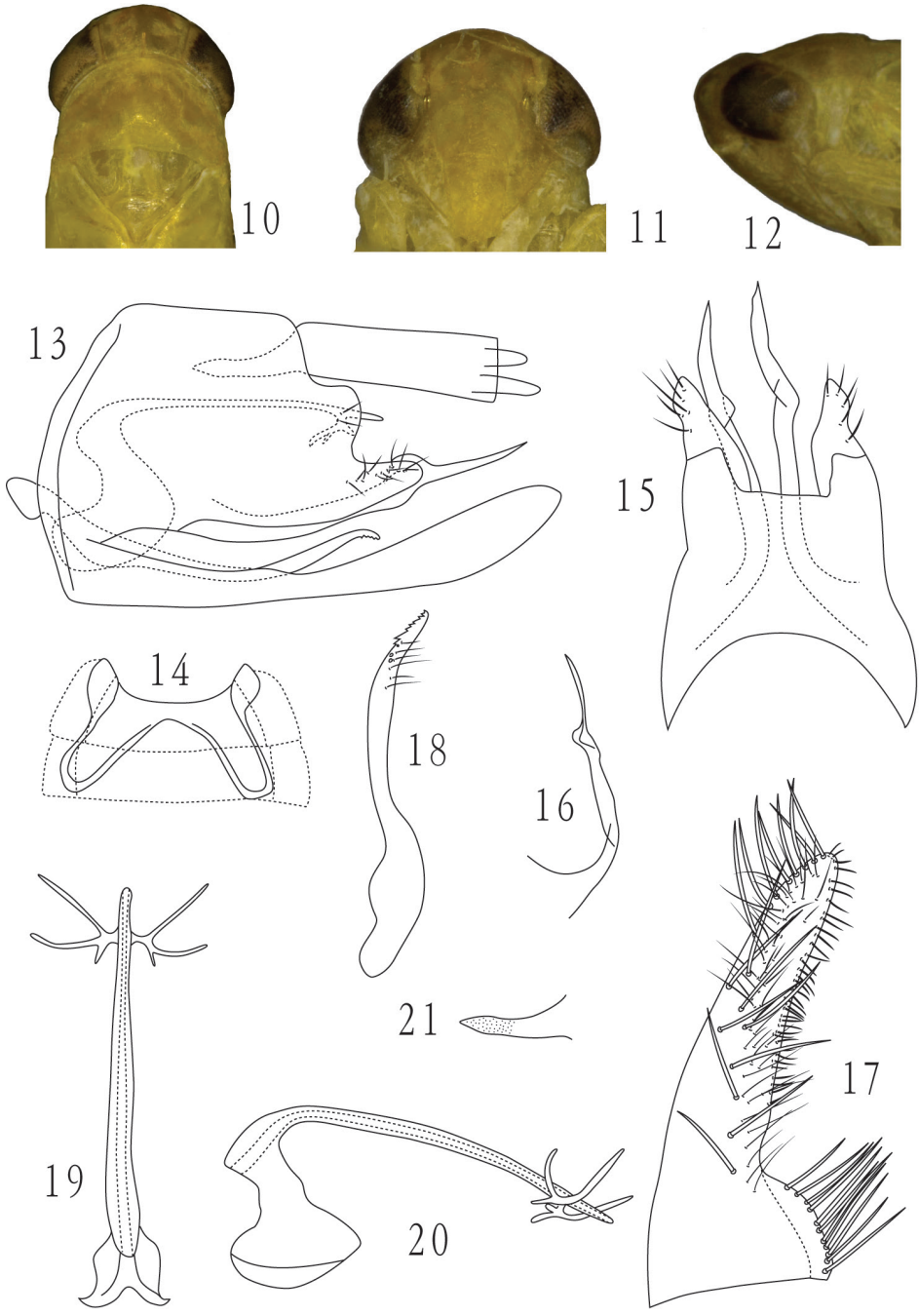
Type material. Holotype male. China: Hainan Province, Wuzhi mountain, 13 April 2009, coll. Zaihua Yang.

Etymology. The new species name is derived from the serrations at midlength of the aedeagal shaft and base of the aedeagal processes.

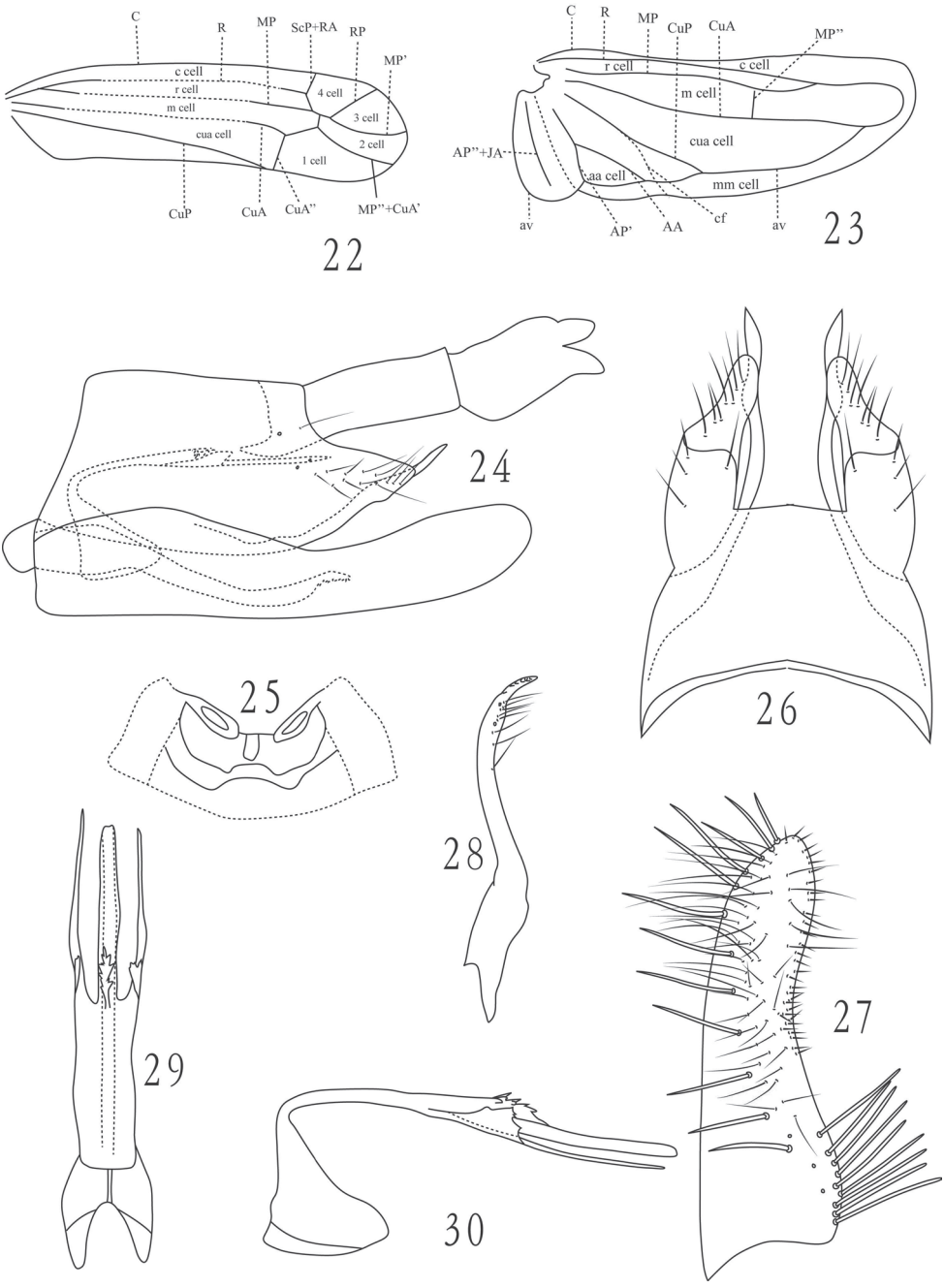
Remarks. The new species can be distinguished mainly by the relatively rather uniform width of the subgenital plate and shape of the aedeagal shaft and configuration of its process as noted in the description.



Figures 1–9. *Dayus bifurcatus* Yu & Yang, sp. n., **1** head and thorax, dorsal view **2** face **3** male genital capsule, lateral view **4** male abdominal apodemes **5** subgenital plate, ventral view **6** paramere **7** aedeagus and connective, dorsal view **8** aedeagus and connective, lateral view **9** anal tube process.



Figures 10–21. *Dayus trifurcatus* Yu & Yang, sp. n., **10** head and thorax, dorsal view **11** face **12** head and thorax, lateral view **13** male genital capsule, lateral view **14** male abdominal apodemes **15** male pygofer, dorsal view **16** ventral pygofer appendage, outside lateral view **17** subgenital plate, ventral view **18** paramere **19** aedeagus and connective, dorsal view **20** aedeagus and connective, lateral view **21** anal tube process.



Figures 22–30. 22–23 *Dayus trifurcatus* Yu & Yang, sp. n., **22** forewing **23** hind wing **24–30** *Dayus serratus* Yu & Yang, sp. n., **24** male genitalia, lateral view **25** male abdominal apodemes **26** male pygofer, dorsal view **27** subgenital plate, ventral view **28** paramere **29** aedeagus and connective, dorsal view **30** aedeagus and connective, lateral view.

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Using seemingly unnecessary illustrations to improve the diagnostic usefulness of descriptions in taxonomy – a case study on *Perochaeta orientalis* (Diptera, Sepsidae)

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Abstract

Many species descriptions, especially older ones, consist mostly of text and have few illustrations. Only the most conspicuous morphological features needed for species diagnosis and delimitation at the time of description are illustrated. Such descriptions can quickly become inadequate when new species or characters are discovered. We propose that descriptions should become more data-rich by presenting a large amount of images and illustrations to cover as much morphology as possible; these descriptions are more likely to remain adequate over time because their large amounts of visual data could capture character systems that may become important in the future. Such an approach can now be quickly and easily achieved given that high-quality digital photography is readily available. Here, we re-describe the sepsid fly *Perochaeta orientalis* (de Meijere 1913) (Diptera, Sepsidae) which has suffered from inadequate descriptions in the past, and use photomicrography, scanning electron microscopy and videography to document its external morphology and mating behaviour. All images and videos are embedded within the electronic publication. We discuss briefly benefits and problems with our approach.

Keywords

Taxonomy, species descriptions, illustrations, bioimaging, videography, Sepsidae

Introduction

Many species descriptions–especially older ones–are very brief: they comprise of discussions and illustrations of diagnostic morphology, geographical distribution, and only occasionally some biology (e.g., see Appendix). The morphology sections are often limited to the most conspicuous features that can be used to differentiate and identify the target species from other species known to the scientific community at the time of description. In the past, this minimalist approach was necessary because journals had tight page restrictions and the cost of including many illustrations was high; this was a particularly serious problem for colour and halftone illustrations. Their high cost contributed to the widespread use of line-drawings in descriptive papers. However, such an exiguous approach towards descriptions is no longer needed given that these restrictions have largely disappeared. While line drawings remain important for clearly illustrating diagnostic features, a description can now afford to include more and different types of data. Electronic journals have fewer limitations on page numbers, and taxonomists now have ready access to high-resolution photography (Ang et al. 2008a) or even μ-CT (Schneeberg et al. 2012), allowing large amounts of data to be acquired quickly. Furthermore, halftone and colour illustrations do not incur additional cost in most electronic publications, and even videos can be embedded in electronic publications, so that primary evidence on the biology of a species can be included (van Achterberg and Durán 2011).

Embracing these new opportunities has many advantages. One is that more data makes it less likely that today's descriptions will be inadequate in the future: a large number of images may serendipitously capture features that will only be revealed to be important in the future. This does not distract from the importance of line drawings, which have the advantage of highlighting important features and can accommodate intraspecific variability (see Discussion). However, line-drawings have the disadvantage that they are unlikely to capture character systems of future importance. For example, 19th and some early 20th century entomologists did not anticipate the importance of genitalia and microtrichosity (pruinosity) patterns in species identification they remained undescribed. Had current-day imaging techniques been available and used by these taxonomists, genitalia [at least "claspers" (hypopygia)] and microtrichosity data would have been captured despite their perceived unimportance at the time of description.

Employing these imaging techniques can also protect against bad taxonomy. For example, Francis Walker (1809–1874), while one of the most prolific taxonomist of his time, was also well known for his poor-quality judgement and descriptions that resulted in numerous synonyms [as his obituary laments; '*More than twenty years too late for his scientific reputation, and after having done an amount of injury almost inconceivable in its immensity, Francis Walker has passed from among us*' (Carrington 1874)]. If the inclusion of large numbers of illustrations and images had been the taxonomic standard in descriptions published in the 19th century, many of his "new species" would not have been published and/or it would have been easier to resolve the taxonomic problems that were caused by his work.

The use of modern imaging is slowly beginning to gain traction in taxonomy (Neusser et al. 2011) because digital images are particularly suitable for dissemination of taxonomic knowledge over the internet. Museum specimen trays can now be accessed virtually (Schmidt et al. 2012), digital reference collections in the form of high-resolution images can be assembled (Ang et al. 2013) and easily curated and updated on wiki sites (Hendrich and Balke 2011). Furthermore, videos, 3D models and other large datasets can be embedded in PDF files or at least linked as supplementary data (Faulwetter et al. 2013). This is advantageous because it encourages the sharing of many kinds of data (e.g., morphology, behaviour, DNA sequences) which can provide different perspectives on difficult taxonomic issues such as cryptic species complexes (Tan et al. 2010).

Here, we present a re-description of *Perochaeta orientalis* (de Meijere 1913) (Sepsidae: Diptera) consisting of morphological, behavioural, DNA sequence, biogeographical, and biological data. We re-describe the species, include comprehensive external morphology data by imaging all views of male and female specimens, and describe their mating behaviour profile along with video data. The re-description of *P. orientalis* is warranted because the two existing treatments (de Meijere 1913, Duda 1926) are both inadequate for reliable species identification. In addition, both are written in German and published in discontinued publications, which limits their accessibility.

Perochaeta is a small Oriental genus, with currently six described species (Ang and Meier 2010, Iwasa and Thinh 2012). This includes *P. orientalis*, *P. cuirassa* Ang, 2010, *P. dikowi* Ang et al., 2008, *P. exilis* Iwasa, 2011, *P. hennigi* Ozerov, 1992 and *P. lobo* Ang, 2010. In order to facilitate the identification of all described species in the genus, we also provide diagnostic differences between all species.

Materials and methods

Collection and rearing of specimens. All new material was acquired from a laboratory culture. This culture was established based on a single female adult specimen collected from a mid-elevation site in Malaysia (Cameron Highlands, 1600m ASL) and reared based on methods described in Ang et al. (2008b). For mating experiments, adult males and females were separated within a day of emergence to obtain virgin flies. These flies were allowed to sexually mature for three days post-eclosion before mating trials began. Specimens (in 70% ethanol) used in this re-description are kept in the Raffles Museum of Biodiversity and Research (RMBR), National University of Singapore, Singapore.

Photography & illustrations. Male and female specimens were extracted from the culture for re-description. The habitus for both sexes were imaged using the Visionary Digital™ Plus Lab System (CF4P3 magnification). Several other structures were also imaged and then digitally transferred into line drawings through tracing with a Wacom® PTZ 630 tablet in Adobe® Photoshop® CS4. Images and illustrations of important diagnostic features are shown in Figs 1 and 2, while images for additional views are

shown in Fig. 3. Images of the holotype (Fig. 4A, B) were provided by the Hungarian Natural History Museum, Budapest, Hungary.

Scanning electron microscopy (SEM). A phallus was dissected and dehydrated in an alcohol series, then critical-point dried with CO2 (Balzers® CPD-030) and mounted on a metal stub and platinum sputter-coated (JEOL® JFC 1600 Pt Fine Coater). SEM was performed at 100× with the JEOL JSM 6510 SEM. The image was then cleaned up with Adobe® Photoshop® CS4, and incorporated into Fig. 1.

Mating experiments. Each mating trial involved two male-female pairs because this species has very low mating success rates. The flies were introduced simultaneously into a small petri-dish and placed under a Leica MZ16A microscope. The mating behaviour was then recorded with an analogue video recorder (36 trials). Recording of behaviour began immediately upon the introduction of specimens into the petri-dish, and ended after 45 minutes if no mounting attempts made, or if they were not successful. The recordings were afterwards digitised and the non-linear editing software Final Cut Pro was used to study the behaviour 'frame by frame' (25 f.p.s.) in order to create a detailed mating profile. This profile was then compared with that of *Perochaeta dikowi* (Ang et al. 2008b). Video clips of relevant behaviours were extracted and put together with Windows Movie Maker (2012 ver.), and embedded as a video object in PDF using Adobe® Acrobat® Pro X.

Online curation of specimens. All images, videos and the appendix in their original resolution are deposited in the species entry for Sepsidnet, an online digital reference collection dedicated to the Sepsidae of the world. These materials are also deposited as a project in Morphobank (Project 1062).

Taxonomic terminology. We adopt the terminology as described by Merz and Haenni (2000) for adult non-terminalia morphology and Sinclair (2000) for genitalia.

Taxonomy and behaviour

***Perochaeta orientalis* (de Meijere, 1913)**
http://species-id.net/wiki/Perochaeta_orientalis
Figs 1–6

Material examined. *Holotype* ♂ (Figs 4A, B). Type locality: "Chip Chip" (Jiji, = 集集) Township, Nantou County (南投), Taiwan ROC [likely, approximate coordinates 23°50'7"N, 120°46'4"E] (type label info: "Formosa Sauter. Chip-Chip 909. III. *Nemopoda orientalis* det de Meijere. Type."). ♂ in the Hungarian Natural History Museum, Budapest, Hungary.

Additional material (Figs 1–3). Locality: Brinchang Jungle Trail, Cameron Highlands, Pahang, Peninsular Malaysia [4°30'9.55"N, 101°23'20.85"E. 1600m ASL]. Isoline culture based on ♀ collected 4.I.2011 (R. Meier). ♂♂♀♀ in the Raffles Museum of Biodiversity Research.

Morphological diagnosis (adult). Male *Perochaeta orientalis* are most easily differentiated from other described *Perochaeta* species based on two large, flattened bristles of

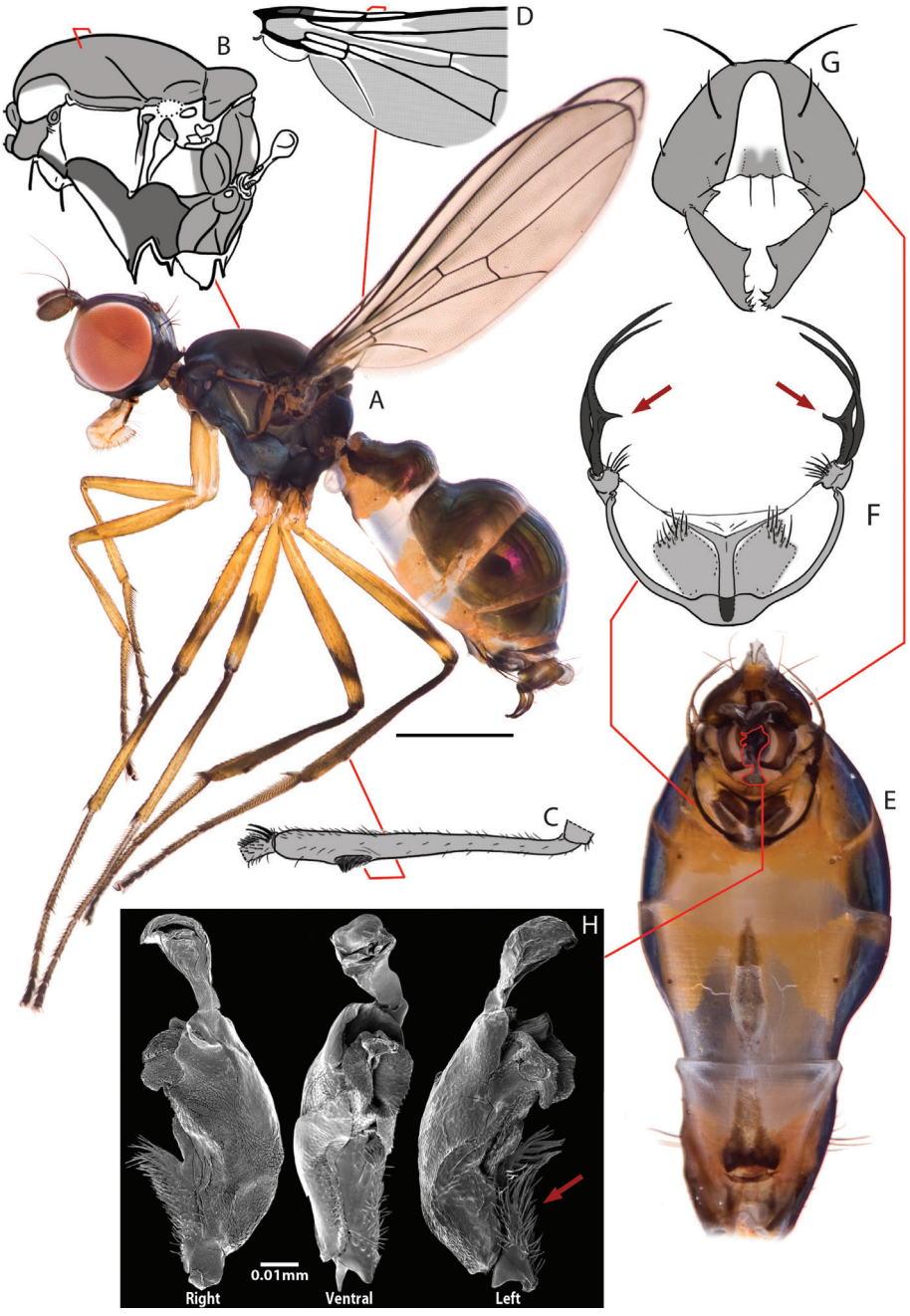


Figure 1. Key views and structures of *Perochaeta orientalis*, Male. **A** Habitus, lateral view **B** Pleural microtomensity pattern; (white = smooth, light grey = lightly microtomentose, dark grey = heavily microtomentose) **C** Rear tibia, with focus on osomterium **D** Basal section of wing showing microtrichosity pattern (white=smooth, light grey=with microtrichia) **E** Whole abdomen, ventral view **F** Sternite appendage **G** Hypopygium, dorsal view **H** Phallus, right, ventral and left views; red arrow indicates basal spiny flap. Scale bars = 0.5mm unless otherwise stated.

the main tuft on the sternite appendage, of which one has a triangular, submedial protrusion (red arrows on Fig. 1F) while all other described *Perochaeta* species have unmodified bristles (Figs 5 with suffix 'A'). The surstylus in *P. orientalis* (Fig. 1G) is also unique in that the median inward protrusion consists of a large, broad-based triangle that spans a third of the surstylus (see Figs 5 with suffixes 'B' and 'C'). The hind tibia of *P. orientalis* also has a distinct, raised osmeterium (Fig. 1C) which is barely visible or missing in other *Perochaeta*. Adult female *P. orientalis* can be distinguished from the females of *P. dikowi* (the only other species with a female description) based on the presence of sternites 3 and 4 (Fig. 2B), which are missing in the latter. For both sexes, the pleural, thoracical microtrichosity for *P. orientalis* (red arrow on Fig. 1B) is most similar to that of *P. exilis* (Fig. 5ED) because it is tomentose on the posterior third of the anepimeron and the dorsal tip of the greater ampulla. In contrast, *P. cuirassa* and *P. lobo* (Fig. 5CD) have a glossy greater ampulla, while *P. dikowi* is pruinose wholly on the greater ampulla and on slightly less than the posterior half of the anepimeron (Fig. 5DD).

Morphological description. *Colour.* Similar in males (Fig. 1A) and females (Fig. 2A). Head capsule black except for face and a connecting thin strip below the eye, which is light-brown. Antennal pedicel dark brown, first flagellomere paler. Proboscis dark-brown with yellow labellum. Thorax wholly black, abdomen with glossy dark-brown tergites and sternites. All femora largely yellow with diffuse obfuscate rings post medially (faint on fore femur). Fore tibia wholly yellow; mid tibia darkened on the basal half; rear tibia entirely dark. All tarsi with first two segments yellow and last three dark-brown. Wing cells clear except for darkened basicostal cell and basal third of costal cell. Veins mostly dark brown. Calypter creamy; haltere whitish with brown base.

Head. Similar in males and females (Figs 1A, 2A). Roundish; facial carina short and shallow, facial area receding. Gena and parafacial region narrow. Ocellar prominence and occipital region lightly microtomentose. Chaetotaxy: *ocellar* longer than divergent *postocellar*; 1 *outer vertical*; *inner vertical* absent; *orbital* very reduced; 2 *vibrissae;* 2–3 weak *postoculars;* Lower fascial margin lined with setulae.

Thorax. Similar in males and females. Scutum, scutellum and subscutellum lightly microtomentose. Mediotergite microtomentose but glossy in the medial region (Figs 3ME, 3FE). Scutellum twice as wide as long (Figs 3MA, 3FA). Pleural pruinosity pattern (Fig. 1B): Protonotopleural lobe glossy on pleural region but microtomentose on dorsal region. Proepisternum fully microtomentose. Anepisternum largely glossy with anterioventral region densely microtomentose. Katepisternum with dense tomentosity except for glossy anterioventral region. Greater ampulla lightly microtomentose on the dorsal tip. Anepimeron glossy with lightly microtomentose strip on posterioventral region. Katatergite, katepimeron, metakatepisterum, meron and metepimeron lightly-dusted. Chaetotaxy: 1 *apical scutellar*, 1 reduced, setulae-like *basal scutellar,* 1 *dorsocentral*, 1 *postalar*, 1 *supraalar*, 2 *notopleural*, 1 *postpronotal*, 1 *anepisternal* and 1 *posterior spiracular*. Postpronotoum, prescutum and anepisternum with few, sporadic setulae.

Legs. Fore legs unmodified in males and females; all femora and tibiae without robust setae except for a longitudinal row of short spines on the anterior basal half of mid femur. Male rear tibia with a small but distinct osmeterium with raised hairs at

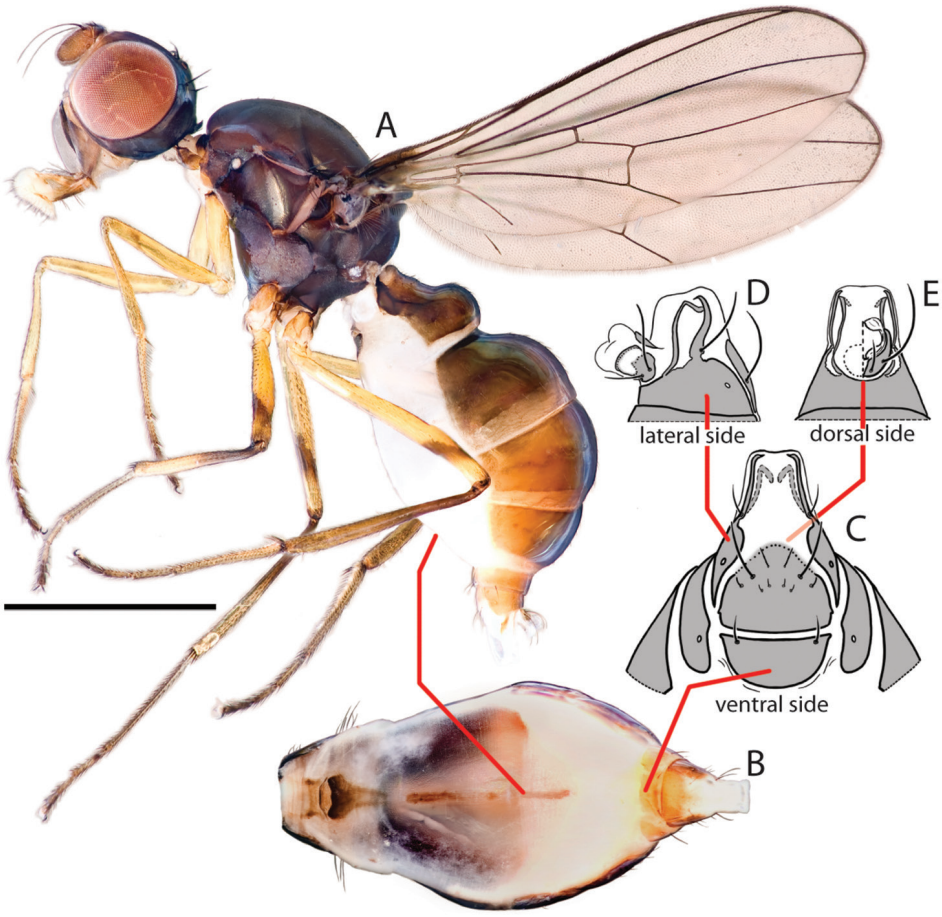


Figure 2. Key views and structures of *Perochaeta orientalis*, Female. **A** Habitus, lateral view **B** Whole abdomen, ventral view **C** Abdominal posterior, ventral view **D** Same, lateral view **E** Same, dorsal view. Scale bar = 0.5mm.

the posteriodorsal region, and with three enlarged ventral setae on basitarsus (Fig. 1C). Females similar but lacking in osmeterium.

Wings. Similar in males and females. Slender. Without apical pterostigma. Veins bare. Wing microtrichia pattern (basal half; Fig. 1D): cells covered with microtrichiae except for subcostal, basal-medial, posterior-cubital cells and alula. Costal, radial 1, radial 2+3, radial 4+5, basal-radial, disco-medial, anterior cubital cells and anal lobe with portions lacking microtrichia. Radial-medial cross-vein divides discal-medial cell by ratio of 2 : 1. Length: 4.4–4.8 mm.

Male abdomen. Ventral view (Figs 1E, F). Syntergite 1+2 to tergite 5 normal, tergite 6 missing, syntergite 7+8 present and extending ventrad as a narrow sclerite. Spiracles 1–4 on intersegmental membrane, spiracle 5 on ventral margin of tergite 5, spiracle 7

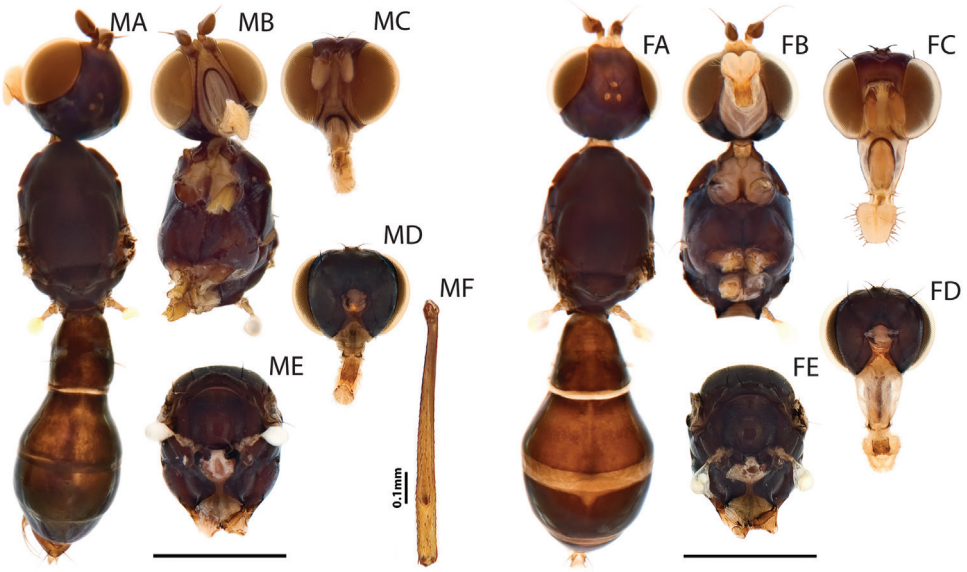


Figure 3. Additional views for *Perochaeta orientalis*, Male (MA-MF) and Female (FA-FE). M and F prefixes refer to male and female specimen respectively. **A** Habitus, dorsal view (sans wings) **B** head and thorax, ventral view **C** Head capsule, anterior view **D** Head capsule, posterior view **E** Thorax, posterior view **F** (male only)–Rear tibia, dorsal view showing osmeterium. Scale bars = 0.5mm unless otherwise stated.

and 8 adjacent on margin of syntergite 7+8. Sternite 1 as a thin lateral band with tapering ends while sternite 2 is triangular, tapering posteriorly; sternite 3 is longitudinally oblong. Sternite 4 heavily modified into paired moveable appendages [Fig. 1F; see Bowsher et al. (2013) for a discussion on the evolution of the appendages and Fig. 5 for sternite appendage illustrations of other *Perochaeta*]: largely desclerotized except for anterior margin as well as two rectangular regions laterally off the median. Two stout moveable appendages branch off laterally, each with a tuft of small short bristles facing the inner side of the sternite and two large, flattened and inward-curving bristles on the apices, of which one is pinched sub medially, resulting in a tooth like furcation on the inward side (red arrows on Fig. 1F).

Hypopygium (Fig. 1G). Cercal plate with two very weak lobes, each with one setae. Hypopygium triangular with a large tooth-like projection originating from the inner base of the surstylus. Surstylus itself fused to hypopygium and branches off dorsally. Each surstylus is curved ventrally, with a large, flattened, inward-facing posteriomedial triangular process; terminus with "teeth" and setulae.

Phallus (Fig. 1H). Basal region with scales on left side and relatively smooth on right side (crinkles and cracks on the surface are artifacts due to drying process). Basal region with large flap adorned with numerous long spines. Distal portion short (ca. 1/3 of basal portion) and membranous. We refrain from assigning terminology, for reasons explained in Discussion.

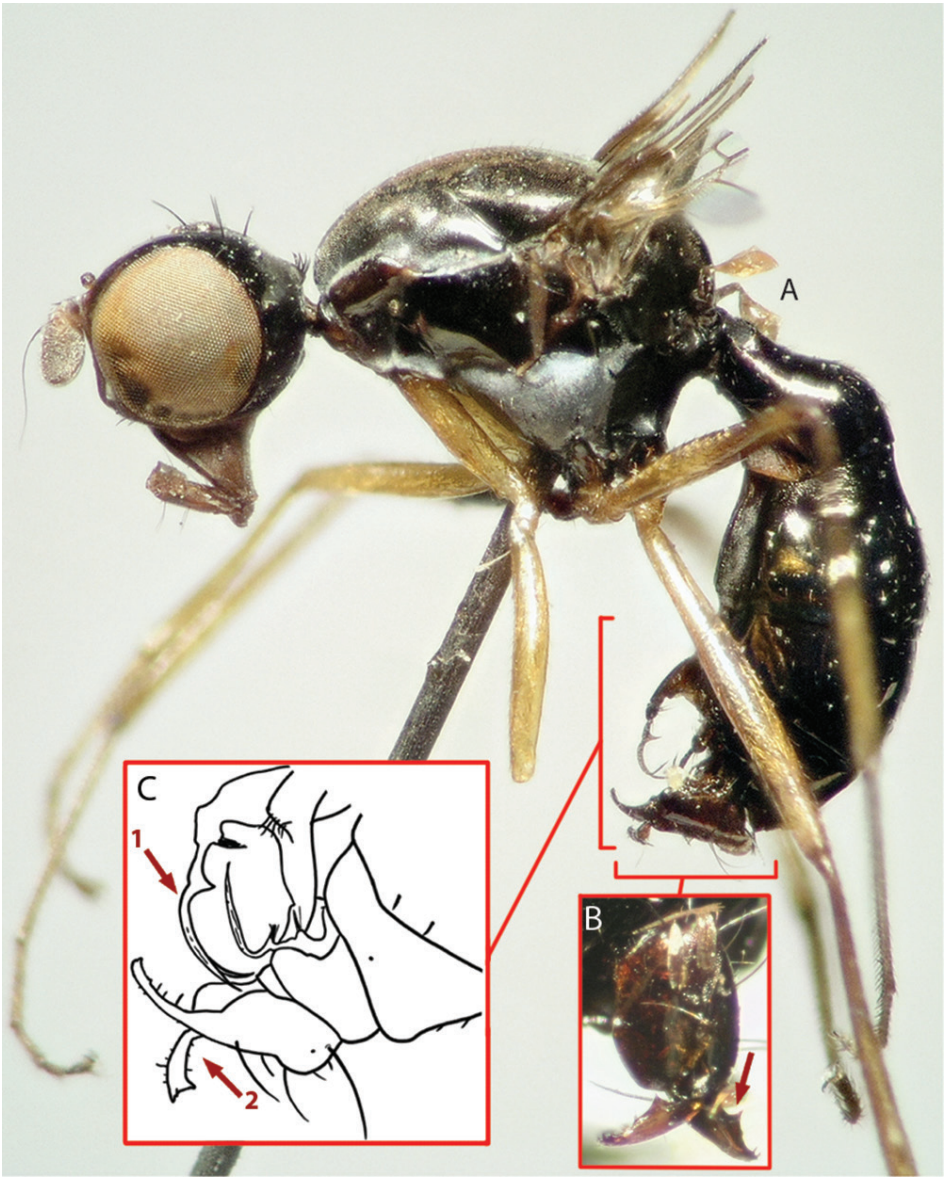


Figure 4. Images of holotype (**A, B**) and drawing (**C**) from description for *Perochaeta orientalis*, male. **A** Image of habitus, lateral view **B** Image of hypopygium, dorsal view; red arrow pointing to the median protrusion on the surstylus **C** Drawing of abdominal posterior (lateral view) as reproduced from Duda (1926); red arrow 1 shows how illustration has fused the two setae into one, red arrow 2 shows how the drawing fails to display the median protrusion as seen in Fig. 1G.

Female abdomen (Fig. 2B–E). Syntergite 1+2–tergite 5 similar to male, tergites 6 and 7 well defined and sclerotized. Spiracles 1–5 in intersegmental membrane while spiracles 6 and 7 are within the tergites. Sternites 1 and 2 similar to male, sternite 3 as a

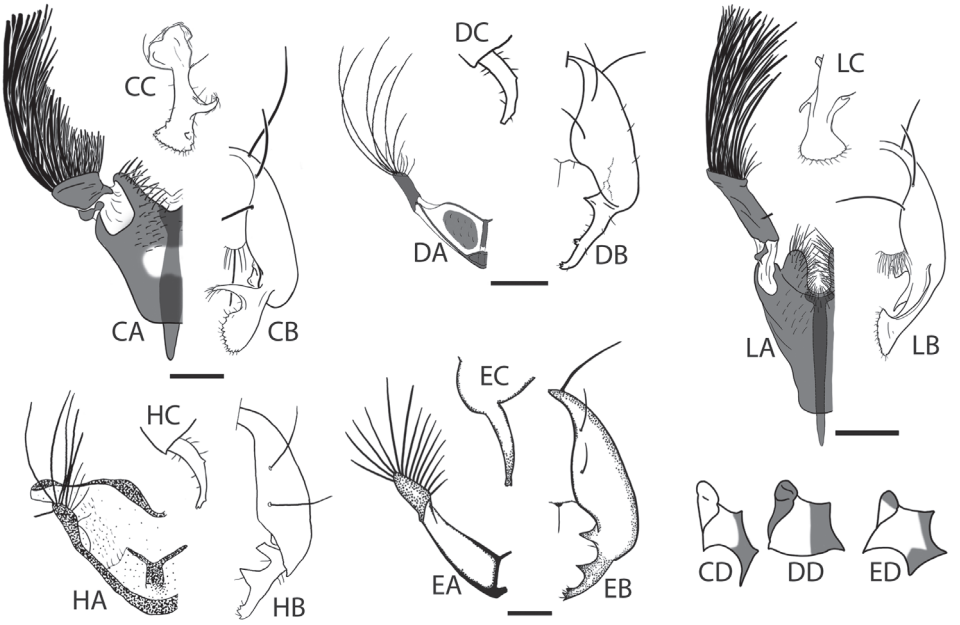
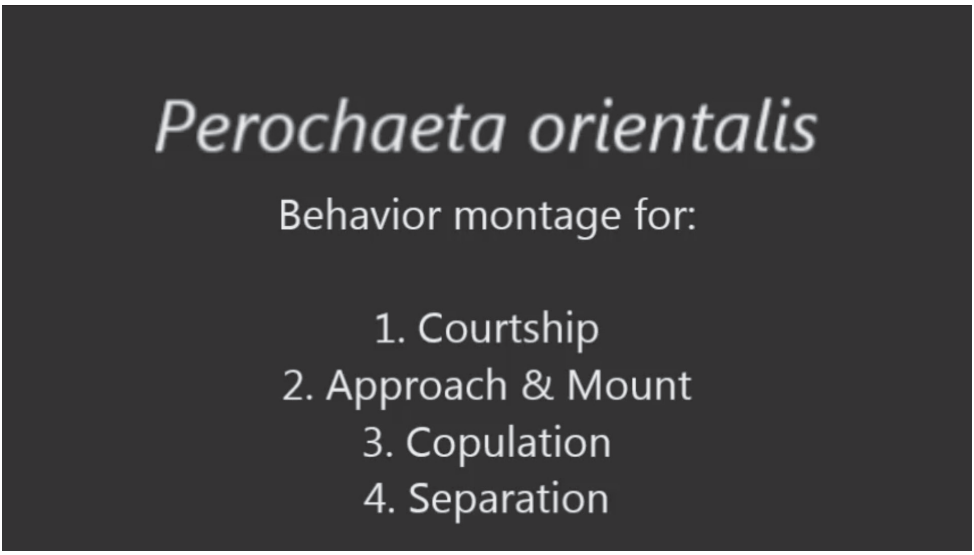


Figure 5. Hypopygia, sternite appendages and anepimeral + greater ampullal microtrichosity of the five other *Perochaeta*: *P. cuirassa* (CA-CC), *P. dikowi* (DA-DC), *P. exilis* (EA-EC), *P. hennigi* (HA-HC) and *P. lobo* (LA-LC); adapted from Ang and Meier (2008; *P. cuirassa* and *P. lobo*), Ang et al. (2008; *P. dikowi*), Iwasa (2011; *P. exilis*) and Ozerov (1992; *P. hennigi*). Suffixes refer to: **A** sternite appendage, left side ventral view **B** hypopygium, right side dorsal view **C** Surstylus, lateral view **D** Anepimeron + greater ampulla [image not available for *P. hennigi* (prefix H)]. *Perochaeta lobo* (prefix L) has a similar anepimeral microtrichosity as *P. cuirassa* (CD). Scale bars = 0.5mm.

very thin longitudinal strip. Sternite 4 also a thin strip with barely visible sclerotization and a diffuse margin, sternite 5 missing. Sternites 6 as a lateral rectangle and sternite 7 tapering posteriorly. Postabdominal segments 6 and 7 with the tergites and sternites separated laterally, the sternites (like the tergites) thus very broad and short; segment 8, when not invaginated, long, extended posteriorly and ventrally, with a ventral element (sternite 8) on each side that remains separated at tip and a dorsal element (tergite 8) that forms the usual pair of ring-like bars that do not quite touch apically. Cercus small and round, with hypoproct present, bare.

Mating behaviour. Here, we conducted 36 mating trials with virgin males and females. Only two of these trials were successful (=5.6% mating success rate), and the copulation time for these two were ca. 75 and 72 minutes. Virgin mating behaviour can be divided into four phases: (1) courtship, (2) approach and mount, (3) copulation and (4) separation. The copulatory profile (section 3) for *Perochaeta orientalis* is shown in Fig. 6, based on a frame-by-frame analysis of one of the trials and documented in Video 1 (time in video given as mm:ss). Where available, we will compare and differentiate the behaviour of *P. orientalis* with *P. dikowi* (Ang et al. 2008b) which is the only other *Perochaeta* species with a known mating profile. Our efforts to provide



Video 1. Video montage for the various behaviours described. Section 1, Courtship: Male wing-flutter dance (00:07). Section 2, Approach and Mount: Failed attempt with female resistance, lateral view (00:15), Successful mount, dorsal view (00:29). Section 3, Copulation: M1 Male fore leg tap to female head (00:41), M2 Male rear leg rub (01:03), M3 Male rear- to mid-leg rub (01:10), M4 Male mid legs tap to female wing (01:18), M5 Male mid legs tap to female abdomen (01:29), F1 Female resistance (mid legs push) (01:39), F2 Female resistance (rear leg push) (01:51), F3 Female grooming (rear leg rub) (02:00), F4 Female grooming (fore leg-head rub) (02:06). Section 4, Separation (02:15). Video available for download in full resolution from http://www.pensoft.net/J_FILES/1/articles/6013/export.php_files/Ang_Wong_Meier_Video_1.avi

detailed mating behaviour for *P. orientalis* is part of a larger series of papers investigating of mating behaviour in sepsids (e.g., Ang et al. 2008b, Puniamoorthy et al. 2008, Puniamoorthy et al. 2009, Tan et al. 2010, Tan et al. 2011). As discussed in Puniamoorthy et al. (2009), attention to detail is important because sepsid mating behaviour is apparently species-specific.

Courtship. When the male detects and shows interest in a female, he courts the female by using a "wing flutter dance"; i.e., he rapidly circles the female from his side while fluttering the wing facing the female (00:07). This behaviour is not observed in *P. dikowi*.

Approach and mount. The male will approach the female from the rear and attempt to mount her. Unlike most sepsid species, *P. orientalis* males lack modified fore legs, and do not clasp the female wing or perform pre-copulatory behaviours when mounted like other sepsids (Puniamoorthy et al. 2008). Instead, he mounts similarly to *P. dikowi;* using his fore tarsi to hold on to the female's abdomen whilst bending his abdomen forward. He then extends his sternite brush to contact the genital region, while the surstylus attempts to clasp the female genitalia (00:15 & 00:29). A crucial difference between the two species is that *P. dikowi* uses his sternite brush to contact the anterior portion of the female abdomen before sliding towards her posterior, while *P. orientalis* immediately contacts the genital region (see attempt

in 00:15). At this stage, females show strong rejection behaviour towards the males which explains the low mating success rate. Males are kicked with mid- and hindlegs and/or the abdomen is raised to prevent genital contact (00:15). All resisting females remained unmated and only those males succeeded in mating that encountered willing females (00:29). In *P. dikowi*, female resistance is much lower and mating success rates were 28.6%.

Copulation (Fig. 6). Once the male locks its genitalia with the female, they copulate for a long time (73.7±1.2 min; based on the two successful trials), which is over 3 times longer than that in *P. dikowi* (22.6±2.48 min). There are periods of rest and activity during copulation. During rest, males place their fore tarsi on the female pronotal callus while mid- and rear legs are splayed out. During active periods, the male displays five types of behaviours: "M1: fore leg head tap"–males using fore tarsi to tap repeatedly on female head(00:41), "M2: rear leg rub"–males rubbing rear legs together (01:03), "M3: rear-mid-leg rub"–males rubbing rear legs with mid legs (01:10), "M4: mid legs wing tap"–males using mid legs to tap repeatedly on female wing (01:18) and "M5: mid legs abdomen tap"–males use mid legs to tap repeatedly on female abdomen (01:29). Behaviours M3 and M4 mostly occur after M1 and M2, suggesting a transfer of substance from the rear tibial osmoteria to the mid legs and then onto the female wing and/or abdomen. Female resistance was recorded even after copulation commenced; the female mostly used her mid legs (F1; 01:39) and only occasionally her hindlegs to push against the male (F2; 01:51). The female also indulged in grooming herself at times, either performing a rear leg rub (F3; 02:00) or a fore leg-head rub (F4; 02:06)

Separation. Just prior to separation, the male performs the "fore leg head tap" as well as the consecutive "rear-mid-leg rub" and "mid legs abdomen tap". The separation event itself is initiated by the male, where he turns 180° and pulls away from the female (02:15). Both males and females will also use their rear legs to push against each other during this time. This is similar in *P. dikowi*.

Distribution, laboratory records and DNA sequence information. *Biogeography. Perochaeta* has been consistently found only in mid- to high-elevation areas [see Ang and Meier (2010) for a discussion on the genus's biogeographical distribution]. *Perochaeta orientalis* itself was first collected by Sauter from two township localities in the central highlands (Nantou County; = 南投縣) of Taiwan: Jiji ("Chip Chip", = 集集) and Puli ("Polisha", = 埔里; approximate coordinates 23°57'56"N, 120°57'57"E) (de Meijere 1913). While the elevation of these two townships are relatively low (ca. 300m for Jiji and 500m for Puli), they are both immediately enclosed by mountain ranges that reach to excesses of 2500m. Specimen collection in Sauter's expedition would likely be from these mountainous regions. It is thus possible that *P. orientalis*–like its other congeners in *Perochaeta*–is a higher-elevation specialist limited to the hills and mountains of the Oriental region. It has been recorded in Taiwan, Indonesia (Sulawesi I.), East and West Malaysia, as well as the Philippines (Luzon I., Mindanao I.) (Ozerov 2005).

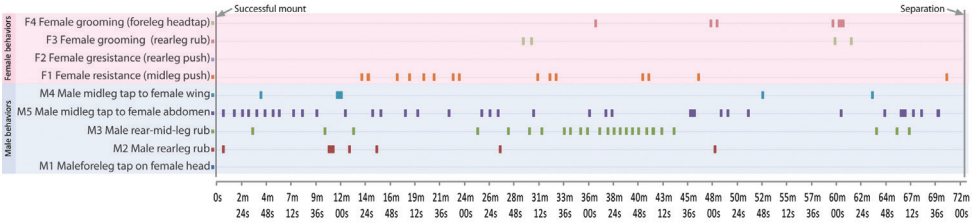


Figure 6. Copulatory profile for *Perochaeta orientalis*, as described in Section 2 (Copulation). Horizontal bars in graph indicate point in time (X-axis) where then the particular behaviour (Y-axis) is performed. The profile begins from when the male mounts the female, and ends when they begin to separate (total time = 72m 30s).

Table 1. A summary of the pairwise distances between the COI of *P. orientalis* with that of *P. cuirassa* (KF199839), *P. dikowi* (KF199840) and *P. lobo* (KF199841). *Perochaeta orientalis* has the most similar sequence to *P. dikowi*'s (3.82%), and all pairwise distances are relatively high.

	P. orientalis	*P. cuirassa*	*P. dikowi*	*P. lobo*
P. orientalis	0.00%	11.44%	**3.82%**	13.15%
P. cuirassa	8.70%	0.00%	12.95%	11.89%
P. dikowi	11.89%	12.95%	0.00%	8.70%
P. lobo	13.15%	**3.82%**	11.44%	0.00%

Laboratory records. Under laboratory conditions, *P. orientalis* has been bred successfully from bovine (cow and gaur) dung. They are also attracted to this substrate in the wild, which makes sampling an area for *Perochaeta* a "bait-and-wait" strategy.

DNA sequence information. Molecular data from our new *P. orientalis* material are presented as part of the updated sepsid phylogeny (Lei et al. 2013). Nine mitochondrial and nuclear genes are sequenced and uploaded to Genbank. Their accession numbers are: 12S - KF199478, 16S - KF199525, COII - KF199667, COI - KF199842, CYTB - KF199714, 18S - KF199572, 28S - KF199618, ATS - KF199795, H3 - KF199739. Genetic distances for COI between existing species with DNA records (*P. cuirassa*, *P. dikowi* and *P. lobo*) were calculated using SpeciesIdentifier (Meier et al. 2006). *Perochaeta orientalis* has the most similar sequence to *P. dikowi* (3.82%; Table 1), a distance that is well in excess of what is normally found between dipteran species (Meier et al. 2008).

Discussion

Concordance with precedent descriptions and holotype

The decision to re-describe *P. orientalis* was based on the quality and accessibility of the two precedent descriptions by de Meijere and Duda (Appendix). de Meijere's de-

scription (1913) was a short paragraph written in German, devoid of illustrations, and published in a journal that has been discontinued; i.e., it was a good case for a relatively inaccessible description that was also insufficient for reliable species identification. Duda's re-description (1926) was much more detailed, but only one illustration was presented which lacked clarity (Fig. 4C): For example, while it did show the two long, flattened setae found in *P. orientalis*, they were drawn fused at the base as a single bifurcated seta (Arrow 1). The hypopygium was also drawn in such a way that it failed to illustrate the large median triangular protrusion on the surstylus (Arrow 2; cf. Fig. 1G). In this case, much effort went into text instead of illustrations, which still resulted in an unclear species diagnosis. It was only through re-imaging of the holotype specimen (Fig. 4A, B) that we were able to determine that our material was indeed *P. orientalis*, based on the lateral thoracical microtrichosity pattern (c.f. Figs 1A, 4A), the bristle morphology on the sternite 4 appendage (square parenthesis on Fig. 4A) as well as the large median protrusion on the surstylus (arrow on Fig. 4B). Overall, there is no doubt that a photograph of the holotype would have been much more informative than the line drawing in Duda (1926).

The phallus as anticipatory data

In this paper we include images of the unlabelled phallus (Fig. 1H). There is still a dearth of information on this structure in Sepsidae, but we anticipate that it will gain in importance in the future. It is well-recognised that insect genitalia evolve rapidly and divergently, and are often the most reliable source of characters to delimit and describe species (Eberhard 1985). However, the phallus is almost never described in Sepsidae because species identification can usually be accomplished using the more exposed genitalia (e.g. hypopygium) and other secondary sexual characters [e.g. fore legs and modified sternites; see Pont and Meier (2002)] while the phallus is often inaccessible and requires dissection and slide preparation. A review of current literature revealed that only two publications included detailed information on sepsid phallus ('aedeagus') and the authors either refrained from a description in the text (Zuska 1965) or used informal terms such as ' spiny tongue', 'long finger' and 'oxtail' (Fig. 7; Eberhard and Huber 1998). Unfortunately, the phallus is very variable between species and genera, and it is difficult to homologise the different parts across species. For example, the large spiny basal flap in *P. orientalis* (red arrow, Fig. 1H) might be homologous to the unlabelled flap in *Archisepsis* Silva, 1993 (red arrow, Fig. 7) but more species need to be studied before this hypothesis can be supported. This problem is not limited to Sepsidae: In his taxonomic review of the kelp fly family Coelopidae (often used as outgroup for Sepsidae), McAlpine (1991) expressed little confidence in the homology of his proposed phallus terminology, and he only applied descriptive terms. For similar reasons, we here only include SEM images for the *P. orientalis* phallus as 'anticipatory' data for a character system that will only become fully available in the future.

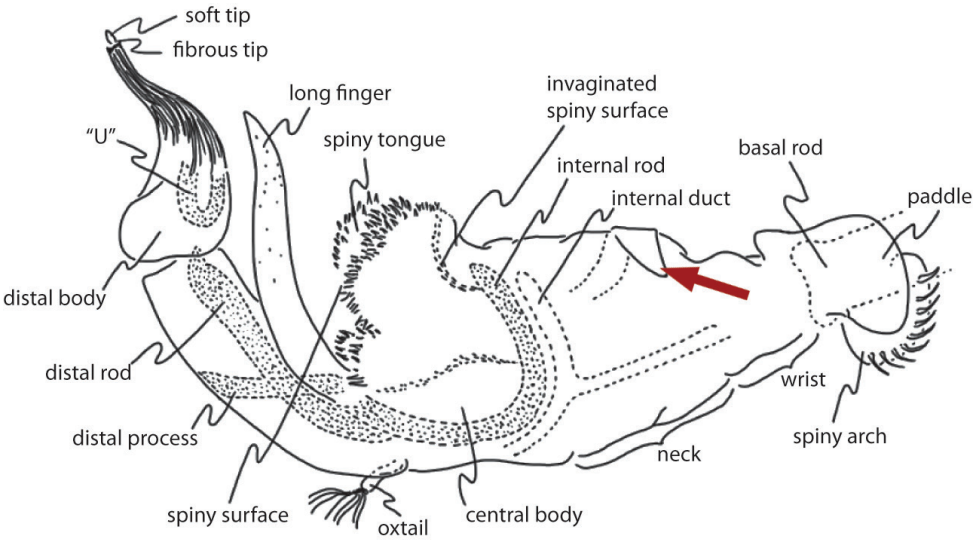


Figure 7. Illustration of *Archisepsis* phallus, as reproduced from Eberhard and Huber (1998). Red arrow indicates region that may be homologous to the basal spiny flap in *P. orientalis*.

Costs and benefits of data-rich descriptions

We here richly illustrate the morphology of *Perochaeta orientalis* with line drawings, photography and SEM images. This may raise the question whether too much effort was invested into a single species. However, all these visual data were acquired within a day, while much more time was needed for getting access to the original type material, literature, and confirming species identity. Of course, one obvious question raised by our proposal is where to stop. While we have covered the external morphology with images, our treatment is far from exhaustive. For example, we did not investigate internal morphology, nor the cuticular hydrocarbon profile (Kather and Martin 2012) and UV reflectance (Shevtsova et al. 2011), etc. We could have also added light-transmission photographs of the phallus which would have distinguished sclerotized and membranous parts. Furthermore, we only imaged one specimen of each sex (in addition to the holotype), which may not represent the intra-specific variability. The amount of data to be presented in a description is ultimately up to the author and determined by the tradeoffs between the costs of acquiring additional information and its potential use. Within the last decade we have seen rapid advancements in digital photography and decreases in the cost of acquiring and publishing imaging data. This has led to the much more widespread use of photographs in taxonomic manuscripts. However, we argue the focus has been too much on illustrating those structures that are already known to be important. Let us be more visionary by illustrating even more structures in anticipation of future needs. This does not only apply to new species, but also to species whose descriptions have become inadequate.

One may argue that this will add to the taxonomic impediment, because future descriptions would require more images. This is a legitimate concern, given that taxon-

omists are already overwhelmed with the amount of undescribed species (Riedel et al. 2013). However, currently much time is invested in long texts which are often of limited value while descriptions that are rich in illustrations can be generated in relatively short amounts of time. For example, with proper equipment, staff can produce 40 high-quality images per day (Tegelberg et al. 2012). Descriptions can then be prepared quickly (e.g., as high-resolution images displayed on a computer screen). Moreover, moving toward descriptions with more images is also an investment into the future. Most taxonomists will concede that it is the processing of inadequate descriptions that are a major reason for the taxonomic impediment. For example, in our case, the main bottle neck in identifying *P. orientalis* was overcoming issues created by previous taxonomic work. These problems could have been avoided if a re-description had been available or the holotype had been properly illustrated at the time of description.

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Appendix

Scan of precedent descriptions of *Perochaeta orientalis* (doi: 10.3897/zookeys.355.6013.app). File format: Adobe PDF file (pdf).

Explanation note: Scanned original and subsequent description of *Perochaeta orientalis* by de Meijere (1913) and Duda (1926). All media have been archived at:

1) Sepsidnet (http://sepsidnet-rmbr.nus.edu.sg/Perochaeta_orientalis.html)
2) Morphobank (http://morphobank.org/permalink/?P1062)

Citation: Ang Y, Wong LJ, Meier R (2013) Using seemingly unnecessary illustrations to improve the diagnostic usefulness of descriptions in taxonomy–a case study on *Perochaeta orientalis* (Diptera, Sepsidae). ZooKeys 355: 9–27. doi: 10.3897/zookeys.355.6013 Scan of precedent descriptions of *Perochaeta orientalis*. doi: 10.3897/zookeys.355.6013.app

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A new genus of metalmark moths (Lepidoptera, Choreutidae) with Afrotropical and Australasian distribution

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Abstract

Niveas Rota, new genus, and its two new species, *N. agassizi* Rota, new species, and *N. kone* Rota, new species, are described and illustrated. *Niveas* is assigned to the subfamily Choreutinae based on morphological and molecular data. *Niveas agassizi* is currently known only from Kenya and only from female specimens. *Niveas kone* has been found on the Solomon Islands and in Papua New Guinea (PNG). In PNG, larvae of this species have been reared from several species of *Ficus* (Moraceae). The two species are superficially quite dissimilar from each other. However, they share features in wing pattern and venation, as well as female genitalia, and the molecular data strongly support the monophyly of *Niveas*.

Keywords

Alpha taxonomy, DNA barcoding, *Ficus* spp., Kenya, *Niveas agassizi*, *Niveas kone*, Papua New Guinea, Solomon Islands, phylogenetics

Introduction

Choreutidae, commonly known as metalmark moths, are a family of micro-moths with a worldwide distribution. The family is most species-rich in the tropics, and, as is the case for numerous other small tropical invertebrates, much of its richness is still unknown to science (unpublished data). Currently, 406 species of choreutids are described (Nieukerken et al. 2011).

Choreutids are medium-sized micro-moths with wingspans ranging from about one to two centimeters, often with bright colors and iridescent markings on their wings (Diakonoff 1986). They are diurnal with only some species attracted to lights at night (personal observation), making them a fairly rare group in museum collections. In our experience, large-scale rearing projects result in finding more species of choreutids than employing light traps.

Through exactly such efforts over the past 20 years in Papua New Guinea (PNG), the Binatang Research Center (BRC), with a large international group of collaborators focusing on the ecology of herbivorous insects and their host plants (Miller et al. 2003; Craft et al. 2010; Novotny et al. 2010; Hrcek et al. 2011; Hrcek et al. 2013; Miller et al. 2013), the number of known species of choreutids and our knowledge of their biology have greatly increased. One of the many new species of choreutids found in PNG during this project is sufficiently different from all described species that it requires a new genus.

Coincidentally, through separate collecting efforts by David Agassiz in Africa, a related species was discovered in Kenya. Herein these two species, as well as the genus to which they belong, are described and illustrated, and the phylogenetic position of the new genus within the family is discussed.

The shared presence of the terminal black band with white spots in the forewing (arrows in Figs 1, 3) was the first indication that *N. kone* Rota, sp. n. and *N. agassizi* Rota, sp. n. might be related. Initially this relationship seemed unlikely because of the disjunct geographical distribution of the two (*N. kone* being distributed in the Australasian Region and *N. agassizi* in the Afrotropical Region) and because their DNA barcodes did not suggest a close relationship. However, once the similarities in wing venation and female genitalia were noticed, and we included nuclear genes in the analysis with a more extensive choreutid molecular dataset, the results strongly supported the close relationship between *N. kone* and *N. agassizi*.

Methods

All material examined is listed in Table 1. Layered photographs of specimens and slides were taken using an Olympus SZX16 microscope with motorized focus drive attached to an Olympus E520 digital camera. The photographs were then combined by using the programs Deep Focus 3.1 and Quick Photo Camera 2.3. The wing venation drawing was made digitally in Adobe Illustrator CS3 overlaid on top of a slide photograph.

Table 1. Material examined.

Species	Type	Country	Province	Locality	Date	Collector	ID number	Host plant	Slide number	GenBank
N. kone	Paratype	PNG	Madang	Baitabag Vill.	04/09/95	BRC	USNM ENT 730507	*Ficus nodosa*		
N. kone	Paratype	PNG	Madang	Baitabag Vill.	08/30/95	BRC	USNM ENT 730558	*Ficus nodosa*		
N. kone	Paratype	PNG	Madang	Baitabag Vill.	08/30/95	BRC	USNM ENT 730572	*Ficus nodosa*		HQ946542
N. kone	Paratype	PNG	Madang	Baitabag Vill.	06/16/95	BRC	USNM ENT 730508	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Baitabag Vill.	03/19/96	BRC	USNM ENT 730513	*Ficus variegata*		HQ946551
N. kone	Paratype	PNG	Madang	Baitabag Vill.	04/09/95	BRC	USNM ENT 730529	*Ficus variegata*		KF714836
N. kone	Paratype	PNG	Madang	Baitabag Vill.	03/19/96	BRC	USNM ENT 730543	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Baitabag Vill.	03/19/96	BRC	USNM ENT 730551	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Kamba (Mis)	10/20/95	BRC	USNM ENT 730576	*Ficus variegata*		HQ946555
N. kone	Paratype	PNG	Madang	Malapau (Riwo)	03/20/95	BRC	USNM ENT 730498	*Ficus variegata*		HQ946554
N. kone	Paratype	PNG	Madang	Malapau (Riwo)	03/20/95	BRC	USNM ENT 730519	*Ficus variegata*		HQ946553
N. kone	Paratype	PNG	Madang	Malapau (Riwo)	03/20/95	BRC	USNM ENT 730535	*Ficus variegata*		HQ946552
N. kone	Paratype	PNG	Madang	Mililat (Riwo)	05/22/95	BRC	USNM ENT 730604	*Ficus nodosa*		HQ946544
N. kone	Paratype	PNG	Madang	Mis Vill.	03/20/96	BRC	USNM ENT 730528	*Ficus nodosa*		HQ946543
N. kone	Paratype	PNG	Madang	Ohu Vill.	04/09/95	BRC	USNM ENT 730560	*Ficus botryocarpa*		HQ946538
N. kone	Paratype	PNG	Madang	Ohu Vill.	05/09/95	BRC	USNM ENT 730602	*Ficus botryocarpa*		HQ946539
N. kone	Paratype	PNG	Madang	Ohu Vill.	12/01/96	BRC	USNM ENT 730542	*Ficus phaeosyce*		KF714835
N. kone	Paratype	PNG	Madang	Ohu Vill.	12/02/94	BRC	USNM ENT 730502	*Ficus pungens*		HQ946546
N. kone	Paratype	PNG	Madang	Ohu Vill.	12/09/95	BRC	USNM ENT 730518	*Ficus variegata*	female genitalia 92352	HQ946549
N. kone	Paratype	PNG	Madang	Ohu Vill.	03/16/95	BRC	USNM ENT 730509	*Ficus variegata*	male genitalia 92355	HQ946550
N. kone	Paratype	PNG	Madang	Ohu Vill.	03/16/95	BRC	USNM ENT 730492	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	03/25/96	BRC	USNM ENT 730493	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	05/09/95	BRC	USNM ENT 730500	*Ficus variegata*		

Species	Type	Country	Province	Locality	Date	Collector	ID number	Host plant	Slide number	GenBank
N. kone	Paratype	PNG	Madang	Ohu Vill.	03/22/95	BRC	USNM ENT 730504	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	12/13/94	BRC	USNM ENT 730510	*Ficus variegata*		
N. kone	Holotype	PNG	Madang	Ohu Vill.	03/13/95	BRC	USNM ENT 730516	*Ficus variegata*		HQ946548
N. kone	Paratype	PNG	Madang	Ohu Vill.	08/09/95	BRC	USNM ENT 730517	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	03/16/95	BRC	USNM ENT 730520	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	05/26/95	BRC	USNM ENT 730521	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	05/09/95	BRC	USNM ENT 730522	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	03/29/95	BRC	USNM ENT 730523	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	06/16/95	BRC	USNM ENT 730524	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	05/11/96	BRC	USNM ENT 730525	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	06/27/95	BRC	USNM ENT 730526	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	06/16/95	BRC	USNM ENT 730531	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	12/13/94	BRC	USNM ENT 730533	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	05/09/95	BRC	USNM ENT 730553	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	12/09/95	BRC	USNM ENT 730564	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	05/09/95	BRC	USNM ENT 730588	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	05/09/95	BRC	USNM ENT 730595	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Ohu Vill.	09/10/95	BRC	USNM ENT 730565	*Ficus wassa*		HQ946545
N. kone	Paratype	PNG	Madang	Pau Vill.	12/13/95	BRC	USNM ENT 730515	*Ficus variegata*		KF714837
N. kone	Paratype	PNG	Madang	Pau Vill.	12/13/95	BRC	USNM ENT 730547	*Ficus variegata*		KF714833
N. kone	Paratype	PNG	Madang	Reinduk	03/28/95	BRC	USNM ENT 730527	*Ficus variegata*		KF714834
N. kone	Paratype	PNG	Madang	Tab Is	01/31/95	BRC	USNM ENT 730506	*Ficus nodosa*		
N. kone	Paratype	PNG	Madang	Tab Is	01/31/95	BRC	USNM ENT 730514	*Ficus nodosa*		KF714832
N. kone	Paratype	PNG	Madang	Tab Is	01/31/95	BRC	USNM ENT 730532	*Ficus nodosa*		HQ946540
N. kone	Paratype	PNG	Madang	Tab Is	01/31/95	BRC	USNM ENT 730538	*Ficus nodosa*		HQ946541
N. kone	Paratype	PNG	Madang	Wanang Vill.	07/31/07	BRC	USNM ENT 660733	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Wanang Vill.	11/05/07	BRC	USNM ENT 660794	*Ficus variegata*		
N. kone	Paratype	PNG	Madang	Wanang Vill.	02/21/06	BRC	USNM ENT 660722	*unknown*		HQ946547

Species	Type	Country	Province	Locality	Date	Collector	ID number	Host plant	Slide number	GenBank
N. kone	Paratype	Solomon Is.	Guadalcanal	Roroni, 35 km E of Honiara; 10 m	05/13/64	R. Straatman	unassigned	*unknown*	wing 137601; female genitalia 137600	
N. kone	Paratype	Solomon Is.	Guadalcanal	Roroni, 35 km E of Honiara; 10 m	05/13/64	R. Straatman	unassigned	*unknown*		
N. kone	Paratype	Solomon Is.	Guadalcanal	Nini Ck., 35 km SE of Honiara	08/05/64	R. Straatman	unassigned	*unknown*		
N. agassizi	Paratype	Kenya	County of Kwale	Mwabungu	08/19/00	David Agassiz	USNM ENT 730794	*unknown*		HQ946715
N. agassizi	Holotype	Kenya	County of Kwale	Mwabungu	08/19/00	David Agassiz	USNM ENT 730793	*unknown*		HQ946716
N. agassizi	Paratype	Kenya	County of Kwale	Mwabungu	08/19/00	David Agassiz	unassigned	*unknown*	female genitalia 137597	
N. agassizi	Paratype	Kenya	County of Kwale	Mwabungu	08/19/00	David Agassiz	unassigned	*unknown*	female genitalia JR2013-02	
N. agassizi	Paratype	Kenya	County of Kwale	Mwabungu	08/19/00	David Agassiz	unassigned	*unknown*	wing JR2013-03	
N. agassizi	Paratype	Kenya	County of Kwale	Mwabungu	08/20/00	David Agassiz	unassigned	*unknown*	female genitalia JR2013-01	

All images were improved in Adobe Photoshop CS3. Genitalic dissections and terminology follow Rota (2008b).

Field sampling and rearing protocols for the PNG material are detailed in Miller et al. (2003; 2013), Craft et al. (2010), and Novotny et al. (2010). The Plant List website (2010) was used for host plant names. Latitude, longitude, and altitude data for the collecting localities is in Table 2.

The molecular phylogeny dataset included three outgroups and 40 species of ingroup taxa, including two individuals each of *Niveas kone* and *N. agassizi* totaling 45 terminal units. We analyzed data from eight genes: COI (mitochondrial), CAD, EF1*a*, GAPDH, IDH, MDH, RpS5, and wingless (all nuclear) (Wahlberg and Wheat 2008). The final alignment was 6187 base pairs long. Molecular sequences for all taxa except *N. kone* and *N. agassizi* are from Rota (2011) and Rota and Wahlberg (2012), and their GenBank accession numbers can be found there. For the specimens of *N. kone* (660733) and *N. agassizi* (Ch_JR44_1), DNA extraction was done from whole abdomens, which were later used for dissection of genitalia. Because the DNA amplification methods described by Wahlberg and Wheat (2008) did not work for obtaining sequences of nuclear genes from these specimens, suggesting that their DNA was too degraded for the standard approach, we used newly-designed primers (Niklas Wahlberg, unpublished) (Table 3) to amplify short fragments of the nuclear genes (see Table 4 for total number of base pairs for each gene fragment amplified and the GenBank accession numbers for fragments longer than 200 base pairs). For sequence storage and manipulation we used the VoSeq application (Peña and Malm 2012). The nexus file with the alignment is available from the Figshare Digital Repository: doi: 10.6084/m9.figshare.811841

Both maximum likelihood (ML) and Bayesian phylogenetic analyses were performed. ML analysis of unpartitioned data was conducted using RAxML blackbox available online (Stamatakis et al. 2008) with the GTR+G model and 100 bootstraps. Bayesian analysis of data partitioned using the program TIGER (Cummins and McInerney 2011) as described in Rota and Wahlberg (2012) was carried out in MrBayes v. 3.2 (Ronquist et al. 2012) for 10 million generations with one cold and three heated chains, sampling trees every 1000 generations. The analyses were run on the freely available Bioportal server (University of Oslo, Norway). The convergence was assessed

Table 2. Locality information.

Locality	m.a.s.l.	latitude	longitude
Baitabag village & Kau Wildlife Area, near Madang, Madang Province, PNG	50	S5°08'	E145°46'
Mis, Madang Province, PNG	50	S5°11'	E145°47'
Ohu Conservation Area, Ohu village near Gum river, Madang Province, PNG	100	S5°13'	E145°41'
Pau, Madang Province, PNG	0	S5°08'	E145°46'
Reinduk, Madang Province, PNG	225	S5°39'	E145°24'
Riwo, Madang Province, PNG	0	S5°09'	E145°48'
Tab Island, Madang Province, PNG	0	S5°10.6'	E145°52.6'
Wanang village, Madang Province, PNG	115	S5°13.9'	E145°10.9'
Mwabungu, County of Kwale, Kenya	0	S4°20.3'	E39°37'

by examining plots of log likelihoods and all model parameters using Tracer v.1.5 (Rambaut and Drummond 2007), as well as potential scale reduction factors and split frequencies, both reported by MrBayes. Branch support is expressed as Bayesian posterior probability (PP) and maximum likelihood bootstraps (ML BS).

DNA barcode sequences (COI) for *Niveas kone* (24 specimens) and *Niveas agassizi* (2 specimens) were obtained at the Biodiversity Institute of Ontario, University of

Table 3. Primers.

COI-1F	GGTCAACAAATCATAAAGATATTGG
COI-1R	GGWGCYCCTARTATTAAAGGWAYTA
EF-1F	CACATYAACATTGTCGTSATYGG
EF-1R	TRSCGGTYTCGAACTTCCA
EF-2F	GAGCGTGARCGTGGTAT
EF-2R	RGCTTCGAACTCACCRGTA
EF-3F	TCAAGAACATGATCACYGG
EF-3R	GARGAYACTTCCTTCTTGA
EF-7F	CAAYGTTGGTTTCAACGT
EF-8R	ACAGCVACKGTYTGYCTCATRTC
GAPDH-1F	AARGCTGGRGCTGAATATGT
GAPDH-1R	AAGTTGTCATGGATRACCTT
GAPDH-2F	GTCATCTCYAATGCYTCYTG
GAPDH-2R	TAACTTTGCCRACAGCYTT
GAPDH-3F	GTGCCCARCARAACATCAT
GAPDH-3R	TCAGCGGCTTCCTTRACCT
IDH-1F	GGWGAYGARATGACNAGRATHATHTGG
IDH-1R	GGACTCTTCCACATTTTYTT
MDH-1F	GAYATNGCNCCNATGATGGGNGT
MDH-1R	TCYTTRCGRGCAACYTTRTC
RPS5-1F	ATGGCNGARGARAAYTGGAAYGA
RPS5-1R	TTGTGWGCRTACCTRCCRGC

Table 4. GenBank accession numbers and the number of base pairs for each gene fragment.

	***N. agassizi* (730793)**	***N. agassizi* (Ch_JR44_1)**	***N. kone* (730509)**	***N. kone* (660733)**
COI	**HQ946716**	-	**HQ946550**	**KF646130**
	609 bp	176 bp	658 bp	610 bp
EF1*a*	-	**KF646128, KF646129**	-	**KF646131, KF646132**
	-	550 bp	-	706 bp
GAPDH	-	-	-	**KF646133**
	-	136 bp	-	430 bp
IDH	-	135 bp	-	-
MDH	-	190 bp	-	-
RpS5	-	155 bp	-	108 bp

Guelph, using their standard methodology (Craft et al. 2010; Hrcek et al. 2011; Wilson 2012). They are deposited in GenBank as accessions listed in Table 1, and their full data including images are in the Barcode of Life Database (http://www.boldsystems.org; see Ratnasingham and Hebert 2007; 2013). These sequences were also analyzed with MrBayes v. 3.2 (unpartitioned dataset, 2 million generations).

Results

Taxonomy

***Niveas* Rota, gen. n.**
http://zoobank.org/F352952E-0F21-464F-BD1E-278C9A0679C1
http://species-id.net/wiki/Niveas
Figs 1–9

Type species. *Niveas kone.*

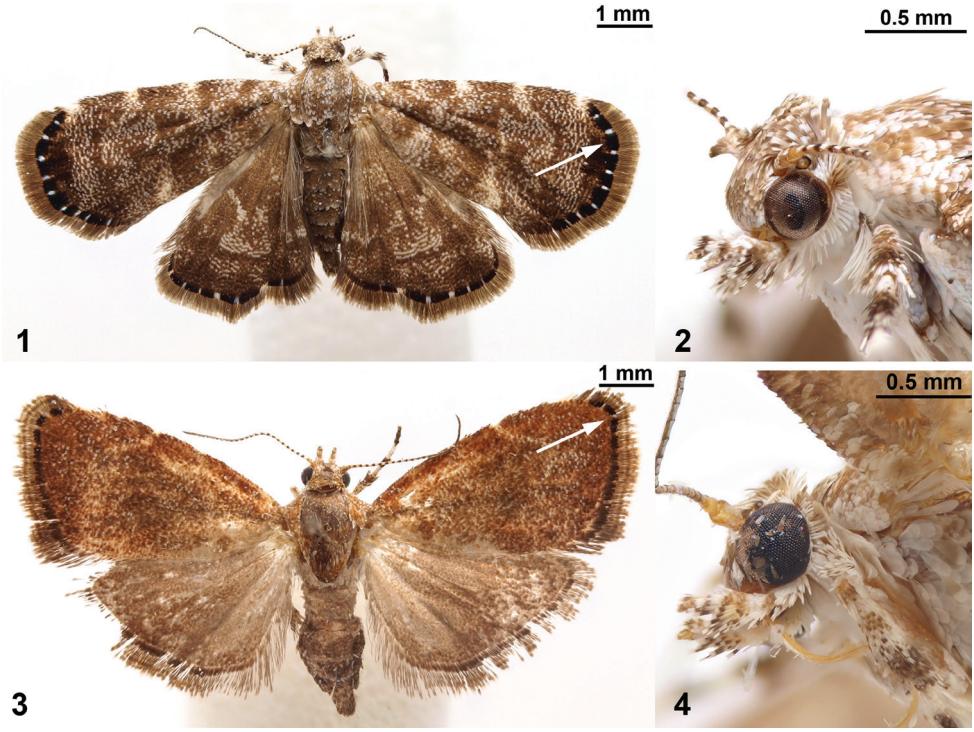
Material examined. See Table 1.

Distribution. Kenya, Papua New Guinea, Solomon Islands.

Diagnosis. *Niveas* can be easily distinguished from most genera of choreutids by the wing pattern (Figs 1, 3). Superficially, species of *Niveas* are similar to some species of *Anthophila* and *Choreutis*, but there is no known species in either of the latter two genera with a black terminal band enclosing white spots in the forewing as in *N. agassizi* and *N. kone*. (Figs 1, 3). Forewing venation with only four radial branches or with R_4 and R_5 fused in the basal half is also diagnostic for the genus. Female genitalia with paired concave sclerotizations on A7 sternite are also unique to *Niveas*.

Description. *Head.* Labial palpi with projecting ventral scale tufts (Figs 2, 4). *Wings.* Forewing veins R four-branched in *N. kone* (Fig. 5), five-branched in *N. agassizi* (Fig. 6), with R_4 an R_5 fused in basal 3/5; CuP present at termen for 1/3 to 1/5 wing length, extending as fold further towards base. Hindwing ten-veined, with M_2 in close proximity to the basally fused M_3 and CuA_1 (*N. agassizi*) or nine-veined, apparently with M_3 and CuA_1 completely fused into a single vein (Figs 5, 6). *Male genitalia.* Tegumen rounded on top, tuba analis extending beyond tegumen; vinculum as inverted trapezoid ventrally emarginate; valva with costal margin straight, ventral margin rounded, ending with a horn-like projection; phallus twice as long as valva (Fig. 7). *Female genitalia.* Apophyses anteriores slightly longer than posteriores; ostium bursae on A7 with a more or less strongly sclerotized antrum; ductus bursae straight, not coiled, with strong lateral sclerotizations; corpus bursae as a single sac (*N. agassizi*) or divided into two sacs (*N. kone*) with one or more signa. A7 sternite with paired, somewhat rounded, concave sclerotizations proximally, clearly visible in *N. kone* (Fig. 8), and slightly less so in *N. agassizi* (Fig. 9).

Host plants. Genus *Ficus* (Moraceae).



Figures 1–4. *Niveas kone*: **1** Habitus **2** Head. *Niveas agassizi*: **3** Habitus **4** Head. (In Figs 1 and 3 arrows point at the terminal black band enclosing white spots.)

Etymology. The generic name is derived from Latin *niveum*, meaning snowy, in reference to speckles of white-tipped scales in the wings of the type species; it is not treated as a Latin word and is feminine in gender.

***Niveas kone* Rota, sp. n.**
http://zoobank.org/9EA367B0-6B92-48FA-8075-D8D0D0BFA566
http://species-id.net/wiki/Niveas_kone
Figs 1, 2, 5, 7, 8

Material examined. See Table 1.

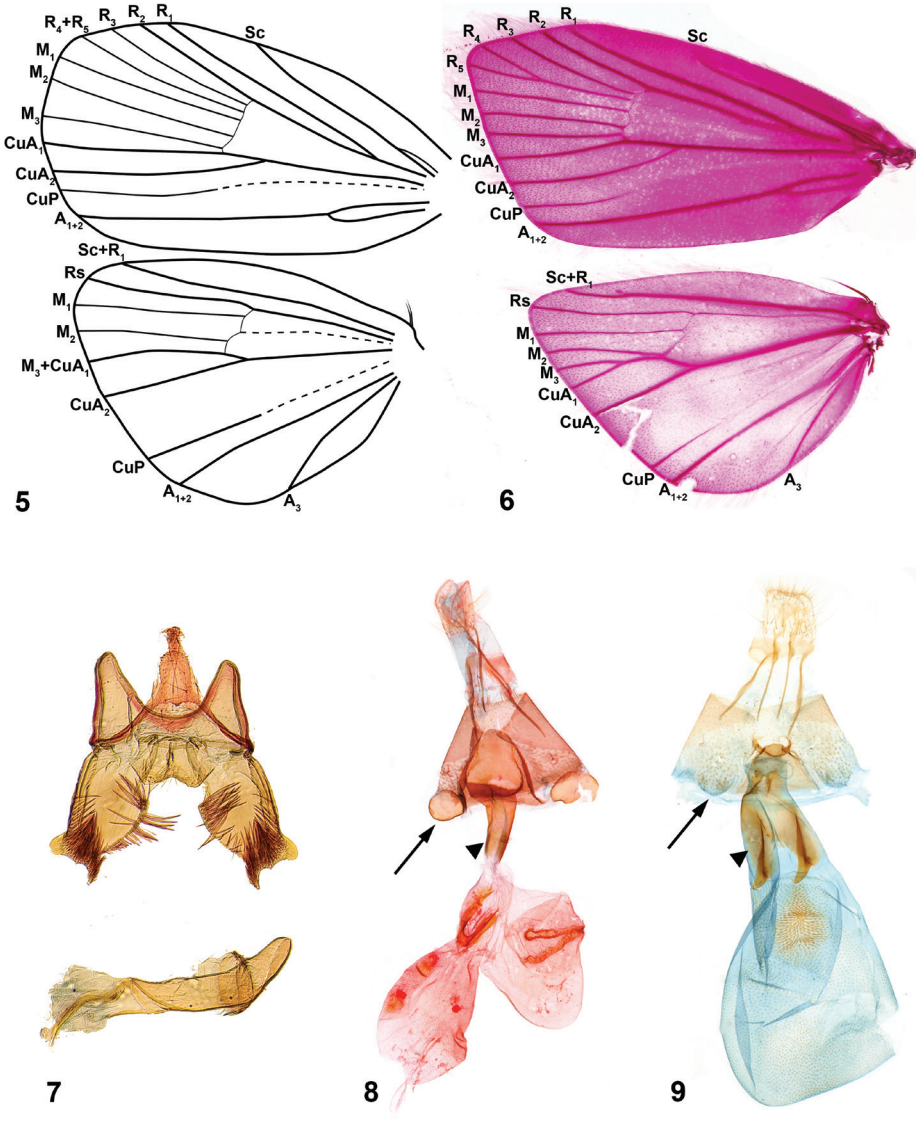
Material deposited. The holotype and most paratypes will be retained at USNM, with paratypes distributed to PNG National Agriculture Research Institute (Port Moresby), BMNH, Bishop Museum, Naturalis (Leiden), and CSIRO (Canberra).

Distribution. Papua New Guinea, Solomon Islands.

Diagnosis. *N. kone* can be separated from all other known choreutids based on its wing pattern (Fig. 1). Superficially, it is similar to a few species of *Brenthia* Clemens, 1860 and *Litobrenthia* Diakonoff, 1978 owing to its background color, but it lacks iridescent

spots along forewing termen, which are always present in those two genera. Both male and female genitalia are very distinct from those of other choreutids (Figs 7, 8).

Description. *Head.* Fig. 2. *Wings.* Fore- and hindwing with brown background color, speckled with white-tipped scales in an irregular pattern; a distinct black band along termen of both wings within which are more or less equidistant white spots (Fig. 1). *Male genitalia.* As for the genus (Fig. 7). *Female genitalia.* Corpus bursae split



Figures 5–9. *Niveas kone*: **5** Wing venation **7** Male genitalia **8** Female genitalia. *Niveas agassizi*: **6** Wing venation **9** Female genitalia. (In Figs 8 and 9 arrows point at the A7 sternite sclerotizations, and triangles point at the lateral sclerotizations on the ductus bursae.)

into two sacs; one sac with a V-shaped signum, the other with two round signa (Fig. 8). *Immature stages.* Fig. 12. See a brief note in text.

Host plants. *Ficus botryocarpa* Miq., *F. nodosa* Teijsm. & Binn., *F. phaeosyce* K. Schum. & Lauterb., *F. pungens* Reinw. ex Blume, *F. variegata* Blume, and *F. wassa* Roxb. (Moraceae).

Etymology. The species is named after the Finnish Kone Foundation (Koneen Säätiö) in appreciation of their funding of this work. The name is a noun in apposition.

***Niveas agassizi* Rota, sp. n.**
http://zoobank.org/7F08322B-C0D2-450C-9DFF-ED9E4FEA5892
http://species-id.net/wiki/Niveas_agassizi
Figs 3, 4, 6, 9

Material examined. See Table 1.

Material deposited. The holotype will be deposited in National Museums of Kenya (Nairobi) (NMK), with paratypes to USNM, BMNH and NMK.

Distribution. Kenya.

Diagnosis. *N. agassizi* can be separated from other known choreutids by the wing pattern (Fig. 3). It is superficially similar to some species of *Choreutis*, but the latter usually have forewings with apparent patterning, and this is absent in *N. agassizi*. Female genitalia are very distinct from those of other choreutids (Fig. 9).

Description. Male unknown. *Head.* Fig. 4. *Wings.* Forewing bronze-brown with speckled white-tipped scales over most of its surface; distinct dark brown to black band along termen with two small white spots at apex; hindwing light brown (Fig. 3). *Male genitalia.* Unknown. *Female genitalia.* Ductus bursae short and wide, opening into large corpus bursae, with one oval signum (Fig. 9). *Immature stages.* Unknown.

Host plants. Unknown.

Etymology. This species is named after David Agassiz, who collected all the known specimens and made many significant contributions to our knowledge of African micro-moths. The name is a noun in the genitive case.

Remarks

We obtained 19 full-length barcodes of *Niveas kone*, as well as 5 shorter fragments. These form cluster AAB7478 in the Barcode of Life Database (accessed 29 August 2013), and using the RESL algorithm as implemented there (Ratnasingham and Hebert 2013), the maximum distance between the COI sequences for members of the species is 0.65%, whereas the distance to the nearest cluster (*N. agassizi*) is 9.22%. In a Bayesian analysis of the COI sequences, all *N. kone* and all *N. agassizi* specimens grouped together with the other members of their species with very high branch support (PP=1) (Fig. 10).

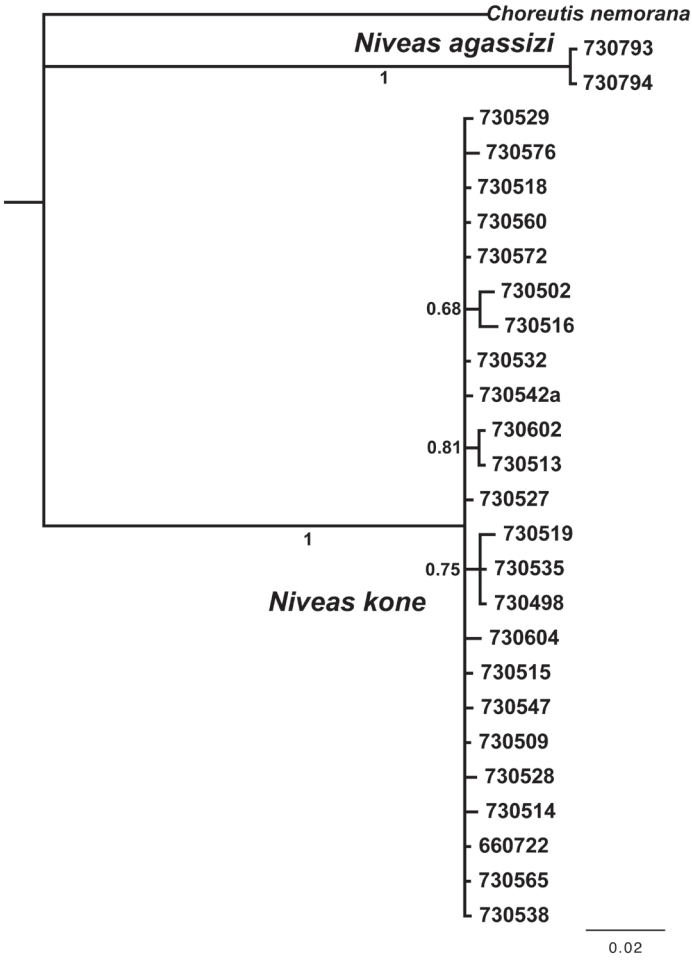


Figure 10. DNA barcode tree from a Bayesian analysis showing low divergence within species and high between species of *Niveas*. Numbers below or next to branches are Bayesian posterior probabilities. Specimen ID numbers are used as labels for the terminal branches.

The placement of *Niveas* in the choreutid generic phylogeny is very strongly supported. *Niveas* clearly belongs within the subfamily Choreutinae (PP=1; ML BS=96), and it appears to be the sister group of *Choreutis* (PP=1.00; ML BS=92) (Fig. 11).

Further comments on the biology of *Niveas kone*: Over the years, BRC field teams have encountered larvae identified as *Niveas kone* (as project morphospecies TORT015) 118 times, of which 62 were reared to adults, usually on *Ficus nodosa* and *Ficus variegata*, but also on four other species of *Ficus* (see full host plant list under *N. kone* description). Larvae have been found in all months except April and November, and are described by BRC staff as being green-clear-whitish in color, with short white hairs, and one spot on the side of the head (Fig. 12). Larvae of *N. kone* share the presence of short hairs with other Choreutinae (Rota 2005), which is unlike Brenthiinae

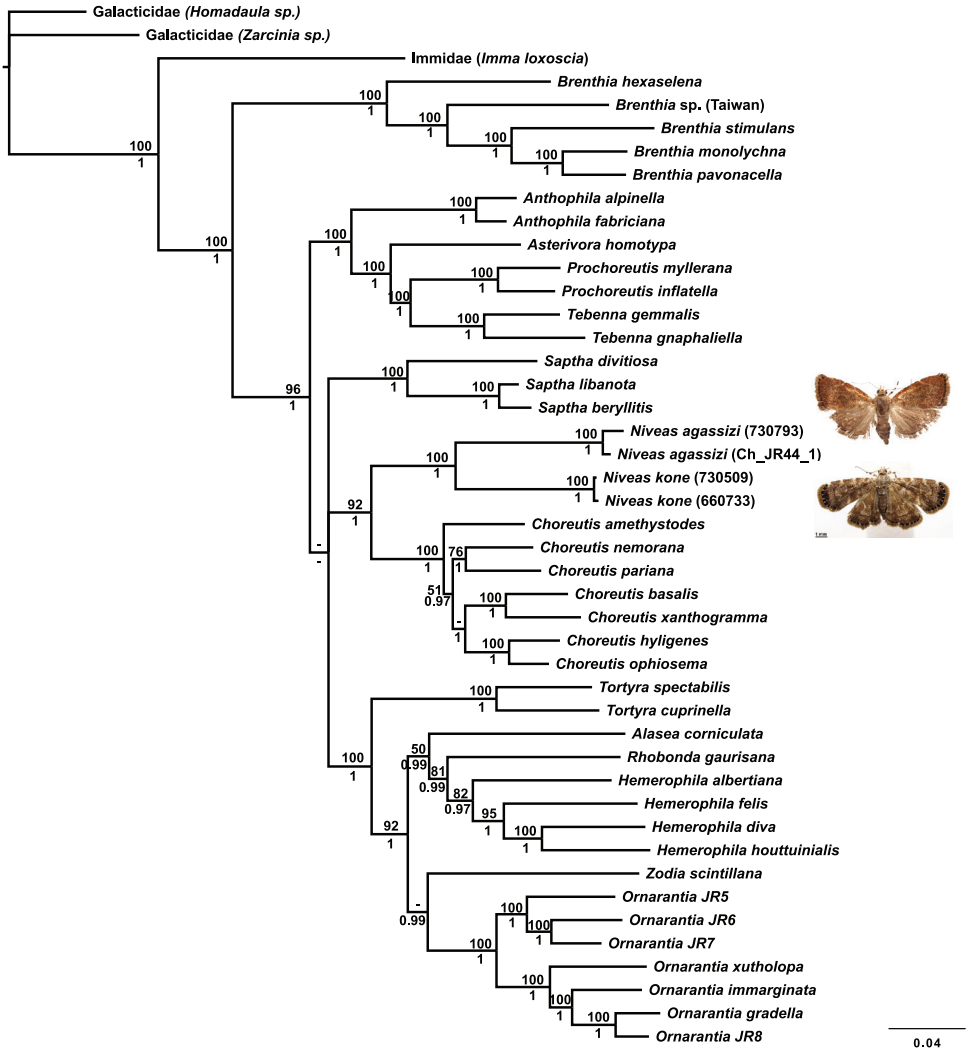


Figure 11. Phylogenetic tree from a Bayesian analysis showing the position of *Niveas* in relation to other choreutid genera. Maximum likelihood (ML) bootstraps are shown above branches, and Bayesian posterior probabilities (PP) are below branches; dashes represent ML bootstraps<50 and PP<0.95.

larvae, which possess very long hairs (Rota 2008a). Project field notes indicate that the shelters are distinct from other local Choreutidae in having strong white webbing. BRC has encountered them most commonly in the lowland coastal areas around Madang (city), but also in the coastal mountains behind Madang (up to about 100 m elevation), and at Wanang in the Ramu River Basin (115 m). The species has been recorded in publications (e.g., supplement to Novotny et al. 2010) and online databases as TORT015, misidentified as *Brenthia* sp. Based on locality information provided by Taylor and Maffi (1978: 185, 212), the Solomon Islands specimens are from lowland and foothill localities near Honiara, Guadalcanal; they were collected in light traps.

Figure 12. A photograph of the *Niveas kone* larvae made in the field.

Taxon descriptions are also organized in tabular format for ease of comparison (see Appendix).

Discussion

The two species of *Niveas* described herein are superficially quite different, but upon closer examination it becomes apparent that they share a number of morphological features. We consider the following as potential autapomorphies of *Niveas*: fusion or reduction in R veins in the forewing (Figs 5, 6); presence of round, concave sclerotizations on the A7 sternite in females (arrows in Figs 8, 9); strong lateral sclerotizations at the base of the ductus bursae (triangles in Figs 8, 9); and the presence of a terminal black band with white spots in the forewing (arrows in Figs 1, 3). In all other Choreutinae genera there are five fully-separated radial veins in the forewing; the A7 sternite in the female, as well as the base of the ductus bursae, are evenly sclerotized; and if present, a black terminal band in the forewing lacks white spots.

The split between *N. kone* and *N. agassizi* has presumably happened a long time ago based on the large COI divergence between them and the length of branches in the phylogenetic analysis including the nuclear genes. We considered assigning each species to its own monotypic genus because of their different external appearance, as well as some of the differences in venation and some aspects of female genitalia. It is unfortunate that *N. agassizi* is known from females only as perhaps the morphology of the male genitalia would help clarify the status of this species. However, we believe that *N. kone and N. agassizi* being each other's closest relatives among the currently known species of choreutids is best conveyed by assigning them to a single genus and therefore we opted for this more conservative approach. It is conceivable that other species of *Niveas* that might bridge this gap in both genetic and morphological variation will be discovered in the future. On the other hand, it is also possible that a new genus will need to be erected to accommodate *N. agassizi* and its currently unknown relatives.

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Appendix

Taxon descriptions organized in tabular format for ease of comparison.

Taxon	*Niveas* Rota, gen. n.	*Niveas kone* Rota, sp. n.	*Niveas agassizi* Rota, sp. n.
Type species	*Niveas kone*		
Material examined	See Table 1.	See Table 1.	See Table 1.
Material deposited		The holotype and most paratypes will be retained at USNM, with paratypes distributed to PNG National Agriculture Research Institute (Port Moresby), BMNH, Bishop Museum, Naturalis (Leiden), and CSIRO (Canberra).	The holotype will be deposited in National Museums of Kenya (Nairobi) (NMK), with paratypes to USNM, BMNH and NMK.
Distribution	Kenya, Papua New Guinea, Solomon Islands.	Papua New Guinea, Solomon Islands.	Kenya.
Diagnosis	*Niveas* can be easily distinguished from most genera of choreutids by the wing pattern (Figs 1, 3). Superficially, species of *Niveas* are similar to some species of *Anthophila* and *Choreutis*, but there is no known species in either of the latter two genera with a black terminal band enclosing white spots in the forewing as in *N. agassizi* and *N. kone*. (Figs 1, 3). Forewing venation with only four radial branches or with R_4 and R_5 fused in the basal half is also diagnostic for the genus. Female genitalia with paired concave sclerotizations on A7 sternite are also unique to *Niveas*.	*N. kone* can be separated from all other known choreutids based on its wing pattern (Fig. 1). Superficially, it is similar to a few species of *Brenthia* and *Litobrenthia* owing to its background color, but it lacks iridescent spots along forewing termen, which are always present in those two genera. Both male and female genitalia are very distinct from those of other choreutids (Figs 7, 8).	*N. agassizi* can be separated from other known choreutids by the wing pattern (Fig. 3). It is superficially similar to some species of *Choreutis*, but the latter usually have forewings with apparent patterning, and this is absent in *N. agassizi*. Female genitalia are very distinct from those of other choreutids (Fig. 9).
Description	Figs 1–9.	Figs 1, 2, 5, 7, 8.	Male unknown. Figs 3, 4, 6, 9.
Head	Labial palpi with projecting ventral scale tufts (Figs 2, 4).	Fig. 2.	Fig. 4.
Wings	Forewing veins R four-branched in *N. kone* (Fig. 5), five-branched in *N. agassizi* (Fig. 6), with R_4 an R_5 fused in basal 3/5; CuP present at termen for 1/3 to 1/5 wing length, extending as fold further towards base. Hindwing ten-veined, with M_2 in close proximity to the basally fused M_3 and CuA_1 (*N. agassizi*) or nine-veined, apparently with M_3 and CuA_1 completely fused into a single vein (Figs 5, 6).	Fore- and hindwing with brown background color, speckled with white-tipped scales in an irregular pattern; a distinct black band along termen of both wings within which are more or less equidistant white spots (Fig. 1).	Forewing bronze-brown with speckled white-tipped scales over most of its surface; distinct dark brown to black band along termen with two small white spots at apex; hindwing light brown (Fig. 3).

Taxon	*Niveas* Rota, gen. n.	*Niveas kone* Rota, sp. n.	*Niveas agassizi* Rota, sp. n.
Male genitalia	Tegumen rounded on top, tuba analis extending beyond tegumen; vinculum as inverted trapezoid ventrally emarginate; valva with costal margin straight, ventral margin rounded, ending with a horn-like projection; phallus twice as long as valva (Fig. 7).	As for the genus (Fig. 7).	Unknown.
Female genitalia	Apophyses anteriores slightly longer than posteriores; ostium bursae on A7 with a more or less strongly sclerotized antrum; ductus bursae straight, not coiled, with strong lateral sclerotizations; corpus bursae as a single sac (*N. agassizi*) or divided into two sacs (*N. kone*) with one or more signa. A7 sternite with paired, somewhat rounded, concave sclerotizations proximally, clearly visible in *N. kone* (Fig. 8), and slightly less so in *N. agassizi* (Fig. 9).	Corpus bursae split into two sacs; one sac with a V-shaped signum, the other with two round signa (Fig. 8).	Ductus bursae short and wide, opening into large corpus bursae, with one oval signum (Fig. 9).
Immature stages		Fig. 12. See a brief note in text.	Unknown.
Host plants	Genus *Ficus* (Moraceae).	*Ficus botryocarpa* Miq., *F. nodosa* Teijsm. & Binn., *F. phaeosyce* K. Schum. & Lauterb., *F. pungens* Reinw. ex Blume, *F. variegata* Blume, and *F. wassa* Roxb. (Moraceae).	Unknown.
Etymology	The generic name is derived from Latin *niveum*, meaning snowy, in reference to speckles of white-tipped scales in the wings of the type species; it is not treated as a Latin word and is feminine in gender.	The species is named after the Finnish Kone Foundation (Koneen Säätiö) in appreciation of their funding of this work. The name is a noun in apposition.	This species is named after David Agassiz, who collected all the known specimens and made many significant contributions to our knowledge of African micro-moths. The name is a noun in the genitive case.

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Treerunners, cryptic lizards of the *Plica plica* group (Squamata, Sauria, Tropiduridae) of northern South America

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Abstract

The arboreal, Neotropical lizard *Plica plica* (Linnaeus, 1758) has been long considered a widespread species with a distribution east of the Andes. A preliminary examination of 101 specimens from about 28 locations mostly north of the Amazon suggests that *Plica plica* is a cryptic species complex with taxa that can be distinguished on the basis of the number of scale rows at mid-body; the arrangement, shape and ornamentation of scales on the snout; the number of lamellae on the fourth toe; the number of subocular plates; as well as other commonly used external morphological traits. The allopatric species discussed here are concordant with northern South American geography. *Plica plica* (Linnaeus,1758) is associated with the Guiana Shield (Suriname, Guyana and Venezuela). A second species, *P. caribeana* **sp. n**. is associated with the Caribbean Coastal Range of Venezuela including Trinidad and Tobago. A third, distinctive species, *P. rayi* **sp. n**. is associated with the middle Orinoco at the eastern edge of the Guiana Shield. Two other species, *P. kathleenae* **sp. n.** and *P. medemi* **sp. n.,** each based upon a single specimen, one from the Sierra Acarai Mountains of Guyana, and the other from southern Meta, Colombia are described. In addition to morphological analyses, we sequenced 12S and 16S rDNA gene fragments from one *Plica plica* from Trinidad to assess its relationship and taxonomy to other mainland *Plica* cf *plica.* The results suggest *Plica caribeana* **sp. n.** likely diverged prior to the separation of Trinidad from northern Venezuela. Isolation in the Caribbean Coastal Range during its rapid uplift in the late Miocene, combined with a marine incursion into northern Venezuela may have contributed to their genetic divergence from other populations.

Resumen

La especie arbórea de lacértido Neotropical *Plica plica* ha sido considerada por mucho tiempo una especie ampliamente distribuida al este de Los Andes. Un examen preliminar de 101 especímenes de más de 28 localidades, mayoritariamante del norte del Amazonas, sugiere que *Plica plica* es un complejo de especies crípticas con taxa que pueden ser distinguidos principalmente por el número de filas de escamas en el centro del cuerpo; la disposición, la forma y ornamentación de las escamas en el hocico; el número de lamellas en el cuarto dígito del pie; el número de placas suboculares; así como por otras características morfológicas comúnmente empleadas. Las especies alopátricas aquí discutidas concuerdan con la geografía del norte de Sur América. *Plica plica* (Linnaeus,1758) está asociada al escudo Guyanés (Surinam, Guyana y Venezuela). Una segunda especie, *P. caribeana* **sp. n.** está asociada a la zona costera montañosa de Venezuela, incluyendo Trinidad y Tobago. Una tercera distintiva especie, *P. rayi* **sp. n.** está asociada al centro del Orinoco. Dos otras especies, *P. kathleenae* **sp. n.** y *P. medemi* **sp. n.** están descritas en base a un solo ejemplar, una para las montañas de la Sierra Acarai de Guyana, y la otra para el sur de Meta (Colombia) respectivamente. Algunas especies amazónicas pueden ser simpátricas basándose en las localidades de los especímenes de museos. Además de análisis morfológicos, secuenciamos una fracción del gen 12S y 16S rDNA de un especimen de *Plica plica* de Trinidad para investigar su relación y taxonomía con *Plica* cf *plica* del continente. Los resultados sugieren que *Plica plica* de Trinidad y de la cordillera costera de Venezuela probablemente divergieron antes de la separación de Trinidad del norte de Venezuela. El aislamiento en la región de la cordillera costera de Venezuela durante el rápido levantamiento en el Mioceno tardío, combinado con una posible inclusión marina en el norte de Venezuela, probablemente contribuyó aún más a la divergencia genética de otras poblaciones.

Keywords

Arboreal lizards, Iguania, Neotropics, new species, systematics

Introduction

Tropidurid lizards are usually scansorial, dwelling on vertical rocks and tree trunks with morphology that includes strongly keeled scales that contribute to their cryptic appearance. The family currently holds eight genera and about 117 species. Townsend et al. (2011) suggest that family last shared and ancestor with the other clades of Neotropical iguanians about 65 million years ago (MYA).

The tropidurid genus *Plica* Gray, 1831 (treerunners) currently contains four species restricted to South America east of the Andes. Two of these are relatively widespread (*Plica plica* and *P. umbra*). The other two species are associated with Pantepuis. *Plica lumaria* is known only from southern Venezuela's Guaiquinima Tepui (Donnelly and Myers 1991), and *P. pansticta* (Myers and Donnelly 2001) from the Yutajé–Corocoro massif of Amazonas, Venezuela. Treerunners are diurnal, medium sized, sit in the open on vertical surfaces, and are often in small colonies that include adults of both sexes and juveniles. The sounds they make scurrying on the bark of trees or rock outcrops draws attention to their presence and thus, they are common in museum collections.

In a review of *Plica*, Etheridge (1970) restricted the type locality for *Lacerta plica* Linnaeus to the vicinity of Paramaribo, Suriname, designating NRM.112 as the lectotype. Hoogmoed (1973) further restricted the locality to the confluence of the Cottica River

and Perica Creek, Suriname. *Plica plica* is known from the countries of Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru, Suriname, and Venezuela, as well as the islands of Trinidad and Tobago, and including the Bocas Island group (Boos 1984; Avila-Pires 1995; Murphy and Downie 2012). Additionally, two specimens collected in the 19th century in the British Museum have the locality data "Grenada" (Henderson and Murphy 2012).

Of the four species of *Plica* currently recognized only *P. umbra* lacks tufts of spines on the neck; and it has 43–69 scales around mid-body (Avila-Pires 1995). *Plica lumaria* is black, the superciliaries are directed laterally, it lacks clusters of spines on the fold below the auditory meatus, and it has 141–156 rows of scales around mid-body and 27–33 lamellae under the fourth toe (Donnelly and Myers 1991). *Plica pansticta* has 143–164 scales around mid-body and 31–39 lamellae under the fourth toe (Myers and Donnelley 2001). However, *P. plica* (sensu Etheridge 1970) has 92–202 scales around mid-body; 21–35 lamellae on the fourth finger and 28–45 lamellae on the fourth toe. The polytypic *P. plica* has been the subject of ecological (Vitt 1991; Brandt and Navas 2011), morphological (Kohlsdorf et al. 2007; Kohlsdorf et al. 2008), and phylogenetic studies (Frost 1992; Harvey and Gutberlet 2000; Frost et al. 2001). Figure 1 shows *Plica plica* from various localities and variation in their overall appearance. Etheridge (1970) seemed to be aware of at least some of the variation. He wrote:

The most deviant sample I have seen is from Puerto Ayachuco (sic) on the Orinoco River, between Venezuela and Colombia. Specimens from this locality differ from others in having a more acuminate snout, larger dorsal snout scales, slightly smaller dorsal body scales, less well developed lateral neck spines, and a less densely spotted throat in males. Specimens from the vicinity of Esmeralda, Venezuela also have large dorsal snout scales but are otherwise typical. Specimens from northern Venezuela, Trinidad, and the Guianas appear to reach the greatest maximum size, and because of greater development of scale mucrons have overall a more spiny appearance.

The intent of this work is not to revise the entire species group but rather to present morphological and molecular evidence that *Plica plica* is a complex of cryptic species, examine the availability of previously proposed names, resolve some of the species that occur in northern South America and suggest avenues for future investigations.

Methods and materials

Molecular methods

Individual DNA was extracted using the Chelex extraction process (Bio-Rad®, Hercules, 12 CA, U.S.A; Walsh et al. 1991) following slight protocol modifications. Muscle tissue (2 mm^3) was cut thoroughly and incubated for 2h at 57°C in 160 µl of 5% Chelex with 40 µl Proteinase K to increase tissue digestion. Following the incubation period it was heated at 100°C for 15 minutes. After a 4 min centrifugation at 12500 rpm, all supernatant was transferred into a 1.5 ml tube. We aimed to amplify a fraction of the mitochondrial 12S and16S rDNA genes with mitochondrial vertebrate universal prim-

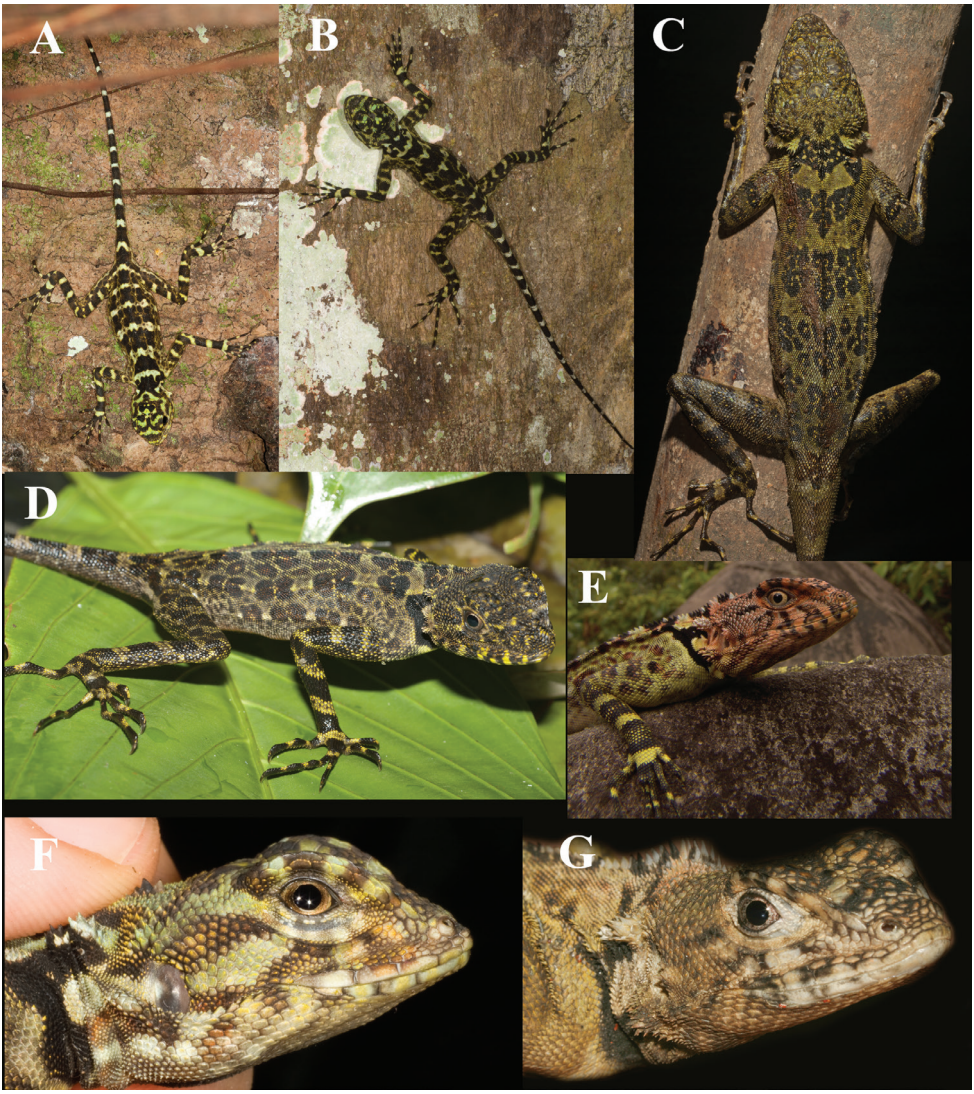

Figure 1. A composite of photographs of lizards in the *Plica plica* Group from various locations: **A** and **F** Tiputini Biodiversity Station, Napo, Ecuador, Ryan Sawby **B** Santa Cruz, Río Mazán near Iquitos (Iquitos, Loreto, Peru), Tobias Eisenberg **C** and **D** Amaila Falls, Guyana, Pedro Bernardo **E** La Laja, Sierra de Lema, 490 m., Estado Bolivar, Venezuela, César Luis Barrio Amorós **G** Cuesa River, Trinidad, JCM.

ers to compare with other *Plica plica* sequences available in Genbank. For each PCR, the 20 µl PCR volume contained approximately 50 ng DNA, 200 µM of each dNTP, 0.15 µM of each primer, 2 µl 10X Buffer, 0.8 µl $Mgcl_2$ and 0.1 unit of taq polymerase (QIAGEN). The thermal cycle profile was as follows: an initial denaturation step of 2min at 94°C; 35 cycles of denaturation at 30s at 94°C, annealing for 30s at 52°C and extension for 45s at 72°C; and a final extension for 5min at 72°C. Following the PCR,

Table 1. Combined matrices for the 12S rDNA and 16S rDNA gene fraction of *p*-uncorrected distances (below) and nucleotide substitutions (above). Specimen Genbank accessions: *P. plica* (unknown); AB218961, *P. plica* (Venezuela); 12S rDNA (L41431) 16S rDNA (L41482), *P. plica* (Guyana?); 12S rDNA (AF362520), *P. plica* (Brazil); 12S rDNA (EF615595) 16S rDNA (EF615664), *T. insulanus*; 12S rDNA (EF615596) 16S rDNA (EF615665), *T. oreadicus*; 12S rDNA (EF615597) 16S rDNA (EF615666).

	Plica (Tri)	*Plica* (unk)	*Plica* (Ven)	*Plica* (Guy)	*Plica* (Bra)	*T. insu*	*T. orea*
(1) *Plica plica* (Trinidad)	-	3	25	15	36	70	72
(2) *Plica plica* (unknown)	0.0043	-	22	13	33	71	73
(3) *Plica plica* (Venezuela)	0.0455	0.0400	-	6	15	49	51
(4) *Plica plica* (Peru)	0.0445	0.0386	0.0282	-	0	43	44
(5) *Plica plica* (Brazil)	0.0518	0.0475	0.0274	0.0000	-	71	74
(6) *Tropidurus insulanus*	0.1013	0.1027	0.0901	0.1284	0.1033	-	29
(7) *Tropidurus oreadicus*	0.1039	0.1053	0.0934	0.1313	0.1074	0.0417	-

excess primers and dNTPs were removed using enzymatic reaction of *E. coli* Exonuclease I, Antartic phosphatase and Antartic phosphatase buffer (all New England Biolabs). Sequencing was carried out in both directions using the BigDye® Terminator v1.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. Labelled fragments were resolved on an automated A3130*xl* genetic analyzer (Applied Biosystems). The primers for the12S rDNA: 12SA 5′-AAACTGGGATTAGATACCCCACTAT-3′, 12SB 5′-GAGGGTGACGGGCGGTGTGT-3′ Kocher et al. (1989) and 16S rDNA: 5′-GCCTGTTTATCAAAAACAT-3′, 16SH 5′-CCGGTCTGAACTCAGATCACGT- 3′ Palumbi et al. (1991) amplified approximately a 400 and 500 respectively base pair fraction (Genbank accessions: 12S rDNA; KU 895880, 16S rDNA; KU 895881). Templates were sequenced on both strands, and the complementary reads were used to resolve rare, ambiguous base-calls in Sequencher v.4.9. After removing PCR primers and incomplete terminal sequences, 337 and 378 base pairs of the 12S and 16S rDNA gene fraction, respectively (715 bp for both concatenated gene fractions) were available for analyses. Sequences were aligned in Seaview v.4.2.11 (Gouy et al. 2010) under ClustalW2 (Larkin et al. 2007) default settings. Nucleotide differences and *p*-uncorrected distances (%) analyses were calculated using MEGA v5 (Tamura et al. 2011).

In order to assess the phylogenetic relationships of *Plica plica* from Trinidad to the mainland, all *Plica plica* partial 12S and 16S rDNA sequences available from Genbank that could be aligned with ours were included in the alignment (Table 1). In addition, to root the phylogenetic tree, we employed as outgroup *Tropidurus oreadicus* and *T. insulanus*, members of a clade that is the sister to the clade containing *Plica* (Pyron et al. 2013).

The most appropriate substitution model for the Bayesian Inference (BI) analysis was determined by the Bayesian Information Criterion (BIC) in jModeltest v.0.1.1 (Posada 2008). The tree was constructed using the Bayesian Inference (BI) optimality criteria under the best fitting model (TIM2+I). MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001) was used with default priors and Markov chain settings, and with random starting trees. Each run consisted of four chains of 5,000,000 generations, sampled each 10,000 generations. A plateau was reached after few generations with 25% of the trees resulting from the analyses discarded as "burn in".

Morphological methods

We examined 101 museum specimens labeled *Plica plica* from: Brazil (n=8), Colombia (n=7), Ecuador (n=9), Guyana (n=2), Peru (n=25), Suriname (n=6), Trinidad (n=17), Venezuela (n=25), and Grenada (n=2). This was done to obtain a broad view of the variation within the species group, while focusing this paper on the Guiana Shield, the Coastal Ranges of northern Venezuela and Trinidad. Specimens examined from the area of study are listed in each species account; specimens examined but not assigned to a name are listed in Appendix 1. Scale counts and nomenclature follow that used by previous workers (Etheridge 1970; Hoogmoed 1973; Frost 1992; Avila-Pires 1995). Measurements of body and tail lengths were taken to the nearest 1 mm; scale measurements were taken to the nearest 0.1 mm and made with dial calipers. Values for paired head scales are given in left/right order. Note the terms spiny and mucronate are used interchangeably in reference to the well-developed keels on scales. Scale counts taken around mid-body were counted from one side of the dorsal crest to the other; ventral counts are scales between the ventrolateral folds. Photographs were taken with a Spot Digital Xplorer™ camera fixed to a Leica compound microscope, and with a Cannon EOS Rebel™ with a 100 mm macro lens. Statistical analysis was done with EXCEL-QI MACROS and DATA LAB 2.6. The PCA used 31 specimens with complete data, damaged specimens, or specimens with incomplete data were not included. Appendix 2 gives factor loadings for 25 meristic and morphometric traits. Institutional abbreviations used are given in the Acknowledgements. Abbreviations used: OUT – operational taxonomic units, PCA – principal component analysis, SAAM – position of spines on anterior margin of auditory meatus, SAB – scales around mid-body, SD – standard deviation, SRN – scales between nasal and rostral, STN – spiny tufts on nape, SVL – snout-vent length. Clarification of the head scale nomenclature used here is illustrated in Fig. 2.

Results

Molecular results

The best-fitting model for the BI tree was the TIM2+I (–lnL=–1567.2450, BIC= 3259.3633). *Plica plica* from Trinidad and one individual from the pet trade (unknown locality, probably collected on Trinidad or in adjacent Venezuela) showed little genetic variation (three substitutions). The Bayesian Inference consensus phylogram recovered three well-resolved monophyletic clades (BPP: 1) where *P. plica* from Trinidad is monophyletic with the pet trade *P. plica* and sister clade to all other *P. plica* available from Genbank (Fig. 3). Genetic divergence estimates for the 12S and 16S rDNA partial gene sequences combined showed contrasting levels of differentiation between localities (Trinidad vs *P. plica* from the pet trade: 0.04% and over 4% between Trinidad and all other *P. plica* mainland localities) (Table 1).

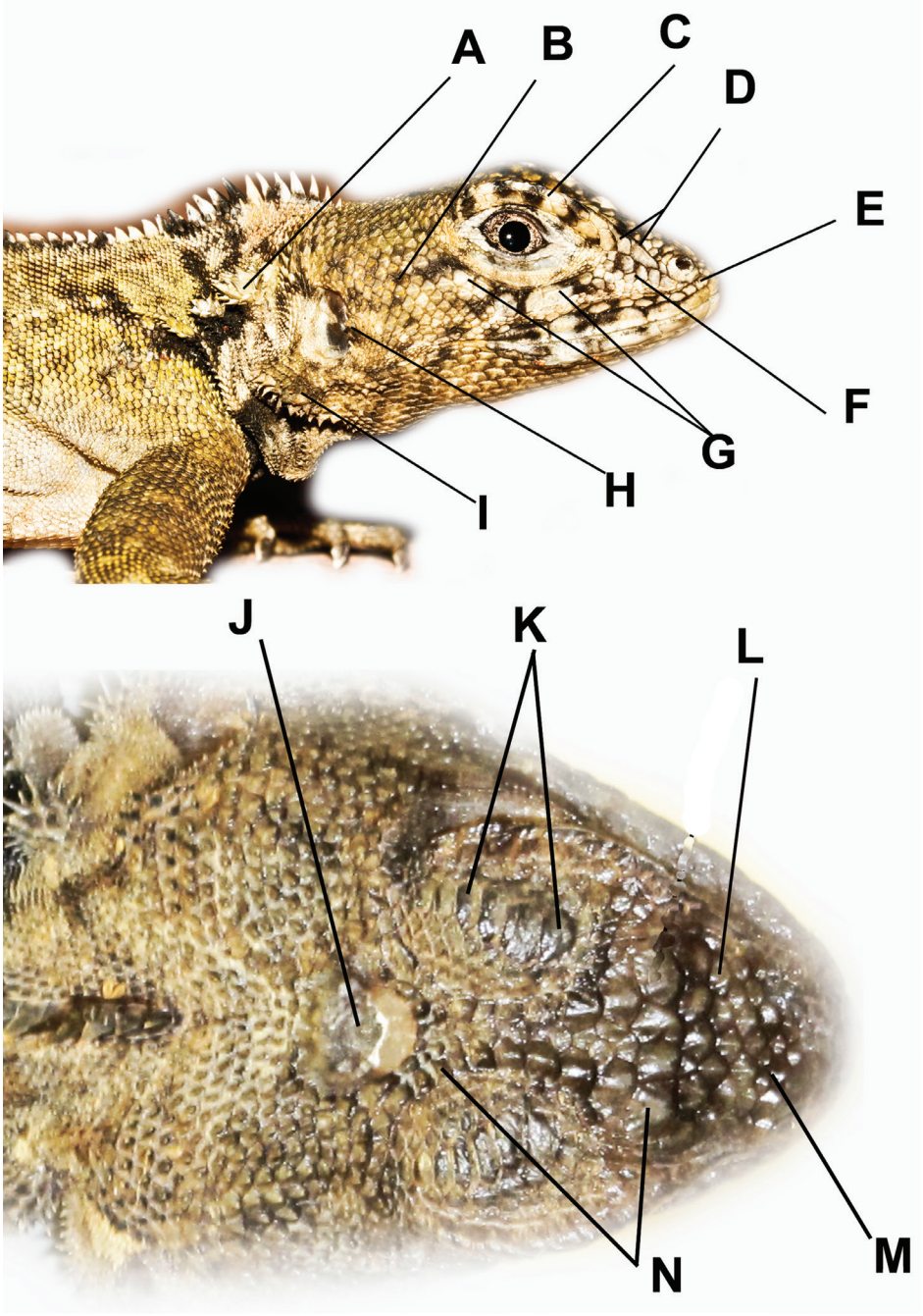

Figure 2. Head scale nomenclature used here. **A** Nape clusters or tufts of mucronate scales **B** Temporal scales **C** Superciliaries **D** Canthal(s) **E** Scale(s) between nasal and rostral **F** Loreals **G** Keeled suboculars **H** Cluster of spines on the anterior edge of the auditory meatus **I** Clusters (or tufts) of spines on the ventral flap of the auditory meatus **J** Occipitoparietal scale **K** Supraocular plates **L** Intercanthal scales **M** Internasal scales **N** Circumorbital scales.

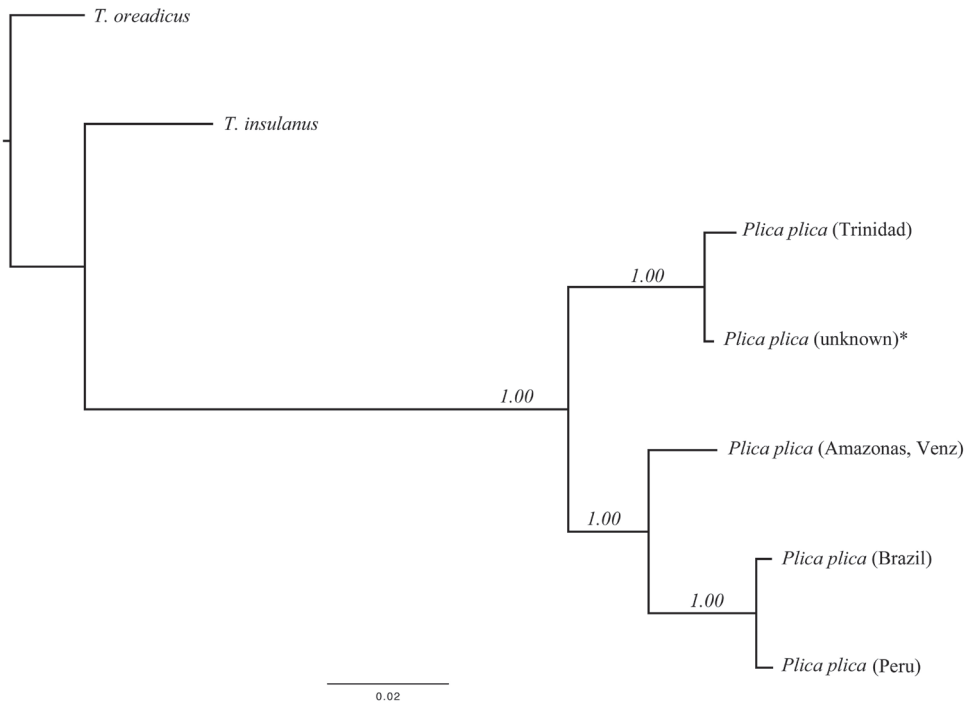

Figure 3. Bayesian Inference phylogram of all *Plica plica* species studied (12S and 16S rDNA partial genes, 715 bp). Values by nodes are the posterior probabilities recovered from the Bayesian Inference analysis. * *Plica plica* (unknown locality) was obtained from the pet trade.

Morphological results

For the morphological analysis we organized specimens geographically and compared them for 25 traits using a PCA (Fig. 4). The results support five morphologically distinct OTUs: one from the Caribbean Coastal Range and Trinidad; two from the Guyana Shield; one from southern Meta, Colombia; and one from the middle Orinoco drainage of Venezuela and Colombia. Table 2 compares the four species of *Plica* previously described to the four new species described here.

Available names

Iguana chalcidica Laurenti (1768:48) has the type locality of "Gallaecia" (= northwest Iberian Peninsula) and the specimen was based upon an illustration in Seba (1734, 2: 65.5). Given that the type locality is in error, and the detailed scale arrangements and counts needed to identify the species are unavailable, this name is *nomen dubia*.

Lophyrus panthera Spix (1825:10) was described from "*sylvis ad pagum Ecga*." The holotype was in the Zoologische Sammlung des Bayerischen Staates, München, and

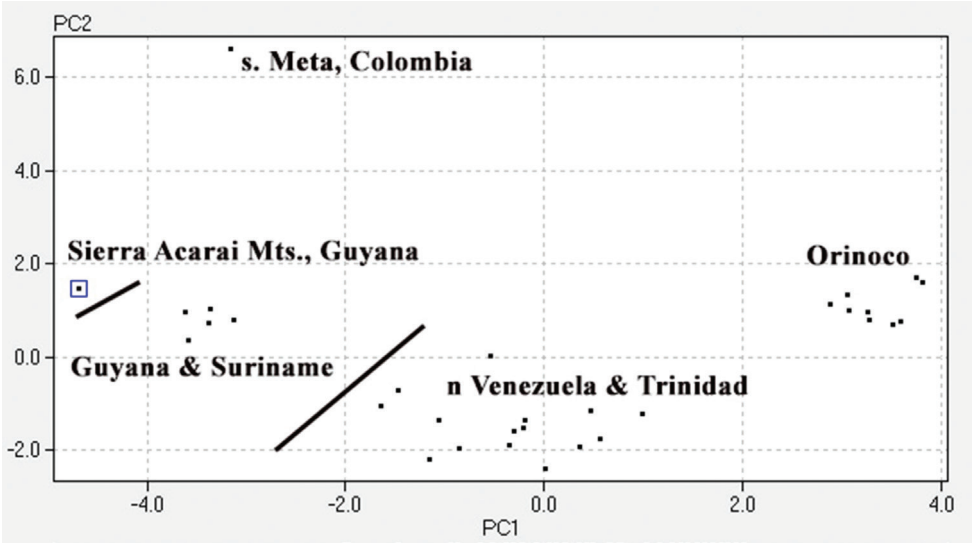

Figure 4. A PCA using 31 specimens labeled *Plica plica*. Scales around mid-body (28.20%), lamellae under fourth toe (13.74%), lamellae under fourth finger (11.09%), number of suboculars (7.14%), number of supraorbital plates (5.87%) accounted for 66.04% of the variation. Other traits used in the PCA included: dorsolateral fold, ventrolateral fold, scales between canthals, scales between nasals, loreals at canthal, scales between nasal and rostral, number of canthals, loreals keeled, upper labials, longest upper labial, lower labials, postmentals, ventral-post auditory tufts of spines, total tufts of spines, length of head, dorsal scale size, dorsal scale keels, dorsal pattern, and antegular fold.

destroyed during World War II. A fact recently reconfirmed by Franzen and Glaw (2007). Etheridge (1970) considered this species based upon a brightly colored juvenile of *Plica plica* because of the description and the colored figure in Spix (1825:11, pl 13, fig. 1). The type locality of *Lophyrus panthera* "Ecga," is currently known as Tefé, Brazil (~03°21'S, 64°42'W). A specimen from Tefé was not located during this investigation. However we did examine specimens from locations within 500 km of Tefé, both localities are close to the Amazon River. One species has 95 scale rows at mid-body and 41 lamellae on the fourth toe; the other has 141 scales at mid body and 29 lamellae on the fourth toe. Therefore the *P. plica* group in the Amazon basin is likely diverse and we take no further action on this name. That said, *P. panthera* (Spix) is undoubtedly a valid name for a species that awaits rediscovery.

Hypsibatus punctatus Duméril & Bibron (1837, 4:258) is based upon MNHN 2387 and it shares a similar number of scale rows around mid-body, subocular plates, and lamellae on fingers and toes with *P. plica*. While the literature suggests *H. punctatus* lacks a type locality (Etheridge 1970; Peters and Donoso-Barros 1970), Duméril and Bibron (1837) wrote that they thought the specimen originated from the same country as the species in the previous account (*Plica umbra* from Guyana or Suriname). Thus it is likely MNHN 2387originated from within the distribution of *Plica plica* as described here.

Table 2. A comparison of morphological traits for the species of *Plica*. Asterisk indicates that mainland Venezuela members of this species have 8–10 circumorbital scales, while Trinidad specimens have 10–12 circumorbital scales. Data for *P. lumaria* from Donnelly and Myers (1991); for *P. pansticta* from Myers and Donnelly (2001), for *P. u. umbra* and *P. u. ochrocollaris* from Avilla-Pires (1995). The ~ symbol denotes numbers taken from drawings. Abbreviations STN (spiny tufts on nape), SAAM (Position of spines on anterior margin of auditory meatus), SAB (scales around mid-body); SRN (scales between rostral and nasal). Numbers in parentheses after range indicate most frequent number.

Distribution	**Caribbean Coastal Range**	**Acarai Mts, Guyana**	**Meta, Colombia**	**Suriname, Guyana, Venezuela**	**Middle Orinoco**	**Guaiquinima Tepui, s. Venezuela**	**Tepuis of northwest Venezuela**	**Widespread Amazonia**	**Bolivia**
name	*caribeana*	*kathleenae*	*medemi*	*plica*	*rayi*	*lumaria*	*pansticta*	*umbra*	*ochrocollaris*
n=	27	1	1	11	12	Literature	Literature	Literature	Literature
STN	2	2	2	2	2	2	2	0	0
Antegular fold	Complete	Interrupted	Complete	Interrupted	Interrupted	complete	Complete	Folds absent	Folds absent
Mite pockets	4	Absent	4	2	4	4	4	Absent	Absent
SAAM	Center	Ventral edge	Entire margin	Center	Ventral edge	Entire margin	Ventral half	None	None
SAB	92–125 x̄=114.72 SD=8.72	158	145	126–140 x̄=133.75 SD=4.57	181–202 x̄=189.75 SD=8.61	141–156 x̄=146.2	143–164 x̄=159	47–69 x̄=60.5	42–56 x̄=49.9
SRN	1–2	3	1/2	2–3	1	1	1–2	0–1	0–1
Lamellae 4th finger	23–29 x̄=26.2 SD=1.89	29	32	25–28 x̄=26.5 SD=0.71	27–35 x̄=29.1 SD=2.39	20–24	23–30 x=26.1	17–25	17–25
Lamellae 4th toe	28–35 x̄=31.26 SD=1.92	35	41	31–36 x̄=35.0 SD=1.41	36–44 x̄=39.40 SD=2.88	27–33	31–39 x̄=34.8	24–33	26–33
Scales on snout	Imbricate, keeled	Imbricate, pyramidal	Juxtaposed, conical, smooth	Imbricate, few keeled	Juxtaposed, flat, smooth	Imbricate, smooth	Imbricate, smooth	Variable	Variable
Intercanthals	6–8	8	8	7–9	7	~5	~7	~8	~5
Suboculars	5–6	6	6–7	5–7 (7)	4–5	1–5	7	1–9	~3
Circumorbitals	8–12*	12/12	11/9	10–13	7–10 (8)	~12	~11	7–11	~7
Internasals	5–6	8	5	7–8	5–6	~6	~6	7–11	7–11
Dorsal pattern	Green with black brown transverse markings	Uniform gray brown	Green, bold dark spots	Green with black brown markings	Dark mottling, white spots	Black w/yellow spots	Green gray with black blotches	Green dorsum	Green dorsum

The identity of *Plica plica* (Linnaeus,1758)

Our comparison of Guiana Shield *Plica* and the lectotype (Fig. 5) to those from across the range are in close agreement with the description reported for Suriname specimens with 5–8 labials, usually six; mental in contact with 3–5 scales on posterior edge; dorsal crest well developed in males, slightly less so in females; ventrals 61–65; fourth finger with 24–28 lamellae; fourth toe with 31–36 lamellae.

Plica plica occurs on the Guiana Shield in Suriname, Guyana, and Bolivar, Venezuela. It is also likely present in French Guyana. Three specimens (BMNH 67.5.4.1–2) from the island of Grenada were reported by Boulenger (1885), Barbour (1914) considered the locality data in error and suggested the specimens were from "New Grenada" (=Colombia). However, these specimens agree well with *Plica plica*. The adult male has 134 scale rows at mid-body; 28 lamellae on the fourth finger, 36 on the fourth toe; 4/5 upper labials; 7/6 suboculars; 13/13 semicircle scales. Thus, the specimens may represent a population now extirpated from the island, or an extant population that remains undiscovered (Henderson and Murphy 2012). The Bolivar, Venezuela specimens (KU167502–03) examined have a lower number of circumorbital scales (9–10) than specimens to the east which have 11–13 circumorbital scales. The locations from which *Plica plica* were examined as well as literature reports that reliably represent this species (Heyer and Barrio-Amorós 2009, Lasso 2009, Barrio-Amorós et al. 2011, Barrio-Amorós and Fuentes 2012) and are included in Fig. 6. For colors in life see Fig. 1c,d.

Plica caribeana sp. n.

http://zoobank.org/F06E2E69-0BD2-474F-827A-8D6F1FA1430B
http://species-id.net/wiki/Plica_caribeana
Caribbean treerunner
Figs 7, 8a, 9a

Hypsibatus agamoides – Court 1858: 440.
Uraniscodon plica – Boulenger 1885, 2: 180 [in part].
Plica plica – Burt and Burt 1931: 282 [in part].
Tropidurus plica – Frost 1992: 1 [in part].

Holotype. FMNH 49838, an adult female, 110 mm SVL, 172 mm tail. Collected in 1947 by Frank Wonder, the Republic of Trinidad & Tobago, Trinidad: San Rafael (~10°34'N; 61°16'W).

Other material. Trinidad: Brickfield (~10°20'N; 61°16'W) 49834–35; Maracas (~10°45'N; 61°25'W) MCZ 60826; Mt. Harris (~10°30'N; 61°06'W) FMNH 49836; Nariva MCZ 60825; St. Annes MCZ 9002; San Rafael (~10°34'N; 61°16'W) FMNH 49837; Tucker Valley (~10°42'N; 61°36'W) FMNH 40448; no specific locality FMNH 25014; Cuesa River, Chagaramas (~10°43.319N; 61°38.6'W) UWIZM

Figure 5. The holotype of *Lacerta plica* Linnaeus, 1758, NRM 112. Note the tuft of spines on the anterior edge of the auditory meatus (**A**); the indistinct spots on the dorsum (**B**) and the double dewlap fold (**C**). Photo credit: Sven Kullander.

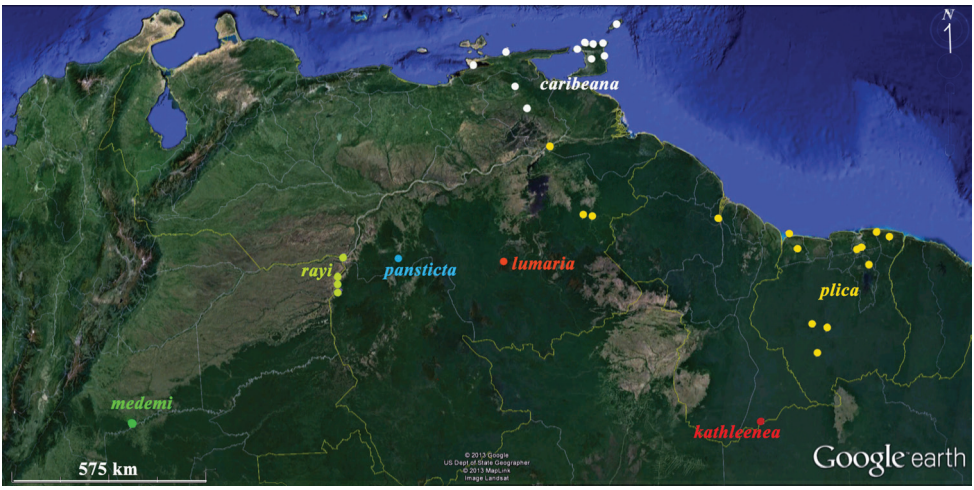

Figure 6. Distributions of *Plica plica* Group species discussed in this paper. Yellow – *P. plica*; white *P. caribeana* (because of scale not all Trinidad localities were plotted); red – *P. kathleenae*; green – *P. medemi*; light blue – *P. rayi*; and the type localities of *P. pansticta* and *P. lumaria* are show in dark blue and orange respectively. A Google Earth map.

2012.27.56. Venezuela: Sucre, Guaranunos (~10°32'N; 63°06'W) KU167497–98, 167500–3; Cumana, Muchorapo (~10°27'N; 64°10'W), KU117088. Sucre, Chacaracual (~9°51'N; 63°42'W) MCZ 43861–65, 81186–89; Monagas: MCZ 88185.

Diagnosis. A *Plica* with dorsal scales in 92–125 (usually 110–125) rows at mid-body; scales on snout imbricate and keeled; dorsolateral and ventrolateral folds well developed; head length 21–22% of the SVL; one longitudinal dewlap fold; and a dorsal pattern of black and green transverse bands. *Plica plica* has 126–140 dorsal scale rows at mid body and two longitudinal dewlap folds. The middle Orinoco species, *P. rayi* sp. n. has 180–202 scale rows at mid-body; flat, juxtaposed scales on the snout; and the ventrolateral fold is weakly spinose. The Sierra Acarai Mountain's (Guyana) *P. kathleenae* sp. n. has 158 scale rows around mid-body; head 29% of SVL; and lacks gular mite pockets (all other species have them). The species from southern Meta, Colombia *P. medemi* sp. n., has 145 scale rows at mid-body, and a dorsal pattern of six rows of small, bold, dark, irregular spots. The two species, associated with the Venezuelan tepuis (*P. lumaria* and *P. pansticta*) have a higher number of scale rows at mid-body (141–164) and smooth scales on the snout.

Description of holotype. Rostral band-like (much wider than tall), contacts five post-rostral scales; nasals positioned over first labial, separated from upper labials by 1–2 scales, separated from each other by six scales; scales on snout imbricate, keeled, in regular rows, with asperities; loreals in seven rows (some keeled); canthals single, contacts three loreals, separated by eight inter-canthals scales; circumorbital scales 11/12, keeled, smaller posteriorly; separated from occipitoparietal by small scales; occipitoparietal broader than long; subocular plates 5/5, with serrated keel; upper labials 5/5, fifth is longest; supraciliaries in three layers, eight scales per layer; auditory meatus has

Figure 7. *Plica caribeana*, from the Arima Valley of Trinidad.

a cluster of spiny scales on anterior margin, none on posterior margin, ventral fold has a cluster of spiny scales; two tufts of spines on neck, anterior tuft largest; antegular and gular folds complete, dewlap fold originates from the antegular fold; four mite pockets present, two under each fold; mental rounded, contacts four postmentals; lower labials 6/6; scales on throat small, quadrangular and smooth; dorsal crest well developed onto tail; dorsolateral fold well developed to thigh; ventrolateral fold present along length of body; scales around mid-body 125; ventrals 53; dorsal surfaces of limbs covered with mucronate scales, ventral surfaces covered with larger, smooth scales; fourth finger with 29 lamellae; fourth toe with 32 lamellae; feet 37% of SVL; tail depressed at base, cylindrical distally.

In alcohol, a uniform brown gray dorsum; tail with alternating, indistinct dark and pale bands; forearms and lower legs also with indistinct bands; chin spotted, black gular center; venter of body and tail cream.

Variation. Rostral makes contact with five or six post-rostral scales; nasals separated from each other by 5–7 scales; canthal single or double, in contact with 2–4 loreals, separated by 6–8 inter-canthal scales; circumorbital scales 11 or 12 in Trinidad

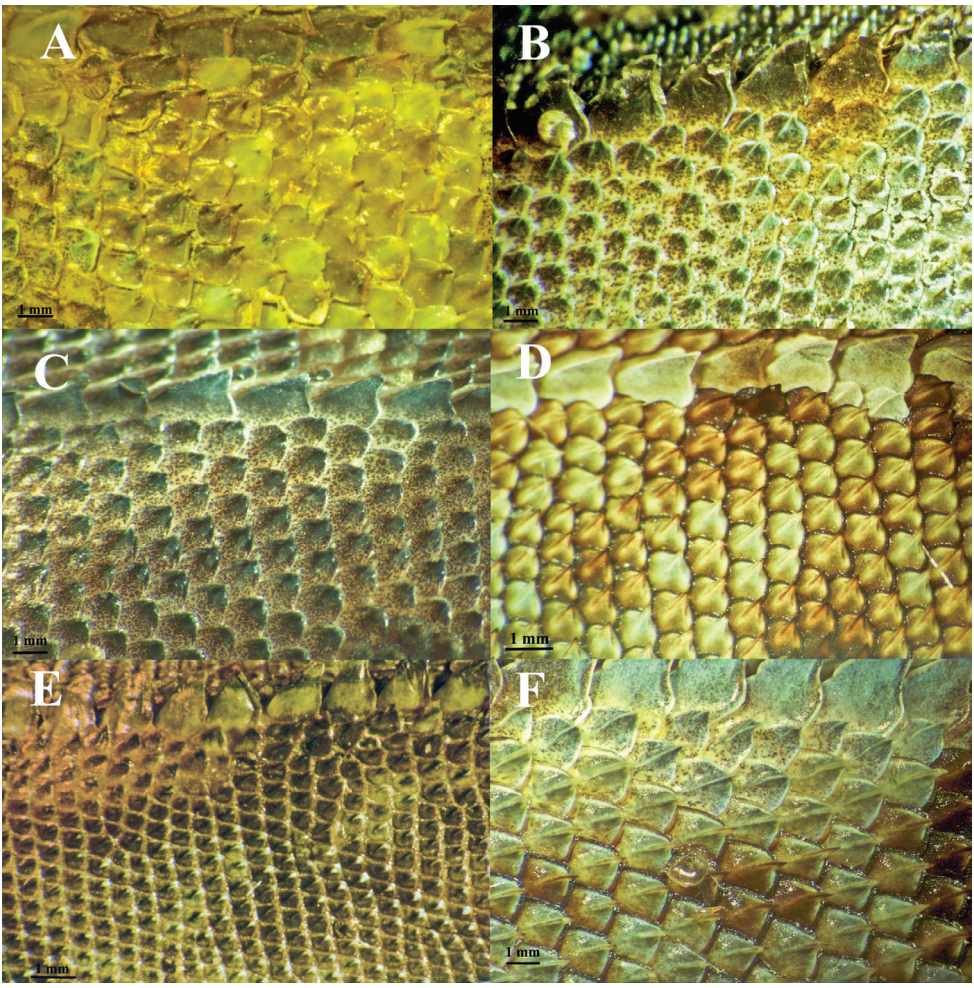

Figure 8. A comparison of dorsal scale morphology at mid-body near the dorsal crest. Anterior is to the left in all images. The scale bar shown in each image is one millimeter. Only large adult specimens were used for scale comparisons. **A** *Plica plica,* FMNH 128950 **B** *Plica caribeana,* FMNH 49838 **C** *Plica kathleenae,* FMNH 30931 **D** *Plica medemi* FMNH 165207 **E** *Plica rayi*, FMNH 177925, and **F** is a *Plica* cf. *plica* from Amazonas, Venezuela, MCZ 101841.

and northern Venezuelan populations, 8 to 10 in Bolivar, Venezuela populations; upper labials usually four, rarely five; loreal region has 6 –9 rows of weakly keeled loreals between canthal and upper labials; suboculars usually five, rarely six; lower labials 5–6, bordered by 3–5 rows of slightly enlarged scales; dorsal scales in 92–125 rows around mid-body (usually 110–125); dorsal crest well developed in males, less so in females.

Color in life (Fig. 7), head mostly green with black markings on supraciliaries, a black postorbital stripe and indistinct black bars on labials; dorsum mostly green with 4–6 transverse brown bands; vertebral crest mostly black but interrupted with green between the

brown bands; limbs and feet with alternating green and brown bands; tail with alternating black and green bands; ventral surface mostly uniform white with brown mottling.

Body and tail sizes. Female's SVL 105–121 mm (n=6, $\bar{x}$=98.8. SD=18.22); undamaged tails 132–205 mm (n=5, $\bar{x}$=170.6, SD=33.83). Male's SVL 81–121(n=12, $\bar{x}$=104.17, SD=12.04); undamaged tails (n=7) 132–205 mm ($\bar{x}$=170.6, SD= 32.63). Hind feet average about 39% of the SVL.

Etymology. Named for its Caribbean Coastal Range distribution.

Distribution. Eastern Coastal Range (Cordillera de la Costa Oriental) of Venezuela south into Bolivar; Trinidad, the Bocas Islands (Huevos, Monos, and Gaspar Grande); Tobago (Murphy and Downie 2012).

Ecology. *Plica caribeana* is a forest and forest-edge species most frequently observed on tree-trunks, rock walls, walls of caves, and buildings. They often occur in small colonies of 6–15 individuals, usually positioned with head downward, but slightly raised off the substrate. They are ambush insectivores feeding on ant columns, beetles, cicadas, spiders, and other arthropods. Females are reported to lay clutches of two eggs; the smallest juvenile measured for this study was 47 mm SVL with a damaged tail; this species is known to be preyed upon by the snake *Siphlophis compressus* (Boos 1977; Murphy 1997). Note that Beebe's (1944) comments on *Plica plica* may be partially applicable to this species.

***Plica kathleenae* sp. n.**
http://zoobank.org/D1F2C377-1FEB-4F02-A017-0C5A4D4AFE0C
http://species-id.net/wiki/Plica_kathleenae
Kathleen's Treerunner
Figs 8b, 9b

Holotype. FMNH 30931. An adult male, 124 mm SVL, tail damaged. Collected by Emmet Reid Blake on the Sewell Avery British Guiana Expedition, in September-October, 1938; Guyana, Boundary Camp, Itabu Creek headwaters (~1°42'N, 57°55'W) in the Sierra Acarai Mountains near the Brazilian border, at an elevation of about 549 m).

Diagnosis. A *Plica* with dorsal scales in 158 rows at mid-body, 6 suboculars; scales on snout mostly imbricate and slightly pyramidal with asperities; head length 29% of the SVL (other species have heads 17–23% of the SVL); gular fold complete, antegular fold incomplete; dewlap originates in the space between the two folds; throat folds in this species are relatively shallow and do not form the mite pockets seen in other species. *Plica plica* has fewer scale rows at mid-body (126–140) as well as complete antegular folds. *Plica caribeana* sp. n. has 92–125 scale rows at mid-body, and scales on snout are keeled and imbricate. *Plica medemi* sp. n. has 145 scale rows at mid-body; seven suboculars; head length of 23% of the SVL. *Plica rayi* sp. n. has 181–202 scale rows at mid-body; and flat, juxtaposed scales on the snout. *Plica lumaria* and *P. pansticta* have smooth imbricate scales on snout and one scale between nasal and rostral.

Figure 9. A comparison of head scalation for five species in the *Plica plica* Group found in northern South America. **A** *Plica caribeana* FMNH 49838 **B** *Plica kathleenae* FMNH 30931 **C** *Plica medemi* FMNH 165207 **D** *Plica plica* FMNH 128950 **E** *Plica rayi* FMNH 177926.

Description of holotype. Rostral in contact with six post-rostrals; nasals separated from rostral by three scales, internasals eight; scales on snout imbricate, slightly pyramidal, with one or two asperities; canthals single; separated by eight inter-canthal scales; circumorbital scales 12, separated from the occipitoparietal by a row of small scales; occipitoparietal slightly broader than long; supraorbital plates 5/6, separated from the superciliaries by two rows of small scales; superciliaries three layers with about eight scales in each layer; suboculars 7/6, first is largest (fourth almost as large), with serrated keels; upper labials five, fifth is longest; six lower labials; loreal scales slightly keeled, in 8–10 rows between canthal and upper labials; auditory meatus with a spiny tuft on anterior margin, rest of margin with smaller mucronate scales; ventral flap with large tuft of spines; two clusters of spiny tufts on nape; mental small, in contact with four tiny

post mental scales; gular fold complete; antegular fold incomplete; dewlap originates anterior to gular fold, transects the antegular fold, mite pockets absent; dorsal crest well developed on anterior body; scales around mid-body 158; ventrals 63; limbs well developed and covered with keeled mucronate scales above, and smooth scales below; lamella under fourth finger 29; lamellae under fourth toe 35. Feet 33% of the SVL.

In alcohol, dark brown dorsum with an irregular row of dark spots with light centers on each side of the mid-line, some of the dark spots are surrounded by smaller white spots; small white spots are also on the front limbs. There is a narrow dark collar that extends laterally. Forelimbs and hind limbs have transverse bands.

Etymology. Named in honor of Kathleen Kelly, Division of Amphibians and Reptiles, Field Museum of Natural History, for her interest and effort on behalf of herpetology.

Distribution. Known only from the type locality in the Acarai Mountains of Guyana.

Ecology. Nothing is known about the ecology of this species, but see the discussion.

***Plica medemi* sp. n.**
http://zoobank.org/C2FCD890-B516-44F5-B728-46AB3F4CEA16
http://species-id.net/wiki/Plica_medemi
Medem's Treerunner
Figs 8c, 9c

Holotype. FMNH 165207 an adult male, 112 mm SVL, with a damaged tail. Collected by Fredrico Medem in 1957. Colombia, Meta, Lower Guayabero, Angostura No. 2, Cerro de las Pinturas (~2°34'N; 72°51'W).

Diagnosis. A *Plica* with dorsal scales in 145 rows at mid-body; scales on snout juxtaposed, smooth and slightly domed; dorsolateral and ventrolateral folds well developed and extend to groin; entire anterior margin of auditory meatus is lined with spiny scales (no distinct cluster of spines); 41 lamellae on fourth toe. It has a green dorsum with black spots, and an orange head and lacks the transverse bands present in other members of the genus. *Plica plica* has 126–140 dorsal scale rows at mid body, imbricate, keeled scales on the snout; and 31–36 lamellae on the fourth toe. *Plica rayi* sp. n. has 180–202 scale rows at mid-body; juxtaposed and flat scales on the snout; and the ventrolateral fold is barely visible. *Plica kathleenae* sp. n. has 158 scale rows around mid-body, and a cluster of spiny scales on the anterior margin of the auditory meatus. *Plica medemi* sp. n. differs from *P. lumaria* and *P. pansticta* in having more lamellae on the fourth finger and fourth toe (32 and 41 respectively), supracilliaries directed dorsally as opposed to laterally; an exceptional spiny texture to its scales, and a distinctly different pattern.

Description of holotype. Rostral band-like, in contact with five post-rostrals; scales on snout juxtaposed, smooth, but bulge to form slight domes, asperities few in number; nasals separated from rostral and upper labials by 1/2 scales; five internasals; nine intercanthals; circumorbitals 11/9, moderately distinct posteriorly, rounded, smooth, me-

dially; six supraocular plates separated from circumorbitals and superciliaries by small scales; occipitoparietal subtriangular, about as wide as broad; canthal short, sharply keeled, contacts two loreals; superciliaries in three layers of 7–9 upturned, keeled scales; loreal region has 7/8 rows of scales between canthal and upper labials (counted diagonally); subocular plates 7/6 each sharply keeled, the first is the longest; five elongated upper labials, the last is the longest; five lower labials similar to upper labials, bordered below by two or three rows of larger scales; mental sub-triangular contacts three small post-mentals; antegular fold complete; gular fold incomplete; the dewlap originates from the antegular fold; the folds form four, lateral mite pockets (two under each fold); gulars small, imbricate, smooth anteriorly, decreasing in size posteriorly, keeled near the transverse gular fold; mid-dorsal crest composed of a row of enlarged spiny scales extending from occiput to proximal half of tail; two tufts of enlarged, spinose scales on side of neck; spiny scales line the entire anterior edge of auditory meatus, a cluster of spinose scales absent; one tuft of spiny scales on auricular flap that covers most of its surface; dorsal scales around mid-body 145; ventrals 44; dorsolateral fold well developed from shoulder to thigh; ventrolateral fold present; caudal scales similar to body scales above and below; scales on upper limbs keeled, very spinose, and imbricate; ventral limb scales mostly smooth, imbricate; scales on dorsal surface of digits keeled ending in a stout spine. Lamellae on fourth finger 32, on the fourth toe 41, each has a median keel ending in a stout spine; hind feet about 34% of SVL.

In alcohol, the head is orange the dorsum of the body is green with dark, bold spots, no transverse blotches on the dorsum. The collar is a network of dark pigment with light colored area surrounded by darker pigment. Upper surfaces of the limbs are similarly patterned with bold spots, but transverse bands are present distally. The venter of the chin is spotted with a black area of pigment around the dewlap; the venter of the body is a uniform cream.

Etymology. The lizard is named in honor of Colombian herpetologist Fredrico Medem.

Distribution. Known only from the type locality at Angostura No. 2, Cerro de las Pinturas, Lower Guayabero, and Meta, Colombia.

Ecology. Nothing is known about the ecology of this species, but see the discussion.

***Plica rayi* sp. n.**
http://zoobank.org/6DE2FD0E-8AA0-4563-8858-B11CFF86277D
http://species-id.net/wiki/Plica_rayi
Ray's Treerunner
Figs 8e, 9e, 10

Plica plica – Etheridge 1970: 242 (in part).

Holotype. FMNH 177926 a male. Collected by Gary Myers in 1962. Venezuela, Amazonas, Puerto Ayacucho (~05°39'N; 67°38'W), on the Orinoco River.

Other material. Colombia– Vichada, Puerto Carreno, (~6°08'N; 67°26'W) MCZ 150179–81; Venezuela – Puerto Ayacucho FMNH 177924–34, MCZ58335–36, 160222–24.

Diagnosis. A *Plica* with greatly reduce mucronate scales, the two clusters on the neck reduced to small knobs; in overall appearance this lizard has a smooth external texture; 182–202 scales around mid-body (more than any other *Plica* species); seven subocular plates; scales on snout juxtaposed and flat; nasal scales separated from rostral by a single scale; spiny scales around auditory meatus greatly reduced or completely absent; lamella under fourth toe 36–45 (more than any other *Plica* species). Males have a well-developed dorsal crest that extends onto the tail; females have this crest greatly reduced. *Plica caribeana* sp. n. has 125 or fewer scales around mid-body and imbricate, keeled scales on the snout. *Plica kathleenae* sp. n. has 158 scales at mid-body and imbricate scales on the snout. *Plica medemi* sp. n. has 145 scale rows at mid-body and well developed spines on the anterior margin of the auditory meatus. *Plica plica* has 140 or fewer scales around mid-body; keeled, imbricated scales on the snout, and has well developed spiny scales. The two tepui associated species (*P. lumaria* and *P. pansticta*) have fewer scale rows around the body (141–164), imbricate scales on the snout and fewer lamellae on the toes.

Description of holotype. FMNH 177926, male, 103 mm SVL, 175 mm tail. Rostral broader than tall, with small asperities, makes contact with five post-rostral scales; nasals small, separated from upper labials by one scale, separated from each other by five scales; scales on snout, juxtaposed, flat with round asperities; canthal single, makes contact with three loreals, separated by seven inter-canthal scales; circumorbital scales weakly keeled, well developed, nine on each side, and maintain their size posteriorly; occipitoparietal broader than long, in direct contact with circumorbital scales; upper labials 4/4, fourth is the longest; lower labials five, bordered by four rows of slightly enlarged scales below; loreals in 8/9 rows between canthal and upper labials, scales weakly keeled; dorsal crest on nape and extends to about mid-tail, scales relatively small, largest spines close to occipital region; dorsolateral fold well developed on anterior body extends passed thigh, ventrolateral fold barely discernible in preserved specimens; dorsal scales exceptionally small; scales around mid-body 190; ventrals 80; auditory meatus with small cluster of reduced spines on anterior margin, none on posterior margin; ventral flap has few spines on margin; two small tufts of spines on neck, reduced to almost smooth knobs, anterior tuft larger than posterior; gular fold complete; antegular fold incomplete; dewlap originates on the gular fold; mental rounded, in contact with four scales on posterior edge; scales on throat small, smooth, subtriangular; ventrals larger than dorsals, imbricate, smooth; fourth finger with 27 lamellae; fourth toe with 37 lamellae; feet about 35% of SVL; tail laterally compressed at base and along most of its length.

In alcohol, dark transverse bands on the snout and back of head, as well as a well-formed shoulder stripe that extends onto neck and throat. Dorsum brown gray with light spots; tail with alternating, indistinct dark and pale bands; forearms and lower legs also with indistinct bands; chin spotted, black gular center; venter of body and tail cream.

Figure 10. *Plica rayi*. A male in breeding coloration. Photographed at Tobogan de la Selva, Puerto Ayacucho. Photo credit Zelimir Cernelic.

Variation. Rostral may or may not have asperities; nasals separated from upper labials by one or two scales, separated from each other by 5–6 scales; circumorbital scales usually 8–9 (rarely 7 or 10), keeled, and may become slightly smaller posterior; loreal region has 7–9 rows between canthal and upper labials, scales weakly keeled; suboculars 4–5, usually four; mental rounded, in contact with 3–4 scales on posterior edge; lower labials 4–6, usually five; scales around mid-body 181–202; ventrals 80–93; fourth finger with 27–35 lamellae; fourth toe with 36–45 lamellae. Preserved specimens show poorly defined dorsal crests, males have greatly enlarged spines in the occipital region, these are barely discernible in females; dorsolateral and ventrolateral folds not obvious in preserved specimens; in life these folds are quite distinct based on Fig 10.

Body and tail. Female's SVL 66–106 (n=8, $\bar{x}$=79.13, SD=17.21); undamaged tails 134–175 (n=4, $\bar{x}$=148.50, SD=15.79). Male's SVL 85–105 (n=3, $\bar{x}$=96.00, SD=8.92), undamaged tails (n=1) 143 mm. Feet 35–39% of SVL; tail depressed at base, becomes more cylindrical distally. Individuals measured for this study ranged from 55–106 mm SVL, and had unbroken tails that were up to 2.1 times the SVL.

In alcohol, a uniform brown gray dorsum; dorsum has some traces of transverse bands, but white spots numerous on some individuals; tail with alternating, indistinct dark and pale bands; forearms and lower legs also with indistinct bands; chin spotted, black gular center; venter of body and tail cream.

In life breeding males have a red-orange face and throat with about five irregular black brown markings extending from the supraorbital crest to the upper labials and some extend onto the throat; crown and face otherwise brown. A black nape blotch extends from the dorsal crest onto the throat where it widens and contains some white pigment; the body is brown-black with indistinct white and black markings; limbs

with indistinct bands. Females are brown black with the head slightly darker in color than the body, and a black gular blotch at the anterior edge of the dorsolateral fold; upper forelegs with light colored mottling, lower forelegs with indistinct bands.

Etymology. This lizard is named in honor of Ray Pawley, former Curator of Reptiles at Brookfield Zoo, for his lifelong interests and work on amphibians and reptiles. Suggested common name: Ray's Treerunner.

Distribution. Known from two localities along the Orinoco River: Puerto Ayacucho, Amazonas, Venezuela and Puerto Carreno, Vichada, Colombia. The distance between these two locations is about 65 km. They have also been observed at Tobogan de la Selva (~5°23'13"N, 67°37'W) and Raudal de Danto at Autana (4°48'N, 67°29'W, 89 m asl).

Ecology. *Plica rayi* is associated with granitic rainforests. It is very abundant in rocky areas; tobogans are granite slabs used as refugia by the lizards. In May, coinciding with the initiation of rains, males have a bright red-orange head coloration not observed in other months (July, September, or December). At night they sleep vertically with the head facing the sky (César Barrio-Amorós, personal communication).

Discussion

Molecular and morphological data support the hypothesis that *Plica plica* is a complex of cryptic species that are concordant with features of South American geography. We identify *Plica plica* (Linnaeus, 1758), *P. kathleenae* sp. n., and *P. rayi* sp. n. as Guiana Shield endemics or near endemics. Hoogmoed (1979) considered 23 of the 65 (35%) Guyana lizards known at the time to be endemic and he viewed the Acarai Mountains as one of the most important refugia for the forest herpetofauna. More recently, Avila-Pires (2005) reported 118 lizards on the Guiana shield, 52 (44%) were listed as endemics. *Plica kathleenae, P. plica* and *P. rayi* can now be added to that list.

The four new species described here all appear to be associated with areas of endemism or specific features of the northern South American landscape.

Plica caribeana is associated with the Eastern Coastal Range bioregion of Rivas et al. (2012), as well as Trinidad and Tobago. Other lizards restricted to this region include: *Ameiva atrigularis*, *Anadia pariaensis*, *Euspondylus monsfumus*, *Gonatodes ceciliae*, *G. seigliei*, *Riama rhodogaster*, and *R. shrevei.* Mitochondrial genetic divergence for gene fragments in Squamata (16S: 0.45% and 12S: 0.5%, per million years, Carranza et al. 2004; Poulakakis et al. 2005, respectively) would suggest a genetic divergence between the mainland *Plica plica* Group and *Plica caribeana* in the Late Miocene. This would have preceded the separation of Trinidad from the mainland (Audemard and Audemard 2002; Moretti et al. 2007; Persad 2009).

Plica kathleenae is known only from the type locality in the Acarai Mountains. The Itabu Creek, is a small tributary entering the New River from the south near its headwaters, and provides a route into the Acarai Mountains which separate the Amazon drainage from that of the Essequibo and Courantyne drainages (Blake 1941). The area

was the subject of a Rapid Biological Assessment in 2006 and the team documented 34 species of reptiles, including "*Plica plica.*" The report suggests the area contains a rich herpetofauna and is considered to be a center of endemism (Señaris et al. 2008). The distribution and natural history of this lizard remains to be determined.

Plica medemi is known only from the type locality in La Macarena National Park at the base of the Colombian Andes, an area transitional between Guyana and Amazonia. Kattan et al. (2004) found the Macarena Range to have the most diverse fauna per unit of area in the Colombian Andes with much of the fauna associated with the Guiana Shield (based upon butterflies, frogs, birds, bats and rodents). It is unclear if this *Plica* is a Macarena endemic, or is more widespread.

Plica rayi is known from the western edge of the Guiana Shield along the middle Orinoco. The Orinoco-Negro white sand forest bird area is nearby and considered a center for endemic bird species. The forest is mostly undisturbed primary lowland humid forest up to about 500 m that follows rivers into the middle Orinoco watershed (BirdLife 2012). Groger and Huber (2007) describe the Puerto Ayacucho area as a true center of endemism for the flora due to an overlap in the northern and southern units of the inselberg vegetation. Additionally, Barrio-Amorós et al. (2010) described a new species of frog in the genus *Anomaloglossus* (Dendrobatidae) from this region and considers the Puerto Ayacucho biodiverse but poorly known.

There is at least one unresolved Venezuelan species of *Plica* from the Amazonas portion of Venezuela. It has a relatively low dorsal scale row count (113–114), two tufts of spines on the auricular fold, and four subocular plates. The survey of the specimens examined here suggests Peru has at least three distinct species, Ecuador has at least one other, and Brazil has several more, one of which will be revalidated as *Plica panthera* (Spix). Distinguishing the species present in the *Plica plica* Group will help clarify the evolution of the forested South America landscape and expand our knowledge of tropidurid biogeography.

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d'Histoire naturelle (MNHN); W. Ronald Heyer and Robert Wilson at the National Museum of Natural History (USNM); and Mike Rutherford at the University of the West Indies Museum of Zoology (UWIMZ). Special thanks also go to Stevland Charles (Howard University), Adrian Hailey at the University of the West Indies and William Lamar (University of Texas at Tyler) for suggests on the content of the manuscript.

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Appendix 1

Specimens examined but not assigned a name. Brazil: FMNH 83595, MCZ 92750. Colombia: MCZ150179–81. Ecuador: FMNH 42502–4, MCZ 37268. Peru: FMNH 45493–5, 56011-20 56104, 83305, 81451–2, 109821, 109826–27, 134427, 228259. Venezuela: MCZ 23166, 101841.

Appendix 2

Factor loadings of 25 meristic and morphometric traits. Eigenvalues and percentage of variance explained by the respective components are at the bottom of the table. Abbreviations: scales around mid-body (SAB); lamellae on 4th finger (LAMF); lamellae on 4th toe (LAMT); number of suborbital plates (SUBO); number of supraorbital plates (SPRO); dorsolateral fold (DF); ventrolateral fold (VF); intercanthals (IC); Internasals (IN), scales separating nasal from rostral (NR); loreals contacting canthal (LC); loreals keeled (LK); upper labials (UL); longest upper labial (LUL); lower labials (LL); postmentals (PM); spiny tuffs on ventral auditory fold (VTS); total tuffs of spines (TT); dorsal scale size (DSS); keels extend over scale apex (KOA); head length (HL); dorsal scales broad (DSB); antegular fold (AF);

	PC1	PC2	PC3
SAB	0.266	0.316	0.164
LAMF	0.224	0.336	0.094
LAMT	0.201	0.175	-0.008
SUBO	0.297	0.065	0.064
SUPRO	0.227	-0.126	-0.188
DF	0	0	0
VF	0.325	-0.196	-0.124
IC	-0.244	0.059	0.13
IN	-0.217	-0.064	0.256
NR	-0.223	0.208	0.353
CAN	0	0	0
LC	0.045	-0.313	0.328
LK	0	0	0
UL	-0.269	0.227	0.07733
LUL	-0.268	0.1943	0.059
LL	-0.197	0.123	0.045
PM	0.077	-0.072	0.102
VTS	-0.074	-0.025	0.106
TT	-0.074	0.319	-0.451
KOA	0.325	0.196	0.124
HL	-0.109	0.122	0.221
DSB	-0.074	0.319	-0.451
DSS	0	0	0

	PC1	**PC2**	**PC3**
DP	0.326	-0.159	-0.001
AF	-0.04	0.392	0.294
Eigenvalues	7.52	3.44	2.72
Variance Explained	30.08	13.78	10.89
F (ANOVA)	45.64	5.22	5.33
Significance (ANOVA)	<0.01	<0.01	<0.01
DF	24	24	24

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RESEARCH ARTICLE

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Two newly recognized species of *Hemidactylus* (Squamata, Gekkonidae) from the Arabian Peninsula and Sinai, Egypt

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† *http://zoobank.org/BCF502BE-88CB-4CEF-B015-1975DB8A8920*
| *http://zoobank.org/BF30E84D-46C5-45EE-9140-E7E2154D9A66*
¶ *http://zoobank.org/D6D37DD5-AC94-4747-8C49-C043831FA591*
# *http://zoobank.org/71D11914-B33C-416A-B38F-C4E52989D29F*
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‡‡ *http://zoobank.org/7D466342-7FF7-4A35-B257-1D92459D390E*
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Abstract

A recent molecular phylogeny of the Arid clade of the genus *Hemidactylus* revealed that the recently described *H. saba* and two unnamed *Hemidactylus* species from Sinai, Saudi Arabia and Yemen form a well-supported monophyletic group within the Arabian radiation of the genus. The name '*Hemidactylus saba* species group' is suggested for this clade. According to the results of morphological comparisons and the molecular analyses using two mitochondrial (*12S* and *cytb*) and four nuclear (*cmos*, *mc1r*, *rag1*, *rag2*) genes, the name *Hemidactylus granosus* Heyden, 1827 is resurrected from the synonymy of *H. turcicus* for the Sinai and Saudi Arabian species. The third species of this group from Yemen is described formally as a new species *H. ulii* **sp. n**. The phylogenetic relationships of the members of '*Hemidactylus saba* species group' are evaluated and the distribution and ecology of individual species are discussed.

Keywords

Reptilia, Gekkonidae, molecular phylogeny, Arabia, Red Sea, *Hemidactylus saba* species group, *Hemidactylus granosus* Heyden, 1827, *Hemidactylus ulii* sp. n.

Introduction

The genus *Hemidactylus* Oken, 1817, the second most species-rich genus of Gekkonidae (122 currently valid species; Uetz 2013), has been witnessing a species-description boom within the last decade. Eighteen species have been described within the last two years, most of them from the Arabian Peninsula and surroundings areas where 13 new species and a new subspecies have been discovered (Busais and Joger 2011a; Moravec et al. 2011; Torki et al. 2011; Carranza and Arnold 2012). Despite the large number of taxa added recently to the Arid clade of *Hemidactylus* [*sensu* Carranza and Arnold (2006)], it has been shown that the real diversity of *Hemidactylus* in Arabia and northeast Africa is still underestimated, with at least seven species remaining to be described (Busais and Joger 2011b; Moravec et al. 2011; Šmíd et al. 2013). A recent study (Šmíd et al. 2013) revealed that two of these newly recognized but still unnamed species, one from Sinai [labelled in accordance to previous works (Moravec et al. 2011; Šmíd et al. 2013) as *Hemidactylus* sp. 1] and one from Yemen (*Hemidactylus* sp. 4), clustered with the recently described Yemeni endemic *H. saba* Busais & Joger, 2011. They form a very well supported clade within the Arabian radiation of the genus (Fig. 1). Although the phylogenetic relationships among these three species were not resolved satisfactorily, it was inferred that they began to diversify approximately 7 million years ago (95% highest posterior density interval 4.3–10), what was followed by a subsequent dispersal of the Sinai species from southern Arabia to the north (Šmíd et al. 2013).

The discovery of a monophyletic species group consisting of one recently described and two newly recognized species calls upon a more thorough study of the nomenclatural status, evolutionary relationships, taxonomy and distribution of its members based on further genetic and morphological data. The present study focuses on this task.

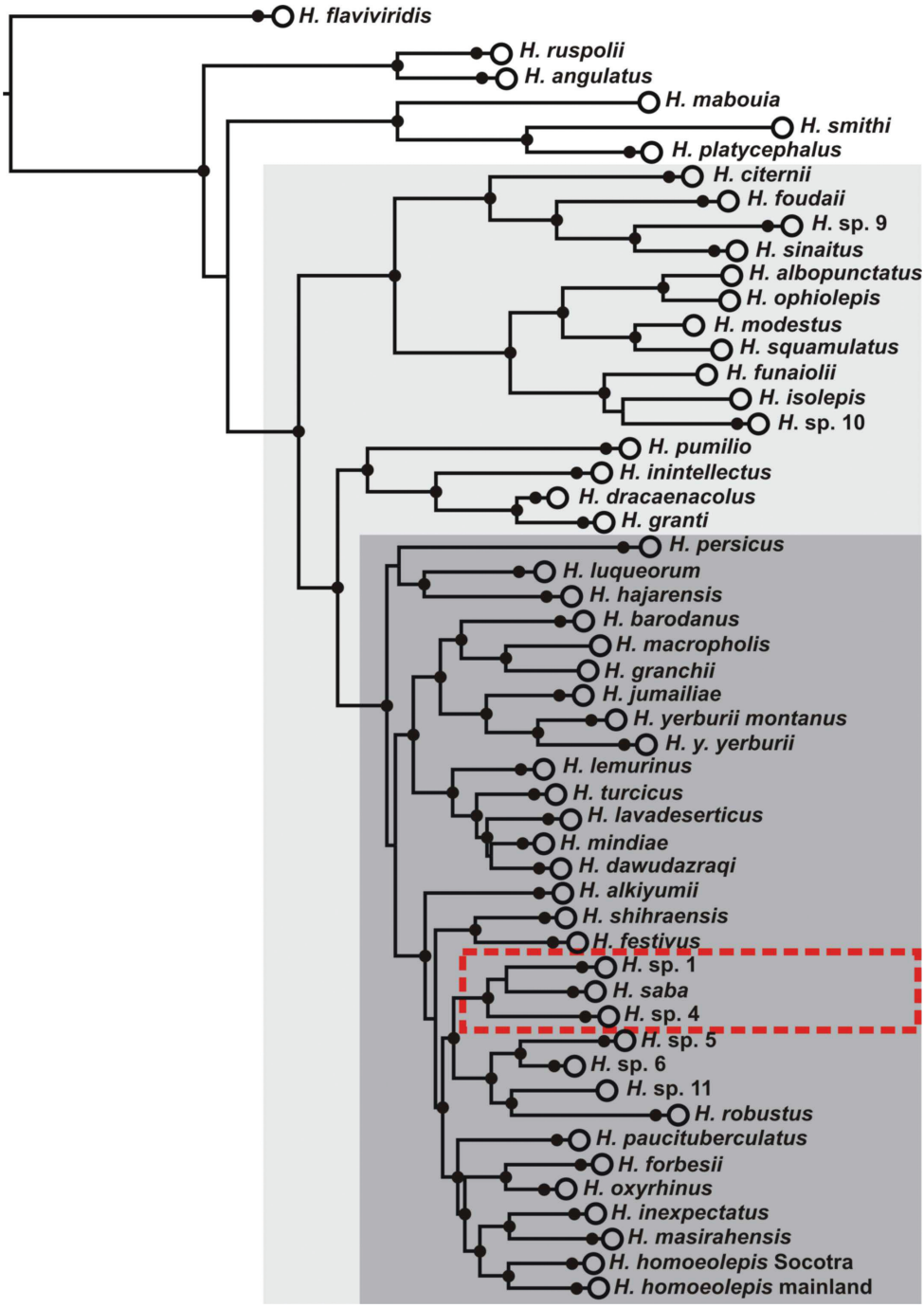

Figure 1. Phylogeny of the *Hemidactylus* Arid clade (light grey rectangle) modified after Šmíd et al. (2013). Dark grey rectangle highlights the Arabian radiation of this clade, dashed red line delimits the '*H. saba* species group' dealt with in this study. Black dots indicate ML bootstrap values ≥ 70 and BI posterior probabilities ≥ 0.95.

Material and methods

Material for phylogenetic analyses

In order to resolve the phylogenetic relationships between the two newly recognized *Hemidactylus* species and *H. saba* based on genetic data, a dataset containing only representatives of these three species was assembled. Apart from the data used by Šmíd et al. (2013), additional sequences of the following specimens were produced (Table 1): the holotype and two paratypes of *H. saba* (the only known existing material), 21 individuals from Sinai and Saudi Arabia belonging to *H.* sp. 1 (Šmíd et al. 2013), and five individuals of the undescribed species from Yemen (*H.* sp. 4; Šmíd et al. 2013), one of which was included in the study by Busais and Joger (2011a) (labelled as 'OTU 7' therein). Total genomic DNA was extracted using DNeasy Blood & Tissue Kit (Qiagen). Subsequently, sequences for up to two mitochondrial (12SrRNA [*12S*] – ca. 400 bp and cytochrome *b* [*cytb*] – 307 bp) and four nuclear (*cmos* – 402 bp, *mc1r* – 666 bp, *rag1* – 1023 bp, *rag2* – 408 bp) were produced using primers and PCR conditions described in details elsewhere (Šmíd et al. 2013). Chromatograms of all newly obtained sequences were checked by eye and assembled in Geneious 5.6.5 (Biomatters, http://www.geneious.com/). All genes were aligned individually using MAFFT (Katoh and Toh 2008) with the iterative refinement algorithm with 1000 iterations. Poorly aligned positions in the alignment of *12S* were eliminated with Gblocks (Castresana 2000) under low stringency options (Talavera and Castresana 2007), producing a final *12S* alignment of 386 bp. Alignments of all coding genes were trimmed so that all started by the first codon position and no stop codons were revealed when translated into amino acids with the appropriate genetic codes.

Phylogenetic analyses and haplotype networks construction

The final dataset consisted of 36 ingroup individuals. Specimen numbers, localities, and GenBank accession numbers of all genes sequenced are presented in Table 1. The alignment of all concatenated genes was 4012 bp long. The software jModelTest 2.1.1 (Guindon and Gascuel 2003; Darriba et al. 2012) was used to assess the best-fitting model of nucleotide substitution for each gene separately under the Akaike information criterion [AIC, Akaike (1973)]. The best-fitting models were selected as follows: *12S* – GTR+G; *cytb* – GTR+I+G; *cmos* – HKY+I; *mc1r* – TIM2+I; *rag1* – HKY+I; *rag2* – TrN+I). Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian inference (BI) methods. In order to detect the potential effect of the nuclear genes on the tree topology and nodal support, independent analyses were run on two datasets: (1) a dataset containing mtDNA genes only (*12S*, *cytb*), and (2) a concatenated dataset of all mtDNA and nDNA genes. Sequences of nuclear genes were not phased; heterozygous positions were coded according to the IUPAC ambiguity codes. Gaps were treated as missing data. Three specimens of *H. flaviviridis* and one

of *H. angulatus*, representatives of two different clades of *Hemidactylus* (Carranza and Arnold 2006), were used to root the trees. Uncorrected genetic distances (*p* distances) were calculated in MEGA 5 (Tamura et al. 2011). Almost complete *cytb* sequences (1127 bp) of the new species from Yemen deposited in GenBank (Šmíd et al. 2013) were used to calculate *p* distances within this species, whereas an alignment of 307 bp was used to obtain intraspecific *p* distances within *H. saba* and the new species from Saudi Arabia and Sinai, and also interspecific *p* distances between these three species.

Maximum likelihood analyses of both datasets were performed in RAxML 7.0.3 (Stamatakis 2006) using raxmlGUI (Silvestro and Michalak 2012) graphical extension with parameters estimated independently for each partition, GTR+I+G model of nucleotide evolution and a heuristic search with 100 random addition replicates. Support of the tree nodes was assessed by bootstrap analysis with 1000 pseudoreplications (Felsenstein 1985).

The BI analyses were run in MrBayes 3.2.1 (Ronquist et al. 2012). Appropriate equivalents of the best-fitting models were specified to each partition (gene) and all parameters were unlinked across partitions. Analyses were performed with two runs and four chains for each run for 10^7 generations, with sampling interval of 1000 generations. Appropriate sampling was confirmed by examining the stationarity of log likelihood (ln*L*) values and the value of average standard deviations of the split frequencies. Convergence between two simultaneous runs was confirmed by the PSRF (potential scale reduction factor) value. From 10^4 sampled trees, 25% were discarded as a burn-in and a majority-rule consensus tree was produced from the remaining ones, with posterior probabilities (pp) of each clade embedded. Nodes with ML bootstrap values ≥ 70% and pp values ≥ 0.95 were considered highly supported (Huelsenbeck and Rannala 2004).

Heterozygous positions in nuclear genes were identified based on the presence of double peaks in chromatograms and using the Heterozygote Plugin in Geneious. For the purpose of haplotype network construction, haplotypes from sequences with more than one heterozygous position were resolved in PHASE 2.1.1 (Stephens et al. 2001). Input data for PHASE were prepared in SeqPHASE (Flot 2010). In order to include as much data as possible, sequences of all *Hemidactylus* species from the Arid clade used in our previous study (Šmíd et al. 2013) were combined with the newly produced sequences and phased together (data not shown). In the case of *rag1*, the original alignment was trimmed to 846 bp, the length at which sequences of all individuals did not contain any N ends that would give misleading results in the allele reconstruction (Joly et al. 2007). PHASE was run under default settings except the probability threshold, which was set to 0.7. Haplotype networks of the four nuclear markers (*cmos*, *mc1r*, *rag1*, *rag2*) were drawn using TCS 1.21 (Clement et al. 2000) with 95% connection limit.

Material for morphological analyses

Material for morphological comparison included 225 specimens of 8 *Hemidactylus* species and one subspecies (Appendix) and was obtained from the following collec-

Table 1. List of material used for the phylogenetic analyses. Holotype of *Hemidactylus ulii* sp. n. and *H. saba* are in bold. The column 'Loc. Nº' refers to the locality number as shown in Fig. 6.

Species	Code	Museum number	Country	Locality	Loc. Nº	Lat	Long	*12S*	*cytb*	*cmos*	*mc1r*	*rag1*	*rag2*
H. granosus	Sher10660	SMB 10660	Egypt	Ayoun Musa	1	29.875	32.649	JQ957071	JQ957216	JQ957148	JQ957282	-	JQ957409
H. granosus	Hd41	NMP6V70163/2	Egypt	Sharm el Sheik; Sinai	2	27.885	34.317	KC818724	HQ833759	JQ957148	-	KC818981	KF647606
H. granosus	Hd96	NMP6V70163/1	Egypt	Sharm el Sheik; Sinai	2	27.885	34.317	KC818724	HQ833759	-	-	-	KF647607
H. granosus	Hd97	NMP6V70163/3	Egypt	Sharm el Sheik; Sinai	2	27.885	34.317	KC818724	HQ833759	-	-	-	KF647608
H. granosus	HSA63	ZFMK 94084	Saudi Arabia	Al Wajh	3	26.208	36.4976	KC818724	HQ833759	KF647576	KF647589	KF647596	KF647610
H. granosus	HSA64	ZFMK 94085	Saudi Arabia	Al Wajh	3	26.208	36.4976	KF647571	-	-	-	-	-
H. granosus	HSA65	ZFMK 94086	Saudi Arabia	15 km S of Al Wajh	4	26.123	36.5689	KF647570	KF647581	KF647574	KF647590	KF647601	KF647610
H. granosus	HSA66	ZFMK 94087	Saudi Arabia	15 km S of Al Wajh	4	26.123	36.5689	KC818724	-	-	-	-	-
H. granosus	HSA67	ZFMK 94088	Saudi Arabia	15 km S of Al Wajh	4	26.123	36.5689	KF647569	-	-	-	-	-
H. granosus	HSA68	TUZC-R8	Saudi Arabia	15 km S of Al Wajh	4	26.123	36.5689	KF647570	-	-	-	-	-
H. granosus	HSA69	ZFMK 94089	Saudi Arabia	15 km S of Al Wajh	4	26.123	36.5689	KF647570	-	-	-	-	-
H. granosus	HSA70	TUZC-R9	Saudi Arabia	72 km N of Umluj	5	25.614	36.9867	KF647569	KF647582	JQ957148	KF647591	KF647600	KF647609
H. granosus	HSA62	TUZC-R10	Saudi Arabia	180 km W of Hail	6	26.883	40.0874	KF647569	KF647585	JQ957148	KF647588	KF647602	KF647609
H. granosus	HSA61	IBES10001	Saudi Arabia	Al Ghat	7	26.054	45.0003	KF647569	KF647585	JQ957148	KF647588	KF647599	KF647610
H. granosus	HSA57	IBES10183	Saudi Arabia	30 km NE of Alhawiyah	8	21.624	40.7094	KF647568	KF647580	-	-	KF647597	KF647610
H. granosus	HSA58	ZFMK 94090	Saudi Arabia	30 km NE of Alhawiyah	8	21.624	40.7094	KF647569	-	-	-	-	-
H. granosus	HSA59	TUZC-R11	Saudi Arabia	30 km NE of Alhawiyah	8	21.624	40.7094	KF647569	-	-	-	-	-
H. granosus	HSA60	IBES10344	Saudi Arabia	30 km NE of Alhawiyah	8	21.624	40.7094	KF647569	KF647583	-	-	KF647598	KF647610
H. granosus	HSA54	IBES10150	Saudi Arabia	20 km S of Ashayrah	9	21.602	40.6911	KF647568	KF647584	KF647576	KF647588	KF647595	KF647609
H. granosus	HSA55	ZFMK 94091	Saudi Arabia	20 km S of Ashayrah	9	21.602	40.6911	KF647569	KF647584	KF647575	KF647588	KF647596	KF647610
H. granosus	HSA56	IBES10363	Saudi Arabia	20 km S of Ashayrah	9	21.602	40.6911	KF647569	-	-	-	-	-
H. granosus	ZFMK 87236	ZFMK 87236	Saudi Arabia	Taif National Wildlife Research Center	10	21.25	40.96	KF647569	-	-	-	-	-
H. saba	BJ27	NHM-BS N41914	Yemen	Marib	17	14.9	45.5	KF647567	-	KF647573	-	-	KF647605
H. saba	BJ28	NHM-BS N41913	Yemen	Marib	17	14.9	45.5	KF647567	KF647579	KF647573	KF647586	-	KF647605
H. saba	**BJ29**	**NHM-BS N41912**	**Yemen**	**Marib**	17	14.9	45.5	KF647567	-	KF647573	KF647587	KF647594	KF647605
H. ulii sp. n.	JS48	NMP6V 74834/1	Yemen	Wadi Zabid	11	14.147	43.517	KC818730	KC818881	KC818789	KC818943	KC819001	KC819062

Species	Code	Museum number	Country	Locality	Loc. Nº	Lat	Long	*12S*	*cytb*	*cmos*	*mc1r*	*rag1*	*rag2*
H. ulii sp. n.	JS49	NMP6V 74834/2	Yemen	Wadi Zabid	11	14.147	43.517	KC818731	KC818882	KC818789	-	KF647603	KF647614
H. ulii sp. n.	JS45	not collected	Yemen	Al Hababi	12	13.333	43.722	KC818728	KC818878	-	-	-	KF647612
H. ulii sp. n.	JS46	NMP6V 74833/1	Yemen	Al Hababi	12	13.333	43.722	KC818728	KC818879	KC818789	-	-	KF647613
***H. ulii* sp. n.**	**JS47**	**NMP6V 74833/2**	**Yemen**	**Al Hababi**	12	13.333	43.722	KC818729	KC818880	KC818789	KC818942	KC819001	KC819061
H. ulii sp. n.	JS37	NMP6V 74832/1	Yemen	3 km S of Najd an Nashamah	13	13.358	43.957	KC818727	KC818876	KF647578	KC818943	-	KF647611
H. ulii sp. n.	JS38	NMP6V 74832/2	Yemen	3 km S of Najd an Nashamah	13	13.358	43.957	KC818727	KC818877	KC818789	KF647593	-	KF647614
H. ulii sp. n.	JS32	NMP6V 74835	Yemen	35 km W of Lahij	14	13.032	44.558	KC818726	KC818875	KC818788	KC818941	KC819000	KC819060
H. ulii sp. n.	BJ09	NHM-BS N41916	Yemen	Radman	15	14.1	45.283	KF647572	-	KF647577	KF647592	-	KC819059
H. ulii sp. n.	JS17	NMP6V 74831/1	Yemen	Al Hadr	16	13.877	45.8	KC818725	KC818874	KC818787	KC818940	KC818999	KC819059
H. ulii sp. n.	JS18	NMP6V 74831/2	Yemen	Al Hadr	16	13.877	45.8	KC818725	-	KC818789	-	KF647604	KC819059
H. angulatus	JS123	NMP6V 74845/2	Ethiopia	Arba Minch	-	6.034	37.564	KC818659	KC818807	KC818747	KC818903	KC818956	KC819018
H. flaviviridis	JS111	not collected	Pakistan	Okara	-	30.811	73.457	KC818676	KC818822	JQ957126	JQ957253	KC818965	KC819026
H. flaviviridis	JS113	not collected	India	Haridwar	-	29.964	78.201	KC818676	KC818823	JQ957126	JQ957253	KC818966	KC819027
H. flaviviridis	JS119	not collected	Oman	Jalan Bani Bu Hassan	-	22.089	59.278	JQ957119	JQ957183	KC818754	KC818911	KC818967	KC819028

tions: National Museum Prague, Czech Republic (NMP); Natural History Museum in Braunschweig, Germany (NHM-BS); Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt, Germany (SMF); Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK); Museo Civico di Storia Naturale "Giacomo Doria", Genova, Italy (MSNG); Museo Civico di Storia Naturale di Milano, Milano, Italy (MSNM); Museo Civico di Storia Naturale, Carmagnola, Italy (MCCI); Università di Firenze, Museo Zoologico "La Specola", Firenze, Italy (MZUF); British Museum of Natural History, London, UK (BMNH); California Academy of Sciences, San Francisco, USA (CAS); Taif University Zoological Collection, Taif, Saudi Arabia (TUZC); Institute of Evolutionary Biology Collection, Barcelona, Spain (IBES); Tomas Mazuch private collection, Dříteč, Czech Republic (TMHC); L. Kratochvíl collection (JEM); J. Šmíd collection (JS); Sherif Baha El Din private collection, Cairo, Egypt (SMB). Names of localities and governorates are spelled according to Google Earth (http://www.google.com/earth/). All coordinates are in WGS84 geographic coordinate system. Table of localities in a CSV text format and high-resolution photographs of all individuals analyzed in this study (397 pictures in total) have been deposited in MorphoBank (Project 1006; http://www.morphobank.org).

Morphological characters

The following measurements were taken with Powerfix digital calliper to the nearest 0.1 mm: snout-vent length (SVL), measured from tip of snout to vent; head length (HL), measured from tip of snout to retroarticular process of jaw; head width (HW), taken at the widest part of the head; head depth (HD), maximum depth of head; left eye diameter (E), measured horizontally; axilla-groin distance (AG), measured from posterior end of front limb insertion to anterior end of hind limb insertion; tail length (TL), measured from vent to tip of original tail. In addition to these metric characters, the following meristic characters were examined using a dissecting microscope: number of upper and lower labials (left/right); contact of nasals; number of infralabials in contact with first postmentals; mutual position of first postmentals; number of longitudinal rows of enlarged dorsal tubercles; number of lamellae under the first and fourth toe including unpaired proximal ones; and number of preanal pores in males. Terminology and diagnostic characters follow Moravec and Böhme (1997) and Moravec et al. (2011).

Results

Phylogenetic analyses of both datasets resulted in trees presented in Fig. 2. Tree topology remains congruent with that showed in Šmíd et al. (2013). The three species form a well-supported monophyletic group (mtDNA: ML bootstrap 85/ Bayesian pp 1; mtDNA + nDNA: 100/1) to which we will refer to as the '*Hemidactylus saba* species group'

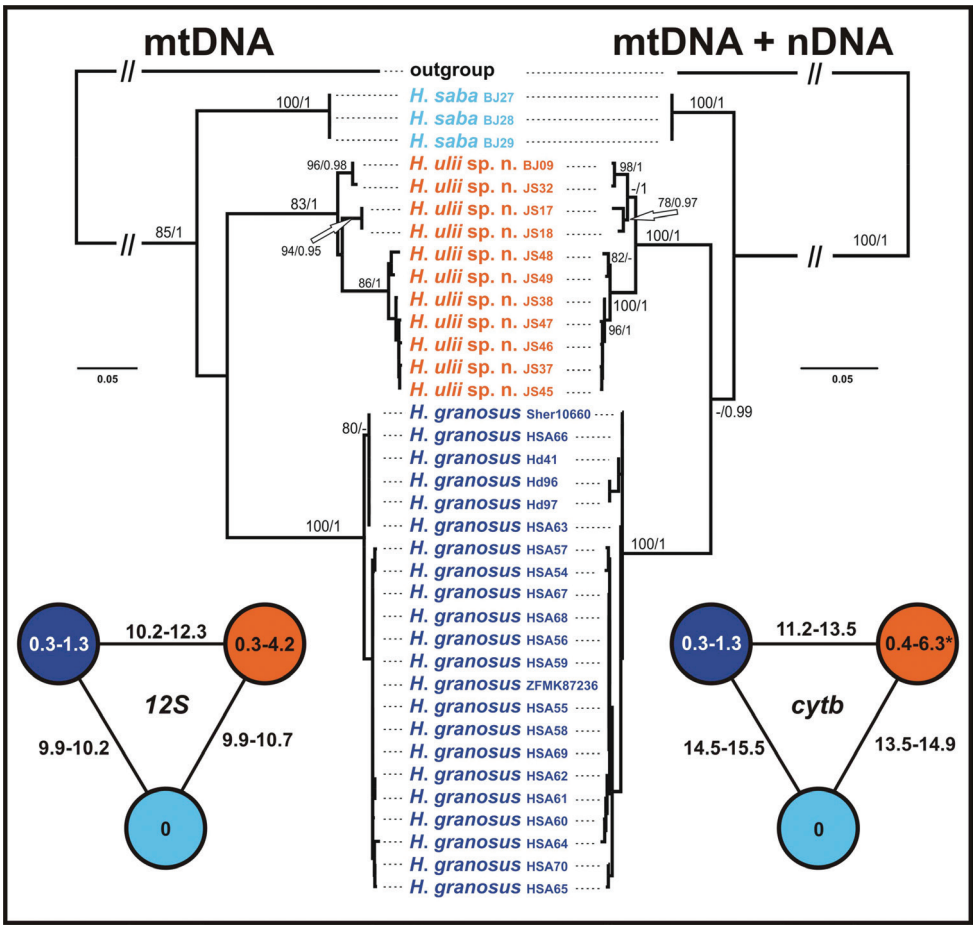

Figure 2. Maximum likelihood trees of mtDNA and mtDNA + nDNA datasets of the '*Hemidactylus saba* species group'. ML bootstrap values/Bayesian posterior probabilities are indicated by the nodes. *Hemidactylus flaviviridis* and *H. angulatus* were used as outgroups. At the sides, schematic networks showing intra- and interspecific uncorrected *p* distances (in %) in the sequences of *12S* and *cytb*. * intraspecific distances within *H. ulii* sp. n. are based on an alignment of 1127 bp, all other values for *cytb* are calculated for an alignment of 307 bp.

[support of individual species: *H. saba* (100/1; 100/1), *Hemidactylus* sp. 1 from Sinai and Saudi Arabia (100/1; 100/1), *Hemidactylus* sp. 4 from Yemen (83/1; 100/1)]. The performed analyses did not resolve the topology within this species group despite the inclusion of more individuals and additional genetic data in comparison with previous works (Moravec et al. 2011; Šmíd et al. 2013). Therefore, with the current knowledge, this group remains polytomic. There is no genetic variability within *H. saba* (all three specimens analyzed originate from the same locality) in both of the studied mtDNA genes and a very little variability in nDNA (*mc1r* and *rag1* only) (Fig. 3). The species from Sinai and Saudi Arabia also shows very little variation in mtDNA (intraspecific *p*

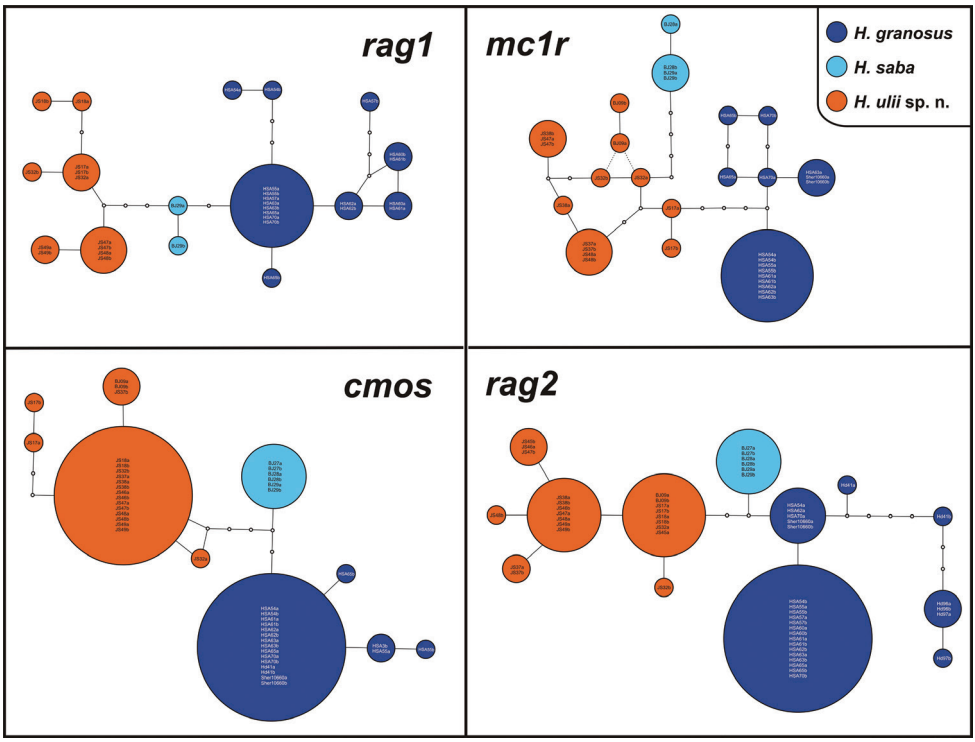

Figure 3. Nuclear allele networks of the four loci analyzed (*cmos*, *mc1r*, *rag1*, *rag2*). Circle sizes are proportional to the number of alleles. Small white circles represent mutational steps. Position of alleles BJ09a and BJ09b in the *mc1r* network is indicated by dashed lines because the sequence of the sample BJ09 (voucher NHM-BS N41916) was 108 bp shorter than the rest of the alignment and haplotype network reconstructions based on both 666 bp and 558 bp alignments linked these alleles to JS32b and JS32a, respectively.

distance max. 1.3% in both *12S* and *cytb*), but it varies in sequences of all the nDNA genes studied (Fig. 3). On the other hand, the unnamed *Hemidactylus* from Yemen exhibits relatively deep intraspecific differentiation into three well supported lineages. Uncorrected genetic distances between these lineages are up to 6.3% in *cytb* and up to 4.2% in *12S* (Fig. 2). Moreover, the nDNA genes show a high level of genetic differentiation (Fig. 3). Intra- and interspecific genetic distances in both mtDNA genes analyzed between all three species are shown in Fig. 2. The results of the nuclear networks indicate that all alleles for all four independent loci are specific for each species.

The results of the molecular analyses, together with a unique combination of morphological features (see below) confirm the earlier conclusion that the newly recognized *Hemidactylus* sp. 1 and *Hemidactylus* sp. 4 represent two separate species, whose taxonomy and nomenclature need to be resolved.

Systematics

Redescription of *Hemidactylus granosus* Heyden, 1827

http://species-id.net/wiki/Hemidactylus_granosus
Figs 4, 5

Hemidactylus granosus Heyden, 1827: p. 17; Tab. 5, Fig. 1. Lectotype SMF 8723 designated by Mertens (1967); collected by E. Rüppell 1827.
Hemidactylus turcicus (Linnaeus, 1758) – Boettger (1893: 29; part.); Anderson (1898: 80; part.); Salvador (1981: 84; part.); Baha El Din (2006: 66; part.).
Hemidactylus turcicus turcicus (Linnaeus, 1758) – Loveridge (1947: 143; part.); Mertens and Wermuth (1960: 79; part.); Baha El Din (2005: 19; part.); Mertens (1967: 55).
Hemidactylus verrucosus (Cuvier, 1829 [*corr*. *H. verrucosus* Gray, 1831]) – Rüppell (1845: 300; part.).
Hemidactylus sp. 1 – Moravec et al. (2011: 24); Carranza and Arnold (2012: 17); Šmíd et al. (2013: 3).
Terra typica (Heyden 1827): "Egypten, Arabien, und Abyssinien".
Terra typica restricta [by lectotype designation by Mertens (1967)]: "Arabia petraea" = Sinai, Egypt.

Material examined. SMF 8723 (lectotype, adult male), Petr. Arabica [Arabia petraea], collected by E. Rüppell in 1827 (MorphoBank M305565–M305594); NMP6V 70163/1 (adult female, MorphoBank M305520–M305528), NMP6V 70163/2 (adult male, MorphoBank M305529–M305542), NMP6V 70163/3–4 (adult females, MorphoBank M305543–M305554, M305555–M305564), Egypt, South Sinai governorate, Sharm el-Sheikh (27.885°N, 34.317°E), ca. 30 m a.s.l., collected by R. Kovář and R. Víta in 1996; ZFMK 94084, ZFMK 94085 (adult females, MorphoBank M305744–M305760, M305761–M305775), Saudi Arabia, Tabuk province, Al Wajh (26.2076°N, 36.4976°E), 5 m a.s.l., 31. V. 2012; ZFMK 94086 (adult female, MorphoBank M305778–M305791), ZFMK 94088, ZFMK 94089 (adult males, M305793–M305799, M305807, M305822–M305827, M305828–M305841), Saudi Arabia, Tabuk province, 15 km S of Al Wajh (26.1226°N, 36.5689°E), 25 m a.s.l., 31. V. 2012; TUZC-R10 (adult female, MorphoBank M305728–M305743), Saudi Arabia, Hail province, 180 km N of Hail (26.8831°N, 40.0874°E), 1020 m a.s.l., 30. V. 2012; IBES10183, TUZC-R11 (adult males, MorphoBank M305656–M305671, M305688–M305701), ZFMK 94090,IBES10344 (adult females, MorphoBank M305672–M305687, M305702–M305717), Saudi Arabia, Makkah province, 30 km NE of Alhawiyah (21.6244°N, 40.7094°E), 1295 m a.s.l., 28. V. 2012; IBES10150, IBES10363 (adult males, MorphoBank M305615–M305628, M305643–M305655), ZFMK 94091 (adult female, MorphoBank M305629–M305642), Saudi Arabia, Makkah province, 20 km S of

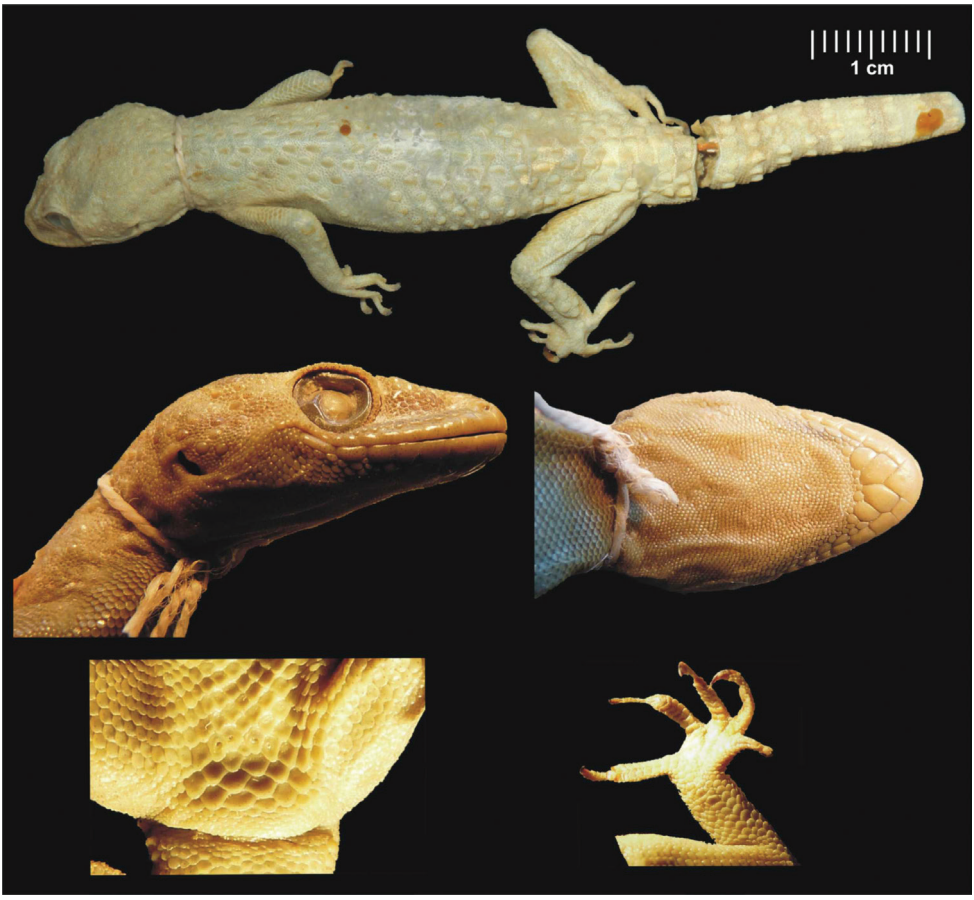

Figure 4. Male lectotype of *Hemidactylus granosus* (SMF 8723) from Sinai, Egypt. General habitus, lateral and ventral view of the head, precloacal region with preanal pores, right hind leg. Scale refers to the uppermost picture only.

Ashayrah (21.6022°N, 40.6911°E), 1316 m a.s.l. , 28. V. 2012. All Saudi specimens were collected by M. Shobrak, S. Carranza and T. Wilms.

Referred material. SMB 10660, Egypt, Suez governorate, Ayoun Musa (29.875°N, 32.649°E), ca. 12 m a.s.l., collected by S. Baha El Din, date unknown; TUZC-R9, Saudi Arabia, Tabuk province, 72 km N of Umluj (25.614°N, 36.9867°E), 19 m a.s.l., 31. V. 2012; IBES10001, Saudi Arabia, Riyadh province, Al Ghat (26.0545°N, 45.0003°E), 776 m a.s.l., 29. V. 2012; ZFMK 94087, TUZC-R8, Saudi Arabia, Tabuk province, 15 km S of Al Wajh (26.1226°N, 36.5689°E), 25 m a.s.l., 31. V. 2012; ZFMK 87236, Saudi Arabia, Makkah province, Taif National Wildlife Research Center (21.25°N, 40.96°E), 25. VI. 2007 by T. Wilms. These specimens were used for the molecular analyses only.

Status and nomenclature. Heyden (1827) described *Hemidactylus granosus* as a new species occurring in Egypt, Arabia and Abyssinia (Ethiopia and Eritrea). Although not explicitly mentioned by the author, the description was apparently based on four specimens

collected by Rüppell currently deposited in the Senckenberg Naturmuseum Frankfurt (collection numbers SMF 8723–8726). Heyden did not diagnose the new species against *H. turcicus* (Linnaeus, 1758) and in respect to our today's knowledge on the morphological variation in *Hemidactylus* the description of *H. granosus* is very general. Traditionally, *H. turcicus* has been considered a common species widely distributed across the Mediterranean and the Middle East. As the general diagnostic characters of *H. granosus* given by Heyden (1827) were also applicable to *H. turcicus* at that time, the name *Hemidactylus granosus* Heyden, 1827 was considered its junior synonym (e.g. Boulenger 1885, Loveridge 1947, Mertens and Wermuth 1960, Mertens 1967, Salvador 1981, Baha El Din 2006).

Recent examination (by JŠ) of four specimens collected by Rüppell (SMF 8723–8726) has shown that one of them [SMF 8723 designated by Mertens (1967) as lectotype of *H. granosus*; for description see below] corresponds morphologically to *Hemidactylus* sp. 1 from Sinai. The other three specimens from this series morphologically correspond to *H. robustus* Heyden, 1827 (SMF 8725, 8726) and *H.* cf. *granosus* (SMF 8724), an animal superficially resembling *H. granosus* but differing from the members of the '*H. saba* species group' in several important characters (see below). These findings lead to the conclusion that *Hemidactylus granosus* Heyden, 1827 is a valid taxon and needs to be resurrected from the synonymy of *H. turcicus*. In the light of current knowledge, the range of *H. turcicus* does not include a large part of Egypt, being restricted mostly to northern Egypt including Sinai and its Red Sea coast. The species is also missing in Arabia (sensu lato) and Ethiopia (Carranza and Arnold 2006; Moravec et al. 2011; Rato et al. 2011; Šmíd et al. 2013).

Diagnosis. *Hemidactylus granosus* is a member of the '*Hemidactylus saba* species group' within the Arabian radiation of the Arid clade as evidenced by the mtDNA and nDNA analyses. The species has the following combination of molecular and morphological characters: (1) Uncorrected genetic distance from *H. saba*: 9.9–10.2% in *12S*, 14.5–15.5% in *cytb*; from *Hemidactylus* sp. 4: 10.2–12.3% in *12S*, 11.2–13.5% in *cytb*; (2) small size, SVL 39.0–53.2 mm in males, 40.6–53.3 mm in females; (3) rather elongated head, head length 24–28% of SVL, head width 68–86% of head length, head depth 33–47% of head length; (4) tail length 107–130% of SVL; (5) uppermost nasals separated by a small shield in 89% of specimens; (6) large anterior postmentals in wide mutual contact, and always in contact with the 1st and 2nd lower labial; (7) 9–11 upper labials; (8) 7–9 lower labials; (9) 14–15 longitudinal rows of enlarged, subtriangular, distinctly keeled dorsal tubercles; (10) 7–8 lamellae under the 1st toe and 10–13 under the 4th toe; (11) ca. 6–8 tail segments bearing 6 pointed tubercles; (12) 4–7 preanal pores in males forming a continuous row on the left and right side; (13) subcaudals enlarged; (14) in life, dorsum pale buff with dark brown spots tending to form transverse bands or X-shaped markings, dark horizontal stripe in prefrontal and temporal region, tail with ca. 10–13 dark brown transverse bands, venter white.

Description of the lectotype. SMF 8723, adult male [erroneously determined as female by Mertens (1967)]. Head and body moderately depressed (Fig. 4). Upper labials (10/10), lower labials (8/7). Nostril between rostral, three subequal nasals and in punctual contact with first upper labial. Uppermost nasals separated by a small inserted scale. Mental triangular, as long as wide. Anterior postmentals long, in a broad contact

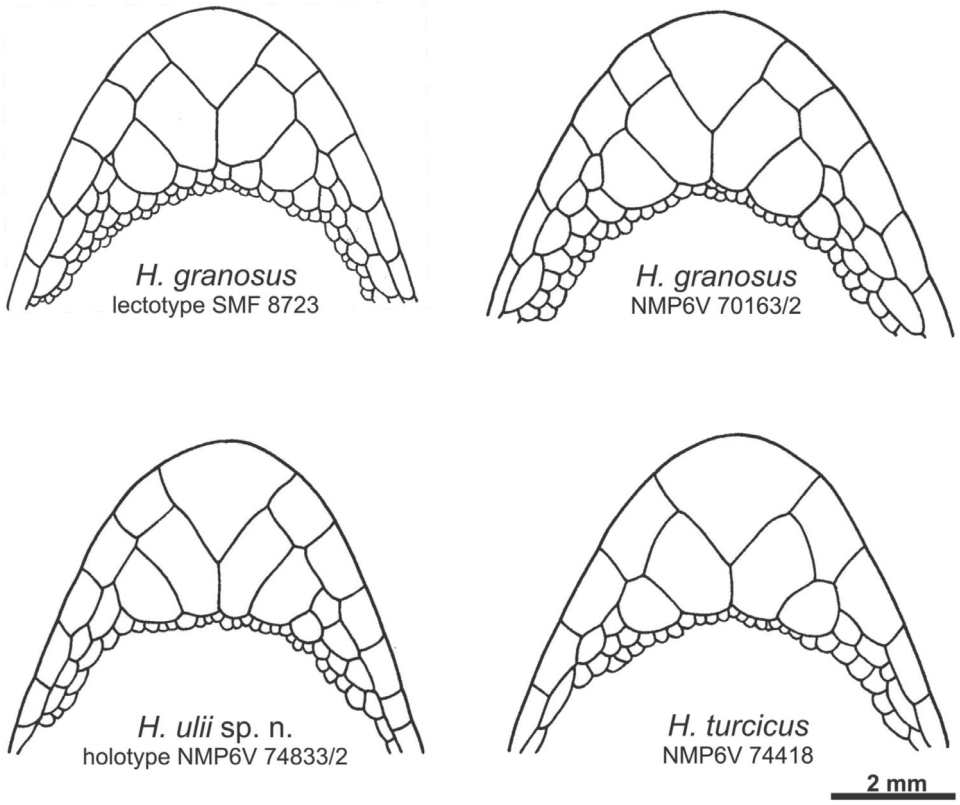

Figure 5. Schematic drawing of the chin region of the lectotype and a new specimen from Sinai of *Hemidactylus granosus*, the holotype of *H. ulii* sp. n., and *H. turcicus* from Sinai.

with each other, both in contact with the 1st and 2nd lower labial reaching in about one fourth of the width of the 2nd labial. Second postmentals almost round, touching only the 2nd lower labial (Fig. 5). Two enlarged scales behind each second postmental, the lateral ones in contact with the 3rd lower labial. Eye moderate (E/HL=0.26). Head long, distinctly separated from body by a slender neck. Crescent-shaped ear opening. Interorbital region, crown of head and temporal area above the level of ear opening covered by round smooth tubercles. Dorsal region of the specimen is slightly scarred so it is not possible to count the enlarged tubercles on both sides precisely, but there are seven longitudinal rows of large, keeled and caudally pointed tubercles on the left side from which we infer there were originally 14 rows on both sides together. Lower arms, thighs and lower legs with prominent tubercles without keels. Tail original with 6 segments bearing 6 pointed tubercles, broken into three pieces, subcaudals enlarged from just after the hemipenial bulges. Lamellae under the 1st toe 7/7, lamellae under the 4th toe 11/11. Four preanal pores in a continuous row. No femoral pores or enlarged femoral scales. Colour (in alcohol) faded due to long fixation.

Measurements (in mm): SVL 51.5, HL 12.9, HW 9.8, HD 6.0, E 3.3, AG 23.7.

Paralectotype SMF 8724 differs from other individuals of *H. granosus* in having relatively high head (HD 50% of HL), lower number of lower labials (6), uppermost nasals in wide contact, first postmentals in contact with 1st lower labials, and 2 preanal pores.

Comparison. *Hemidactylus granosus* can be distinguished from other member of the '*Hemidactylus saba* species group' and from other congeners distributed in Sinai and the Red Sea coast by the following set of characters (see also Table 2).

From *H. saba* by having distinctly keeled dorsal tubercles (smooth in *H. saba*), and lower number of lamellae under the 1st toe (7–8 vs. 8–9).

From *Hemidactylus* sp. 4 (described below) by its larger size (max. SVL 53.2 mm vs. 40.4 mm in males, 53.3 mm vs. 40.7 mm in females), in having more frequently separated uppermost nasals (100% vs. 60% of specimens), lower number of preanal pores in males (4–7 vs. 8), and higher number of lamellae under the 1st (7–8 vs. 5–6) and 4th (10–13 vs. 8–9) toe.

From *H. flaviviridis* by its smaller size (max. SVL 53.2 mm in males and 53.3 mm in females vs. up to 90 mm [Anderson (1999); sexes not distinguished]), by the presence of enlarged dorsal tubercles, and the absence of femoral pores in males.

From *H. mindiae* by the lower number of supralabials (9–11 vs. 10–12), by having anterior postmentals in wide contact (punctual in *H. mindiae*) and keeled dorsal tubercles (smooth in *H. mindiae*).

From *H. robustus* by the larger size of males (max. SVL 53.2 mm vs. 43.7 mm), longer tail (tail length 53.0–64.8 mm vs. 40.9–48.7 mm), and lower number of preanal pores in males (4–7 vs. 5–8).

From *H. turcicus* by its higher number of upper labials (9–11 vs. 7–10), in having anterior postmentals more frequently in contact with 2nd lower labial (100% vs. 12.1%), in having anterior postmentals in wide mutual contact behind the mental scale (contact punctual in 67% specimens of *H. turcicus*), and by the lower number of preanal pores in males (4–7 vs. 6–10).

Variation. Specimens with intact tail vary in number of tail segments bearing 6 pointed tubercles (7–8). The original portion of the tail of the female NMP6V 70163/4 is very wide at the base, separated from cloacal region by a basal constriction. One specimen (IBES10212) is the only animal with 15 longitudinal rows of enlarged tubercles. Another one (IBES10284) has uppermost nasals in wide contact. Most striking is the variation in the number of preanal pores in males. Whereas the lectotype and the only male from Sinai (NMP6V 70163/2) have both 4 pores, all males from Saudi Arabia have 6–7 pores. There seems to be clinal variability in this character, males from NW of the known range (Fig. 6) possess only 4 preanal pores, all animals from the eastern Red Sea coast in Saudi Arabia have 6 pores and a single individual from the southern limit of the range has 7 pores.

Coloration (in life) pale buff dorsally (Fig. 7). Conspicuous dark brown horizontal stripe in loreal and temporal area, terminated at the level of ear from where it continues in a series of dark patches on the neck. Four barely visible X-shaped markings on dorsum formed mainly by dark brown enlarged tubercles (first on nape, second across scapulae, third in lumbal region, and fourth just in front of the anterior insertion of hind limbs). Isolated dark brown stripe runs across body in the place of posterior insertion of hind

Table 2. Morphological comparison among members of the '*Hemidactylus saba* species group' and with other *Hemidactylus* species from Sinai and SW Yemen. The values are given as follows: sample size, mean ± standard deviation above, min. – max. value below.

Species / Character	*H. saba* species group						*H. robustus*		*H. turcicus*		*H. mindiae*		*H. jumailiae*		*H. y. yerburii*		*H. y. montanus*	
	H. granosus		*H. saba*		*H. ulii* sp. n.													
Upper labials	18	9.4 ± 0.5	3	9.3 ± 0.8	10	9.3 ± 0.8	27	9.4 ± 0.7	33	8.2 ± 0.5	5	10.8 ± 0.8	18	9.8 ± 0.7	51	10.3 ± 0.7	57	10.2 ± 0.7
		9–11		8–10		8–10		8–11		7 - 10		10 - 12		8–12		9–12		8–12
Lower labials	18	7.4 ± 0.4	3	7.7 ± 0.6	10	8.0 ± 0.6	27	7.7 ± 0.6	33	6.7 ± 0.5	5	8.1 ± 0.4	18	8.2 ± 0.6	51	7.9 ± 0.5	57	7.8 ± 0.6
		7–9		7–8		7–9		6–9		6–8		7–9		7–10		6–9		6–10
Nasals in contact (%)	18	11	3	33.3	10	40	27	22.2	33	21.2	5	0	18	5.5	51	7.8	57	5.3
1st postmental in contact with 2nd lower labial (%)	18	100	3	33.3	10	100	27	70.3	33	12.1	5	80	18	83.3	51	98	57	89.5
Rows of dorsal tubercles	18	14.1 ± 0.2	3	14 ± 0.0	10	14.1 ± 1.0	27	14.8 ± 1.2	33	13.8 ± 0.7	5	12.4 ± 0.9	15	14 ± 1.4	46	15.3 ± 1.1	53	15.2 ± 1.2
		14–15		14–14		12–16		13–18		12–16		12–14		12–16		13–18		12–18
Pores	8	5.6 ± 1.1	1	6	2	8 ± 0.0	9	6.1 ± 0.8	13	7.2 ± 1.4	1	4	9	7.2 ± 1.1	23	13.7 ± 2.2	27	11.2 ± 1.1
		4–7				8 - 8		5–8		6–10				6–9		10–18		9–13
Lamellae under 1st toe	18	7.4 ± 0.5	3	8.2 ± 0.3	10	5.4 ± 0.5	27	6.1 ± 0.5	32	6.5 ± 0.5	5	6.2 ± 0.3	18	6.9 ± 0.7	51	6.7 ± 0.4	57	6.3 ± 0.4
		7–8		8–9		5–6		5–8		6–7		6–7		6–8		6–8		5–7
Lamellae under 4th toe	18	11.5 ± 0.7	3	11.2 ± 0.3	10	8.6 ± 0.5	27	10.1 ± 0.7	32	9.7 ± 0.6	5	10 ± 0.0	18	10.9 ± 0.8	51	10.4 ± 0.6	57	10.2 ± 0.5
		10 - 13		11–12		8–9		8–12		8–11		10–10		9–12		9–12		9–11
SVL (males)	8	46.8 ± 5.9	1	58.3	2	38.6 ± 2.6	8	41.8 ± 2.3	13	46.0 ± 5.8	1	49.3	8	48.4 ± 4.1	23	58.5 ± 7.1	25	56.5 ± 5.7
		39.0–53.2				36.8–40.4		37.0–43.7		37.3–54.1				40.0–54.2		43.6–74.9		45.2–65.3
SVL (females)	10	49.0 ± 3.5	2	53.5 ± 7.9	2	40.1 ± 0.9	16	43.6 ± 4.7	18	49.2 ± 5.1	4	46.2 ± 11.4	8	48.6 ± 3.3	23	55.7 ± 5.3	30	52.6 ± 5.1
		40.6–53.3		47.9–59.1		39.4–40.7		32.7–50.1		39.4–56.2		35.6–56.6		43.1–54.0		43.6–62.1		42.4–64.1

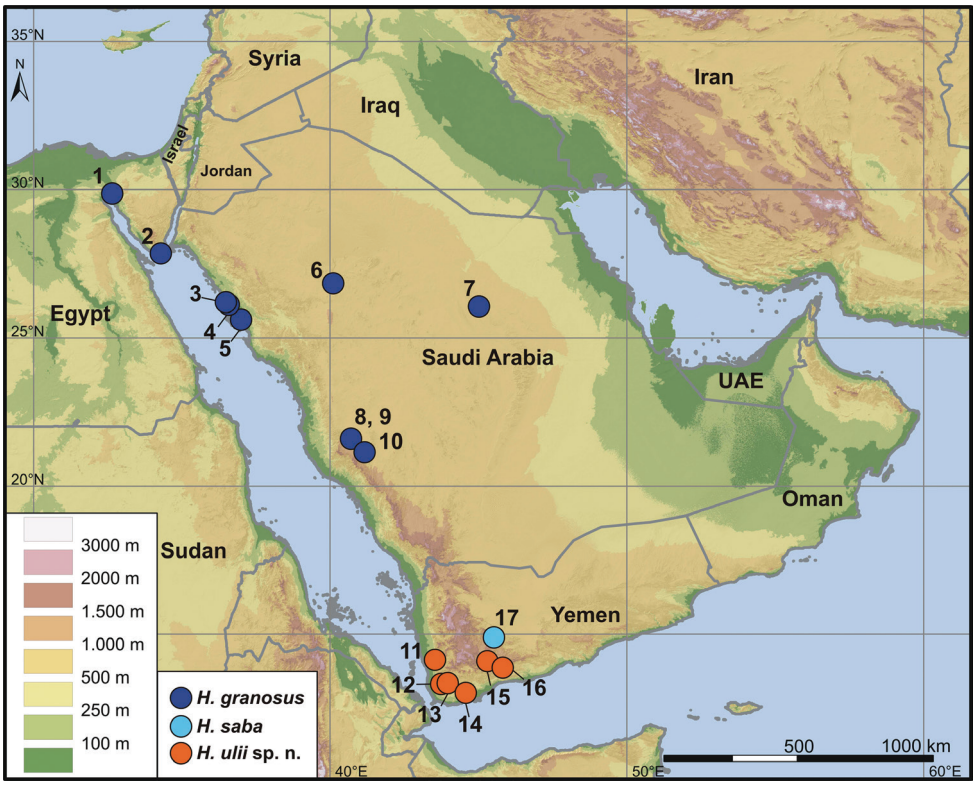

Figure 6. Distribution map of *Hemidactylus granosus*, *H. saba* and *H. ulii* sp. n. For the list of locality names and their corresponding numbers in the map see Table 1.

limbs. Regenerated tails are uniformly buff from above. Dorsum, sides of chin, underside of front and hind limbs and underside of tail with faint stipple visible under magnification. Belly white. Tips of fingers and toes black behind insertion of terminal phalanges. Coloration is consistent among all specimens and varies only in distinctness of the markings.

There is a very low variation in mtDNA between specimens from Sinai and Saudi Arabia (max. 1.3% in both *12S* and *cytb*). All animals from Sinai share the same haplotypes in *12S* and also *cytb* gene. All four nuclear loci studied show some degree of intraspecific variation (Fig. 3).

Distribution and ecology. Eduard Rüppell collected the original series in 1827 when he began his marine biological studies of the Red Sea and travelled from Egypt to Eritrea. There is no specific information that he went to Arabia as well (Rüppell 1826–1828; Klausewitz 2002; Wagner 2008); therefore the original distribution of *H. granosus* described as "Egypt, Arabia, and Abyssinia [Ethiopia and Eritrea]" by Heyden (1827) was probably too general and incorrect. Because there were no other specimens assignable with certainty to *H. granosus* apart from the four individuals collected in Sinai (SMF 8723–8726, for their current status see 'Status and nomenclature' section) (Boettger 1893), one of which became the lectotype after Mertens' (1967) designation, Sinai could

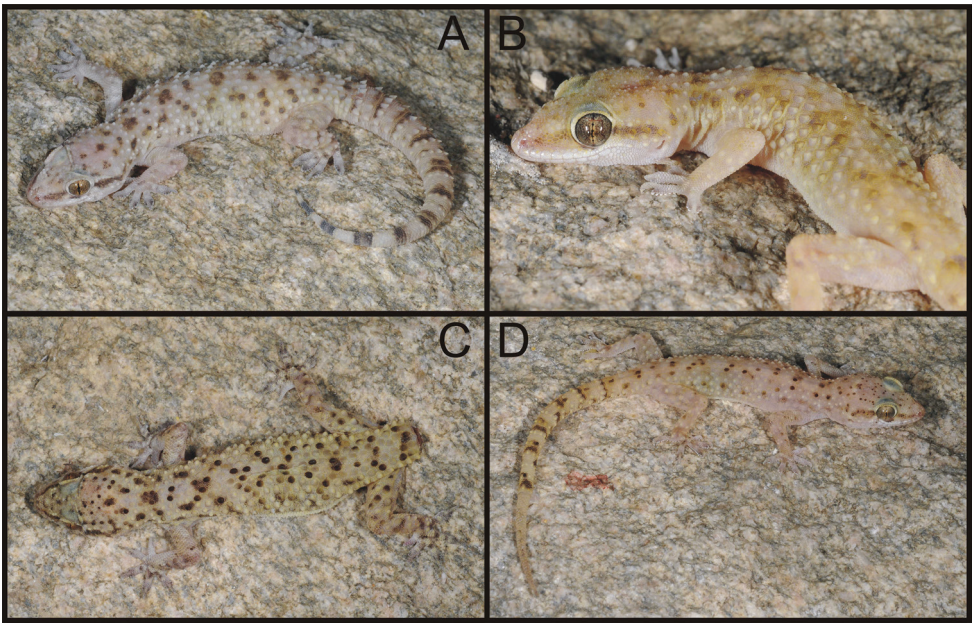

Figure 7. Live specimens of *H. granosus* from Saudi Arabia. **A** IBES10344, 30 km NE of Alhawiyah (loc. number 8) **B** TUZC-R10, 180 km W of Hail (6) **C** ZFMK 94091, 20 km S of Ashayrah (9) **D** ZFMK 94086, 15 km S of Al Wajh (4).

be considered the only reliable locality for *H. granosus*. Here, *H. granosus* is also confirmed from two coastal localities in south and west Sinai and from coastal and inland regions in western and central Saudi Arabia (Fig. 6). Nevertheless, a wider distribution of the species along the Red Sea coast can be expected. According to Baha El Din (2005), *Hemidactylus* geckos inhabiting the interior lowland of Sinai and the Eastern Desert in Egypt stand out in having notably coarse scalation. Interestingly, the areas with occurrence of animals with coarse scalation correspond with the presence of individuals with low numbers of preanal pores (Baha El Din 2005), which is typical for the Sinai populations of *H. granosus*.

In 1996, when the NMP specimens were collected, the locality in Sharm el-Sheikh was formed by a crop field supplied with drain water from nearby habitations. Geckos were found during the day under unused empty barrels and also inside buildings. Other species syntopic with *H. granosus* in Sharm el-Sheikh were: *Hemidactylus turcicus*, *Chalcides ocellatus* (Forskål, 1775), *Stenodactylus sthenodactylus* (Lichtenstein, 1823), and *Ptyodactylus hasselquistii* (Donndorff, 1798) (R. Víta in litt, 2013). However, when visited again in 2010, the locality had changed dramatically (R. Víta in litt, 2013). The whole area was under heavy development and the irrigation channels had disappeared. The current conditions at the place are unknown to us. In 2011 JM surveyed a neighbouring urban area east of this locality. It was covered by a mosaic of tourist resorts and abandoned ruderal plots. In dry anthropogenic habitats (e.g. rubbish dumps, road ditches, old walls and buildings, abandoned construction sites, natural but heavily disturbed open areas, etc.) dominated two very abundant gecko species. *Ptyodactylus*

hasselquistii occupied primarily various vertical surfaces whereas *Cyrtopodion scabrum* (Heyden, 1827) prevailed on the ground. *Tropiocolotes nattereri* Steindachner, 1901 was found in dry and relatively well-preserved natural places. *Hemidactylus turcicus* was occasionally encountered in more humid artificial habitats in parks and hotel gardens. Specimens from Saudi Arabia were mostly collected during the day inside concrete tunnels under roads. In some of the tunnels they were syntopic with *Ptyodactylus hasselquistii*. One specimen was also collected on the walls of the Taif National Wildlife Research Centre, where it was also syntopic with *Ptyodactylus hasselquistii*.

***Hemidactylus ulii* sp. n.**

http://zoobank.org/8E15D1BC-5D4D-4A55-AFEB-2E20FAD40112
http://species-id.net/wiki/Hemidactylus_ulii
Figs 5, 7, 8

Hemidactylus turcicus – Rösler and Wranik (1998: 120; part.).
Hemidactylus sp. 'OTU7' – Busais and Joger (2011a: 27); Busais and Joger (2011b: 268); Carranza and Arnold (2012: 95).
Hemidactylus sp. 4 – Moravec et al. (2011: 25); Šmíd et al. (2013: 3).

Holotype. NMP6V 74833/2, adult male (MorphoBank M305892–M305902), Yemen, Ta'izz governorate, Al Hababi (13.333°N, 43.722°E), 463 m a.s.l.; collected by L. Kratochvíl, 28. X. 2007.

Paratypes. NMP6V 74833/1 (adult male, MorphoBank M305884–M305891), same collecting data as holotype; NMP6V 74831/1–2 (one adult and one subadult female, MorphoBank M305854–M305863, M305864–M305870), Yemen, Abyan governorate, Al Hadr (13.877°N, 45.8°E), 1151 m a.s.l., collected by L. Kratochvíl on 22. X. 2005; NMP6V 74832/1–2 (two subadult females, MorphoBank M305871–M305875, M305876–M305883), Yemen, Ta'izz governorate, ca. 3 km S of Najd an Nashamah by road (13.358°N, 43.957°E), 1182 m a.s.l., collected by L. Kratochvíl on 26. X. 2007; NMP6V 74834/1–2 (one adult and one subadult female, MorphoBank M305903–M305911), Yemen, Dhamar governorate, Wadi Zabid (14.147°N, 43.517°E), 292 m a.s.l., collected by L. Kratochvíl on 29. X. 2007; NHM-BS N41916 (juvenile, MorphoBank M305842–M305852), Yemen, Al Bayda' governorate, Radman (14.1°N, 45.283°E), collected by W. Mustafa on 13. XI. 2007.

Referred material. NMP6V 74835 (juvenile), Yemen, Lahij governorate, wadi 35 km W of Lahij (13.032°N, 44.558°E), 297 m a.s.l., collected by L. Kratochvíl on 25. X. 2007; JEM476 (juvenile), same collecting data as holotype; All juvenile specimens were used for comparison of meristic characters and included in the molecular analyses.

Diagnosis. A small species of the '*Hemidactylus saba* species group' within the Arabian radiation of the Arid clade of *Hemidactylus*, as evidenced by the mtDNA and nDNA analyses. The new species is characterized by the following combination of molecular and morphological characters: (1) Uncorrected genetic distances from *H. saba*:

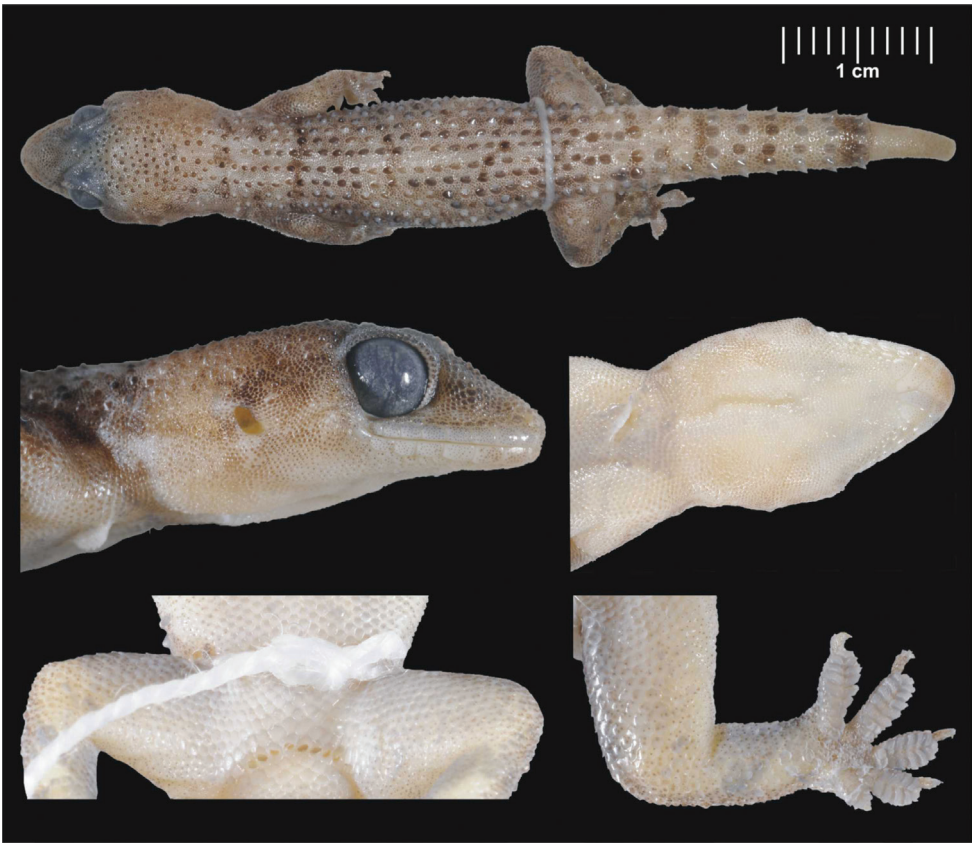

Figure 8. Holotype of *Hemidactylus ulii* sp. n. (NMP6V 74833/2, male) from Al Hababi, Yemen. General habitus, lateral and ventral view of the head, precloacal region with preanal pores, right hind leg. Scale refers to the uppermost picture only.

9.9–10.7% in *12S*, 13.5–14.9% in *cytb*; from *H. granosus*: 10.2–12.3% in *12S*, 11.2–13.5% in *cytb*; (2) small size with a maximum recorded SVL 40.7 mm (36.8–40.4 mm in males, 39.4–40.7 mm in females); (3) moderately robust head, head length 28–30% of SVL, head width 70–75% of head length, head depth 37–46% of head length; (4) tail length 116% of SVL (only 1 specimen with intact tail); (5) uppermost nasals separated by a small shield (60% specimens) or in wide contact (40%); (6) large anterior postmentals in wide mutual contact in 90% of individuals, and in contact with the 1st and 2nd lower labial (scarcely and unilaterally with the 1st lower labial only); (7) 8–10 upper labials; (8) 7–9 lower labials; (9) dorsum with 12-16 longitudinal rows of enlarged, slightly keeled, conical tubercles; (10) 5–6 lamellae under the 1st toe and 8–9 lamellae under the 4th toe; (11) ca. 6–8 tail segments bearing 6 tubercles; (12) 8 preanal pores in one continuous row in males; (13) subcaudals enlarged; (14) in alcohol dorsum brownish grey with a pattern of more or less conspicuous dark transverse bands starting on the nape, tail with 9 dark brown transverse bands.

Comparison. *Hemidactylus ulii* sp. n. can be distinguished from the other members of the '*Hemidactylus saba* species group' and from all other congeners distributed in the region by the following combination of characters (see also Table 2):

From *H. granosus* by its smaller size (max. SVL 40.4 mm vs. 53.2 mm in males, 40.7 mm vs. 53.3 mm in females), by having less frequently separated uppermost nasals (60% vs. 89% of specimens), higher number of preanal pores in males (8 vs. 4–7), and lower number of lamellae under the 1st (5–6 vs. 7–8) and 4th (8–9 vs. 10–13) toe.

From *H. saba* by its smaller size (max. SVL 40.4 mm vs. 58.3 mm in males, 40.7 mm vs. 59.1 mm in females), higher number of preanal pores in males (8 vs. 6), and lower number of lamellae under the 1st (5–6 vs. 8–9) and 4th (8–9 vs. 11–12) toe.

From *H. flaviviridis* by its smaller size (maximum SVL 40.4 mm in males, 40.7 mm in females vs. up to 90 mm [Anderson (1999); sexes not distinguished]), the presence of enlarged dorsal tubercles, and the absence of femoral pores in males.

From *H. jumailiae* by its smaller size (max. SVL 40.4 mm vs. 54.2 mm in males, 40.7 mm vs. 54.0 mm in females), lower frequency of separated uppermost nasals (60% vs. 95%), in having conical and at least slightly keeled dorsal tubercles (vs. non-protruding and smooth tubercles), and lower number of lamellae under the 1st (5–6 vs. 6–8) and 4th (8–9 vs. 9–12) toe.

From *H. robustus* by its smaller size (max. SVL 40.4 mm vs. 43.7 mm in males, 40.7 mm vs. 50.1 mm in females), and lower number of lamellae under the 4th toe (8–9 vs. 8–12).

From *H. sinaitus* by the presence of enlarged tile-like subcaudals and in having separated uppermost nasals (60% vs. 9% of specimens).

From *H. yerburii montanus* by its smaller size (maximum SVL 40.4 mm vs. 65.3 mm in males, 40.7 mm vs. 64.1 mm in females), lower number of preanal pores in males (8 vs. 9–13), and lower number of lamellae under the 4th toe (8–9 vs. 9–11).

From *H. yerburii yerburii* by its smaller size (maximum SVL 40.4 mm vs. 74.9 mm in males, 40.7 mm vs. 62.1 mm in females), lower number of supralabials (8–10 vs. 9–12), lower frequency of having separated uppermost nasals (60% vs. 92%), lower number of preanal pores in males (8 vs. 10–18), and lower number of lamellae under the 1st (5–6 vs. 6–8) and 4th (8–9 vs. 9–12) toe.

Description of holotype. NMP6V 74833/2, adult male. Body slightly depressed to cylindrical (Fig. 8). Upper labials 8/8, lower labials 7/7. Nostril between rostral, three nasals and in punctual contact with the first upper labial. Uppermost nasals separated by a small inserted shield. Mental almost triangular. Anterior postmentals large and very long, in wide mutual contact behind mental, in contact with the 1st lower labial (left) and the 1st and 2nd lower labials (right) (Fig. 5). Posterior postmentals smaller, in contact with the 1st and 2nd (left) and the 2nd (right) lower labial. Eye moderate (E/HL=0.24). Supraciliar granules with prominent projections, which form a comb-like structure above the eyes. Parietal and temporal region covered with round pointed regularly distributed tubercles. Ear opening oval. Dorsum with 14 longitudinal rows of enlarged, prominent, caudally pointed tubercles bearing distinct longitudinal keels. Thighs and lower legs with scattered enlarged tubercles. Tail partially regenerated from about half of its original

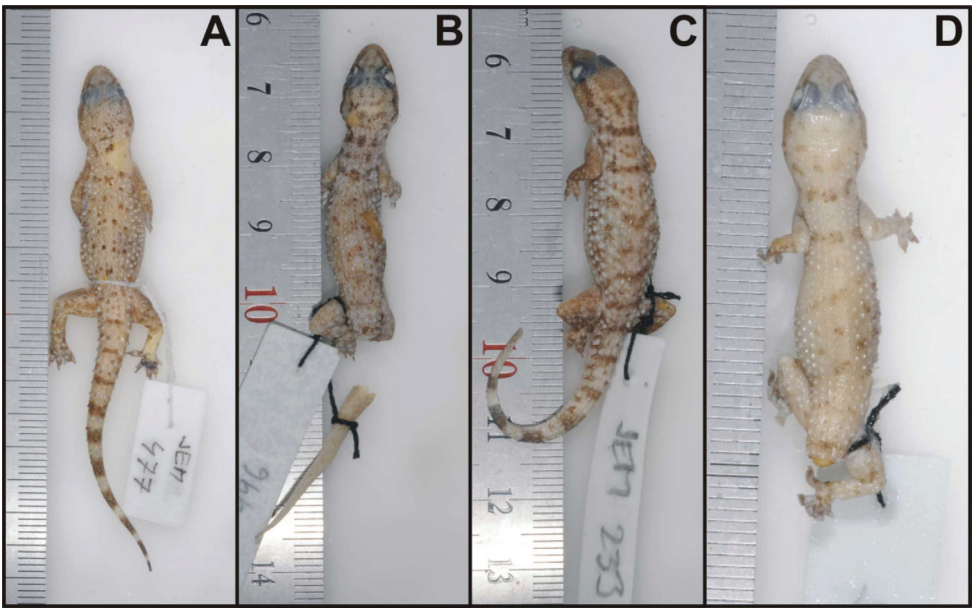

Figure 9. Four (out of eight) paratypes of *Hemidactylus ulii* sp. n. **A** NMP6V 74833/1, male **B** NMP6V 74834/1, female **C** NMP6V 74831/1, female **D** NMP6V 74832/1, subadult female.

length (estimate), original part relatively thick without basal constriction. Conical and keeled tail tubercles on tail segments forming regular whorls. Each whorl separated from the next one by four small scales. Subcaudals enlarged, tile-like. Regenerated part of the tail with small uniform scales without tubercles. Lamellae under the 1st toe 6/6, lamellae under the 4th toe 8/8. Eight preanal pores, no femoral pores or enlarged femoral scales.

Measurements (in mm): SVL 40.4, HL 11.5, HW 8.6, HD 5.2, E 2.8, AG 16.2.

Coloration of holotype in preservative. Overall dorsal coloration brownish grey. An indistinct dark horizontal stripe in loreal and temporal area. Seven dark brown transverse bands across the nape and body, the one in scapular region being the most conspicuous. Dark brown bands also on the original part of the tail. Belly whitish.

Variation. The paratypes (Fig. 9) differ from the holotype in the following features: number of upper labials 8–10; number of lower labials 7–9; four paratypes (NMP6V 74831/1, NMP6V 74832/1–2, NMP6V 748333/1) have uppermost nasals in wide contact; anterior postmentals in contact with 2nd lower labials on both sides (except of NMP6V 74832/1 where the arrangement is the same as in the holotype); longitudinal rows of enlarged tubercles 12–16; lamellae under the 1st toe 5–6, lamellae under the 4th toe 8–9. The intact tail of the paratype NMP6V 74833/1 has 7 segments bearing at least six enlarged spine-like tubercles and 9 dark brown transverse bands widening towards the tail tip.

Measurements of paratypes (in mm): NMP6V 74831/1: SVL 40.7, HL 11.5, HW 8.2, HD 4.9, E 3.0, AG 19.0; NMP6V 74831/2: SVL 32.0, HL 9.3, HW 6.6, HD 3.7, E 2.1, AG 12.7; NMP6V 74832/1: SVL 32.7, HL 9.7, HW 7.0, HD 3.4, E 2.3, AG 14.3; NMP6V 74832/2: SVL 32.9, HL 9.3, HW 6.7, HD 3.6, E 2.4, AG 13.5;

NMP6V 74833/1: SVL 36.8, HL 10.7, HW 8.0, HD 4.5, E 2.4, AG 14.1, TL 42.5; NMP6V 74834/1: SVL 39.4, HL 11.1, HW 8.1, HD 4.4, E 2.7, AG 16.7; NMP6V 74834/2: SVL 32.0, HL 9.5, HW 6.7, HD 3.9, E 2.5, AG 13.8; NHM-BS N41916: juvenile, not measured.

As already mentioned (Results), the level of genetic variability within *H. ulii* sp. n. is very high. The species is divided into three well supported sublineages which reflect the geographic origin of the samples. Although there is a certain geographic separation corresponding with these sublineages, the exact limits are not distinct and also morphological variation among paratypes is not congruent with geography.

Etymology. The species epithet "*ulii*" is a patronym for Prof. Ulrich Joger, a German herpetologist known as Uli among friends, in recognition of his important contribution to the knowledge of the herpetofauna of the Western Palearctic.

Distribution and ecology. *Hemidactylus ulii* sp. n. is known from inland mid-altitude areas (292–1182 m) of southwestern Yemen (Fig. 6). Most specimens were collected in open dry wadis with scattered rocks and boulders, in stony deserts and also in the vicinity of villages in gardens and irrigated cropland fields.

The following reptile species were found to occur in sympatry with *H. ulii*: *Bunopus spatalurus* Anderson, 1901; *Hemidactylus y. yerburii* Anderson, 1895; *Pristurus crucifer* (Valenciennes, 1861); *P. flavipunctatus* Rüppell, 1835; *P. rupestris* Blanford, 1874; *Ptyodactylus* sp.; *Tropiocolotes scorteccii* Cherchi and Spano, 1963; *Acanthodactylus* sp.; *Chamaeleo arabicus* Matschie, 1893; *Pseudotrapelus sinaitus* (Heyden, 1827); *Trapelus flavimaculatus* Rüppell, 1835; and *Pelomedusa subrufa* (Bonnaterre, 1789).

Discussion

Previous phylogenetic studies of the Arid clade of *Hemidactylus* disclosed an extraordinarily rich diversity within this genus in the Arabian Peninsula (Moravec et al. 2011; Carranza and Arnold 2012; Šmíd et al. 2013). The latter work, besides of showing the phylogenetic relationships among individual species of the Arid clade, highlighted the high level of genetic differentiation and existence of several yet undescribed taxa within this genus. The '*Hemidactylus saba* species group' as defined herein represents one of the monophyletic groups within the Arabian radiation. All three species forming this group – *H. granosus*, *H. saba*, and *H. ulii* sp. n. – are well defined and distinguishable both genetically and morphologically from each other, as well as from other *Hemidactylus* species that occur in the same area. Geographically, *H. saba* and *H. ulii* sp. n. are confined to the foothills and submontane areas of southwestern Yemen, where they occupy mid-altitude elevations (292–1182 m in *H. ulii* sp. n., 1180 m in *H. saba*). In comparison, *H. granosus* has a much wider distribution, spanning from northeastern Egypt to central Saudi Arabia. It was found from the sea-level up to almost 1600 m in the Asir Mountains, which stretch along the eastern Red Sea coast of the Arabian Peninsula. Its occurrence in eastern Egypt is also likely based on observations of Baha El Din (2005, 2006), who reported morphologically variable populations of *H. turcicus* (sensu lato) in these regions attribut-

able to *H. granosus* (see Distribution and ecology). The distribution of *H. granosus* in the coastal Sinai and Saudi Arabia near important marine junctions together with the genetic uniformity of this species indicates extensive gene flow between these populations. It may be the result of recent colonization event(s), their inadvertent human-mediated transportation or perpetual contact of populations in a continuous range. The continuous range of *H. granosus* along the Hijaz and Asir Mountains in western Arabia confirms that these mountain ranges can serve as a corridor providing connection between the eastern Mediterranean and southern Arabia (Scott 1942; Gvoždík et al. 2010).

The highlands of southwestern Saudi Arabia and Yemen are known to host a high number of endemic taxa (Balletto et al. 1985; Arnold 1986; Gasperetti 1988; Harrison and Bates 1991; Gasperetti et al. 1993). The genus *Hemidactylus* also shows a high rate of speciation and endemicity in the area. Currently, there are eight species and one subspecies known from the Yemen highlands, which makes *Hemidactylus* one of the most specious reptile genera in the area (Fritz and Schütte 1987; Busais and Joger 2011b; Šmíd et al. 2013; Uetz 2013). As new genetic and morphological data are becoming available from Arabia even more new species are to be expected (Moravec et al. 2011; Šmíd et al. 2013), thus fulfilling the prognosis of Baha El Din (2005) and the models of Ficetola et al. (2013) which suggested that the Red Sea region is likely to contribute significantly to the diversity of *Hemidactylus*.

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Appendix

Specimens examined

H. flaviviridis (8 individuals) - NMP6V 74858 (Oman, Jalan Bani Bu Hasan); NMP6V 74859/1–5 (Pakistan, Multan); NMP6V 74856 (Pakistan, Rakhni); NMP6V 74857 (Pakistan, Sukkur)

H. jumailiae (18 individuals) - NMP6V 74818/1 (Yemen, near Al Bayda [At Dageeg]); NMP6V 74819 (Yemen, Sana'a); NHM-BS N41788, NHM-BS N41890 (paratype), NHM-BS N41891, NHM-BS N41893 (holotype), NHM-BS N41894 (paratype), NHM-BS N41897 (paratype) (Yemen, Ibb); NHM-BS N41898 (paratype, the same number as one of *H. y. montanus* paratypes, Busais and Joger 2011b), NHM-BS N41899 (paratype) (Yemen, Thamar); BMNH1982.1143–44 (Yemen, Al Nabi Shuaib, 30 Km W. of Sana'a); BMNH1982.1145 (Yemen, Sana'a); BMNH1982.1146 (Yemen, Wadi Ahger, 45 Km. W. of Sana'a); BMNH1952.1.3.52 (Yemen, Sana'a); MSNG-YEM02, MSNG-YEM03 (Yemen, El Menghil); MCCI-R814 (Yemen, Hababah)

H. mindiae (5 individuals) - NMP6V 71323/1–2 (Jordan, Jabal Ghazali); NMP6V 72739/1–3 (Jordan, Wadi Ramm Nughra Radet Salem)

H. robustus (27 individuals) - SMF 8720 (lectotype), SMF 8721 ("Abyssinia" [Ethiopia and Eritrea]); SMF 8725–8726 – redetermined from *H. granosus* (Egypt, Sinai); JS210, TMHC2012.07.092, TMHC2012.07.100 (Ethiopia, Jijiga), CAS130512 – redetermined from *H. macropholis* as it is in the CAS catalogue (Kenya, vicinity of Mandera); NMP6V 74820 (Iran, Bandar Lengeh); NMP6V 74821/1–2 (Yemen, Wadi Zabid); NMP6V 74829 (Yemen, Bir Ali); JS144 (Kenya, Garissa); NMP6V 74867/1–3 (Oman, Muscat); NMP6V 74868 (Oman, Salalah); NMP6V 74869/1–7 (Oman, Mughsayl); NMP6V 74870/1–2 (Oman, Shisr); MCCI–R815 (Yemen, Zabid)

H. saba (3 individuals) - NHM-BS N41912 (holotype, MorphoBank M305478–M305492), NHM-BS N41913 (paratype, MorphoBank M305493–M305504), NHM-BS N41914 (paratype, MorphoBank M305505–M305519) (Yemen, Marib)

H. sinaitus (23 individuals) - BMNH82.8.16.27 (holotype, probably from Suakin, Sudan); BMNH97.10.28.83–85 (Sudan, Durrur, N of Suakin); BMNH97.10.28.87 (Sudan, Wadi Haifa); BMNH1974.3931 (Ethiopia, Mule River?, Danakil); BMNH1937.12.5.293–294 (Somalia, Borama district); BMNH95.5.23.7 (Yemen, Sheikh Osman, near Aden); BMNH1945.12.12.14 (Yemen, Bir Fadhl, Aden); NMP6V 74809/1–4 (Sudan, Wad Ben Naga); NMP6V 74810 (Sudan, 15 km SE Atbara); MZUF28645–646 (Yemen, Moka); MZUF10914, MSNM521 (Eritrea, Isola [island] Sheik-Said); MSNM523–524 (Eritrea, Ailet); CAS174021–022 (Sudan, Assalaya)

H. turcicus (33 individuals) - NMP6V 34747 (Syria, Baniyas); NMP6V 34748/1–3 (Syria, Palmyra); NMP6V 34749 (Syria, Salkhad); NMP6V 70648/1–4 (Turkey, Kaş); NMP6V 70668 (Greece, Kastellorizo, St. Georgies); NMP6V 71056

(Egypt, Bahariya); NMP6V 71587/1–3 (Cyprus, Famagusta); NMP6V 71592/1–2 (Cyprus, Yali); NMP6V 72497 (Syria); NMP6V 74046/1–2 (Syria, Cyrrhus); NMP6V 74047/1–2 (Turkey, Antakya); NMP6V 74050 (Greece, Crete, Kavros); NMP6V 74131/1–3 (Syria, Palmyra); NMP6V 73626/1–3 (Turkey, Finike); NMP6V 70269 (Italy, Sardinia, Cagliari); NMP6V 72073 (Greece, Korfu, Nicos); NMP6V 74167 (Greece, Crete, Kavros); NMP6V 70667 (Greece, Kastellorizo); NMP6V 70163/5 (Egypt, Sharm el-Sheikh)

H. yerburii yerburii (51 individuals) - NMP6V 74827/1–4 (Yemen, Jabel Habeshi); NMP6V 74825/1–2 (Yemen, Al Turbah); NMP6V 74826 (Yemen, N of Lahij, Wadi Tuban); NMP6V 74823/1–3 (Yemen, 14 km NW of Al Turbah); NMP6V 74824/1–2 (Yemen, 3 km S of Najd an Nashamah); NMP6V 74828/1–3 (Yemen, Al Hababi); NMP6V 74822/1–5 (Yemen, near Zinjubar); MSNG-YEM01 (Yemen, Ta'izz); MSNG-YEM05, MSNG-YEM06 (Yemen, Vahren); NHM-BS N41856–59, NHM-BS N41861–64, NHM-BS N41866, NHM-BS N41868–69, NHM-BS N41888 (Yemen, Tour Albaha); NHM-BS N41860 (Yemen, Lahij); NHM-BS N41871–72 (Yemen, Radfan); NHM-BS N41873 (Yemen, Shihr); NHM-BS N41875 (Yemen, Ariab); NHM-BS N41876–77, NHM-BS N41879–86 (Yemen, Lowder); NHM-BS N41887 (Yemen, Aden)

H. yerburii montanus (57 individuals) - NMP6V 74802 (Yemen, Jabal Bura); NHM-BS N41751–52 (paratypes), NHM-BS N41758 (paratype), NHM-BS N41762–63, NHM-BS N41765–66, NHM-BS N41768–69, NHM-BS N41770 (paratype), NHM-BS N41772–74, NHM-BS N41779, NHM-BS N41783 (paratype), NHM-BS N41785 (paratype), NHM-BS N41791 (paratype), NHM-BS N41793 (paratype), NHM-BS N41797–800 (paratypes), NHM-BS N41802–06 (paratypes), NHM-BS N41807 (paratype), NHM-BS N41809 (paratype), NHM-BS N41811–15 (paratypes), NHM-BS N41818 (paratype), NHM-BS N41821 (paratype), NHM-BS N41823 (paratype), NHM-BS N41836 (holotype), NHM-BS N41839, NHM-BS N41840 (paratype), NHM-BS N41842 (paratype), NHM-BS N41843, NHM-BS N41844 (paratype), NHM-BS N41846, NHM-BS N41848, NHM-BS N41851–52, NHM-BS N41867 (paratype) (Yemen, Ibb); NHM-BS N41771 (paratype) (Yemen, Yareem); NHM-BS N41789–90 (Yemen, Thamar); NHM-BS N41833–34 (paratypes) (Yemen, Wadah); NHM-BS N41853–55 (paratypes) (Yemen, Sana'a).