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Sylphella puccoon gen. n., sp. n. and two additional new species of aquatic oligochaetes (Lumbriculidae, Clitellata) from poorly-known lotic habitats in North Carolina (USA)

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Abstract

Three new species of Lumbriculidae were collected from floodplain seeps and small streams in southeastern North America. Some of these habitats are naturally acidic. *Sylphella puccoon* **gen. n.**, **sp. n.** has prosoporous male ducts in X–XI, and spermathecae in XII–XIII. Muscular, spherical atrial ampullae and acuminate penial sheaths distinguish this monotypic new genus from other lumbriculid genera having similar arrangements of reproductive organs. *Cookidrilus pocosinus* **sp. n.** resembles its two subterranean, Palearctic congeners in the arrangement of reproductive organs, but is easily distinguished by the position of the spermathecal pores in front of the chaetae in X–XIII. *Stylodrilus coreyi* **sp. n.** differs from congeners having simple-pointed chaetae and elongate atria primarily by the structure of the male duct and the large clusters of prostate cells. Streams and wetlands of Southeastern USA have a remarkably high diversity of endemic lumbriculids, and these poorly-known invertebrates should be considered in conservation efforts.

Keywords

Lumbriculids, biodiversity, acidic waters, pocosin soils, North America

Introduction

In contrast to larger streams and rivers, aquatic habitats such as wetlands, small headwater tributaries or springs have received little attention. As these habitats are not directly connected to each other by flow, and their physical structure can vary greatly from one location to another, they may be expected to support a distinct invertebrate fauna, demonstrate greater variation in taxonomic composition, and have patterns of taxa richness and assemblage variation that do not correlate with adjacent main stream habitats. Riverine wetlands studies in North America indicate high macroinvertebrate taxonomic richness, and greater assemblage variation compared to nearby riffle communities (Curry et al. 2012).

Biological assessment of swamp streams in the southeastern USA has been particularly difficult, especially in naturally acidic areas, since the unusual conditions result in a distinctive fauna with relatively low diversity and abundance of taxa commonly used to indicate excellent water quality (e.g., Ephemeroptera, Plecoptera, Trichoptera – EPT). The North Carolina Division of Environmental Management has struggled with ratings for swamp streams in North Carolina for many years, and for some regions of the state there is still insufficient information to assign water quality ratings (NCDENR 2013). Separate criteria (Lenat 2003) have been established for some swamp streams, taking into account their much lower EPT and total taxa richness relative to more typical streams. Documenting the presence of rare or endemic taxa is one way to address the requirement for conservation of these habitats. A reasonable assessment of such habitats will only be possible with a better understanding of their little-known fauna.

The southeastern region of North America has revealed a great degree of endemicity in the oligochaete family Lumbriculidae. Recent collections from the Sandhills and Coastal Plain ecoregions of North Carolina have resulted in the description of new, and probably endemic, lumbriculid species and genera (Fend and Lenat 2007, 2012). The objective of present study is to contribute to the knowledge of the oligochaete fauna inhabiting poorly known aquatic habitats in that region, with the description of three new lumbriculid species, one of which is assigned to a new genus. Knowledge of the communities of these insufficiently studied habitats is essential to undertake any conservation plan of biodiversity in fluvial catchments.

Material and methods

Oligochaetes were usually collected by disturbing the substrate and then sweeping through this area with a 300 μm mesh net. Samples were elutriated to remove the heavier sediments. Most collections were live-picked at the sample site, but some material was fixed whole in 10% buffered formalin and brought back to the laboratory for sorting. Field-picked specimens were relaxed by the addition of small amounts of alcohol, and then they were fixed in formalin and/or Bouin's solution. Formalin-preserved specimens were transferred to 70% alcohol after one day, for long term storage.

Most worms were whole-mounted or longitudinally dissected, stained with Harris' hematoxylin or borax carmine, dehydrated through an alcohol series, transferred to methyl salicylate and slide mounted in Canada balsam. A few specimens were sagitally sectioned at 7 μm, slide mounted, and stained in hematoxylin and eosin Y.

Drawings of the reproductive system and chaetae were made using a camera lucida. In the descriptions of the male duct and spermatheca, the term *ental* indicates a position that is inner or deep within the body, as opposed to *ectal* for an outer or near-surface position. In the descriptions of chaetae, *proximal* is used to describe a position near the symmetry axis of the body, as opposed to *distal*. Segment numbers are shown in Roman numerals; intersegments are given as Arabic numerals; e.g., "9/10" to represent the intersegment of IX and X. Holotype and paratype specimens are deposited in the U.S. National Museum of Natural History, Smithsonian Institution (USNM), Washington D.C., USA; California Academy of Sciences (CASIZ), San Francisco, California, USA; and Museo Nacional de Ciencias Naturales (MNCN), Madrid, Spain. Non-type specimens are in the authors' collections.

Abbreviations in the figures

a: atrium, aa: atrial ampulla, ad: atrial duct, ae: atrial epithelium, am: atrial muscle layer, b: brain, bv: blood vessel, cc: chloragogen cells, cg: chaetal gland, ch: chaeta, dv: dorsal blood vessel, e: efferent duct of nephridium, ff: female funnel, fp: female pore, g: gut, i: intestine, mp: male pore, o: ovary, oc: oocytes, pg: pharyngeal glands, ph: pharynx, pr: prostate, prj: prostate junction, p: penis, ps: penis cuticular sheath, pt: prostomium, sa: spermathecal ampulla, sd: spermathecal duct, sf: sperm funnel, siv: supraintestinal blood vessel, sp: spermatheca, spp: spermathecal pores, t: testis, vd: vas deferens.

Study area

The new species were collected at five North Carolina locations: one site in the central Piedmont region (UT Pokeberry Creek), two sites in the eastern Coastal Plain (Pettiford Creek and Lake Run) and two sites in the Sandhills (Drowning Creek and Anderson Creek). The Sandhills area is located between the Piedmont and Coastal Plain regions, in the southeastern part of North Carolina. The Coastal Plain and Sandhills sites are humic ("brown-water") systems. Coordinates for sampling sites are given in WGS84.

The unnamed tributary (UT) to Pokeberry Creek was sampled near the town of Pittsboro in Chatham County, N35.8267, W79.1013. This is part of a floodplain complex of seeps and pools that have surface water only during fall, winter and spring months. The clay soils in the Pokeberry Creek catchment produce small streams (seeps) with a limited hyporheic zone and reduced groundwater storage, causing them to go dry during summer months. These shallow seeps are about 0.5 m wide, with a substrate of clay and decomposing leaves. They originate at the base of a steep hill, largely

fed by groundwater, and flow for about 200 m into Pokeberry Creek, within a totally forested area. There were no water chemistry samples from the Pokeberry seeps, but samples from nearby streams suggest pH values close to 7.

Pettiford Creek drains a pocosin area (nutrient-poor, forested or shrub wetland) of the Croatan National Forest in Carteret County. The sampling site (N34.7471, W77.0221) was both upstream and downstream of Forest Service Road 128, also known as Millis Road. Pettiford Creek is about 5 m wide in constricted areas (bridges), but has a much wider braided channel elsewhere (>100 m). The substrate is mostly detritus over a fine sand base. This stream has been frequently used as a reference location by the North Carolina Division of Water Quality. Water pH values from this stream were 3.6 in 2004 and 3.4 in 2010, and conductivity was low (50–85 µS/cm) (NCDENR 2005, 2011). The dominant invertebrates were isopods, amphipods and chironomids, as expected for a swamp stream in this geographic area, but EPT taxa richness was higher than expected for such a low pH stream, with about 10 species per collection (NCDENR 2011). In addition to the new species described here, the lumbriculid fauna in Pettiford Creek is relatively rich, including *Rhynchelmis croatanensis* Fend & Lenat (type locality), *Martinidrilus arenosus* Fend & Lenat, *Altmanella lenati* Fend (type locality), *Eclipidrilus lacustris* (Verrill), *E. breviatriatus* Fend & Lenat, *E. microthecus* Fend & Lenat, and *E.* cf. *fontanus* Wassell (Fend 2009, Fend and Lenat 2007, 2010, 2012).

Lake Run drains Little Singletary Lake in Bladen County; samples were collected at State Road (SR) 1325, N34.7773, W78.6646. This stream was sampled for benthic macroinvertebrates in 1981, as part of a study of naturally acidic streams (Lenat, unpublished). At that time, the pH was found to be consistently less than 3.8, with a substrate of fine sand and clay overlain by leaves and woody debris. Stream width was 2–4 m, with a maximum depth of 1.2 m. Conductivity was low, with a range of 45–56 µS/cm. Lake Run also supported 11 EPT species usually considered to be intolerant (Lenat, unpublished data from 1981). In addition to the *Cookidrilus* species described here, the present collection included the lumbriculids *A. lenati* and *M. arenosus*.

Drowning Creek was sampled at SR 1004 on the Richmond County/Moore County border, N35.0662, W79.5496. A NCDWQ collection in July 2006 recorded a pH of 5.6, and conductivity was 26 µS/cm in the main channel. This site is about 2.5 km upstream of a reach classified as Outstanding Resource Water (NCDENR 2007), which received an Excellent classification based on high EPT taxa richness (29–30) and low NC Biotic Index values (≤ 4.5). However, the collections cited in this paper are limited to floodplain seeps and pools. Seeps were usually less than 2 m wide, although often forming a braided channel; the substrate was fine sand and detritus, sometimes with patches of aquatic plants or filamentous algae. Water quality in the floodplain is assumed to be similar to that of the main channel of Drowning Creek, which supports a diverse hyporheic oligochaete fauna, with 15 lumbriculid species known from a small (about 200 m) stream segment (Lenat and Fend, unpublished). In addition to the new *Cookidrilus* and *Stylodrilus* species described herein, lumbriculids collected from these seasonally inundated habitats included *A. lenati*,

E. cf. *fontanus*, *Martinidrilus carolinensis* Fend & Lenat and an as-yet undescribed athecate species.

Anderson Creek is a small tributary to the Lower Little River at SR 2031 in Harnett County, N35.2661, W78.8192. Based on earlier studies, conductivity is low (49 µS /cm) and pH slightly acidic (5.0–5.9); the stream has a sand-gravel substrate and is classified as "Good" based on a moderate EPT species richness (NCDENR 2004). Lumbriculid collections from this site included a single specimen of the new *Cookidrilus* species, *A. lenati*, *E.* cf. *fontanus*, *M. carolinensis*, and at least three undescribed species.

Results

***Sylphella* gen. n.**
http://zoobank.org/06570C13-DB67-418B-B1CC-EC2815B80DFF

Diagnosis of the genus *Sylphella* gen. n. Simple-pointed chaetae. Two pairs of testes and one pair of ovaries. Ovaries in first segment behind testes. Male pores paired in X and XI, female pores paired in the intersegment 12/13. Male ducts prosoporous. Atrial duct forms a penis within a penial sac, distally covered by a cuticular sheath. Two pairs of spermathecae, beginning in the ovarian segment.

Type species. *Sylphella puccoon* sp. n.

***Sylphella puccoon* sp. n.**
http://zoobank.org/3D6F9393-7C28-4FBF-A362-37A042364047
Figs 1–3

Holotype. USNM 1251692: a dissected worm, stained in Harris' hematoxylin, mounted in Canada balsam (collected 23 Jan 2009).

Paratypes all from the type locality. USNM 1251693-1251698: 7 Jan 2009, 1 whole mount; 23 Jan 2009, 3 dissected; 30 Jan 2009, 2 sectioned (1 sagittal, 1 transverse). MNCN 16.03/3083: 14 Jan 2009, 2 dissected. CASIZ 197898: 23 Jan 2009, 3 dissected.

Type locality. An unnamed, very small tributary (seep) to Pokeberry Creek, Chatham Co., North Carolina, USA.

Etymology. The genus name refers to *Sylph*, the Latin name of an elemental spirit of the air that suggests the Latin *silva*, for woodland, followed by the Latin diminutive *-ella*. The specific name *puccoon* is the Algonquian Indian word which means pokeberry (*Phylolacca* species).

Other material. 7 Jan 2009, 2 whole mounts. 14 Jan 2009, 3 dissected and 3 whole mounts; 11 in alcohol. 23 Jan 2009, 9 dissected and 1 whole mount; 3 in alcohol. 30 Jan 2009, 2 sectioned for histological study. All specimens (including the type series) collected by D.R. Lenat from the type locality.

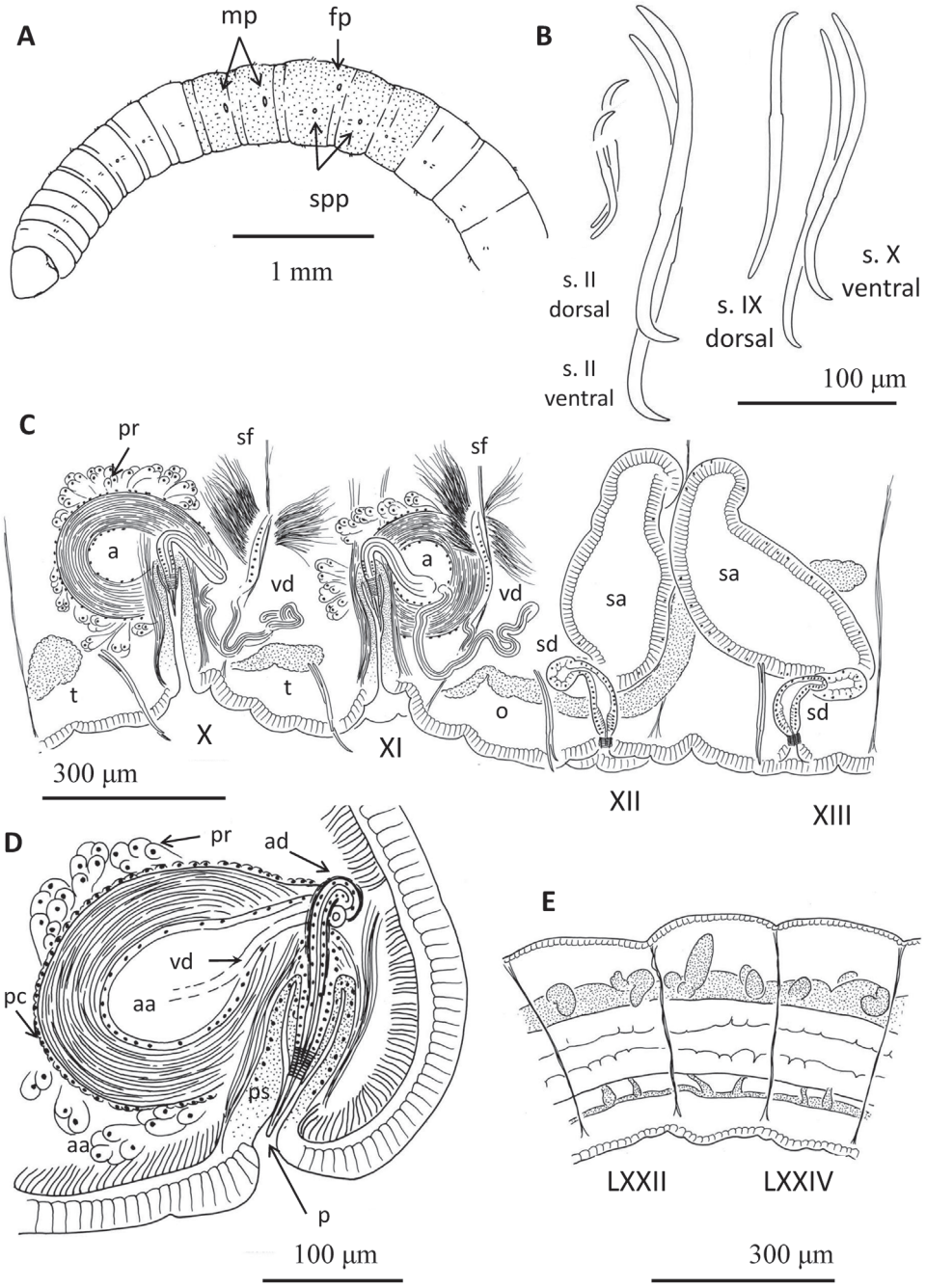

Figure 1. Drawings of *Sylphella puccoon* gen. n., sp. n. **A** Anterior part of the body showing secondary annulations, clitellum and position of genital pores **B** chaetae of segment II and clitellar region **C** schematic drawing of reproductive organs (female funnel obscured by ovary) **D** detail of atrium **E** posterior lateral blood vessels.

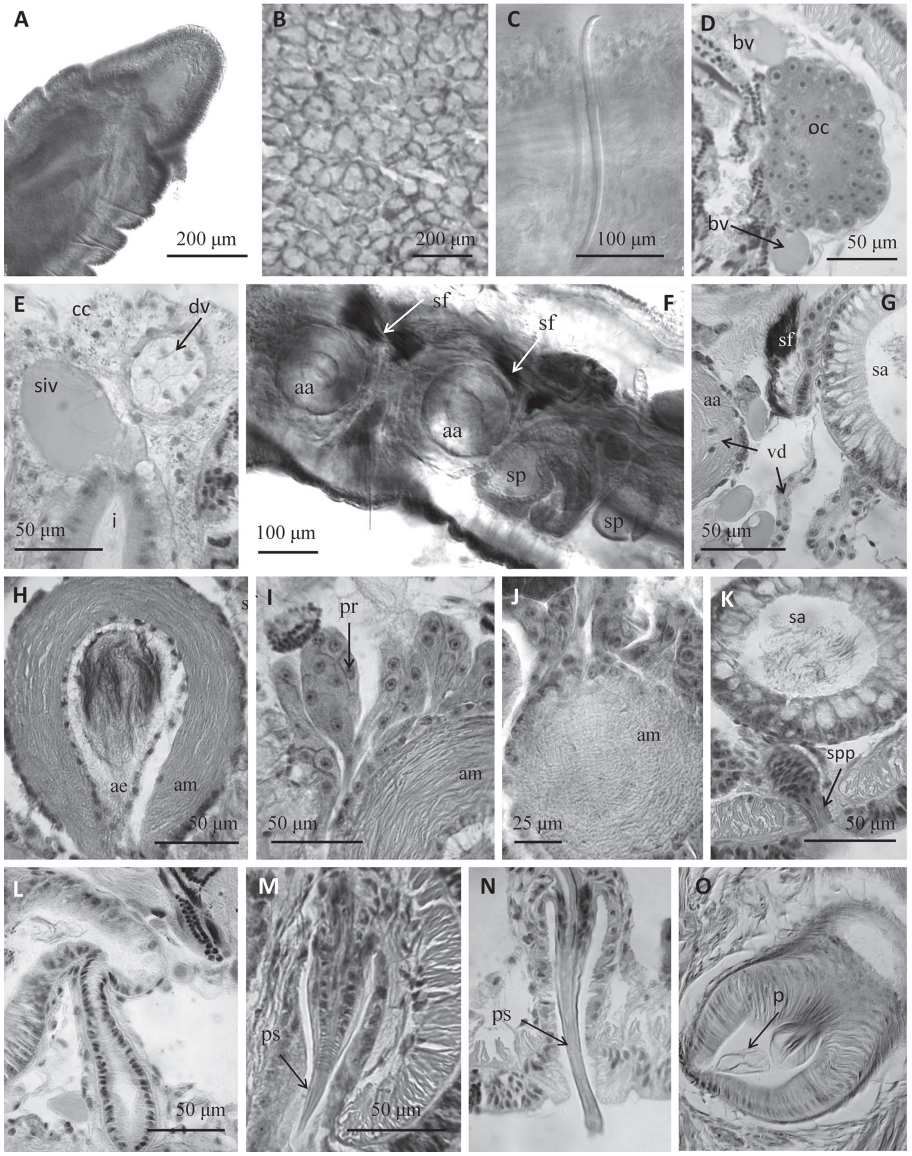

Figure 2. *Sylphella puccoon* gen. n., sp. n. **A** Anterior part of the worm, showing prostomium **B** clitellar epidermis **C** chaeta in XII **D** egg sac containing oocytes and some blood vessels **E** dorsal vessel showing the cardiac cells and supra-intestinal vessel, dorsal to the intestine in segment XVII **F** reproductive segments, showing two atria, with their respective sperm funnels, and spermathecae of an unmated specimen **G** sperm funnel on the septum behind the atrium **H** atrial ampulla with sperm in the lumen, showing the several layers of musculature **I** prostatic cells forming small clusters over the atrial ampulla **J** cross-hatched muscular fibers shown at the surface of the atrial ampulla **K** Spermathecal ampulla with loose sperm in the lumen **L** spermathecal duct **M** penis within the penial sac, with conical penial sheath. For comparison **N** penis with tubular cuticular sheath in *Styloscolex japonicus*, and **O** penis with a soft cuticular layer in *Lumbriculus japonicus*. **D, E, G–O** histological sections of reproductive organs, other photographs from stained whole mounts or dissected specimens.

Table 1. Length (μm) of chaetae in *Sylphella puccoon* gen. n., sp. n. (measurements on one whole-mounted specimen from Pokeberry Cr., North Carolina, USA 14 Jan 2009).

Segment	II	III	IV	V	VI	VII	VIII	IX	X	Posterior
Dorsal	63	–	88	90	103	94	118	120	120	99–121
Ventral	167	141	140	141	162	154	140	141	132	95–124
Ventral/dorsal length	2.6	–	1.6	1.6	1.6	1.6	1.2	1.2	1.1	1.0

Description (based on mated specimens). Number of segments 65–83. Length of fixed worms 15–25 mm. Diameter of the body from 14 unmounted worms in lateral aspect (measured to 0.01 mm): 0.44–0.66 mm in VIII (mean 0.51 mm), 0.45–0.68 mm at clitellum (mean 0.54 mm), and 0.50–0.76 mm (mean 0.61 mm) at mid-body.

Prostomium rounded-conical, 270–400 μm long, width about the same as length. Secondary annulation (narrow ring in anterior part of segment) from segment V; present but weak in post-clitellar segments (Fig. 1A). Epidermis 10–15 μm high in anterior segments. Clitellum annular, from segment X to XIV, with epithelium up to 25–35 μm high, formed by unordered, glandular cells (Fig. 2B). Longitudinal muscles up to 32 μm thick in anterior segments. Chaetae sigmoid, simple-pointed with strongly curved distal tip; ventral chaetae larger than corresponding dorsal chaetae in anterior segments (Table 1). Ventrals largest in II to about XIII (126–204 μm long, up to 7 μm thick), anterior dorsals distinctly smaller and thinner, (60–130 μm long, 4 μm thick) (Fig. 1B); maximum ventral chaeta length about 1.6 that of dorsals in preclitellar segments. Ventral chaetae only slightly larger than dorsals in post-citellar segments. Nodulus at about 0.32–0.46 (mean = 0.40) from the distal end.

Transverse, oval male pores are in line of ventral chaetae of segment X and XI, about midway between chaetae and posterior septum (Fig. 1A). Female pores open just below the lateral line, in intersegment 12/13. Inconspicuous, round spermathecal pores open behind, and in line with the ventral chaetae in XII–XIII.

Pharynx developed mainly dorsally and laterally, in segments II and III. Pharyngeal glands well developed dorsally and ventrally in IV–VI, usually extending ventrally into VII. Chloragogenous tissue well developed from VII backwards. A supra-intestinal vessel may appear differentiated from the perivisceral sinus (Fig. 2E) beginning in XIV; this is not evident after the dorsal vessel joins the gut in about XX. One pair of simple commissural blood vessels join dorsal and ventral vessels in anterior segments to about XV; those in XII may loop into the egg sacs (Fig. 2D). Lateral blood vessels absent from posterior segments except for 1–2 very short lobes on dorsal vessel in about the posterior 1/4 of the body (Fig. 1E). Nephridia usually paired in VII and VIII, and paired, single, or absent in segments posterior to XIII; efferent ducts simple, mostly limited to ventral half of body, without vesicles at nephridiopores. Sperm sacs extend anteriorly to VIII or IX, and backwards as far as XXII. Egg sacs may extend to 2 or 3 segments beyond sperm sac; when eggs have partially completed vitellogenesis, egg sacs shorter, not extending beyond sperm sacs, to XIII or XIV.

Two pairs small testes, in segments X and XI; one pair elongate ovaries in XII, extending through XIII. Female funnels large, attached to the septum and opening in intersegment 12/13. Two pairs spermathecae, the first in the post-atrial segment (typically XII), and the second in the post-ovarian segment (typically XIII) (Figs 1C, 2F).

A single vas deferens per atrium (prosoporous condition), sperm funnels located on the septa of intersegments 10/11 and 11/12 (posterior septa of atrial segments), but folded back into the next segment. Vasa deferentia long (about 700 μm), penetrating the posterior septa, and forming a long, convoluted loop within each post-atrial segment (Fig. 1C). Vasa deferentia narrow (12–16 μm diameter) and transparent, each joining the atrium at the ectal (or basal) part of the ampulla (adjacent to the atrial duct), and running under the atrial musculature to about the middle part of the ampulla, where it opens into the atrial lumen (Fig. 1C, D). Atria petiolate, extending medially from male pore, with nearly spherical ampulla (140–210 μm diameter, slightly longer than wide) and tubular ectal duct (Fig. 1D). Ampullar musculature very thick (40–50 μm), organized in many intercrossing layers (Fig. 2H–J). Atrial ampulla with very thin epithelium, and covered by a thin (up to 5 μm) layer of cells and prostate glands formed by elongate-petiolate clusters of cells; each gland is pedunculate with a narrow extension penetrating the atrial musculature (Fig. 2I). Atrial duct tubular (17–24 μm diameter, 90–110 μm long), composed of an epithelium surrounded by loose, indistinct musculature, extending into a type-1 penis (Fig. 17, in Rodriguez and Giani 1994) within a deep penial sac (120–230 μm deep) (Figs 1C, D, 2M), and associated with retractor muscles extending dorso-laterally to the body wall. Penis length 90–110 μm; the broad, ental part forms a distinct epithelial tube which disappears ectally; the middle portion is surrounded by a ring of what appears to be circular musculature; and the ectal part is sharply acuminate, covered by a thin (ca.1 μm), cuticular layer.

The spermathecae have a narrow duct and an irregular, sacciform ampulla. Spermathecal duct fusiform, (30–45 μm maximum diameter), formed by columnar epithelium, a thin (about 2 μm) muscle layer, and with a wide lumen except at the pore; ental end of the duct prolonged into a narrow neck (12–20 μm diameter) which joins the ampulla (Figs 1C, 2L). Duct sharply narrowed at the pore, with a short sphincter surrounded by a circular muscle layer (Figs 1C, 2K). Ampulla in two parts, a short ectal section (60–90 μm long by 35–46 μm wide), lined with irregular epithelium, and a much larger ental part (320–480 μm by 130–250 μm), which is lined by columnar, vacuolated epithelium, up to 35 μm thick (Fig. 2G, K). Sperm within the spermathecae loose and unordered; epithelial vacuoles not obviously containing resorbed sperm. All spermathecae similar in size; ampullae of mated worms may extend into adjacent segments.

Anomalies. Two specimens had the entire sequence of reproductive organs in segments VII–X, with the clitellum in VII–XI instead of the usual X–XIV; apparently an anterior shift of three segments. These aberrant worms appeared normal in other respects, except that nephridia were not present in VII and VIII.

Taxonomic remarks. The combination of multiple atrial segments, prosopore male ducts in the testicular segments (GI and GII, see Brinkhurst 1991), and postatrial spermathecae in *Sylphella puccoon* gen. n., sp. n. is shared with the monotypic genera *Lamprortus* Rodriguez, 1994 (in Brinkhurst et al. 1994) and *Wsewolodus* Semernoy,

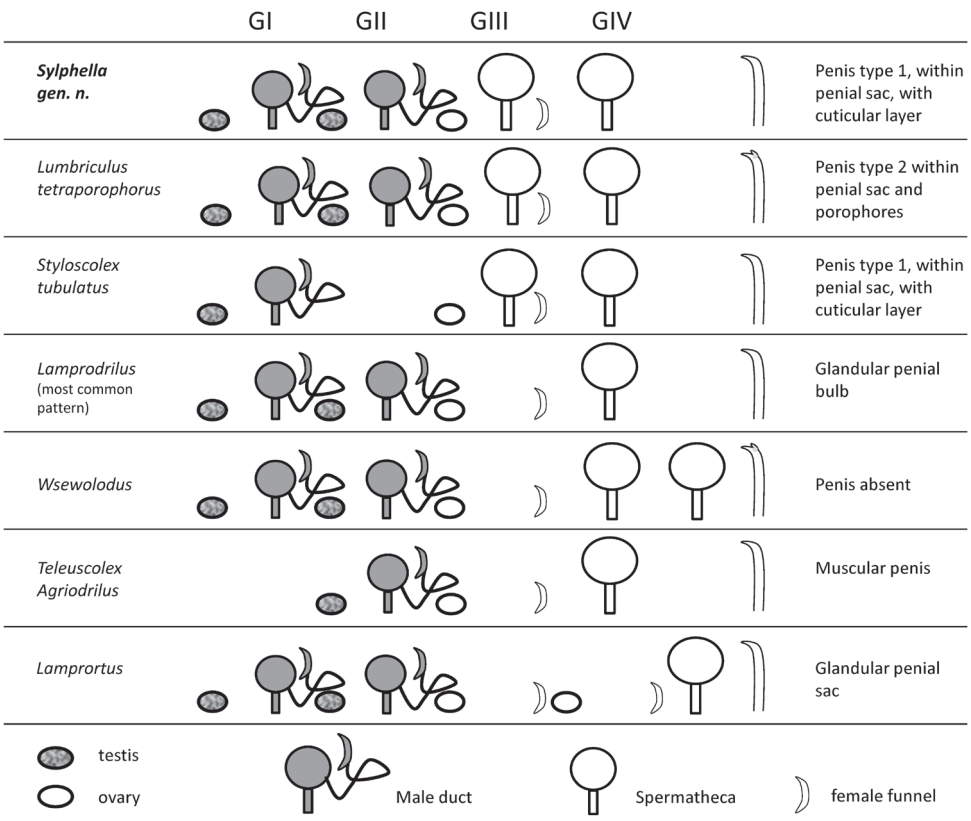

Figure 3. Comparative schema of the reproductive system and chaetae in the new genus *Sylphella* and other related prosoporous lumbriculid genera. Type-1 and type-2 penes as described by Rodriguez and Giani (1987) (see text).

2004 (Fig. 3). Additionally, this arrangement of reproductive organs occurs in some species (or variants) of *Lumbriculus* Grube, 1844 and *Lamprodrilus* Michaelsen, 1901 (*Teleuscolex* and *Agriodrilus* included). *Lamprortus* is well distinguished from other lumbriculids by its hologyny, i.e., by the possession of 2 pairs of testes and 2 pairs of ovaries. *Lamprortus* and most *Lamprodrilus* species have only one spermathecal segment, although variants of *L. mrazeki* Hrabě, 1928 and *L. satyriscus* Michaelsen, 1901 may have two or more pairs of spermathecae. Almost all lumbriculids with two atrial segments differ from *Sylphella* gen. n. in having one intervening segment between the last atrial segment and the first spermathecal segment (Fig. 3). Thus, relative to the gonads, the first spermathecal segment is in the first post-ovarian segment (GIV in *Lamprodrilus* and *Wsewolodus*, and behind GIV in *Lamprortus*). In *Sylphella*, the first spermathecae are in the ovarian segment (GIII). The genus *Lumbriculus* is highly variable not only in number but also in the position of the spermathecae, but usually more than two pairs open laterally, either at the dorsal or ventral side of the body. The closest match to *Sylphella* is *L. tetraporophorus* Popchenko, 1976a, but that species is

Table 2. Taxonomic characters of the reproductive system that distinguish *Lumbriculus tetraporophorus* Popchenko, 1976 from *Sylphella puccoon* gen. n., sp. n.

Characters	*Lumbriculus tetraporophorus*	*Sylphella puccoon*
Male porophores	2 pairs, prominent, conical (210 μm high), formed by concentric muscle ridges	Absent
Atrial ampulla	Pear-shaped (250×510 μm)	Nearly spherical (140–210 μm Ø)
Atrial duct	170 μm long, wider in the middle	Cylindrical (90–110 μm long). Penial sac 120–130 μm
Atrial musculature	48–51 μm, in 2 orthogonal layers, circular muscle 34 μm thick	40–50 μm intercrossing fibers in many indistinct layers
Penis	140–170 μm long, with tapered end (probably typical *Lumbriculus* type of extrudable lining cells in the atrial duct)	90–110 μm long, tapered end of atrial duct sharply acuminate, ectally covered by cuticle
Prostatic cells	Diffuse (*rykhlym*)	In petiolate clusters of cells
Vas deferens	Prosoporous, 18–20 μm Ø, joining entally, barely penetrating next segment	Prosoporous, (12–16 μm Ø, joining ectally, forming a loop in next segment
Female pores	ventral	lateral

distinguished from *Sylphella* by typical *Lumbriculus* characters, including bifid chaetae, a plexus of anterior commissural blood vessels, and *Lumbriculus*-type male reproductive organs, with a pyriform atrium and penial sac ending in a porophore (see Table 2).

The tetrathecate condition, with paired spermathecae in the first two postatrial segments, is a feature shared with some species in the semiprosoporous lumbriculid genera *Trichodrilus* Claparède, 1862 and *Eremidrilus* Fend & Rodriguez, 2003. However, the presence of two pairs of prosoporous atria in *Sylphella* suggests that a close phylogenetic relationship with these genera is improbable.

The general form of the atria bears a slight resemblance to some Palearctic species of the genus *Trichodrilus* having petiolate atria, spherical and very muscular atrial ampullae, and two pairs of spermathecae (e.g., *T. aporophorus* Popchenko, 1976b, *T. claparedei* Hrabě, 1937). *Bichaeta sanguinea* Bretscher, 1990 also has a spherical and very muscular atrium, but lacks an atrial duct. In contrast, genera resembling *Sylphella* in the arrangement of reproductive organs (*Lamprodrilus*, *Lamprortus*, *Wsewolodus* and *Lumbriculus*) tend to have elongate atria.

The atrial musculature in *Sylphella* consists of many small, cross-hatched layers, similar to some other lumbriculids, such as *Trichodrilus longipenis* Giani & Rodriguez, 1994. Details of atrial musculature are usually not given in lumbriculid diagnoses, but where described, the atrial muscle fibers show a simpler organization (parallel or two opposing layers) in the related genera. The *Sylphella* arrangement of atrial muscles should be distinguished from the simple crossed musculature in *Lumbriculus* species, which consists of only two perpendicular layers; however, it bears some resemblance to the many diagonally arranged layers in some *Eclipidrilus* Eisen species (Fend 2005).

The penis in *Sylphella puccoon* is similar to that described for *Styloscolex japonicus* Yamaguchi, 1937 in its basic structure, as well as in the presence of a smooth, rigid

cuticular layer (sheath) on the ectal end (Fig. 2M, N). *Styloscolex* Michaelsen, 1901 has an intervening segment between the testicular and the ovarian segments, an autapormorphy that separates this genus from other lumbriculids. Several other *Styloscolex* characters, including pre-atrial spermathecae in most species, elongate atria in a single segment, and a forward shift in reproductive organs (Brinkhurst 1989) suggest that *Styloscolex* is probably not closely related to *Sylphella*.

Lamprortus and most *Lamprodrilus* species also have a type-1 penis (i.e., an extension of the atrial duct within a fold of the ventral body wall, see Rodriguez and Giani 1994), but these usually have a characteristic structure, being associated with a large mass of glands. Some *Lamprodrilus* species also have muscular penial bulbs. *Lumbriculus* species have a type-2 penis (i.e., formed in part by elongation of atrial lining cells) within a penial sac formed by very thick, columnar epithelium (see Hesse 1902 for *L. variegatus*). Penes are absent in *Wsewolodus*.

Enlarged ventral chaetae in anterior segments occur to some degree in many lumbriculids, but the difference is well marked in several *Trichodrilus* species (see Rodriguez and Giani 1994), *Lamprodrilus inflatus* Michaelsen, 1905, and *Stylodrilus mirus* (Chekanovskaya, 1956).

Ecological remarks. *Sylphella puccoon* gen. n., sp. n. has only been collected during winter months from a single, small seep that is a tributary of Pokeberry Creek, North Carolina. A large number of similar seeps were investigated by one of the authors (D. Lenat) adjacent to Pokeberry Creek, but *Sylphella* was limited to a 10-m reach of the largest seep (1 meter wide). The small streams in this area go completely dry during summer months, due a combination of clay soils and seasonal rainfall patterns. The dominant macroinvertebrates in these seeps were the isopod *Caecidotea forbesi* (William), the amphipod *Crangonyx* sp. Bate, and chironomids. The mayfly genera *Callibaetis* Eaton and *Leptophlebia* Westwood can be abundant, but other EPT taxa were sparse. Other oligochaetes at this site include *Rhynchelmis bolinensis* Fend & Lenat (the type locality), *Eclipidrilus* cf. *fontanus*, *Rhyacodrilus propiporus* Rodriguez & Fend, and an undescribed lumbriculid of unknown generic attribution.

Cookidrilus pocosinus sp. n.

http://zoobank.org/D72DE213-2FCC-4B0C-A696-FEED022939D0
Figs 4 and 5

Holotype. USNM 1251699: a dissected specimen, stained in Harris' hematoxylin and mounted in Canada balsam (collected 4 March 2011).

Paratypes. USNM 1251700-1251702: from the type locality, 22 Feb 2011, 1 dissected; 4 Mar 2011, 1 whole-mounted; Pettiford Creek, at Millis Road, Carteret County, North Carolina, USA, 15 Mar 2007, 1 whole mount. MNCN 16.03/3084: from the type locality, 22 February 2011, 1 dissected, stained in Harris' hematoxylin and mounted in Canada balsam, and 1 histologically sectioned, stained with hematoxylin-eosin. CASIZ 197899: Pettiford Creek, 15 Mar 2007, 1 whole mount.

Type locality. Lake Run, outlet stream draining Little Singletary Lake at SR 1325, in Bladen County, North Carolina, USA.

Etymology. The specific name refers to *pocosin*, "swamp-on-a-hill" in the Algonquin Indian language. Most specimens were collected in two sites draining pocosin areas.

Other material. From the type locality, 22 Feb 2011, 4 whole mounts, 1 dissected, 1 sagittally sectioned. Pettiford Creek, at Millis Road, Carteret County, North Carolina, USA, 22 Apr 2008, 1 whole mount; 5 Apr 2010, 2 dissected. Drowning Creek at State Road 1004, Moore County, North Carolina, 12 Jan 2009, 1 whole mount. Anderson Creek at SR 2031, Harnett County, North Carolina, 27 Jun 2011, 1 whole mount. All specimens (including the type series) collected by D.R. Lenat.

Description. Number of segments 53–71. Diameter of the body 279–342 μm in segment VIII and 360–441 μm at the clitellum. Prostomium round, 120–154 μm long. Brain back to intersegment 2/3. Secondary annulation (narrow ring in anterior part of segment) well marked from segment VI to IX, not always visible in the postclitellar region, but evident in the caudal region of the body (Figs 4A, 5B). Epidermis in anterior segments 10–16 μm high. Clitellum from segment X to XII, with epithelium 16–34 μm high, formed by small glandular cells arranged in regular transverse rows (Fig. 5E). Chaetae sigmoid, simple-pointed (Fig. 5C), length about equal in dorsal and ventral bundles, shortest in segment II (56–62 μm), progressively longer to the middle of the body (68–82 μm long), and gradually shorter to the end of the body (down to 66 μm). A chaetal gland behind chaetal bundles, conspicuous in anterior segments, smaller posteriorly (Figs 4B, 5D). Nodulus at about 0.3–0.4 from the distal end. Pygidium normally formed (Fig. 5B). Male pores located behind and in line of ventral chaetae of segment X (Fig. 5F). Female pores open in the line of ventral chaetae, in intersegment 11/12. Spermathecal pores opening midway between ventral chaetae and anterior septum, in line with the ventral chaetae, in atrial and 2 postatrial segments. In most sexually mature, fixed individuals, the ventral region of clitellar region is concave with prominent lateral margins (saddle shaped clitellum).

Pharynx developed mainly dorsally, in segments II and III. Pharyngeal glands in last part of IV, and well developed in V and VI, dorsally and laterally. Chloragogenous tissue starting in the hind part of VI and well developed from VII backwards. Nephridia present on at least one side in VII in some specimens; most specimens have at least one nephridium in XIII, and in a few posterior segments. Nephridiopores inconspicuous, without vesicles; nephridial duct very thin and transparent. Sperm sac extends forward to VIII, and backward to XII–XV. Egg sac back to XIII–XVII.

Two pairs testes, in segments IX and X, and one pair ovaries in segment XI. Two vasa deferentia per atrium (semiprosoporous condition), originating in sperm funnels located in the septa of intersegment 9/10 and 10/11, respectively. Posterior vas deferens not entering segment XI. Vasa deferentia very narrow (8–14 μm diameter), joining the atrium in the ectal (or basal) part of the ampulla, and running through the atrial musculature to the apical part of the atrium, where they open to the atrial lumen (Figs 4B, C, 5H, J). Atrium tubular, with elongated ampulla

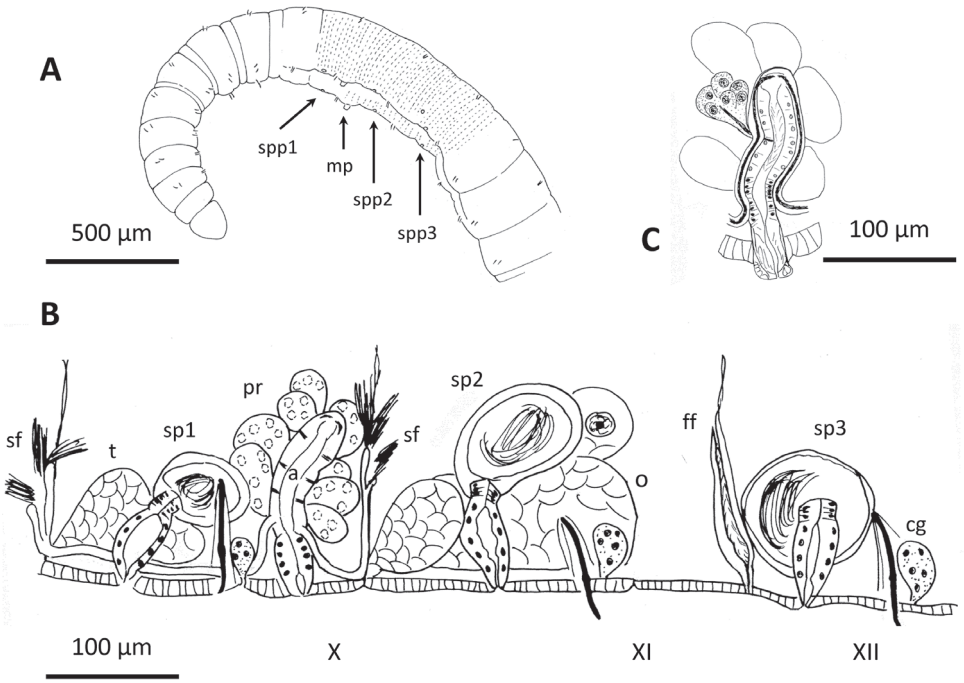

Figure 4. Drawings of *Cookidrilus pocosinus* sp. n. **A** anterior body region **B** reproductive organs **C** detail of the atrium showing the vasa deferentia junction and prostatic cell clusters.

(86–120 µm long, 26–36 µm diameter) and short duct (34–40 µm long, 24–26 µm diameter). Atrial muscle layer thin, 2–3 µm thick. The columnar inner epithelium of the atrial duct can extend beyond the male pore forming a short, protrusible penis (less than 40 µm long, when protruded) formed by the extension of lining cells of the atrial duct (Figs 4C, 5G, K), extended cells may appear vacuolated. Atrial ampulla with ciliated lumen, and covered by 8–10 well-separated prostate glands formed by clusters of cells, each of which tapers to form a narrow stalk before joining the ampulla (Figs 4C, 5I, J).

Female funnels large, attached to the septum and opening in intersegment 11/12 (Fig. 4B). Three pairs of spermathecae, the first in the atrial segment (X), and the next in the ovarian (XI) and post ovarian (XII) segments. The spermathecae are formed by a bottle-shaped duct (34–86 µm long, 22–38 µm maximum diameter) and an oval ampulla (54–120 µm maximum diameter and 34–90 µm minimum diameter), filled by loose sperm (Figs 4B, 5L). In several of the examined specimens, the ampullar epithelium appeared very much vacuolized, with some vacuoles containing resorbed sperm.

Taxonomic remarks. *Cookidrilus pocosinus* sp. n. has been ascribed to the genus *Cookidrilus* Rodriguez & Giani, 1987 based on the main diagnostic characters of the genus: 2 pair testes and one pair ovaries, two (anterior and posterior) vasa deferentia joining each atrium, one pair spermathecae in the atrial segment, and subsequent pairs

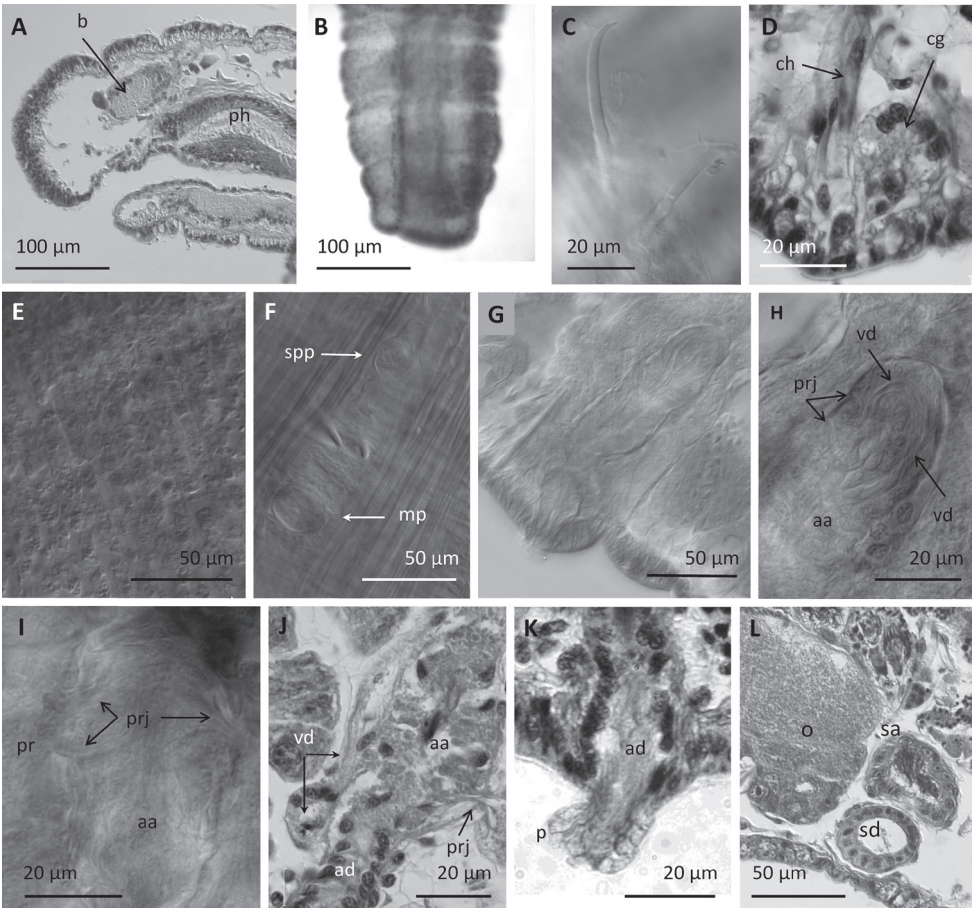

Figure 5. *Cookidrilus pocosinus* sp. n. **A** Anterior region of the body **B** caudal region with pygidium **C** chaetae **D** chaetal gland behind ventral bundle of chaetae **E** clitellum **F** spermathecal and male pores in front of and behind ventral chaetae of segment X **G** atrium **H** atrial ampulla showing apical junction of vasa deferentia **I** atrial ampulla showing junction of prostatic cell clusters **J** basal junction of vas deferens to atrial ampulla **K** detail of atrial duct and protruded penis **L** third spermatheca behind the female segment. **A, D, J, K, L** histological sections, other photographs from stained whole mounts or dissected specimens.

of spermathecae in postatrial segments (Rodriguez and Giani 1987). The groundwater lumbriculid genus *Cookidrilus* was originally described from the Labouiche Cave in southern France. Since then, another species has been described from the hyporheos of Lachein Creek, a karstic stream in the same geographic region (Route et al. 2004). In the present study, a third species is described from coastal plain habitats of North Carolina (USA), mostly from acidic swamp streams.

In Table 3, we have summarised the main morphological features that distinguish the three species of the genus *Cookidrilus*. The new species is closer to the type species of the genus, *C. speluncaeus* Rodriguez & Giani, 1987, based on the presence of three pairs of spermathecae instead of only two pairs in *C. ruffoi* Giani et al., 2004 (in Route et al. 2004).

Table 3. Morphological characters of the three known species of the genus *Cookidrilus* Rodriguez & Giani, 1987.

Cookidrilus	***speluncaeus***	***ruffoi***	***pocosinus* sp. n.**
Body diameter	350 µm at clitellum	583–633 µm	360–441 µm at clitellum
Double annulation begins	In III	–	In VI
Prostomium form and length	Round, 76–83 µm	Often wrinkled, 305–400 µm	Round, 120–154 µm
Clitellum	X–XII	Poorly developed, in X–XII	X–XII
Pharynx	Dorsal and ventrally well developed, in II–IV	Dorsal and ventrally well developed, back to VII, VIII	Typical dorsal pad, in II–III
Pharyngeal glands	IV–VIII	III–VII (VIII)	IV(posterior)–VI
Chaetae, length	73–82 µm in ante-clitellar region	105–112 µm in II, 174–236 µm in anterior to middle region	56–62 µm in II, 68–82 µm in anterior to middle region
Posterior body region	Not modified	Evaginable tube	Not modified
Spermathecae, number and position of pores	3 pairs, pores behind ventral chaetae	2 pairs, pores behind ventral chaetae	3 pairs, pores in front of ventral chaetae
Spermathecal ducts, form and length	Tubular, short ducts 44–76 µm	Bottle shaped, 115–143 µm	Bottle shaped, short ducts, 34–86 µm
Spermathecal ampulla	The first is small, the third penetrates XIII	Do not penetrate other segments	The first smaller. Do not penetrate other segments
Atrium	In X	In X	In X
Atrial ampulla, shape and size	Pyriform, 71 µm long, 48 µm Ø	Pyriform, 207 µm long, 161 µm Ø	Tubular, 86–110 µm long, 26–30 µm Ø
Atrial duct length	29 µm	84 µm	34–40 µm
Penis	Simple pore	Protrusible penis	Protrusible penis
Prostate layer	Dense, diffuse layer	3–4 clusters of cells	8–10 clusters of cells
Atrial muscular layer	4 µm thick	16–20 µm thick	2–3 µm thick
Vas deferens diameter/ junction to the atrium	11 µm/ apical	15–20 µm/ lateral	8–14 µm/ at the base of the ampulla (opening apically)
Posterior vas deferens	Penetrates 10/11	Penetrates 10/11	Does not penetrate 10/11

However, it resembles *C. ruffoi* in the structure of the prostatic cell layer, which is organised in well-separated clusters that join the atrial ampulla by distinct stalks. The new Nearctic species *C. pocosinus* is distinguished from both European species by the singular position of the spermathecal pores in front of the ventral chaetae, instead of behind the chaetae (the most common position in lumbriculids). The genus *Cookidrilus*, previously amended by Route et al. (2004), is now further amended to include some additional diagnostic features.

In the original description of the genus, Rodriguez and Giani (1987) discussed the taxonomic relationships of *Cookidrilus* with other lumbriculids (*Kincaidiana* Altman, 1936 and *Guestphalinus* Michaelsen, 1933) having a pair of spermathecae in the atrial segment. *Kincaidiana hexatheca* Altman, 1936 is endemic to North America where there are also representatives of the genus *Guestphalinus* (S. Fend, unpublished data). However, although the former has a similar arrangement of spermathecae to *C. speluncaeus*, a combination of characters clearly distinguishes it from *Cookidrilus*: a proboscis, a forward shift of reproductive organs, a single pair of testes, and one prosoporous vas deferens per atrium (Fend 2009). In addition, morphology of the atria, spermathecae, and chaetae does not

resemble that of the known *Cookidrilus* species. *Guestphalinus* is semiprosoporous, but has only one pair of spermathecae, and like *Kincaidiana*, has a proboscis. *Guestphalinus* also has a forward shift in reproductive organs relative to the position in *Cookidrilus*.

The presence of penis may be a common generic character in *Cookidrilus*, since it is only absent in the type species, *C. speluncaeus*. On the other hand, *C. ruffoi* differs in the number of spermathecae. The analysis of lumbriculid genera performed by Brinkhurst (1989) stated that characters related to number and placement of the spermathecae (characters 7 and 9 in that analysis) were subject to changes/reversals in the resulting phylogenetic tree, and such variations are probably not highly significant. Thus, variation in number of spermathecal segments within *Cookidrilus* (3 in two species, versus 2 in *C. ruffoi*) is not extraordinary, as similar variation occurs in other lumbriculid genera such as *Trichodrilus* and *Rhynchelmis* Hoffmeister, 1843 (see Cook 1971).

The position of spermathecal pores in front of the ventral chaetae is an unusual feature of the new *Cookidrilus* species. Spermathecal pores in lumbriculids are usually placed behind the chaetae of the corresponding segment, and in the other 2 species of *Cookidrilus*, even the first spermatheca opens in the narrow space between the ventral chaetae and the male pores. This character is shared with several Nearctic lumbriculids: *K. hexatheca* (for the first pair of spermathecae), some *Rhynchelmis* species (in Fend and Brinkhurst 2000), and *Eclipidrilus pacificus* Fend, 2005.

Ecological remarks. *Cookidrilus pocosinus* sp. n. appears to have a life cycle adapted to seasonal drying of surface flow. This is the first record of *Cookidrilus* in North America, and it is also the first report of the genus in a non-subterranean habitat. This species has been found so far in four North Carolina streams, but almost all specimens were from Lake Run and Pettiford Creek. These streams are both located in the southern Coastal Plain, in relatively undisturbed watersheds, and drain pocosin areas with peat soils. Both streams have extremely low pH values (often less than 4.0), very low conductivity, and dry up completely during summer droughts. In Lake Run, most specimens were found in shaded sections, in midstream areas with both good flow and a fine sand substrate. In Pettiford Creek, the substrate consisted of fine sand covered by a layer of organic debris. These data indicate that *C. pocosinus* is usually associated with very low pH, although single specimens were collected from a seasonally inundated side channel of Drowning Creek, and from the main channel of Anderson Creek. Both of those streams have average pH values near 5.5.

Diagnosis of the genus (amended by Route et al. 2004, and modified here with additions in italics): **Type species.** *Cookidrilus speluncaeus* Rodriguez & Giani, 1987.

Chaetae sigmoid, simple-pointed. One pair male pores in segment X (*the second testis-bearing segment*), behind and in line with the ventral chaetae. *Two or three pairs spermathecae; first pair in the atrial segment, anterior to male pores, and* one pair *in the first, or in the first and second postatrial segments*. Two pair testes, in IX and X. *One pair atria in the second testicular segment. Semiprosoporous male duct, two vasa deferentia per atrium. Prostatic cells either in a simple diffuse layer or forming discrete clusters*. One pair ovaries located in the first postatrial segment (XI).

***Stylodrilus coreyi* sp. n.**
http://zoobank.org/361604D8-0420-44CE-8F91-BFBB133A1510
Figs 6 and 7

Holotype. USNM 1251703: A whole-mounted specimen in Canada balsam (collected 19 Jan 2010).

Paratypes. USNM 1251704-1251707: 17 Feb 2007, 1 whole mount; 19 Jan 2010, 2 whole mounts; 5 Apr 2010, 1 dissected. MNCN 16.03/3085: 19 Jan 2010, 1 dissected and 1 histologically sectioned; 5 Apr 2010, 1 whole mount, stained in borax carmine. CASIZ 197900: 16 Feb 2011, 2 dissected. All from the type locality.

Type locality. Pettiford Creek at Millis Road, Carteret County, North Carolina, USA.

Etymology. This species is named in honor of Jesse Edward (Ed) Corey III, an Inventory Biologist at the North Carolina Division of Parks and Recreation. We celebrate Ed's unwavering interest in all animals and plants, including our beloved oligochaete worms.

Other material. From the type locality: 17 Feb 2007, 1 dissected. 30 Sep 2009, 7 whole mounts, 1 dissected. 19 Jan 2010, 5 whole mounts, 2 dissected, 3 sectioned (2 sagittal, 1 transverse), 2 in alcohol. 5 Apr 2010, 6 whole mounts, 2 dissected. 16 Feb 2011, 1 whole mount, 10 in alcohol. Floodplain seeps in Drowning Creek floodplain at State Road 1004, Moore County, North Carolina: 31 Dec 2008, 2 whole mounts. 12 Jan 2009, 2 whole mounts, 5 dissected. 17 Feb 2011, 1 whole mount. All specimens (including the type series) collected by D.R. Lenat.

Description. Number of segments 53–69. In 27 unmounted specimens, body length 11.7–14.2 mm, diameter of the body in segment VIII, 240–585 μm (mean 379 μm); maximum diameter in the clitellar region to 760 μm (mean 467 μm); midbody diameter 330–630 μm (mean 429 μm). Prostomium round or conical, 142–196 μm long (Figs 6A, 7A). Brain deeply lobed, back to septum 2/3. Clitellum saddle-shaped, formed by cells in distinct rows (Fig. 7C), extending from the anterior part of segment X (from the level of chaetae) to the end of segment XII. Epidermis 6–17 μm high in anterior segments, and up to 23–34 μm in the clitellum; 25–32 μm high in the prostomium. Secondary annulation (a narrow ring in anterior part of segment) usually in IV to IX.

Chaetae simple-pointed (Fig. 7B), nodulus at 0.3 (rarely at 0.4) from the ectal end, of similar size in dorsal and ventral bundles or slightly longer in ventral bundles; smaller in segment II (63–70 μm), length increasing in the anterior segments to segment VIII (73–116 μm), and usually smaller in the posterior part of the body (71–111 μm, down to 63 μm).

One pair spermathecal pores in segment IX and one pair male pores in segment X, in line with and behind the ventral bundles of chaetae (one specimen from Drowning Creek regenerating the anterior part of the body, with spermathecal pores in VII and male pores in VIII). One pair female pores in the intersegment 11/12.

Pharyngeal pad well-developed dorsally, usually extending through IV; pharyngeal glands from the posterior part of segment IV back to VIII, dorso-lateral and ventral to

the gut in segments V to VIII (Fig. 6B). Chloragogen cells covering the gut from the posterior part of segment VI onwards. Nephridia with long efferent ducts observed in segment VII and in some postclitellar segments, tubular shaped, running ventrally through several segments (Fig. 7D); nephridiopores without vesicles, in front of ventral chaetae. Lateral blood vessels absent in posterior segments. Two pairs testes, in anterior part of segments IX and X, and one pair ovaries in segment XI. Sperm sacs back to segment XV or XVI (never observed extending forward), and egg sacs back to XVI or XVII.

Semiprosoporous male ducts, with one anterior vas deferens attached to the sperm funnel in intersegment 9/10, and the posterior one to the funnel in 10/11, the anterior being longer (280–480 μm) than the posterior (215–300 μm). Both funnels appear deflected backward, somewhat behind their respective septa when full of sperm. Vasa deferentia (15)20–28 μm in diameter, to 34 μm close to the sperm funnel. Posterior vas deferens does not enter postatrial segment (Figs 6D, 7G). Atrium elongate (176–390 μm total length, including the penis), with the ampulla (120–184 μm long, 43–70 μm maximum diameter) usually restricted to segment X, but in some individuals passing into segment XI. Several discrete clusters of prostatic cells (44–100 μm high) join the atrium by distinct stalks that traverse the atrial musculature (Fig. 7E–I). Atrium length 0.45–0.70 (usually c. 0.50) times the body diameter at the clitellum. Short atrial duct not distinctly separated from the ampulla, narrowing to about 24 μm wide, the male pore on a short penis (25 μm long), in a shallow fold of the body wall. Several dorso-ventral muscular strands are associated with the male pore. Atrial epithelium very granulated, 14–19 μm high, and atrial lumen ciliated; atrial musculature thin (4–6 μm thick). Vasa deferentia join the atrium at about the basal one third, and run under the atrial musculature to the most apical part of the ampulla, where they open to the atrial lumen (Figs 6C, 7G).

One pair spermathecae, with ampullae typically located in segments IX and X, oval to nearly spherical (174–331 μm diameter, 205–348 μm long), containing a mass of loose sperm in the ectal part, sometimes together with amorphous material (Figs 6D, 7J). Ampulla with thin epithelium in ectal part (about 5–10 μm); epithelium with large, irregular cells (to over 50 μm), which may fill the lumen in ental part; no sorptive vacuoles were observed. Spermathecal duct long (150–247 μm) and relatively thin (22–31 μm diameter), slightly widening at ectal end up to 42 μm; with narrow, columnar cells and thin (<5 μm) muscle coat. One pair female funnels open ventrally in intersegment 11/12 (Figs 6D, 7L).

Worms from Downing Cr are generally larger than those from Pettiford Cr (see Table 4), but morphology is otherwise similar.

Taxonomic remarks. *Stylodrilus coreyi* sp. n. conforms to the general diagnosis of the genus *Stylodrilus* Claparède, 1862 (see Rodriguez and Coates 1996), which includes most known lumbriculid species with 2 pairs of testes and one pair of ovaries, one pair of spermathecae in the first testicular segment, and one pair of semiprosoporous male ducts in the second testicular segment. According to Hrabě (1929, 1970), the genus *Bythonomus* Grube, 1880, which had the same arrangement of reproductive organs, was restricted to those species with all chaetae simple-pointed, 2 pairs of branched lat-

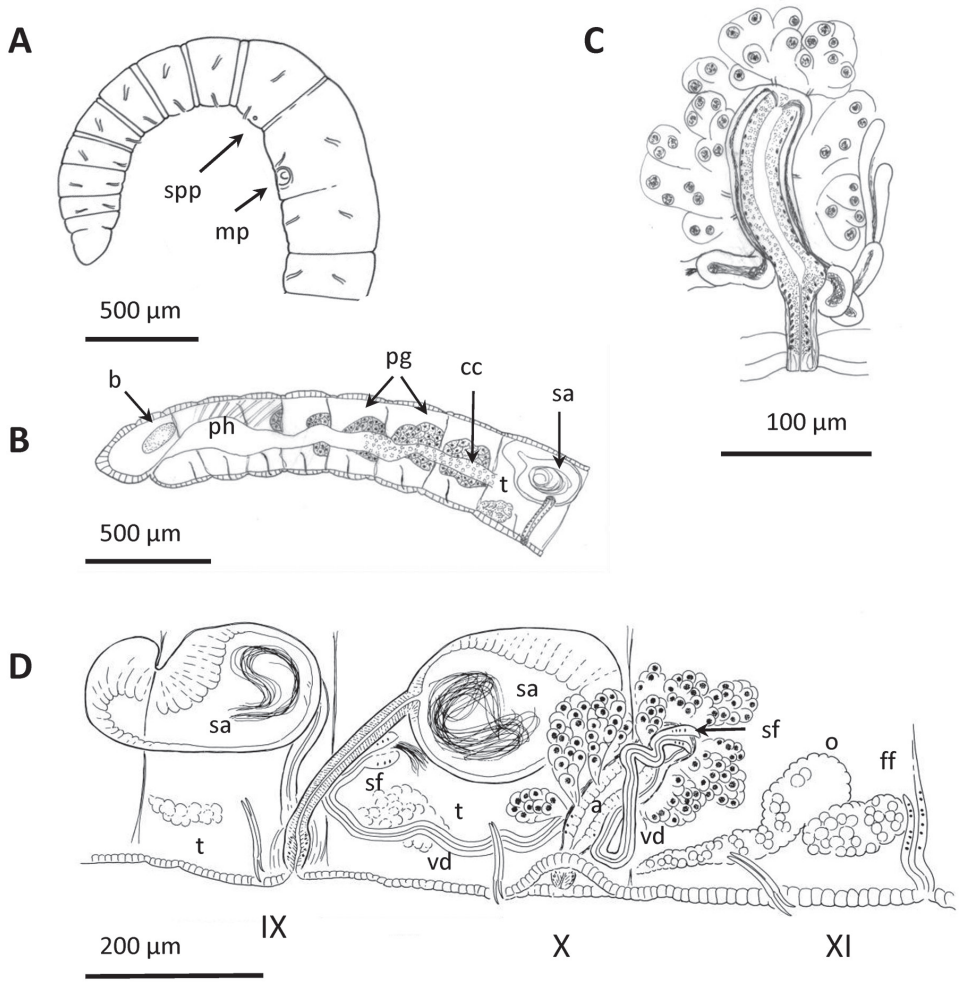

Figure 6. Drawings of *Stylodrilus coreyi* sp. n. **A–B** Anterior part of the body showing double annulation and genital pores (**A**) and digestive tract with associated glands (**B**) **C** details of male duct **D** reproductive organs.

Table 4. Comparison of morphological features in two study populations of *Stylodrilus coreyi* sp. n.

Population	No. segments	Body Ø in VIII µm	Chaetae length µm	Atrium length µm	Atrium Ø µm	Spth duct length µm	Spth ampulla length µm	Spth ampulla Ø µm
Pettiford Creek	53–65	240–420	63–105	176–248	40–63	c.163–225	205–206	174–179
Drowning Creek	55–69	390–585	81–120	266–390	54–74	150–247	239–348	198–331

eral blood vessels (sometimes only bifurcate or absent), tubular or oval atria, and vasa deferentia opening basally (ectally) to the atrium. *Bythonomus* was classified as junior synonym of *Stylodrilus* by Brinkhurst (1965), a decision that was refuted by Hrabě

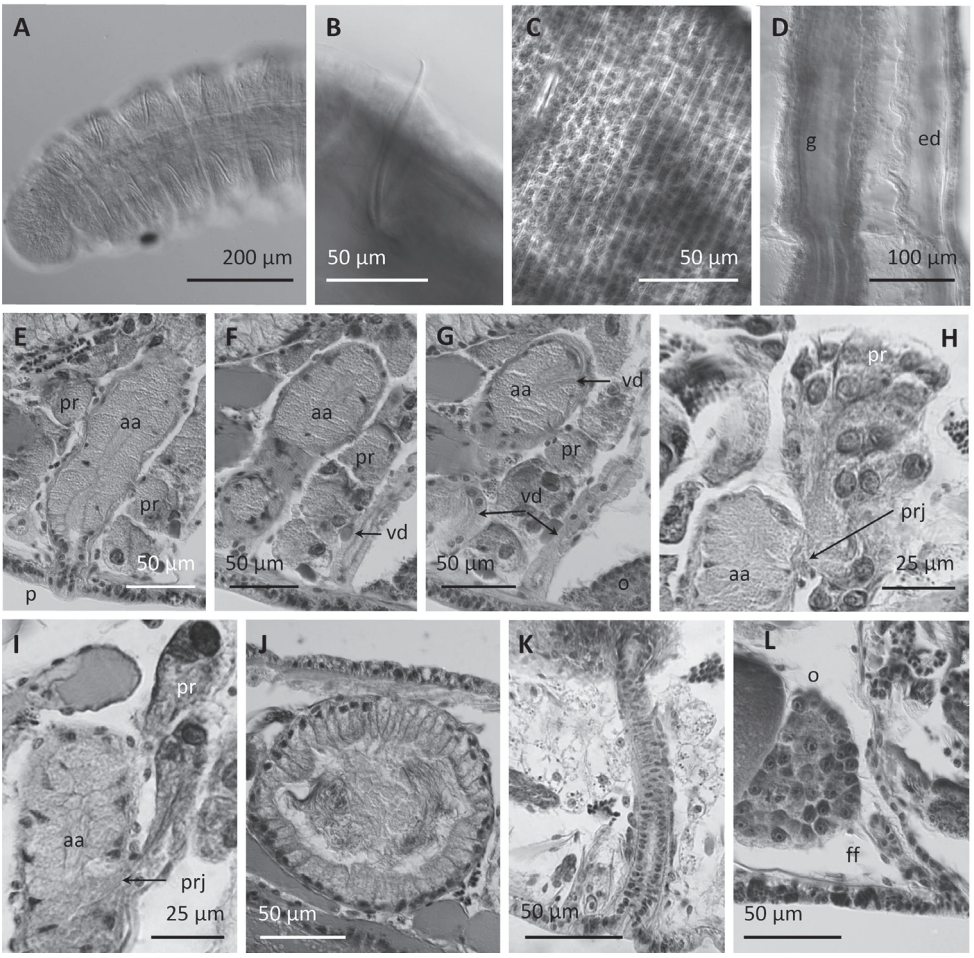

Figure 7. *Stylodrilus coreyi* sp. n. **A** Anterior part of the body, B: simple-pointed chaeta **C** clitellum **D** nephridial efferent duct in ventral part of posterior segment (anterior part facing up) **E–G** consecutive histological sections of male duct **H–I** details of prostatic glands and connection to atrial ampulla **J** spermathecal ampulla **K** spermathecal duct **L** female funnel. **E–L** histological sections, other photographs from stained whole mounts or dissected specimens.

(1970), and still divides taxonomists in the present. For example, Giani and Martínez-Ansemil (1984) accepted the synonymy, and used the characters of *S. glandulosus* as an example to invalidate *Bythonomus* as a genus; this view is actually supported by most authors (e.g., Rodriguez 1988, Timm 2009). However, Kaygorodova and Martin (2008), still supported a distinction between *Bythonomus* and *Stylodrilus*, based on the shape of chaetae. Until the taxonomic status of *Stylodrilus* is clarified by molecular analyses, we accept the synonymy, since several species of the *Stylodrilus* complex have a mixture of *Bythonomus*-like and *Stylodrilus*-like characters. In the present case, the new species has simple-pointed chaetae and tubular atria, but no posterior lateral blood vessels have been observed, and although the junction of vasa deferentia is basal,

the opening to the atrial lumen is completely apical. The junction of the vasa deferentia to the atrium is not always easily defined as basal or apical in the "*Bythonomus* group", but rather it occurs in every possible position from basal to apical (Brinkhurst and Wetzel 1984). Besides, the atrial shape can be difficult to categorize in species with long atrial ducts that gradually widen toward the ampulla.

Stylodrilus coreyi sp. n. belongs to a group of *Stylodrilus* species with simple-pointed chaetae, elongate atrium, and posterior vas deferens not forming a loop in the postatrial segment (Table 5). Within this group, *S. coreyi* is distinguished by several features of the male duct: atrium length about half (0.5–0.7) times the diameter of the body at the clitellum; a very short, barely differentiated atrial duct forming a short penis within a fold of the body wall; the atrial ampulla covered by large clusters of discrete prostatic glands, entering the atrium through narrow passages; vasa deferentia joining the atrium in the basal third of its length, and opening to the atrial lumen at the apical end.

Among this group of species, *S. wahkeenensis* Rodriguez & Coates, 1996 can be distinguished from *S. coreyi* not only by the remarkable shape of the chaetae (proximal nodulus, hair-like in dorsal bundles, and enlarged, hook-shaped in ventral bundles of segment II), but also by the position and structure of the atrium (in segment IX, small and covered by a simple, diffuse layer of prostatic cells, with no duct or penis observed). Of the other species in that group, *S. glandulosus* Giani & Martínez-Ansemil, 1984 and *S. curvithecus* Collado et al., 1993 are separated from congeners by clear apomorphies, such as a muscular, bulbous penial sac with associated glandular complex, and a long atrial duct. *Stylodrilus beattiei* Cook, 1975 and *S. tschaunensis* Morev, 1982 also have simple-pointed chaetae and vas deferens not penetrating the postatrial segment, but they are well distinguished from this species group by the distinctly petiolate atrium with oval or pyriform ampulla, short atrial duct, and absence of a penis. Other species of the genus in which the posterior vas deferens does not penetrate the post-atrial segment are *S. cernosvitovi* Hrabě, 1950, *S. mirandus* (Hrabě, 1982), and *S. aclotudi* Kaygorodova & Martin, 2008, but they all have bifid chaetae.

Stylodrilus species with simple chaetae and an elongate to tubular atrium with short atrial duct include *S. absoloni* (Hrabě, 1970), *S. lemani* and *S. chukotensis* Sokolskaya, 1975, but in these species, the vas deferens penetrates the post-atrial segment, forming a loop. Another species in this group is *S. sulci* (Hrabě, 1934), distinguished from *S. coreyi* by the median junction of vasa deferentia to the atrium, the entrance of the posterior vas deferens into the postatrial segment, and the absence of a penis.

In North America, there are only five *Stylodrilus* species known so far, one of which is cosmopolitan (*S. heringianus* Claparède, 1862). *Stylodrilus beattiei* (Cook, 1975) was the first Nearctic *Stylodrilus* species described, from a cave in West Virginia. Subsequently, *S. sovaliki* (Holmquist, 1976) was described from lakes in Alaska. Later, *S. californianus* Rodriguez, 1996 was discovered in subterranean waters in eastern California, and *S. wahkeenensis* Rodriguez & Coates, 1996, was described from hyporheic waters and small streams associated with subterranean waters of Oregon and southeastern USA.

The low number of *Stylodrilus* species in North America may be related in part to the tendency of researchers in this area to erect new genera for those taxa with very distinct

Table 5. *Stylodrilus* species with simple-pointed chaetae, elongate to tubular atrium, vas deferens not entering postatrial segment.

Species	Atrium position (segment)	Atrial ampulla/ duct size	Prostate	Vas deferens junction to atrium	Penis	Spermatheca	Spermathecal ampulla/duct size	Posterior lateral blood vessels
S. curvithecus Collado et al., 1993	X–XI	Atrium elongate, ampulla pyriform, length > duct	Diffuse, poorly developed	At the base of the ampulla, but open subapically to atrial lumen	Conical, in a fold of the body wall, with muscular bulb and associated glands	Restricted to segment IX	Ampulla folded, length > duct	Absent
S. glandulosus Giani & Martínez-Ansemil, 1984	X–XI(XII)	Ampulla elongate, length ≈ duct	Diffuse	At the base of the ampulla, but open subapically to atrial lumen	Conical, in a fold of the body wall, with muscular bulb and associated glands	Restricted to segment IX	Ampulla oval, length < duct	Present (not branching)
S. tschaunensis Morev, 1982	X	Ampulla pyriform, length>duct	Diffuse	At the base of the ampulla, but open apically to the atrial lumen	Absent	In IX	Sac-shaped ampulla, length > duct	Present, short and slightly branching
S. wahkeenensis Rodriguez & Coates, 1996	IX	Atrial duct absent	Diffuse	Medially	Absent	Ampulla in VII–VIII (genitalia shifted forward)	Ampulla length > duct (both elongate)	Absent
S. coreyi sp. n.	X–XI	Ampulla tubular, >> duct	In petiolate clusters	At the base of the ampulla, but open apically to atrial lumen	Short penis	Ampulla in IX–X	Ampulla oval, length < duct	Absent

apomorphies (e.g., *Spelaedrilus* Cook, 1975, *Phagodrilus* McKey-Fender & Fender, 1988, *Tenagodrilus* Eckroth & Brinkhurst, 1996), despite a general arrangement of the reproductive system that fits the *Stylodrilus* pattern. This situation indicates the need for a sound revision of the genus, since some *Stylodrilus* species can in fact be closer to other genera.

Ecological remarks. *Stylodrilus coreyi* sp. n. has been collected from seeps and pools in humic coastal plain streams (Drowning and Pettiford Creeks), most commonly outside of the main channel. These habitats have a temporary flow regime, with seasonal drying during summer months. *S. coreyi* was mostly collected in detritus over a layer of fine sand. Both streams have very high water quality (NCDENR 2007, 2011), but pH values are higher in Drowning Creek (usually about 5.5) than in Pettiford Creek (< 4.3). This suggests that *S. coreyi* tolerates extremely low pH values, but does not require such conditions. Interestingly, lumbriculids found in this kind of habitat have also congeneric relatives in groundwaters (three of five described *Stylodrilus* species in the Nearctic region are subterranean; see also *Cookidrilus pocosinus* remarks, above).

Discussion

The new taxa show several characters that are interesting in the context of taxonomy of the family Lumbriculidae, and are worth a more general discussion.

Spermathecal position and number

In the present paper, we describe three species that differ in number and position of spermathecae. The phylogenetic analysis of the family Lumbriculidae performed by Brinkhurst (1989) suggested that these characters were subject to many reversals and have low value in the phylogeny; however, this result contradicts the central role that number and position of spermathecae have played in the taxonomy of the family. Still, only two recognized lumbriculid genera include species with spermathecae both anterior and posterior to the atrial segments, namely, *Styloscolex* and a single *Lumbriculus* species. *Lumbriculus alexandrovi* Popchenko, 1976, is remarkable in having spermathecae in front of, in, and behind atrial segments. Future phylogenetic analyses based on both morphological and molecular data will provide more light on the importance of these characters and their validity in the classification of lumbriculids.

With respect to the spermathecal pores, it is interesting to note that the most common (and thus probably ancestral) position in aquatic oligochaetes is in the anterior part of the segment, in front of the ventral chaetae or even very close to the anterior septum. This is also the most common position within the family Naididae (sensu Erséus et al. 2008), although in the Tubificinae and Limnodriloidinae, the spermathecal pores are usually located just in front of, or at about the level of the chaetal bundles. There are exceptions where the spermathecae are well behind ventral chaetae, such as *Branchiura sowerbyi*, at present classified in Rhyacodrilinae. Thus, it is remarkable that the position

of the pores is behind the ventral chaetae in most lumbriculids. It is also noteworthy that among the lumbriculids, several Nearctic taxa have spermathecal pores in front of the ventral chaetae (see taxonomic remarks in *Cookidrilus pocosinus*), whereas this is extremely rare in Palearctic species (Timm and Popchenko 1978 reported an abnormal spermatheca opening in the anterior part of segment VIII in *Tatriella slovenica* Hrabě, 1939).

Prostatic cells

The organization of prostatic cells into petiolate bundles has been reported before in several lumbriculid genera, but to date this character has not been considered diagnostic for genera. Therefore, species with either diffuse or clustered prostatic cells are found within *Trichodrilus* (see Rodriguez and Giani 1994), and *Stylodrilus* (e.g., *S. mirus* Chekanovskaya, 1956, or the North American *S. sovaliki* (Holmquist, 1976)). Multicellular prostate glands seem to be present in most North American lumbriculids, e.g., species of *Eclipidrilus*, *Rhynchelmis*, *Eremidrilus*, *Kincaidiana* and *Altmanella* Fend, 2009. Clusters of glandular cells connected to the atrium through a single passage have also been reported in some East Asian lumbriculids (e.g., *S. mirus*, *Hrabea ogumai* Yamaguchi, 1936, *Yamaguchia toyensis* Fend & Ohtaka, 2004), as well as in a few European taxa (e.g., *Pseudorhynchelmis paraolchonensis* (Giani & Martínez-Ansemil, 1984), and several *Trichodrilus* species in Rodriguez and Giani 1994). The structure of prostatic glands may be subject to interpretation due to fixation or cell density; however, this character has played an important role in the classification of other higher oligochaete taxa, and does require more attention in the Lumbriculidae.

Penial sheath

"Cuticular penis sheath" has been a confusing term, since different structures may be fundamentally homologous as presumably derived from ectodermal secretions of the developing penis. Holmquist (1985) discussed the problem with reference to tubificids, and defined different resultant structures, reserving the term "sheath" for a rigid structure that disassociates from the soft tissue (thus the penis is free within it). Other authors use the term "penis sheath" for any cuticular covering. In the Lumbriculidae, this has generally been restricted to the well-defined structure that covers the ectal part of the penis of some species of *Styloscolex* (e.g., Cook 1971, Semernoy 2004), and we have adopted this broader definition.

In *Lumbriculus variegatus*, Hesse (1902) described a tubular cuticular penis, and Holmquist (1976) also referred to the presence of a "slender cuticular penis" in *Lumbriculus inconstans* (Smith, 1895), *L. genitosetosus* (Holmquist, 1976) and *L. ambiguus* (Holmquist, 1976). Our observations indicate the presence of a soft cuticular layer on the penis of a sectioned *L. japonicus* Yamaguchi from Yamaguchi's collection (Fig. 2O), although it appears to be present in some, but not all, dissected *Lumbriculus*

specimens in S. Fend's collections. In contrast, in *Styloscolex*, a rigid cuticular layer encloses the attenuated epithelial tube of the penis within a non-cuticular penial sac (Fig. 2N), thus resembling penes of *Sylphella* (Fig. 2M). This external cuticular layer should not be confused with the internal cuticular lining described for penes of other lumbriculids, such as *Eclipidrilus frigidus* Eisen (see Fend 2005).

The importance of the unusual aquatic habitats in systematics and conservation

Biomonitoring programs are well developed for larger streams and rivers (Lenat 1988, 1993, Lenat and Barbour 1994), but evaluation of smaller streams, temporary streams and swamp streams can be more difficult (Lenat 2003). Many biological monitoring systems use the taxa richness of intolerant EPT groups (Ephemeroptera, Plecoptera, Trichoptera) as an important metric, but these groups may be relatively sparse in temporary streams or acid waters. In this situation, it may be more informative to evaluate other macroinvertebrate groups, including oligochaete worms. Studies in North Carolina (Lenat and Fend, in preparation) suggest that Lumbriculidae can be abundant and diverse in temporary streams and swamp streams, such that the identification of lumbriculid species can make an important contribution to both water quality assessments and consideration of conservation value. An inventory of unaltered (reference) sites from all kinds of aquatic habitats is needed to complement information given by more typical riffle sampling, in order to conserve an acceptable level of regional species richness (Curry et al. 2012).

The presence of lumbriculids can be particularly useful when the diversity of the macroinvertebrate community is limited by low pH (Lake Run, Pettiford Creek), lack of water during summer months (Lake Run, Pettiford Creek, Drowning Creek floodplain, UT Pokeberry Creek) or small size (UT Pokeberry Creek). UT Pokeberry Creek presents a very interesting example where the larger creek was severely affected by nonpoint source runoff, but the small seeps (which supported a variety of rare invertebrates) were shown to worthy of environmental protection (Lenat, unpublished data). The study and mapping of unusual aquatic habitats (including pools, seeps, and swamps) will bring interesting novelties to the field of biodiversity and ecology, since the range of environmental conditions and microhabitats differs from those commonly studied in rivers. Future collections from these poorly studied habitats can also give light to the fields of systematics and zoogeography. For example, springs or swamps in southeastern North America constitute the only known habitat for three recently-described, monotypic lumbriculid genera (*Sylphella*, *Pilaridrilus*, *Pararhynchelmis*), and have also provided dramatic range extensions for such genera as *Rhynchelmis*, *Cookidrilus*, and *Altmanella*.

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Appendix 1

Key of the known North American *Stylodrilus* species

1 Chaetae bifid, with short upper tooth (some chaetae in anterior segments can be simple-pointed) **2**

– All chaetae simple-pointed **3**

2 Penis long, permanently protruded, atrium oval, spermathecal duct long .... ***S. heringianus*** **Claparède, 1862**
Medium size worms (0.6–1 mm diameter). Prostatic cells forming clusters, thick (to 30 μm) vasa deferentia join atrial ampulla apically, posterior vas deferens penetrates the postatrial segment. 2 pairs short, unbranched lateral blood vessels in posterior segments. Widespread in Northern USA and Canada, including the Great Lakes.

– Penis short, atrium elongate, spermathecal duct very short .... ***S. californianus*** **Rodriguez, 1996**
Small worms (0.2–0.5 mm diameter). Vasa deferentia join the atral ampulla medially and open to large lumen apically after running through the atrial musculature, posterior vas deferens penetrates the postatrial segment. Ridgecrest, eastern California, phreatic waters (in a well).

3 Chaetae with markedly proximal nodulus, dorsals with very long (hair-like) distal end ..... ***S. wahkeenensis*** **Rodriguez & Coates, 1996**
Small worms (0.3–0.4 mm diameter). Atria in IX, tubular, posterior vas deferens not penetrating the postatrial segment, atrial duct and penis absent, spermathecal duct long and thick. Oregon, Alabama and Tennessee; hyporheic and small rivers associated with subterranean waters.

– Chaetae with distal nodulus **4**

4 Atrium elongate, duct weakly separated from ampulla and of similar diameter... ..... ***S. coreyi*** **sp. n.**
Small worm (0.3–0.4 mm diameter). Posterior vas deferens not penetrating the postatrial segment. North Carolina, pocosin, acidic waters.

– Atrium pedunculate, duct clearly separated from ampulla, and much narrower **5**

5 Posterior vas deferens does not enter the postatrial segment; vasa deferentia join atrial ampulla and open to the lumen basally .... ***S. beattiei*** **Cook, 1975**
Medium size worm (0.7–0.9 mm diameter). Prostatic cells small and disappearing soon after mating; lateral blood vessels absent in posterior segments. Tub Cave, West Virginia.

– Posterior vas deferens penetrates the postatrial segment; vasa deferentia join atrial ampulla basally and open to the lumen medially .... ***S. sovaliki*** **(Holmquist, 1976)**
Medium to large size (about 1 mm diameter). Prostatic cells in bundles, posterior lateral blood vessels branched. Alaska, rivers.

Appendix 2

Location of study sites (all in North Carolina, USA), and species described in present paper.

Stream	Latitude	Longitude	Ecoregion	Species described
Unnamed tributary to Pokeberry Cr	N35.8267	W79.1013	Piedmont	*Sylphella puccoon* gen. n., sp. n
Pettiford Cr	N34.7471	W77.0221	Coastal Plain	*Cookidrilus pocosinus* sp. n. *Stylodrilus coreyi* sp. n.
Lake Run	N34.7773	W78.6646	Coastal Plain	*Cookidrilus pocosinus* sp. n.
Anderson Cr tributary to the Lower Little River	N35.2661	W78.8192	Sandhills	*Cookidrilus pocosinus* sp. n.
Drowning Cr	N35.0662	W79.5496	Sandhills	*Cookidrilus pocosinus* sp. n. *Stylodrilus coreyi* sp. n.

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The mitochondrial genome of the land snail *Camaena cicatricosa* (Müller, 1774) (Stylommatophora, Camaenidae): the first complete sequence in the family Camaenidae

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Abstract

The complete mitochondrial (mt) genome of the snail *Camaena cicatricosa* (Müller, 1774) has been sequenced and annotated in this study. The entire circular genome is 13,843 bp in size and represents the first camaenid mt genome, with content of 31.9%A, 37.9%T, 13.5%C and 16.7%G. Gene content, codon usage and base organization show similarity to a great extent to the sequenced mt genome from Stylommatophora, whereas, gene order is different from them, especially the positions of $tRNA^{Cys}$, $tRNA^{Phe}$, *COII*, $tRNA^{Asp}$, $tRNA^{Gly}$, $tRNA^{His}$ and $tRNA^{Trp}$. All protein coding genes use standard initiation codons ATN except for *COII* with GTG as start signal. Conventional stop codons TAA and TAG have been assigned to all protein coding genes. All tRNA genes possess the typical clover leaf structure, but the TψC arm of $tRNA^{Asp}$ and dihydrouridine arm of $tRNA^{Ser(AGN)}$ only form a simple loop. Shorter intergenic spacers have been found in this mt genome. Phylogenetic study based on protein coding genes shows close relationship of Camaenidae and Bradybaenidae. The presented phylogeny is consistent with the monophyly of Stylommatophora.

Keywords
Camaena cicatricosa, Camaenidae, Stylommatophora, mitochondrial genome, secondary structure

Introduction

The mitochondrial (mt) genome of metazoa usually comprise 37 genes, including 13 protein coding genes (PCGs) (*COI–COIII*, *Cytb*, *ND1–ND6*, *ND4L*, *ATP6* and *ATP8*), two ribosomal RNA (rRNA) genes, and 22 transfer RNA (tRNA) genes (Boore 1999). Additionally, it also contains noncoding regions, such as the AT-rich region and short intergenic spacers (Wolstenholme 1992). The mt genome is characterized by small size (13–36 kb), maternal inheritance, lack of recombination, conserved genomic organization and rapid evolutionary rate compared with the nuclear genome (Avise 1994). It has been widely used in studies of systematics, phylogenetic analysis, phylogeography, population structure at diverse taxonomic groups (White et al. 2011; Gaitán-Espitia et al. 2013; Menegon et al. 2014). The mt genome is the most popular genetic marker though there are numerous debates on their utilization in systematic research (Delsuc et al. 2003; Cameron et al. 2004; Talavera and Vila 2011; Simon and Hadrys 2013; Cameron 2014). Over the last years, next generation sequencing technologies have accelerated further developments of mt genomics. The mt genomes of many vertebrates and insects are well sequenced and studied (Boore 1999; Hahn et al. 2013; Wang et al. 2014). However, studies on molluscan mt genomes are poor relatively (Kurabayashi and Ueshima 2000; Boore et al. 2004; Grande et al. 2008). Only 80 mt genomes of Gastropoda snails have been deposited in GenBank (up to 2014.9.20).

Camaenidae, one of the most diverse families, was erected by Pilsbry in 1893 (Pilsbry 1893–1895). The camaenids mainly feed on green plants and humus, and often harm a large number of crops, landscape plants and forest, leading to a depression in yield and a reduction in quality. Besides, they can spread zoonotic food borne parasitic disease and have great damage to human and animal health (Zhou et al. 2007). When humans are infected by ingesting snails, the nervous system can be injured (Liang and Pan 1992). The camaenids also play an important part in agricultural production and human activities as food, drug, arts, crafts, etc. (Chen and Gao 1987). *Camaena cicatricosa* (Müller, 1774), the type species of the type genus *Camaena* (Albers, 1850), occurs only in China, distributing in Guangdong, Guangxi, Guizhou, Yunnan and Hainan. Adult shell is large, thick and depressed conic. This snail usually feeds a broad range of fruits, vegetables, leaves and weeds (Xiao 1989).

The mt genome of land snail is similar to other invertebrates in containing 37 genes. Since the first mt genome of *Albinaria caerulea* was obtained in 1995 (Hatzoglou et al. 1995), only ten mt genomes from eight species in the order Stylommatophora were determined prior to this study, consisting of three species in Helicidae (Terrett et al. 1995; Groenenberg et al. 2012; Gaitán-Espitia et al. 2013), two in Bradybaenidae (Yamazaki et al. 1997; Deng et al. 2014), one in Clausiliidae (Hatzoglou et al. 1995), one in Succineidae (White et al. 2011) and one in Achatinidae (He et al. 2014).

Although researchers have done some phylogenetic studies on Camaenidae, they often pay much attention to analyses of shell morphology or single gene fragment (Scott 1996; Wade et al. 2007). Complete mt genome evidence is still limited. We select *C. cicatricosa* as subject because of not only relatively wide distribution and varied morphology but also acting as type species of the type genus *Camaena*. We have analyzed nucleotide composition, codon usage, compositional biases, and constructing models of the secondary structure of tRNAs. Besides, we also discussed the phylogenetic relationships with other representative gastropods. This snail mt genome is the first model in the family Camaenidae, thus it can offer worthwhile information to other camaenids.

Materials and methods

Genomic DNA extraction, PCR amplification and DNA sequencing

Adults of *C. cicatricosa* were collected from Xishan Park in Guiping (23°23'58"N, 110°3'46"E), Guangxi, China in November 2, 2013. Specimens were initially preserved in 100% ethanol in the field, and then stored at -20 °C at Fujian Entry-Exit Inspection & Quarantine Bureau (FJCIQ). Total genomic DNA was extracted from the pedal muscle tissue of single individual using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions. Voucher specimen (FJCIQ 18483) is deposited at the Key Laboratory of Molluscan Quarantine and Identification of AQSIQ, Fujian Entry-Exit Inspection & Quarantine Bureau, Fuzhou, Fujian.

The entire genome was successfully amplified by polymerase chain reaction (PCR) in overlapping fragments with four pairs of mitochondrial universal primers from previous works (Palumbi et al. 1991; Folmer et al. 1994; Merritt et al. 1998; Hugall et al. 2002), and four pairs of perfectly matched specific primers designed from sequenced short fragments in this study (Table 1). Short PCRs (< 2 kb) were performed using Takara Taq DNA polymerase (TaKaRa, Dalian, China), with the following cycling conditions: 30 s at 94 °C, followed by 35 cycles of 10 s at 94 °C, 50 s at 40 °C or 45 °C, and 1 min at 72 °C. The final elongation step was continued for 10 min at 72 °C. Long range PCRs (> 4 kb) were performed using Takara Long Taq DNA polymerase (TaKaRa, Dalian, China) under the following cycling conditions: 1 min at 94 °C, followed by 40 cycles of 10s at 98 °C, 50 s at 60 °C, 4–8 min at 68 °C, and the final elongation step at 72 °C for 6 min. The PCR products were checked by spectrophotometry and 1.0% agarose gel electrophoresis.

Short fragments were sequenced from both directions after purification using the BigDye Terminator Sequencing Kit (Applied Biosystems, San Francisco, CA, USA) and the ABI PRIMER^{Tm}3730XL DNA Analyzer (PE Applied Biosystems) with internal primers for primer walking. For the long fragments, the shotgun libraries of *C. cicatricosa* were constructed, and then the positive clones were sequenced using above kit and sequenator with vector-specific primers *Bca*Best primer M13-47 and *Bca*Best Primer RV-M.

Table 1. Primer pairs used for PCR amplification.

No. of fragment	Primer name	Nucleotide sequence (5'–3') and location	Size (bp)	Reference
1	LCO-1490	GGTCAACAAATCATAAAGATATTGG		Folmer et al. 1994
	HCO-2198	TAAACTTCAGGGTGACCAAAAAATCA		Folmer et al. 1994
2	Fcoi	TGAACTGTTTATCCTCCAC (364–382)	1908	Present study
	RL	TAGGGTCTTCTCGTCTTT (2254–2271)		Present study
3	16Sar-L	CGCCTGTTTATCAAAAACAT		Palumbi et al. 1991
	16Sbr-H	CCGGTCTGAACTCAGATCACGT		Palumbi et al. 1991
4	FL2	CGATGTTGGATTAGGAAGTTGA (2415–2436)	4267	Present study
	Rcb2	TAAAGGATTTGTTGACCCACG (6661–6681)		Present study
5	144F	TGAGSNCARATGTCNTWYTG		Merritt et al. 1998
	272R	GCRAANAGRAARTACCAYTC		Merritt et al. 1998
6	Fcb	GTGGGTCAACAAATCCTT (6662–6679)	816	Present study
	Rcoii	ATGAACACCTCGGGTAGT (7460–7477)		Present study
7	FCOII	AAATAATGCTATTTCATGAYCAYG		Hugall et al. 2002
	RCOII	GCTCCGCAAATCTCTGARCAYTG		Hugall et al. 2002
8	SF1F	AAATTCCATTAGAGGGGCTTATACGCCGCC (6984–7013)	6957	Present study
	SF1R	CAAGAGATAGTCCCGTACCAACTATGCCGC (68–79)		Present study

Genome annotation and inference of secondary structure

Raw sequences were proof-read and aligned into contigs with BioEdit v.7.0.5.3 (Hall 1999). The tRNA genes were identified with tRNAscan-SE Search Server v.1.21 (Lowe and Eddy 1997) and DOGMA (Wyman et al. 2004), while others that could not be determined by these two tools were predicted by similarity comparison with other published land snails (Terrett et al. 1995; Yamazaki et al. 1997; Groenenberg et al. 2012; Gaitán-Espitia et al. 2013; He et al. 2014; Deng et al. 2014). The PCGs and rRNA genes were annotated by BLAST in Genbank with published available mitochondrial sequences of terrestrial snails.

PCGs were aligned with Clustal X (Thompson et al. 1997). The nucleotide composition and codon usage were analyzed with MEGA 5.0 (Tamura et al. 2011). Strand asymmetry was denoted by skew values, which were calculated according to the formulas: AT skew = [A–T]/[A+T] and GC skew = [G–C]/[G+C] (Perna and Kocher 1995).

Phylogenetic analyses were performed based on 11 representative gastropod mt genomes from GenBank (Table 2) using maximum likelihood (ML) and maximum parsimony (MP) methods. One species of Opisthobranchia was selected as outgroup. A DNA alignment with 9,892bp length was inferred from the amino acid alignment of 13 PCGs using MEGA 5.0 (Tamura et al. 2011). The selection of best-fit-substitution model for ML estimation was performed using MEGA 5.0 with corrected Akaike information criterion (AIC). Node supports for ML and MP analyses were calculated through 1000 bootstrap replicates. All other settings were kept as default.

Table 2. Summary of samples information used in this study.

Subclass /order	Family	Species	Accession number	Reference
Stylommatophora				
	Camaenidae	*Camaena cicatricosa*	KM365408	Present study
	Bradybaenidae	*Euhadra herklotsi*	Z71693–Z71701	Yamazaki et al. 1997
		Mastigeulota kiangsinensis	KM083123	Deng et al. 2014
	Helicidae	*Cornu aspersum*	JQ417195	Gaitán-Espitia et al. 2013
		Cepaea nemoralis	CMU23045	Terrett et al. 1995
		Cylindrus obtusus	JN107636	Groenenberg et al. 2012
	Succineidae	*Succinea putris*	JN627206	White et al. 2011
	Clausiliidae	*Albinaria caerulea*	X83390	White et al. 2011
	Achatinidae	*Achatina fulica*	NC024601	He et al. 2014
Basommatophora	Lymnaeidae	*Galba pervia*	JN564796	Liu et al. 2012
Opisthobranchia	Aplysiidae	*Aplysia californica*	AY569552	Knudsen et al. 2006

Results and discussion

The complete mt genome of *C. cicatricosa* was a double-stranded circular molecule of 13,843 bp in length (GenBank: KM365408). It contained 13 PCGs, 22 tRNA genes, two rRNA genes, similar to other mt genomes of land snails from the order Stylommatophora. All genes were divided into two groups, encompassing 24 genes on the majority coding strand (J strand) and others on the minority coding strand (N strand) (Fig. 1). However, the gene arrangement differed from that of the known land snails in the order Stylommatophora, specially the locations of $tRNA^{Cys}$, $tRNA^{Phe}$, *COII*, $tRNA^{Asp}$, $tRNA^{Gly}$, $tRNA^{His}$ and $tRNA^{Trp}$ (Fig. 2). Gene overlaps with a total of 242 bp were found at 16 gene junctions, and the longest overlap (50 bp) existed between *ND6* and *ND5*. Besides, there were 144 nucleotides dispersed in 14 intergenic spacers with the shortest 1 bp and the longest 29 bp. The 29 bp long noncoding region was situated between *COIII* and $tRNA^{Ile}$; the shortest 1bp in three gene spacers (Table 3).

Protein coding genes

The length of PCGs was 10,941bp, accounting for 79.04% of the whole mt genome (Table 4). Most PCGs started with ATN as initiation codons (four with ATG, three with ATT, and five with ATA) except for *COII* gene with GTG (Table 3), while ATC, TTA, TTG, CTT and TCG as unconventional start signals have been found in other invertebrates (Raay and Crease 1994; Crease 1999; Yamazaki et al. 1997; Yu et al. 2007; Groenenberg et al. 2012). Conventional stop codons TAA and TAG had been assigned to all PCGs (Table 3). However, an incomplete terminator signal (T) has been found in other snails (Terrett et al. 1995; Hatzoglou et al. 1995; Yamazaki et al. 1997; White et al. 2011; Groenenberg et al. 2012; Gaitán-Espitia et al. 2013).

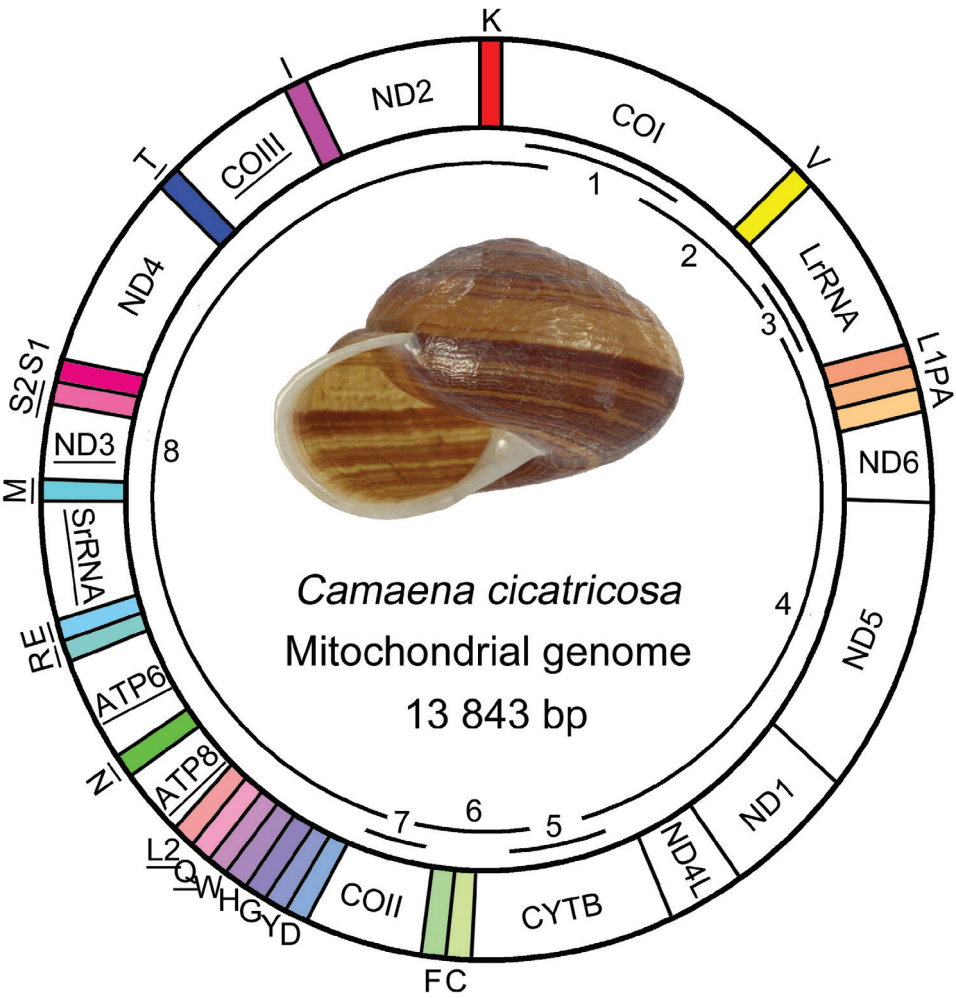

Figure 1. The mt genome of *Camaena cicatricosa*. The tRNA genes are labeled based on the IUPACIUB single letter amino acid codes. Genes with underline illuminate the direction of transcription from 3' to 5', and without underline illuminating from 5' to 3'. Numbers and overlapping lines within the circle indicate PCR fragments amplified for sequencing (see Table 1).

Transfer RNA genes

The 22 tRNA genes typically found in metazoan mt genomes were also discovered in *C. cicatricosa*, and 18 of them were determined by tRNAscan-SE (Lowe and Eddy 1997) and DOGMA (Wyman et al. 2004). The other four tRNA genes that could not be detected by the two programs were identified and drawn through comparison with known patterns of previous researches (Terrett et al. 1995; Grande et al. 2002; Groenenberg et al. 2012; Gaitán-Espitia et al. 2013). Fourteen tRNA genes were encoded on the J strand and the remainings on the N strand. Most

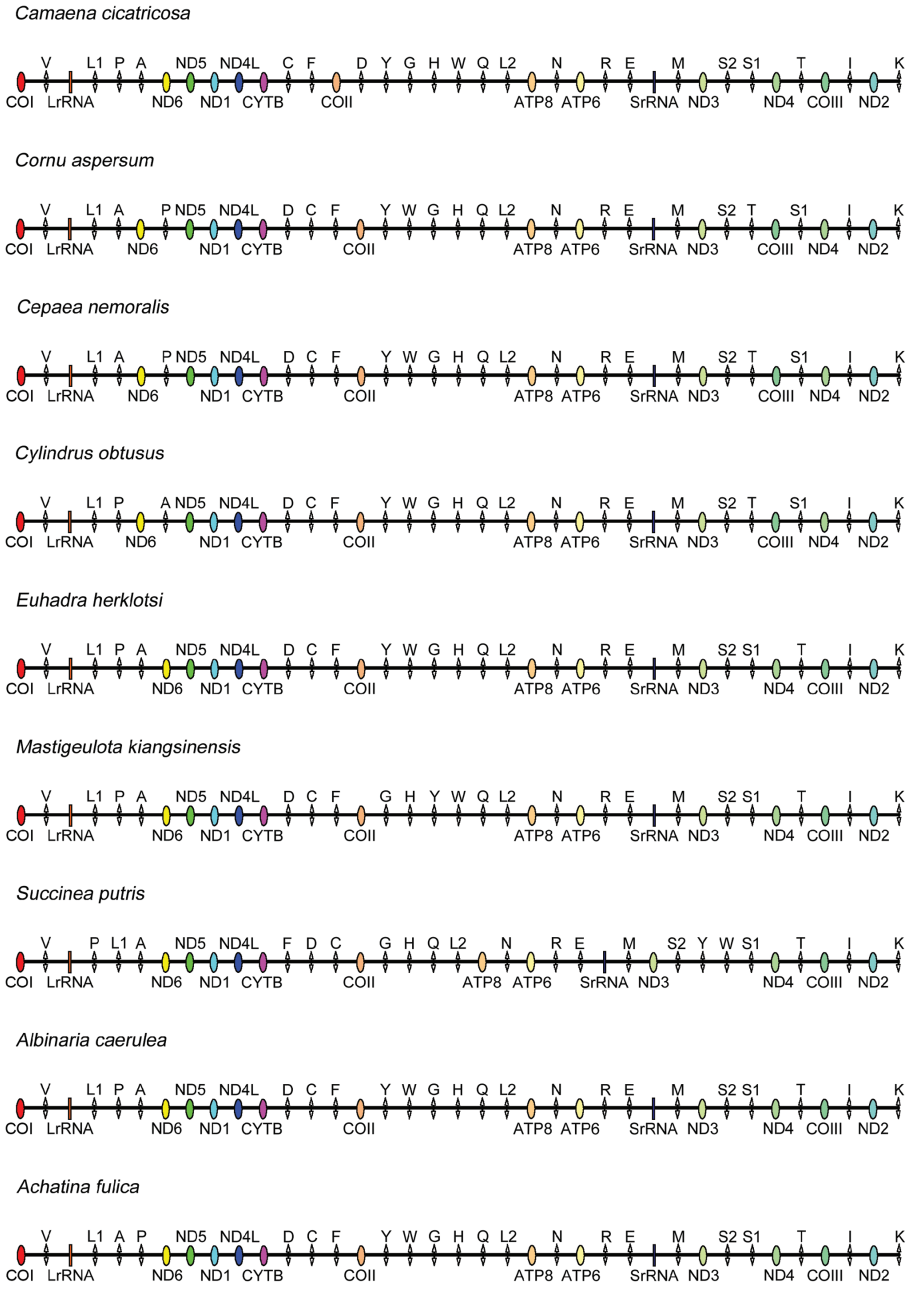

Figure 2. Gene arrangement of nine mt genomes in the order Stylommatophora.

tRNA genes could be folded into classic clover leaf structures exclusive of $tRNA^{Asn}$ and $tRNA^{Ser(AGN)}$, in which their TψC arm and dihydrouridine (DHU) arm simply formed a loop (Fig. 3).

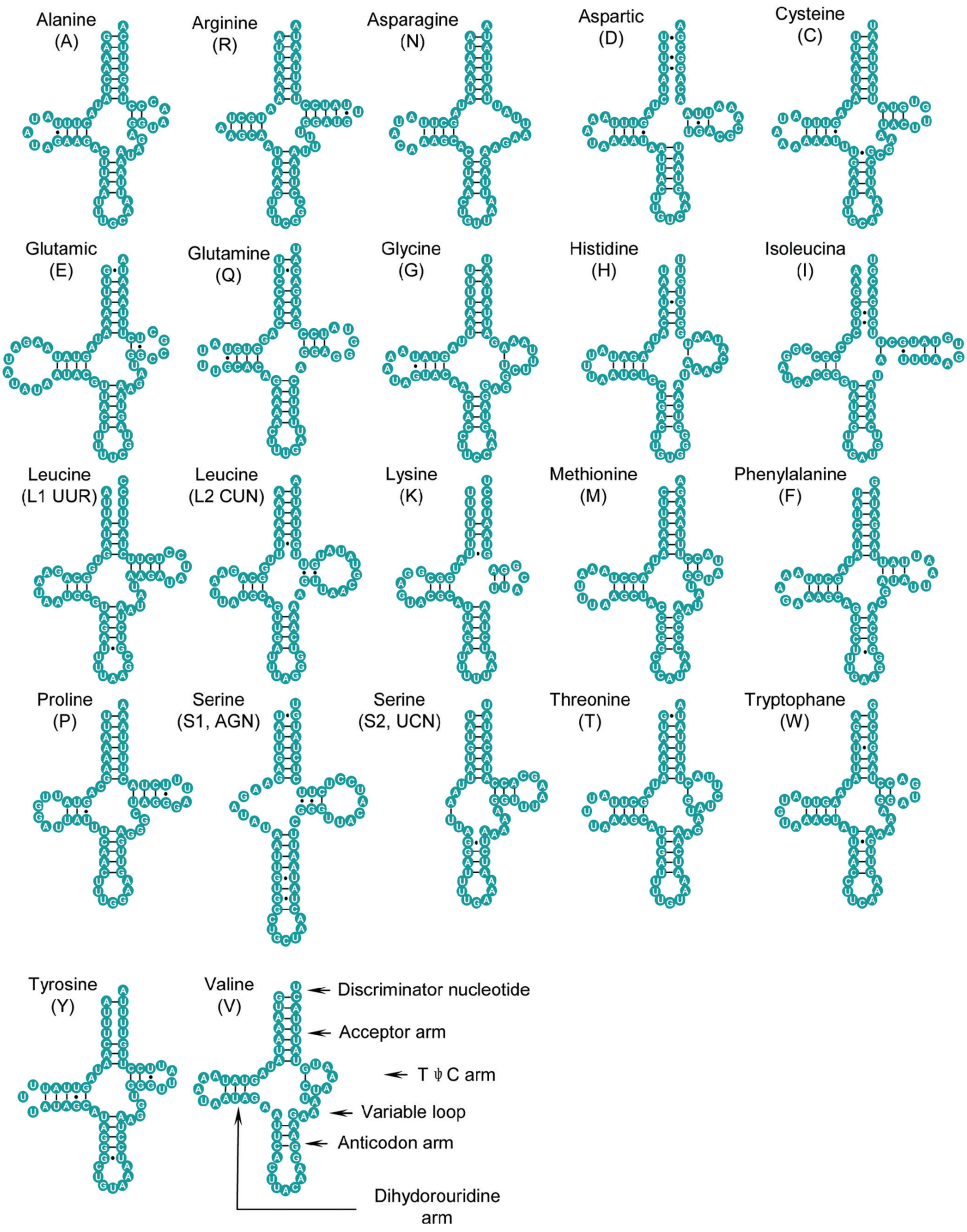

Figure 3. Inferred secondary structures of 22 tRNA genes in *Camaena cicatricosa*. Dashed (-) indicates Watson-Crick base pairing and (•) indicates G-U base pairing.

The length of tRNA genes ranged from 53 to 65 bp (Table 3). All amino acid acceptor (AA) arms (7 bp), anticodon (AC) loops (7 bp) and arms (5 bp) were almost invariant. However, other arms and loops changed considerably in size. Additionally, in some tRNA genes, non-Watson-Crick matches and aberrant loops

Table 3. Organization of the *Camaena cicatricosa* mt genome.

Gene	Direction	Location	Size (bp)	Anticodon	Start codon	Stop codon	Intergenic nucleotides
COI	F	1–1527	1527		ATG	TAG	
tRNA^{Val}	F	1527–1585	59	1557–1559 TAC			–1
lrRNA	F	1586–2582	997				0
tRNA$^{Leu(CUN)}$	F	2583–2642	60	2611–2613 TAG			0
tRNA^{Pro}	F	2640–2702	63	2669–2671 TGG			–3
tRNA^{Ala}	F	2705–2764	60	2735–2737 TGC			2
ND6	F	2784–3251	468		ATA	TAA	19
ND5	F	3202–4893	1692		ATT	TAA	–50
ND1	F	4914–5786	873		ATA	TAA	20
ND4L	F	5797–6072	276		ATA	TAA	10
CytB	F	6076–7188	1113		ATG	TAA	3
tRNA^{Cys}	F	7185–7246	62	7215–7217 GCA			–4
tRNA^{Phe}	F	7249–7309	61	7279–7281 GAA			2
COII	F	7310–7984	675		GTG	TAG	0
tRNA^{Asp}	F	7989–8048	60	8019–8021 GTC			4
tRNA^{Tyr}	F	8075–8136	62	8105–8107 GTA			26
tRNA^{Gly}	F	8132–8191	60	8162–8164 TCC			–5
tRNA^{His}	F	8188–8246	59	8218–8220 GTG			–4
tRNA^{Trp}	F	8255–8314	60	8282–8284 TCA			8
tRNA^{Gln}	R	8311–8369	59	8338–8340 TTG			–4
tRNA$^{Leu(UUR)}$	R	8366–8429	64	8398–8400 TAA			–4
ATP8	R	8431–8595	165		ATG	TAA	1
tRNA^{Asn}	R	8597–8652	56	8620–8622 GTT			1
ATP6	R	8652–9332	681		ATT	TAA	–1
tRNA^{Arg}	R	9309–9366	58	9339–9341 TCG			–24
tRNA^{Glu}	R	9366–9430	65	9393–9395 TTC			–1
SrRNA	R	9431–10112	682				0
tRNA^{Met}	R	10113–10174	62	10140–10142 CAT			0
ND3	R	10165–10524	360		ATA	TAA	–10
tRNA$^{Ser(UCN)}$	R	10517–10569	53	10548–10550 TGA			–8
tRNA$^{Ser(AGN)}$	F	10570–10629	61	10594–10596 GCT			0
ND4	F	10648–11988	1341		ATA	TAA	18
tRNA^{Thr}	R	11940–11999	60	11967–11969 TGT			–49
COIII	R	11965–12792	828		ATT	TAA	–35
tRNA^{Ile}	F	12822–12885	64	12852–12854 GAT			29
ND2	F	12887–13828	942		ATG	TAA	1
tRNA^{Lys}	F	13790–13843	54	13819–13821 TTT			–39

Note: Negative numbers indicate adjacent gene overlap.

had been found. For example, a total of 73 unmatched base pairs existed in some tRNAs, and 38 of them were G-U pairs, situated in the AA stem (13 bp), the T stem (10 bp), the AA stem (8 bp) and the DHU stem (7 bp). The remaining five base pairs included U-U mismatches, U-C mismatches, A-C mismatches, A-G

mismatches and A-A mismatches (Fig. 3). Nevertheless, the post-transcriptional RNA-editing mechanism can rectify these mismatches to maintain tRNA functions (Tomita et al. 2001).

Ribosomal RNA genes

The rRNA genes comprising large rRNA subunit (*lrRNA*) and small rRNA subunit (*srRNA*) are presumed to block in the spaces of flanking genes (Boore 2001; 2006). The *lrRNA* gene was situated between $tRNA^{Val}$ and $tRNA^{Leu(CUN)}$ revealing 78.23% consistency with *Euhadra herklotsi* and *Mastigeulota kiangsinensis*. The *srRNA* gene was located between $tRNA^{Glu}$ and $tRNA^{Met}$ (Fig. 1). The length of them were determined to be 997 bp and 682 bp respectively (Table 3).

Base composition and codon usage

Like other snail mt genomes, the nucleotide composition of the *C. cicatricosa* mt genome was obviously biased toward adenine and thymine (A = 31.90%, T = 37.90%, C = 13.50%, G = 16.70%). The entire mt genome had a high A+T content of 69.80%, by the composition of 69.32% in PCGs, 71.41% in tRNA genes, 72.42% in rRNA genes. Nucleotide bias can also be reflected by codon usage. Evidently, we can see that NNA and NNU were applied frequently in most PCGs. Furthermore, codons TTT (phenylalanine), TTA (leucine), ATT (isoleucine) and ATA (methionine) which were used widely were all composed of A and T. Especially, more and more codons were biased in favor of those codons with A or T in the third position (Fig.4).

The nucleotide composition of metazoan mt genomes usually demonstrate an obvious strand bias (Hassanin et al. 2005; Hassanin 2006) that can be described as AT and GC skews (Perna and Kocher 1995). The PCGs skew statistics of *C. cicatricosa* showed a great TA skew and nearly equal G and C on the N strand, whereas a great GC skew on the J strand. The nucleotide composition of tRNAs on the J strand were GC and TA skews, evidently exceeding values on the N strand (Table 4). AT and GC skews of *C. cicatricosa* mt genome differ from the strand biases of metazoan mtDNA (generally positive AT skew and negative GC skew for the J strand, contrary to the N strand for most metazons).

Noncoding regions

The noncoding regions of *C. cicatricosa* mt genome contained some short intergenic spacers. These short sequences possibly acted as splicing recognition sites during the process of transcription (He et al. 2005). In the sequenced complete mt genome of the order Stylommatophora, the short intergenic spacers range from 1 bp to 65 bp (Hatzoglou et al. 1995; Terrett et al. 1995; Yamazaki et al. 1997; White et al. 2011;

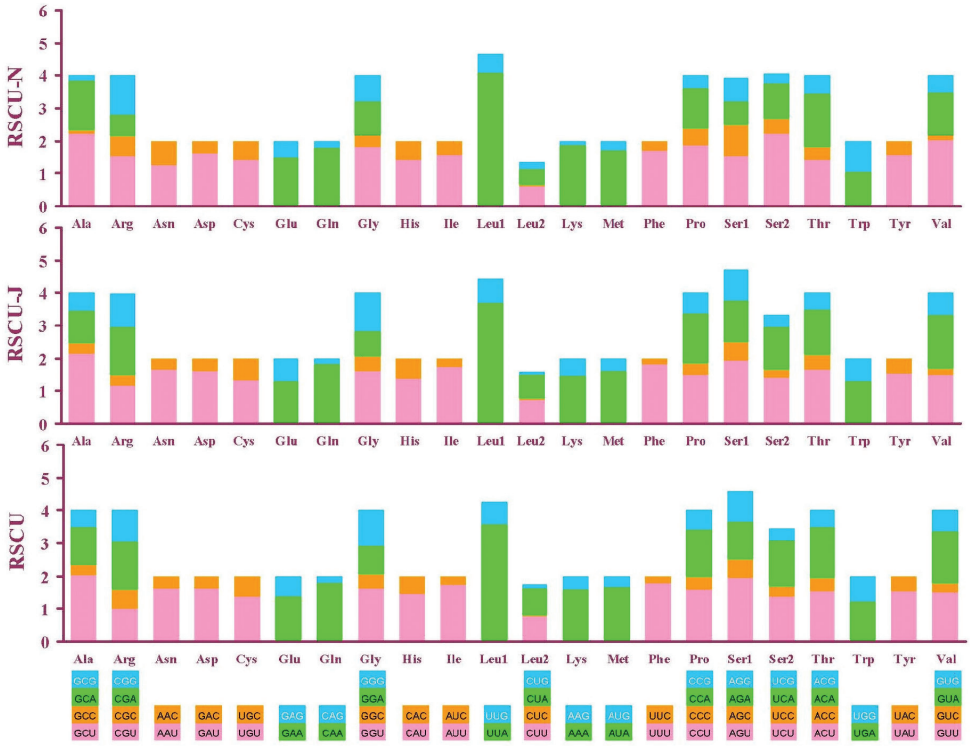

Figure 4. Relative synonymous codon usage (RSCU) in the *Camaena cicatricosa* mt genome. Codon families are provided on the x axis.

Groenenberg et al. 2012; Gaitán-Espitia et al. 2013; Deng et al. 2014) except *Achatina fulica* with 551 bp long noncoding region (He et al. 2014). However, the longest noncoding region was only 29 bp in *C. cicatricosa*. The shorter lengths of noncoding regions indicated that the mt genome of stylommatophorans are quit compact.

A large noncoding region called control region or AT-rich region is commonly seen in metazoan mt genomes (Boore 1999). In fact, variation of size for the entire mt genome can be chalked up to the presence of a number of tandem repeats (Zhang and Hewitt 1997) in control region, which may be caused by replication slippage (Levinson and Gutman 1987; Fumagalli et al. 1996). Nevertheless, putative control region (POR) was not aligned confidently in gastropods (Groenenberg et al. 2012) except *A. fulica* having a 551 bp POR between *COI* and $tRNA^{Val}$ (He et al. 2014). Other eight stylommatophoran species may possess short POR regions located adjacent to *COIII* (Hatzoglou et al. 1995; Terrett et al. 1995; Yamazaki et al. 1997; White et al. 2011; Groenenberg et al. 2012; Gaitán-Espitia et al. 2013; Deng et al. 2014). The POR regions of three helicid species and *M. kiangsinensis* were located between *COIII* and $tRNA^{Ser}$ with lengths of 158–189 bp, whereas in the other three species were located between *COIII* and $tRNA^{Ile}$ with lengths of 42–47 bp. The 29 bp noncoding region of *C. cicatricosa* was located between *COIII* and $tRNA^{Ile}$, but its length was shorter than other stylommatophorans.

Table 4. Nucleotide composition and skew of the *Camaena cicatricosa* mt genome.

	Proportion of nucleotides							
Feature	**%A**	**%T**	**%G**	**%C**	**%A+T**	**AT Skew**	**GC Skew**	**No. of nucleotides**
Whole genome	31.90	37.90	16.70	13.50	69.80	–0.09	0.11	13843
Protein coding genes	31.18	38.14	17.05	13.64	69.32	–0.10	0.11	10941
Protein coding genes (J)	28.83	40.41	17.54	13.23	69.24	–0.17	0.14	8907
Protein coding genes (N)	28.22	41.45	15.44	14.90	69.67	–0.19	0.02	2034
tRNA genes	34.95	36.46	15.81	12.78	71.41	–0.02	0.11	1322
tRNA genes (J)	33.96	36.80	17.51	11.72	70.77	–0.04	0.20	845
tRNA genes (N)	35.85	36.69	14.68	12.79	72.54	–0.01	0.07	477
rRNA genes	35.14	37.28	14.83	12.75	72.42	–0.03	0.08	1679

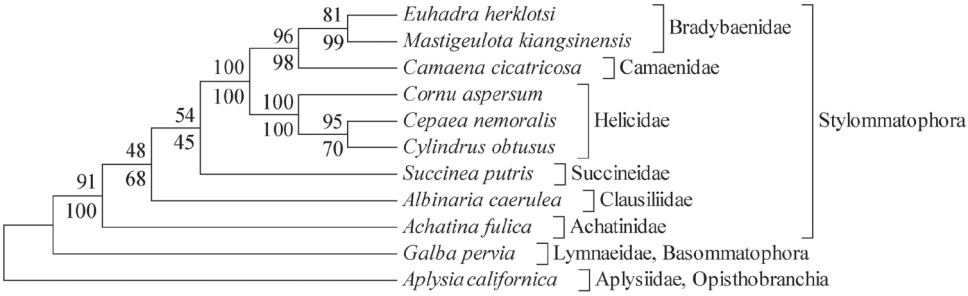

Figure 5. Phylogenetic tree inferred by maximum likelihood (ML) and maximum parsimony (MP) methods based on 13 protein genes. The tree is rooted with *Aplysia californica*. Numbers on or under the nodes represent bootstrap values of MP and ML respectively.

Phylogenetic analysis

ML tree was estimated according to the GTR+G+I substitution model selected by AIC. The ML and MP trees (Fig. 5) displayed the same topologies and presented eight major clades corresponding to the families Bradybaenidae, Camaenidae, Helicidae, Succineidae, Clausiliidae, Achatinidae, Lymnaeidae and Aplysiidae. The monophyly of Stylommatophora was approved. Species in Helicidae were sister groups and congruent with previous works (Gaitán-Espitia et al. 2013). *C. cicatricosa* and *M. kiangsinensis* from China and *E. herklotsi* from Japan are monophyletic. However, the systematics of the families Camaenidae, Helicidae and Bradybaenidae are complicated and not fully resolved. Systematic and phylogenetic studies based on analyses of morphological versus molecular markers have produced inconsistent results (Scott 1996; Cuezzo 2003; Wade et al. 2007; Hirano et al. 2014). A final assessment of the systematic relationships of the three families is pending requiring a more complete taxon sampling.

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DATA PAPER

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Distributional records of Ross Sea (Antarctica) Tanaidacea from museum samples stored in the collections of the Italian National Antarctic Museum (MNA) and the New Zealand National Institute of Water and Atmospheric Research (NIWA)

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Abstract

Here we present distributional records for Tanaidacea specimens collected during several Antarctic expeditions to the Ross Sea: the Italian PNRA expeditions (“V”, 1989/1990; “XI”, 1995/1996; “XIV”, 1998/1999; “XIX”, 2003/2004; “XXV”, 2009/2010) and the New Zealand historical (New Zealand Oceanographic Institute, NZOI, 1958-1961) and recent (“TAN0402 BIOROSS” voyage, 2004 and

"TAN0802 IPY-CAML Oceans Survey 20/20" voyage, 2008) expeditions. Tanaidaceans were obtained from bottom samples collected at depths ranging from 16 to 3543 m by using a variety of sampling gears. On the whole, this contribution reports distributional data for a total of 2953 individuals belonging to 33 genera and 50 species. All vouchers are permanently stored in the Italian National Antarctic Museum collection (MNA), Section of Genoa (Italy) and at the National Institute of Water and Atmospheric Research (NIWA Invertebrate Collection), Wellington (New Zealand).

Keywords
Antarctica, Ross Sea, Crustacea, Peracarida, Tanaidacea, MNA, NIWA

Purpose

The aim of the present study is to provide new distributional data for the tanaidaceans collected during past and recent scientific expeditions conducted in the Ross Sea, Antarctica, and now stored at the Italian National Antarctic Museum (MNA), Section of Genoa, Genoa (Italy) or at the National Institute of Water and Atmospheric Research (NIWA Invertebrate Collection), Wellington (New Zealand). The dataset is the second Italian contribution to the Antarctic Biodiversity Information Facility (ANTABIF – http://www.biodiversity.aq) based on materials stored at the Italian National Antarctic Museum (MNA), Section of Genoa, Italy. The first published MNA contribution was by Ghiglione et al. (2013).

Project details

Project title: Ross Sea Tanaidacea in the collections of the Italian National Antarctic Museum (MNA) and National Institute of Water and Atmospheric Research (NIWA)

Curator and Promoter: Stefano Schiaparelli

Personnel: Magdalena Błażewicz-Paszkowycz, Paola Piazza, Claudio Ghiglione, Maria Chiara Alvaro, Kareen Schnabel, Graham Bird, Stefano Schiaparelli

Funding: The tanaidaceans were collected during different Italian and New Zealand research expeditions to the Ross Sea funded by the Italian National Antarctic Research Program (PNRA) and the New Zealand Government, the Ministry for Primary Industries (formerly the Ministry of Fisheries) and the Ocean Survey 20/20 CAML Advisory Group, listed below:

Italian PNRA Project 3.2.1 (Oceanography) ("V" expedition, 1989/1990, R/V "*Malippo*").

Italian PNRA Project 2a and 2d.2 (Ecology and Biogeochemistry of the Southern Ocean) ("XI" expedition, 1995/1996, R/V "*Italica*").

Italian PNRA Project 2b.3 (Ecology and Biogeochemistry of the Southern Ocean) ("XIV" expedition, 1998/1999, R/V "*Malippo*").

Italian PNRA Project Program 2002/8.6 ("The costal ecosystem of Victoria Land coast: distribution and structure along the latitudinal gradient") ("XIX" expedition, 2003/2004, R/V "*Italica*" 2004).

Italian PNRA Project 2006/08.01 ("The coastal ecosystem of Terra Nova Bay" in the Latitudinal Gradient Program (LGP)) ("XXV" expedition, 2009/2010).

The Ross Sea Endeavour surveys (1958-59 and 1959-60, HMNZS "*Endeavour II*" and 1960–1961, "*Endeavour III*") conducted by the New Zealand Oceanographic Institute (NZOI, now NIWA) – founded by the New Zealand government.

New Zealand BIOROSS voyage (TAN0402, 2004, R/V "*Tangaroa*") – funded by NIWA and the New Zealand Ministry of Primary Industries (formerly the Ministry of Fisheries).

New Zealand IPY-CAML voyage (TAN0802, 2008, R/V "*Tangaroa*") – Census of Antarctic Marine Life programme – funded by the Government of New Zealand and administered by the Ocean Survey 20/20 CAML Advisor Group (Land Information New Zealand and the Ministry of Fisheries, Antarctica New Zealand, Ministry of Foreign Affairs and Trade and NIWA).

Study area descriptions/descriptor: The 2953 individuals belonging to 33 genera and 50 species of Tanaidacea were collected in the Ross Sea sector of the Southern Ocean. The bathymetric range was from 16 to 3543 m.

Design description: The data was gathered by assembling distributional records for the Ross Sea Tanaidacea species stored at the Italian National Antarctic Museum collection (MNA), Section of Genoa, Genoa (Italy) and at the National Institute of Water and Atmospheric Research (NIWA Invertebrate Collection), Wellington (New Zealand). These samples were obtained in the framework of different past research expeditions, which had different aims and geographical scopes. The earliest records are derived from the NZOI Ross Sea Oceanographic Surveys conducted during three consecutive years between 1958 and 1961 that were part of the New Zealand Antarctic Research Programme with the aim to study the hydrology, geology and biology of the Ross Sea (Bullivant and Dearborn 1967). The main purpose of the "V" (1989/1990), "XI" (1995/1996) and "XIV" (1998/1999) Italian PNRA expeditions was to investigate the distribution and structure of coastal communities in the Terra Nova Bay area. The "XIX" (2003/2004), "XXV" (2009/2010) Italian PNRA expeditions and the New Zealand TAN0402 BIOROSS voyage (2004) aimed at understanding the complex ecosystems along the Victoria Land coast under the Latitudinal Gradient Program framework (LGP, http://www.lgp.aq/). The New Zealand TAN0802 IPY-CAML voyage (2008) aimed at assessing a reference baseline in the Ross Sea, fulfilling the CAML research targets (Schiaparelli et al. 2013).

Methods

Method step description: See sampling description below and flowchart of Figure 1.

Figure 1. Flowchart depicting major steps in dataset development and publishing.

Study extent description: The Tanaidacea distributional data considered here originated from 50 sampling stations located in the Ross Sea (comprising its northern archipelagos and seamounts), between 16 and 3543 metres of depth (Fig. 2) and investigated

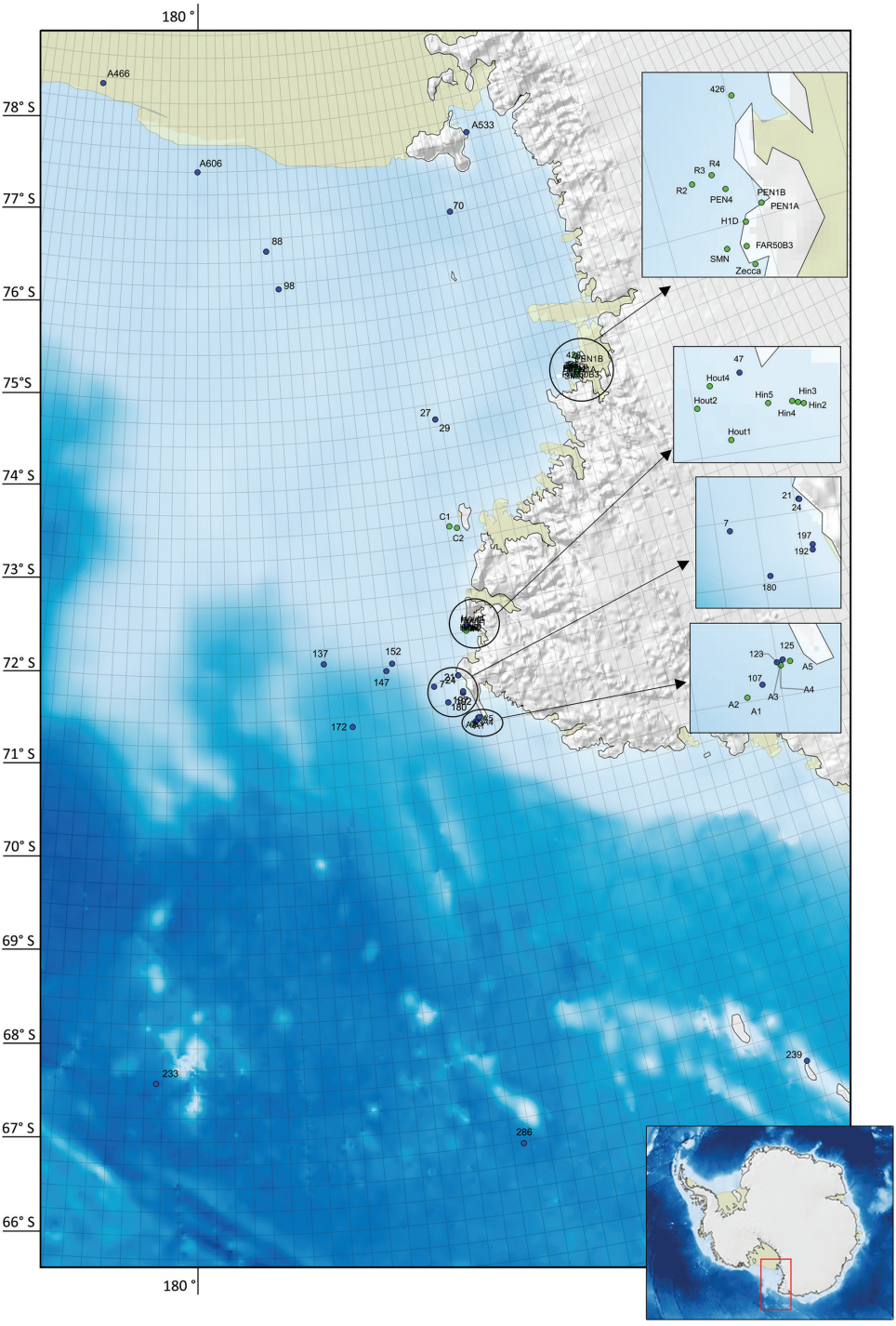

Figure 2. Map of sampling stations. Green dots: samples stored at the MNA. Blue dots: samples stored at the NIWA.

in the framework of different research expeditions from 1958 to 2010. Specifically, these were five PNRA and five New Zealand scientific voyages:

4 species (corresponding to 4 specimens) from three different New Zealand Oceanographic Institute (NZOI) Ross Sea Oceanographic Surveys (stations: A466, A533, A606)
2 species (corresponding to 17 specimens) from the "V" Italian PNRA expedition at Terra Nova Bay (station H1D) on board the R/V "*Malippo*"
2 species (corresponding to 11 specimens) from the "XI" Italian PNRA expedition at Terra Nova Bay (stations 426, PEN 1A, PEN 1B, FAR 50B3) on board the R/V "*Italica*"
1 species (corresponding to 1 specimens) from the "XIV" Italian PNRA expedition at Terra Nova Bay (station PEN 4) on board the R/V "*Malippo*"
40 species (corresponding to 2813 specimens) from the "XIX" Italian PNRA expedition along the Victoria Land Coast (Four different areas: Cape Adare with stations A1, A2, A3, A4, A5; Cape Hallett with stations Hout1, Hout2, Hout4, Hin2, Hin3; Hin4, Hin5; Coulman Island with stations C1, C2; Terra Nova Bay and Cape Russell with stations SMN, R2, R3, R4) on board the R/V "*Italica*"
7 species (corresponding to 80 specimens) from the TAN0402 BIOROSS voyage in the Ross Sea (stations: 7, 21, 24, 47, 107, 123, 125, 180, 192, 197, 239) on board the R/V "*Tangaroa*"
6 species (corresponding to 21 specimens) from the New Zealand TAN0802 IPY-CAML voyage in the Ross Sea (stations: 27, 29, 70, 88, 98, 137, 147, 152, 172, 233, 286) on board of the R/V "*Tangaroa*"
1 species (corresponding to 6 specimens) from the "XXV" Italian PNRA expedition at Terra Nova Bay (stations "zecca")

Sampling description: The material was collected in the framework of several PNRA and NIWA (formerly New Zealand Oceanographic Institute) Antarctic scientific expeditions, through the deployment of a variety of sampling gears. NIWA historical samples (NZOI surveys) were collected by using Devonport dredge, beam trawl and Agassiz trawl. Coastal sampling under the Italian National Antarctic Research Program (PNRA) ("V", "XI", "XIV" expeditions) was mainly performed by using Charcot dredge, Van Veen grabs of different sampling volume. Off-shore sampling along the Victoria Land coast under the PNRA *aegida* ("XIX" expedition) took place by using a Rauschert dredge (Rehm et al. 2006, Ghiglione et al. 2013, Błażewicz-Paszkowycz and Siciński 2014). NIWA more recent expeditions (TAN0402 BIOROSS, 2004 and TAN0802 IPY-CAML, 2008) used VanVeen grab, epibenthic sled, Orange Roughy trawl, multicorer, and hyperbenthic sled. Samples from the "XXV" PNRA expedition originated form bottom samples collected by SCUBA divers by scraping the rock in cryptic environments such as crevices and holes present along the rocky cliff of Tethys Bay ("zecca" station). In more recent cruises (from 2003 on wards), all the collected specimens were fixed on board in at least 90% ethanol and brought back to the collections. After a general sorting, all the specimens were classified to the lowest taxonomi-

cal resolution by two expert taxonomists: Magdalena Błażewicz-Paszkowycz (Department of Polar Biology and Oceanobiology, University of Łódź, Poland) and Graham Bird (Independent, Kāpiti, New Zealand). The present tanaidaceans dataset has been formatted in order to fulfil the standards (Darwin Core) required by the OBIS scheme (http://iobis.org/data/schema-and-metadata) according to the SCAR-MarBIN Data Toolkit (available at http://www.scarmarbin.be/documents/SM-FATv1.zip). The dataset was uploaded in the ANTOBIS database (the geospatial component of SCAR-MarBIN).

All vouchers are now preserved in 75% ethanol and stored at the Italian National Antarctic Museum (MNA), Section of Genoa, Genoa (Italy) and at the National Institute of Water and Atmospheric Research (NIWA Invertebrate Collection), Wellington (New Zealand). Given the proper fixation of the material for molecular studies, a barcoding survey of the Tanaidacea from the Ross Sea is planned in the next future. The dataflow illustrating sampling, storing procedures and data/metadata availability is reported in Fig. 1.

Quality control description: Specimens were classified at the lowest possible taxonomic level and only those that have been classified at least at the genus level were included in the present dataset. During all the phases of sorting, classification and storage of samples, both at the Italian National Antarctic Museum and at the NIWA Invertebrate Collection, quality controls and data cleaning have been undertaken at various steps in order to produce quality data and make consistent cross-references between the database and samples' labels (Fig. 1). Both MNA and NIWA use an SQL-based database (Specify 6) to manage their collections and link all the data (photos, sequences, etc.) to the physical samples. Georeferencing on board the R/V "*Italica*" is based on the interpolation of GPS satellite receivers (models 3S Navigation and Glonass ASHTECH GG24) and a gyrocompass. Station coordinates and sampling events were recorded during sampling activities through the "*Italica*" NetNav WEB system, which is based on the above GPS systems. On board the R/V "*Tangaroa*" a wide-area differential GPS system (models Seastar 9200 DGPS, Seastar 8200 DGPS) was used. Pre-GPS data have been reported as they appeared in original data reports (e.g. Bullivant and Dearborn 1967).

Taxonomic coverage

General taxonomic coverage description: This dataset focused on the Order Tanaidacea (Kingdom Animalia, Phylum Arthropoda, Subphylum Crustacea, Superorder Peracarida) and include a total of 2953 specimens belonging to 33 genera and 50 different species. In the Southern Ocean, the order Tanaidacea numbers 160 species (Błażewicz-Paszkowycz 2013, De Broyer et al. 2014), thus representing the second most diverse group of benthic crustaceans after Isopoda and before Amphipoda (Appeltans et al. 2012, Błażewicz-Paszkowycz et al. 2012). In the Ross Sea, before Błażewicz-Paszkowycz and Siciński (2014), only eight tanaid species were known:

seven of these reported by Sieg (1983, 1986) (*Nototanais dimorphus* (Beddard, 1886), *Andrognathia plumosa* Sieg, 1983, *Pseudoparatanais antarcticus* Sieg, 1983, *Typhlotanaoides insolitus* Sieg, 1983, *Akanthophoreus antarcticus* (Vanhöffen, 1914), *Typhlotanais greenwichensis* Shiino, 1970, *Cryptocopoides antarctica* (Vanhöffen, 1914)) and one, *Exspina typica* (Vanhöffen, 1914) reported by Alvaro et al. (2011). It is worthy to mention that the taxonomical status of *Andrognathia plumosa* has been recently questioned, as the species is only known from a male and it might represent the male of another species, namely *C. antarctica* (see Błażewicz-Paszkowycz et al. 2014 for further details). Błażewicz-Paszkowycz and Siciński (2014), studying the materials collected with a Rauschert dredge (Rehm et al. 2006), reported 40 species for the area, of which only 5 had been previously recorded in the area, 14 represented new species and the remaining species were new records for the area.

Taxonomic ranks

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Superorder: Peracarida
Order: Tanaidacea
Genera: *Akanthophoreus*, *Bathytanaissus*, *Chauliopleona*, *Collettea*, *Cryptocopoides*, *Exspina*, *Insociabilitanais*, *Leptognathia*, *Leptognathiella*, *Meromonakantha*, *Mimicarhaphura*, *Mirandotanais*, *Molotanaissus*, *Neotanais*, *Nototanais*, *Obesutanais*, *Parafilitanais*, *Paragathotanais*, *Paraleptognathia*, *Paranarthrura*, *Paratyphlotanais*, *Peraeospinosus*, *Protanaissus*, *Pseudoleptognathia*, *Pseudotanais*, *Pseudonototanais*, *Pseudoparatanais*, *Singula*, *Tanaella*, *Tanaopsis*, *Typhlotanais*, *Typhlotanaoides*, *Zeuxo*
Species: *Akanthophoreus antarcticus* (Vanhöffen, 1914); *Akanthophoreus australis* (Beddard, 1886); *Akanthophoreus multiserratus* (Hansen, 1913); *Akanthophoreus* sp.; *Bathytanaissus* sp.; *Chauliopleona nickeli* Guerrero-Kommritz, 2005; *Collettea antarctica* (Vanhöffen, 1914); *Cryptocopoides antarctica* (Vanhöffen, 1914), *Cryptocopoides* sp. A; *Exspina typica* Lang, 1968; *Insociabilitanais* sp.; *Leptognathia* sp.; *Leptognathia* aff. *microcephala* Kudinova-Pasternak, 1978; *Leptognathia* cf. *breviremoides* Sieg, 1986; *Leptognathia glandiceps* Shiino, 1978; *Leptognathia* cf. *lineata* Shiino, 1978; *Leptognathiella* sp.; *Meromonakantha* aff. *macrocephala* (Hansen, 1913); *Mimicarhaphura immanis* Sieg, 1986; *Mirandotanais vorax* Kussakin & Tzareva, 1974; *Molotanaissus makrotrichos* (Sieg, 1986); *Neotanais* sp.; *Nototanais antarcticus* (Hodgson, 1902); *Nototanais* cf. *antarcticus* (Hodgson, 1902); *Nototanais dimorphus* (Beddard, 1886); *Nototanais* cf. *dimorphus* (Beddard, 1886); *Obesutanais* sp. A; *Parafilitanais* sp. A; *Paragathotanais* sp. A; *Paraleptognathia multiserratoides* Guerrero-Kommritz, 2004; *Paranarthrura fortispina* Sieg, 1986; *Paratyphlotanais armatus* (Vanhöffen, 1914); *Peraeospinosus*

emergensis Błażewicz-Paszkowycz, 2005; *Peraeospinosus subtigaleatus* Błażewicz-Paszkowycz, 2005; *Protanaissus longidactylus* (Shiino, 1970); *Pseudoleptognathia setosa* Sieg, 1986; *Pseudonototanais* (*Pseudonototanais*) *bransfieldensis* Sieg, 1986; *Pseudoparatanais brachycephalus* Sieg, 1986; *Pseudotanais* sp. A; *Singula* sp.; *Tanaella unisetosa* Sieg, 1986; *Tanaopsis kerguelenensis* Shiino, 1978; *Typhlotanais* sp.; *Typhlotanais* sp. B; *Typhlotanais* sp. C; *Typhlotanais* aff. *mixtus* Hansen, 1913; *Typhlotanais* aff. *cornutus* Sars, 1879; *Typhlotanais greenwichensis* Shiino, 1970; *Typhlotanaoides rostralis* (Tzareva, 1982); *Zeuxo* (*Parazeuxo*) *phytalensis* Sieg, 1980.

Spatial coverage

General spatial coverage

Ross Sea, Antarctica (Figure 2)

Coordinates

PNRA V expedition: -74.748611S; 164.0875E
PNRA XI expedition: -74.7166 and -74.9065 S; 163.97467 and 164.12333E
PNRA XIV expedition: -74.78448S; 164.03895E
PNRA XIX expedition: -71.25833333 and -74.82166667S; 164.1916667 and 170.6983333E
PNRA XXV expedition: -74.69026667S; 164.10255E
NZOI expeditions: -77.5000 and -78.43330S; 166.16670 and 180.0000W
New Zealand TAN0402 BIOROSS voyage: -66.9136657 and -72.3153305S; 163.2246704 and 171.8278350E
New Zealand TAN0802 IPY-CAML voyage: -66.724000 and -76.775000S; 176.756000 and 178.828500E

Temporal coverage

PNRA V expedition (1989/1990): January 5, 1990
PNRA XI expedition (1995/1996): January 31, 1996 – February 6, 1996
PNRA XIV expedition (1998/1999): February 4, 1999
PNRA XIX expedition (2003/2004): February 9, 2004 – February 21, 2004
PNRA XXV expedition (2009/2010): December 9, 2009 – December 26, 2009
NZOI expeditions (1958/1961): January 24, 1959 – January 31, 1961
New Zealand TAN0402 BIOROSS voyage (2004): February 04, 2004 – March 04, 2004
New Zealand TAN0802 IPY-CAML voyage (2008): February 11, 2008 – March 12, 2008

Natural collections description

Parent collection identifier: Italian National Antarctic Museum (MNA Section of Genoa, Italy), and National Institute of Water and Atmospheric Research (NIWA Invertebrate Collection), Wellington (New Zealand)
Collection name: MNA (Section of Genoa) and NIWA Invertebrate Collection - Ross Sea Tanaidacea
Collection identifier: http://www.mna.it, http://niwa.co.nz/nic
Specimen preservation method: Recent material (i.e. those collected from 2003 on wards) was fixed in ethanol immediately after isolation, then sorted into morphospecies and placed in "Screw Thread Vials" (National Scientific, USA) or similar quality tubes and vials for further studies. Samples are now maintained in ethanol in the collections of the Italian National Antarctic Museum (MNA Section of Genoa, Italy) and at the National Institute of Water and Atmospheric Research (NIWA Invertebrate Collection, Wellington). Historical material (i.e. specimens collected before 2003) were generally fixed in formalin and then passed into ethanol for long-term storage. This latter group of species/specimens is therefore not usable for molecular analyses.

Datasets

Dataset description: This dataset contains data about the Phylum Arthropoda, Subphylum Crustacea and Order Tanaidacea from the Ross Sea area. Combined, it includes 50 different species corresponding to a total of 2953 specimens. The validity and synonyms of each species name were checked in WoRMS (World Register of Marine Species; http://www.marinespecies.org; last accessed on 2014-07-23). The Darwin Core elements included in the dataset are: catalogue number (i.e. MNA and NIWA catalogue number), scientific name, station, latitude (DD), longitude (DD), date of collection (year, whenever possible), time of collection (day, whenever possible), event date, gear, institution code (i.e. the name of the institution where the samples are kept), collection code (i.e. MNA and NIWA acronyms), individual counts, basis of record and status. At present, the dataset does not include GenBankID codes referred to the samples, since sequencing will be done as a future step. Sequences will also be deposited in BOLDSYSTEMS (http://www.boldsystems.org/). Images at the electron microscope (SEM) will be made available through the ANTABIF "Antarctic Field Guide" project (http://afg.biodiversity.aq/) in the next months.
Object name: MNA (Section of Genoa) and NIWA Invertebrate Collection - Ross Sea Tanaidacea
Character encoding: UTF-8
Format name: Darwin Core Archive format
Format version: 6 (latest)
Distribution: http://ipt.biodiversity.aq/resource.do?r=mna_database_tanaidacea
Language: English

Metadata language: English
License of use: This dataset [MNA (Section of Genoa) and NIWA Invertebrate Collection - Ross Sea Tanaidacea] is made available under the Open Data Commons Attribution License: http://www.opendatacommons.org/license/by/1.0
Date of metadata creation: 2014-08-08
Hierarchy level: Dataset

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RESEARCH ARTICLE

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On the identity of some weevil species described by Johann Christian Fabricius (1745–1808) in the Museum of Zoology of Copenhagen (Coleoptera, Cucujoidea, Curculionoidea, Tenebrionoidea)

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Abstract

The types of thirty-two nominal weevil species described by Johann Christian Fabricius are reviewed and lecto- and paralectotypes are designated for twenty-two of them. A neotype is designated for *Curculio sticticus* Fabricius, 1777. *Protapion varipes* (Germar, 1817) is declared a *nomen protectum* over *Curculio flavipes* Fabricius, 1775. Based on a study of syntypes, *Rhinomacer curculioides* Fabricius, 1781 is confirmed as a member of *Mycterus* (Mycteridae), *Bruchus undatus* Fabricius, 1787 is tentatively transferred to Erotylidae, *Curculio fulvirostris* Fabricius, 1787 and *Anthribus roboris* Fabricius, 1798 are confirmed as members of *Salpingus* (Salpingidae), and *Brachycerus cristatus* Fabricius, 1798 is transferred to Tenebrionidae. Based on lectotype designation, *Curculio caninus* Fabricius, 1792 is confirmed as a synonym of *Sitona lineatus* (Linnaeus, 1758) and *Curculio innocuus* Fabricius, 1802 as a synonym of *Cneorhinus barcelonicus* (Herbst, 1797). *Bruchus rufipes* Fabricius, 1792 is not considered an available species name, but a later use of *Bruchus rufipes* Olivier, 1790. *Cossonus incisus* Pascoe, 1885 is reinstated as valid from synonymy under *Cossonus illigeri* Champion, 1909 and *Cossonus vulneratus* Illiger, 1805 from synonymy under *Cossonus canaliculatus* (Fabricius, 1792) (a primary homonym of *Curculio canaliculatus* Olivier, 1791). *Cossonus canaliculatus* Fabricius, 1802 is a secondary homonym of the former and is replaced with *Cossonus incisus*. *Salpingus fulvirostris* (Fabricius, 1787) is reinstated as valid from synonymy under *Salpingus planirostris* (Fabricius, 1787), a primary homonym of *Curculio planirostris* Piller & Mitterpacher, 1783. The following new combinations are proposed: *Brachysomus erinaceus* (Fabricius, 1802) (from *Curculio*), *Bronchus ferus*

(Gyllenhal, 1840) (from *Hipporhinus*), *Bronchus glandifer* (Fabricius, 1792) (from *Curculio*), *Bronchus nivosus* (Sparrman, 1785) (from *Curculio*), *Bronchus sparrmani* (Gyllenhal, 1833) (from *Hipporhinus*), *Coelocephalapion atrirostre* (Fabricius, 1802) (from *Attelabus*), *Nerthops sticticus* (Fabricius, 1777) (from *Curculio*), *Piezotrachelus crotalariae* (Fabricius, 1802) (from *Attelabus*), and *Poropterus granulatus* (Fabricius, 1802) (from *Curculio*). The junior homonym *Brachycerus uva* Fabricius, 1792 (non Sparrman, 1785) is replaced by *Brachycerus fabricii* **nom. n.** The following new synonymies are established: *Brachycerus obesus* (Fabricius, 1775) = *Curculio scalaris* Fabricius, 1777, **syn. n.**, *Brachyderes lusitanicus* (Fabricius, 1781) = *Curculio moratus* Fabricius, 1798, **syn. n.**, *Brachypera* (*Brachypera*) *crinita* (Boheman, 1834) = *Curculio striatus* Fabricius, 1787, **syn. n.**, *Brachysomus erinaceus* (Fabricius, 1802) = *Brachysomus villosulus* (Germar, 1824), **syn. n.**, *Bronchus abruptecostatus* (Gyllenhal, 1833) = *Curculio spectrum* Fabricius, 1802, **syn. n.**, *Bronchus nivosus* (Sparrman, 1785) = *Curculio recurvus* Fabricius, 1802, **syn. n.**, *Camptorhinus tibialis* (Sparrman, 1785) = *Rhynchaenus alienatus* Fabricius, 1802, **syn. n.**, *Coelocephalapion atrirostre* (Fabricius, 1802) = *Coelocephalapion luteirostre* (Gerstäcker, 1854), **syn. n.**, *Cyrtoderes cristatus* (DeGeer, 1778) (Tenebrionidae) = *Brachycerus cristatus* Fabricius, 1798, **syn. n.**, *Desmidophorus hebes* (Fabricius, 1781) = *Curculio tuberculatus* Fabricius, 1792, **syn. n.**, *Donus salviae* (Schrank, 1789) = *Curculio denticornis* Fabricius, 1798, **syn. n.**, *Exomias holosericeus* (Fabricius, 1802) = *Exomias chevrolati* (Boheman, 1842), **syn. n.**, *Nerthops sticticus* (Fabricius, 1777) = *Nerthops guttatus* (Olivier, 1807), **syn. n.**, *Phyllobius oblongus* (Linnaeus, 1758) = *Curculio mali* Fabricius, 1782, **syn. n.**, and *Rhinocyllus conicus* (Froelich, 1792) = *Bruchus punctatus* Fabricius, 1798, **syn. n.** *Bronchus synthesys* **sp. n.** is described to represent the concept of *Hipporhinus spectrum* sensu Marshall, 1904, a misidentification.

Keywords

Weevils, Attelabidae, Brentidae, Curculionidae, Erotylidae, Mycteridae, Salpingidae, Tenebrionidae, *Bruchela*, *Chlorophanus*, *Holotrichapion*, *Temnocerus*, Johann Christian Fabricius, new species, new combinations, new synonymies, morphology, systematics

Introduction

Johann Christian Fabricius (1745–1808), also known as the "Prince of Entomology", was the most prominent entomologist of his time. His authority was enormous and so highly influential that later authors used to cite him as the author of others' species (e.g., Linnaeus' species). This favoured the establishment of the so-called "principle of authority", which endangered the universality required by the scientific naming of animals and which was fought by Hugh E. Strickland and others; eventually this reaction led to the creation of the International Commission on Zoological Nomenclature in 1895 and the adoption of an International Code of Zoological Nomenclature since 1905 (as the *Règles* in the beginning) (Melville 1995). Fabricius was a prolific describer for the standards of his time. He published 696 available species names and 302 new combinations (mostly with genera of his own). Some of these are still in their original combination and have never been reviewed.

In recent times, it has become clear that some kind of catalogue of the living beings on Earth should be prepared, not only for primary scientific use but also as a powerful tool for conservation management, pest control, etc. (Wilson 2003). Several initiatives are working towards this goal (e.g., Species 2000, GBIF, Encyclopedia of Life) even if

the dispersal of energies and funds is regrettable. In the framework of the two first of these mentioned initiatives, Christopher H. C. Lyal (Natural History Museum, London) and I united efforts towards the creation of an electronic catalogue of all taxa of Coleoptera Curculionoidea (the Electronic Catalogue of Weevil names), now dubbed "WTaxa" and reachable at URL http://wtaxa.csic.es. This database is still being built and improved, containing now some 167000 names, including those of fossils, and deemed to contain almost all names published by 31st December 2012.

The database was originally compiled from secondary sources and we are currently checking the original sources, a task not without its surprises as we found several cases of species absent from the currently cited original source, species available from the original source but never recorded later, and species that at a given moment in the story of entomology disappeared from the catalogues. The aim of the present paper is to clarify the identity of several of these "lost species" published by J.C. Fabricius by examination of their type specimens. These species are either not recorded in the Junk-Schenkling Coleopterorum Catalogus or are recorded with uncertainty about their identity (usually by the use of a question mark or a placement *incertae sedis*). This list is not complete, as several problems compound the location and identification of syntypes, and Fabrician type specimens in British collections have been studied only fragmentarily. In one case the discovery of a Fabrician type was communicated to an appropriate specialist and he proceeded to a proper identification (Prena 2005). In a few cases, Apionidae and Nanophyidae specimens were also checked to confirm their identity, as this was required for the first volume of Curculionoidea of the Catalogue of Palaearctic Coleoptera (Alonso-Zarazaga 2011b,c). The study of two of the species was advanced because of coincident interests (Caldara et al. 2012).

Materials and methods

The holding of Fabricius' specimens in Copenhagen is, for historical reasons, divided into two different collections, although these are now placed one after the other in the same drawer, following the page order of the Systema Eleutheratorum. The "Kiel collection" is on permanent loan, and the "Copenhagen collection" is basically the Sehestedt & Tønder Lund collection. Zimsen (1964) provided more information on this aspect.

In this paper, 32 species are studied, based primarily on specimens located at the Museum of Zoology of the University of Copenhagen and the results published to be incorporated to WTaxa. Lectotypifications are made when considered necessary to fix the concept of a nominal species. I have extensively used the information given by Zimsen (1964), although in some cases this was found to be inexact or incomplete. I have also included isolated comments in square brackets, when needed. Any author revising a genus containing Fabrician species should verify that the type specimens correspond to the current concept of these species. This may not hold in all cases, as it has happened in the genus *Bronchus* Germar, 1817.

The treatment of every species includes the original reference, the original statement of locality or other data related to type specimens, comments and a summary of the present status of the species. Label lines are separated by a slash (/). Dates provided are those recorded in WTaxa, which may differ from dates given in other works.

Specimens were photographed with an Olympus C7070WZ camera mounted on a photographic frame Kaiser RA1. Extended focus images were generated using the software CombineZP. The programs Adobe Illustrator CS5.0 and Adobe Photoshop CS5.0 were used for image postproduction and mounting.

Results

Potentially valid names

Curculio flavipes Fabricius, 1775

Curculio flavipes Fabricius, 1775: 133.

Location. Habitat frequens primo vere locis apricis calidioribus.

Comments. This nominal species has been currently placed in synonymy of *Apion varipes* Germar, 1817 or another member of genus *Protapion* Schilsky, 1908. Schilsky (1901, 1902) studied the single specimen and identified it as *A. varipes*. However, he failed to give precedence to the Fabrician name, perhaps in the erroneous belief that *Curculio flavipes* DeGeer, 1775 (now in *Polydrusus*) has precedence. For the time being, it has been impossible to date DeGeer's (1775) work and a date of 31st December 1775 is to be assumed for it (Art. 21.3.2), whereas Fabricius's (1775) tome has been dated as of 30th April 1775 (Evenhuis 1997). However, the nominal species *Apion varipes* has been in constant use since its original publication (while the Fabrician one has been considered in synonymy of different species), and I therefore reverse their precedence under Arts. 23.9.1 and 23.9.2 of the Code.

Zimsen (1964) recorded the presence of one specimen in the "Kiel collection". It carries an identification label as *Apion varipes* Germ. by Schilsky, who mentioned the characteristic apically curved front tibiae of the males of this species. The specimen is in a very poor state now, lacking the head, pronotum and elytra. Examination of the remaining parts supports the identification made by Schilsky, which is here considered correct because of the coloration of the mid and hind trochanters and tibiae, and the apex of penis not recurved in side view and slightly rounded-subtruncate in dorsal view. I designate this specimen as the lectotype and have added a white label with red margins and black writing: LECTOTYPUS / *Curculio flavipes* F. / Alonso-Zarazaga des. / 2014.

Present status. A *nomen oblitum* and a synonym of *Protapion varipes* (Germar, 1817), *nomen protectum*. The reversal of precedence is here made in accordance with Art. 23.9.2 by stating that, to my knowledge, *Curculio flavipes* Fabricius, 1775 (and its combinations) meet the requirements of Art. 23.9.1.1 and *Apion varipes* Germar, 1817

(and its combinations) meet those of Art. 23.9.1.2, quoting the following references: Abbazzi and Maggini 2009; Alonso-Zarazaga 1990; Alonso-Zarazaga 2011b; Braunert 2006; Cholokava 2008; Giovanleonardo and Osella 2001; Gønget 1997; Gurrea Sanz and Pérez Barroeta 1994; Hayat et al. 2002; Heijerman and Alders 2010; Khrolinskij 1965; Kocs 2010; Korotyaev et al. 1993; Mazur 2002; Merkl 2008; Morris 2003; Podlussány 1996; Scherf 1964; Schneider and Gruschwitz 2004; Sergeev 1977; Silfverberg 2004; Solodovnikova 1969; Ter-Minasian 1972; Wanat 2001; Zimsen 1964.

***Curculio sticticus* Fabricius, 1777**

Curculio sticticus Fabricius, 1777: 227

Location. Habitat ad Cap. B. Spei.

Comments. This nominal species has been treated subsequently by Fabricius (1781: 191), Olivier (1791: 541), Fabricius (1792: 473, repeating the original description), Herbst (1795: 506), Fabricius (1802: 531) and Thunberg (1813: 382).

Zimsen (1964) stated the specimens are missing, which I can corroborate. However, in the ZMUC General Collection there is an old specimen labelled *Curculio sticticus*, which is not a type and belongs to *Nerthops guttatus* (Olivier, 1807), a most plausible identification, as it could have been compared to some syntype. I hereby designate this specimen as the neotype of this species and have added a handwritten red label: NEOTYPUS / Curculio / sticticus F. / Alonso-Z. 2008. The specimen is pinned through the fore half of the right elytron by a very fine needle, lacks the left fore leg from the middle of the femur and the whole left middle leg, but otherwise it is in a good state of preservation. It carries an old, whitish handwritten label: ff. [H?] stictica (underlined) / Cap. b. Spei. It is a small female, ca. 4 mm in length, with well separated dorsal patches, contrary to the original description, which mentions a pre-apical fascia, formed by the conjunction of several patches, as can be seen in some other specimens of this species. This neotypification is made here to fix the uncertainty about the use of *C. sticticus*, for taxonomic purposes (Art. 75.1).

Present status. This species is here accordingly transferred to the genus *Nerthops* Schoenherr, 1826, as *Nerthops sticticus* (Fabricius, 1777), comb. n. *Nerthops guttatus* (Olivier, 1807) is a new synonym of the former. The latter name has been used in the last 50 years only in a few catalogues, and does not qualify as a *nomen protectum* under Art. 23.9.2.

***Curculio scalaris* Fabricius, 1777**

Curculio scalaris Fabricius, 1777: 228

Location. Habitat ad Cap. Bon. Spei. Dr. Schulz.

Comments. This nominal species was subsequently mentioned by Olivier (1790: 183) and by Herbst (1797: 87). Haaf (1957b: 552) treated *Brachycerus scalaris* (Fabricius) as a species unknown to him, while stating correctly that the types are in the Museum of Copenhagen. He cited Olivier's treatment of *Brachycerus scalaris* as a misidentification in synonymy of *B. obesus* (Fabricius, 1775), following Schoenherr's (1833b: 391) opinion.

I have found two syntypes in the "Kiel collection", thus corroborating Zimsen's (1964) statement. One carries a red label with TYPE printed on it, and a white label, partly printed and partly handwritten: Brachycerus / obesus F. / det. E. Haaf 1957. The other has a similar white label, only the determination date is 1960. I have compared them again with the single type specimen of *Curculio obesus* Fabricius, 1775 and found all three to be conspecific. The single type specimen of *C. obesus* also carries an identification label by Haaf dated 1960. It seems that Haaf checked these specimens after the publication of his monograph, but he never published any correction, as far as I know. I designate the first mentioned specimen as the lectotype and have added a white label with red margins and black writing: LECTOTYPUS / *Curculio scalaris* F. / Alonso-Zarazaga des. / 2014. The second specimen is a paralectotype and has a similar label.

Present status. This species is a new synonym of *Brachycerus obesus* (Fabricius, 1775). Olivier's treatment is neither a different taxon nor a misidentification, just a transfer of the Fabrician species to the genus *Brachycerus* Olivier, 1789.

Rhinomacer curculioides Fabricius, 1781

Rhinomacer curculioides Fabricius, 1781: 199

Location. Habitat in Italia. Dr. Allioni.

Comments. This nominal species was later treated by Fabricius (1802: 428), who established the synonymy with *Mycterus griseus* [Clairville], 1798, and *Curculio rhinomacer* Paykull, 1792.

Zimsen (1964) reported four specimens in the "Kiel collection" and one in the "Copenhagen collection". I have been able to study all five, which are to be considered syntypes. The "Copenhagen collection" specimen carries a label: Fabricius ded[it] / Mus. S. & T. L. / Rhinomacer / curculiono_ / des Fabr. All these specimens belong to the genus *Mycterus* [Clairville], 1798. Lectotypification should be made by a specialist in the group.

Present status. This is the species currently known as *Mycterus curculioides* (Fabricius, 1781) (Mycteridae).

Curculio mali Fabricius, 1782

Curculio mali Fabricius, 1782: 499

Location. Habitat Lipsiae. Dom. Prof. Leske.

Comments. This nominal species was subsequently treated by Fabricius (1792: 487), who indicated a synonymy with *Curculio padi* Bonsdorff, 1785 (a synonym of *Phyllobius pyri* (Linnaeus, 1758)) and by Herbst (1795: 261), Fabricius (1802: 542) and Olivier (1807: 415). Billberg (1820: 45) transferred it to the genus *Polydrusus*.

Zimsen (1964) reported the presence of two specimens in the "Kiel collection", which I have studied. One belongs to *Polydrusus* (*Metallites*) *marginatus* Stephens, 1831, but it has a more modern green label with this correct name and does not match the original description, thus it is here deemed not to belong to the type series. The other belongs to *Phyllobius oblongus* (Linnaeus, 1758) (phenotype with dark elytra: ab. *floricola* Herbst, 1784) and matches the description, and I here designate it as the lectotype and have added a handwritten red label: LECTOTYPUS / Curculio / mali F./ Alonso-Z. 2008.

Present status. *Curculio mali* is a new synonym of *Phyllobius oblongus* (Linnaeus, 1758).

Bruchus undatus Fabricius, 1787

Bruchus undatus Fabricius, 1787a: 41

Location. Habitat in Africae floribus Dom. Vahl.

Comments. The description reads: "B. niger elytris fuscis: strigis undatis albis. Corpus medium, nigrum, immaculatum. Elytra laevia, fusca strigis tribus aut quatuor undatis fuscis." Manuel (1797: 606) placed the species in the genus *Macrocephalus* Olivier, 1789 (Anthribidae), an invalid homonym, currently in synonymy of *Platystomos* Schneider, 1791.

Zimsen (1964) reported the presence of one specimen in the "Copenhagen collection" and of another in the "Kiel collection". I have checked both. They each carry a label with the name *Tritoma undatum* and they are neither chrysomeloids nor curculionoids. The placement suggested by the labels seems to be a good approximation.

Present status. Apparently a member of Cucujoidea, probably in Erotylidae, that should be studied by a specialist. The species is not included in the recent volumes of the Catalogue of Palaearctic Coleoptera.

Curculio flavescens Fabricius, 1787

Curculio flavescens Fabricius, 1787a: 112

Location. Habitat in America meridionali Dom. Schreber [error, Wibmer and O'Brien 1986: 22]. A Western Palaearctic species.

Comments. This nominal species was later discussed by Olivier (1791: 528), Fabricius (1792: 454), Herbst (1795: 135) and Fabricius (1802: 512). Billberg (1820: 45) transferred it to *Brachyrhinus* and Schoenherr (1826: 54) to *Chlorophanus*, adopting,

however, Herbst as author of the species. Schoenherr (in Ménétriés 1832: 214) described *Chlorophanus graminicola* and placed *Curculio flavescens* Herbst as a synonym of it, a fact reflected in the treatment given to the species by Günther and Zumpt (1933: 70). Thus the synonymy has been known for a long time.

Zimsen (1964) recorded one specimen in the "Kiel collection", which is a male of the species usually known as *Chlorophanus graminicola*, pinned, in a good general condition, but lacking the fore right tarsus, the onychium of the mid right tarsus and that of the hind left tarsus. I here designate it as the lectotype and have placed a red handwritten label: LECTOTYPUS / Curculio / flavescens F./ Alonso-Z. 2008, on its pin.

Present status. A senior synonym of *Chlorophanus graminicola* Schoenherr, 1832. The nominal species *Chlorophanus graminicola* Gyllenhal, 1834 is a homonym and synonym of the former (synonymy by Schoenherr 1840b: 429). To my knowledge, Schoenherr's name does not meet the requirements of Art. 23.9.2 to be declared a *nomen protectum*, so the correct name is *Chlorophanus flavescens* (Fabricius, 1787). This name has already been used as valid by Ren et al. (2013).

Curculio fulvirostris Fabricius, 1787

Curculio fulvirostris Fabricius, 1787b: 381

Location. Habitat in Scania Dom. de Paykull.

Comments. This nominal species was later treated by Fabricius (1792: 377) as a synonym of his *Curculio planirostris* Fabricius, 1787, when he transferred the latter to the genus *Anthribus*. Paykull (1800: 167) transferred *C. fulvirostris* to the genus *Anthribus*, and synonymized *A. planirostris* under it.

Zimsen (1964) did not mention any type specimen and I have been unable to identify any either, probably because they are merged with the type material of *Curculio planirostris* Fabricius, 1787. This name is an invalid primary homonym of *Curculio planirostris* Piller & Mitterpacher, 1783 [a synonym of *Tropideres albirostris* (Schaller, 1783)].

Present status. A species belonging to Salpingidae. *Salpingus planirostris*, being an invalid primary homonym, has been incorrectly used as valid by Pollock and Löbl (2008). The correct name for this species is *Salpingus fulvirostris* (Fabricius, 1787).

Bruchus rufipes Fabricius, 1792

Bruchus rufipes Fabricius, 1792: 373

Location. Habitat Parisiis. Mus. Dom. Bosc.

Comments. The original description reads "*Bruchus rufipes* Oliv. Ins. tab. fig.", so there is at least some doubt whether this is to be considered a different nominal species from *Bruchus rufipes* Olivier, 1790. This corroborates my suspicion that the plates of

Olivier's *Entomologie* (at least those for the weevils) were available with names before 1792 and used by Fabricius. The Fabrician "species" was synonymized by Germar (1819: 119) with *Anthribus sericeus* Fabricius, 1802 and placed in *Bruchela* by Dejean (1821: 78).

Zimsen (1964) did not treat this "species", supporting my assessment that it is to be treated just as a later use of Olivier's. Wolfrum (1929) also treated it as such.

Present status. A later use of *Bruchus rufipes* Olivier, 1790 (now in *Bruchela* Dejean, 1821), not a different nominal species.

Brachycerus uva Fabricius, 1792

Brachycerus uva Fabricius, 1792: 383

Location. Habitat ad Cap. Bon. Spei Dom. de Paykull.

Comments. This nominal species was later treated by Herbst (1797: 86), Fabricius (1802: 416), Thunberg (1813: 397) (who synonymized it with his *Brachycerus uva* Thunberg, 1799), and Billberg (1820: 39). Schoenherr (1833: 402) treated all the descriptions and previous uses of *B. uva* and *Curculio uva* Sparrman, 1785 as a single entity, with a description by Gyllenhal. However, Schoenherr (1840a: 673) later determined that his previous treatment of *B. uva* and its former synonyms involved different species and he named his own 1833 species as *Brachycerus racemus* Gyllenhal, 1840 and the Thunbergian species as *Brachycerus labrusca* Gyllenhal, 1840, keeping the name *B. uva* for the Fabrician species, which included the synonym *C. uva* Sparrman. However, Haaf (1957a: 119) did not consider Fabricius' species a different nominal species from that of Sparrman, even if in the original description the latter is not mentioned as the source for either the name or the material, which was credited to Paykull.

Zimsen (1964) mentioned one specimen being in the "Kiel collection". This specimen is a black, small (ca. 7.5 mm) *Brachycerus* that cannot be identified with any of the species included in Haaf's (1957b) keys. It is close to the group of species related to *Brachycerus uva* (Sparrman, 1785) by the characters of the head and rostrum, however it differs from the species in this group by the absence of the characteristic patches of elongate yellow scales. In addition, it differs from *B. uva* (Sparrman) by the interstriae 3 and 5 being more prominently tuberculate than the others, which have almost obsolete tubercles, and the outer distal angle of fore and mid tibiae strongly prominent, knife-like. I have compared this specimen with representatives of the other species in the Copenhagen museum collection. I here designate this specimen as the lectotype and have placed a red, handwritten label: LECTOTYPUS / Brachycerus / uva F. / Alonso-Z. 2008, on its pin.

Present status. Being apparently a valid species, the name *Brachycerus uva* Fabricius, 1792 is a **new homonym** of that of Sparrman's species, and is here replaced with *Brachycerus fabricii* Alonso-Zarazaga, nom. n.

Curculio caninus Fabricius, 1792

Curculio caninus Fabricius, 1792: 467

Location. Habitat in Germania Dom. Smidt.

Comments. This nominal species was subsequently treated by Herbst (1795: 496), Paykull (1800: 308) (who considered it to be a 'variety' of *Curculio lineatus* Linnaeus, 1758), Fabricius (1802: 524), Germar (1824: 416) (also as a 'variety' of *Sitona lineatus*) and Schoenherr (1826: 135) (who combined it with the genus *Sitona* as a valid species). However, Schoenherr (1834a: 110) placed it as a 'variety' of *Sitona lineatus* and Emden and Emden (1939) placed it in synonymy of *Sitona flavescens* (Marsham, 1802), with doubt.

According to Zimsen (1964) there are four specimens in the "Kiel collection", which I have studied. Three of these belong to *S. lineatus* and one to *S. obsoletus* (Gmelin, 1790). Of the three *S. lineatus* specimens, one has a rather uniform yellowish brown, non-banded scaling and is here designated as the lectotype, as it closely matches the original description, except for the antennae not being black (an illusory character, as it often occurs in Fabricius' descriptions), which in any case no other specimen has. The two other specimens are heavily banded and do not match the original description, and they are here deemed not to belong to the type series. The specimen of *S. obsoletus* has also a similar yellowish brown, non-banded scaly pattern and is considered to be a paralectotype. I have added handwritten red labels to each specimen as follows: lectotype, LECTOTYPUS / Curculio / caninus F./ Alonso-Z. 2008; paralectotype, similar, except for PARALECTOTYPUS. I have also added white identification labels.

Present status. The lectotype designation is made to avoid nomenclatural changes. Thus, this nominal species is confirmed as a synonym of *Sitona lineatus* (Linnaeus, 1758), as it has been recently treated by Velázquez de Castro (2013).

Curculio glandifer Fabricius, 1792

Figs 1–3

Curculio glandifer Fabricius, 1792: 483

Location. Habitat ad Cap. Bon. spei Mus. Dom. Lund.

Comments. This nominal species was subsequently treated by Herbst (1795: 511), Fabricius (1802: 537), Olivier (1807: 390), Thunberg (1813: 388) and Billberg (1820: 45), the last transferring it to his genus *Hipporhis*. Schoenherr (1833: 469) synonymized this species with *Hipporhinus spiculosus* Gyllenhal, 1833, with doubt. This doubtful placement was adopted by Schenkling and Marshall (1929).

Zimsen (1964) mentioned one specimen in the "Copenhagen collection". I have studied it (ZMUC 00022544) and found it to be a male *Bronchus* pinned with a long, thin pin, in rather good state but lacking part of the right antenna and of the left front

Figures 1–6. *Curculio glandifer* Fabricius lectotype **1** Dorsal view **2** Lateral view **3** Labels *Rhynchaenus granulatus* Fabricius lectotype **4** Labels **5** Lateral view **6** Dorsal view.

leg, and the whole left hind leg, and smeared with glue apparently used to stick the pronotum to the elytra. It carries a small, green, square label, a red label with TYPE printed and a handwritten label: Cap. bon. sp. / Mus. S. & T. L. / Glandifer / F. The specimen is similar to *B. ferus* (Gyllenhal, 1840) (comb. n.) using Marshall's (1904) key, but it differs from this species by the more elongate body outline, the oblong pronotum with fewer and larger tubercles, the elytral tubercles being devoid of scales on the apical half and well separated, not fused by the bases into a continuous crest, those on interstria 2 starting level with front margin of the white band or a little behind it, the fore tibiae curved at the apical third, the rostrum with the lateral keels low, rounded, and the epistome continuous with the dorsum of rostrum, not separated from it by a V-shaped sulcus. I have used for comparison specimens of *B. ferus* identified by Marshall in the NHM collection. I designate this specimen as the lectotype and have

added a white label with red margins and black writing: LECTOTYPUS / *Curculio glandifer* F. / Alonso-Zarazaga des. / 2014.

Present status. I consider that this is a valid species, *Bronchus glandifer* (Fabricius, 1792), comb. n., for the time being, until a modern revision may reveal its true affinities or identity.

Bruchus punctatus Fabricius, 1798

Bruchus punctatus Fabricius, 1798: 158

Location. Habitat - - -.

Comments. This species was subsequently treated only by Fabricius (1802: 397) and Schoenherr (1835: 153), who synonymized it with *Nerthops guttatus*, with doubt.

Zimsen (1964) reported the presence of two specimens in the "Kiel collection", which I have been able to study. Both belong to the common species *Rhinocyllus conicus* (Froelich, 1792). I have selected the small one (apparently a male) as the lectotype and added the following handwritten red label to its pin: LECTOTYPUS / Bruchus / punctatus F./ Alonso-Z. 2008, and the larger one (apparently a female) as paralectotype, with a similar label except for the word PARALECTOTYPUS. They carry also white identification labels.

Present status. This is a new synonym of *Rhinocyllus conicus* (Froelich, 1792), and is removed from synonymy under *Nerthops guttatus* (Olivier, 1807) [erroneously as *guttula* in Klima (1935)]. Alonso-Zarazaga and Lyal (1999: 80) used *Bruchus punctatus* as the valid name for the type species of *Nerthops*, following the synonymy given in Klima (1935), but this is incorrect. See the treatment of *Curculio sticticus* above for more information.

Anthribus roboris Fabricius, 1798

Anthribus roboris Fabricius, 1798: 161

Location. Habitat in Kiliae Robore.

Comments. In the original description, Fabricius (1798: 161) pointed out that this was the same as *Attelabus ruficollis* Linnnaeus *sensu* Herbst, 1784, which is the case.

Zimsen (1964) reported a single specimen in the "Kiel collection", which I have studied. It is a rather immature specimen of *Salpingus ruficollis* (Linnaeus, 1761). Lectotypification should be made by a specialist in the group.

Present status. This nominal species is a synonym of *Salpingus ruficollis* (Linnaeus, 1761) in Salpingidae. The current synonymy (cf. Pollock and Löbl 2008) is thus confirmed.

***Brachycerus cristatus* Fabricius, 1798**

Brachycerus cristatus Fabricius, 1798: 161

Location. Habitat ad Cap. Bon. spei Mus. Dom. Lund.

Comments. This species was subsequently mentioned by Thunberg (1813: 399) and Schoenherr (1833b: 441), who transferred it to the genus *Sepidium* Fabricius, 1775 and listed as its synonyms *Tenebrio cristatus* DeGeer, 1778, *Brachycerus areolatus* Thunberg, 1799 and *Sepidium lacunosum* Thunberg, 1787.

Zimsen (1964) mentioned one specimen in the "Copenhagen collection". I have checked it and, pending confirmation by a specialist in Tenebrionidae, consider it to be a syntype, and the nominal species to be an overlooked synonym of *Cyrtoderes cristatus* (DeGeer, 1778). This species is not recorded in the Coleopterorum Catalogus [Gebien (1910) did not include the name *Cyrtoderes* but did include as valid the genus *Phligra* Laporte, 1840, a junior synonym of *Cyrtoderes* Dejean, 1834 (Bousquet and Bouchard 2013); if present the species name *cristatus* would have been included under this genus].

Present status. A **new synonym** and secondary homonym of *Cyrtoderes cristatus* (DeGeer, 1778) (Tenebrionidae).

***Attelabus coeruleus* Fabricius, 1798**

Attelabus coeruleus Fabricius, 1798: 163

Location. Habitat in Germania Dom. Daldorff.

Comments. This nominal species was later treated by Schoenherr (1833a: 232) as a synonym of *Rhynchites pauxillus* Germar, 1824. Dalla Torre and Voss (1937) placed it as a synonym of *Pselaphorhynchites tomentosus* (Gyllenhal, 1839). Legalov (2002) transferred the species to the genus *Temnocerus* Thunberg, 1815, without explanation.

Zimsen (1964) mentioned one specimen in the "Kiel collection". I have studied what remains of it, identified as a *Temnocerus* and designated as lectotype by Legalov (2007: 124); it lacks the head and pronotum, left elytron, apical half of the right elytron and the hind left leg. The remains do not allow verification of the identification made by Legalov, he did not state whether he saw the whole specimen or just these remains.

Present status. Considered by Alonso-Zarazaga (2011a) as a species in the genus *Temnocerus*, *T. coeruleus* (Fabricius, 1798) (*fide* Legalov). However, the species is not recognisable from its lectotype and a neotype should be designated if considered necessary.

Curculio moratus Fabricius, 1798

Curculio moratus Fabricius, 1798: 171

Location. Habitat in Isle de France D. Billardiere

Comments. Later mentioned only by Fabricius (1802: 511).

Zimsen (1964) mentioned one specimen in the "Kiel collection", which I have studied. It is a male of *Brachyderes lusitanicus* (Fabricius, 1781) in a very good state of preservation, pinned with a minuten pin and lacking the right antenna and the onychia of the right fore and hind tarsi. This specimen closely matches the original description, and I have selected it as the lectotype and added a red, handwritten label: LECTOTYPUS / Curculio / moratus F./ Alonso-Z. 2008 to its pin. Evidently the type locality given is a mistake, the species occurring in Spain, Portugal and western France.

Present status. *Curculio moratus* is a new synonym of *Brachyderes lusitanicus* (Fabricius, 1781).

Curculio denticornis Fabricius, 1798

Curculio denticornis Fabricius, 1798: 173

Location. Habitat ad Cap. Bon. spei Dom. Daldorff.

Comments. This nominal species has been later treated by Fabricius (1802: 539), who modified the provenance as: "Habitat in India orientali. Dom. Daldorff."

Zimsen (1964) reported one specimen in the "Kiel collection". This specimen (ZMUC 00022538) matches the original description and belongs in Hyperinae. It is pinned with a short, moderately thick, headless, somewhat bent pin to a piece of white plastic, which in turn is pinned with a long pin. The apical projection of the pedicel that gives its name to this species is an illusion. I have selected this specimen as the lectotype and placed the following red, handwritten label on its pin: LECTOTYPUS / Curculio / denticornis F./ Alonso-Z. 2008. It belongs to the genus *Donus* Jekel and is a normal specimen of *D. salviae* (Schrank, 1789). The original locality and the subsequent correction have to be erroneous, as this is a Southern European species and not known to have been artificially dispersed beyond its original range.

Present status. A junior synonym of *Donus salviae* (Schrank, 1789), syn. n.

Attelabus atrirostris Fabricius, 1802

Attelabus atrirostris Fabricius, 1802: 424

Location. Habitat in America meridionali D. Smidt. Mus. D. de Sehestedt.

Comments. This nominal species was transferred by Schoenherr (1833a: 271) to the genus *Apion* Herbst, 1797 and has remained in it since then as an *incertae sedis* species.

Zimsen (1964) mentioned three specimens in the "Copenhagen collection" (seen by Kuschel) and one in the "Kiel collection". I have been able to study all four, which are conspecific and belong in the genus *Coelocephalapion* Wagner, 1914. Of the three "Copenhagen collection" specimens, which are glued to the apex of small triangular card pieces, pinned with thin, long pins, the first (ZMUC 00523238) carries a label reading: Essequibo / Smidt / Mus: do. Sehestedt / Attelabus / atrirostris. This specimen is a female with a tubiform shiny rostrum, lacking the right part of the hind body. The second (ZMUC 00523239) is a male and lacks the abdomen and left elytron. The third (ZMUC 00523240) consists only of a pronotum, a mesosternum and some legs. The "Kiel collection" specimen (ZMUC 00523288), pinned with a thin, short pin going through a plastic piece, in turn pinned with a long pin, is in a better condition (even if the elytra and abdomen are parted), with a short brown rostrum, all legs and antennae intact, yellow, the latter inserted at the very base of the rostrum, big eyes, a very wide prothorax and the apex of the elytra slightly yellowish. This specimen cannot be considered a syntype, as it is not in the Sehestedt Collection. From the three syntypes in the Sehestedt Collection, I have selected the female carrying the Essequibo label as the lectotype and added a white label with red margins and black writing: LECTOTYPUS / *Attelabus atrirostris* F. / Alonso-Zarazaga des. 2014. The two other specimens are designated as paralectotypes and have accordingly ben fitted with similar labels. The type locality is here restricted to Essequibo, according to the label of the lectotype.

Present status. This is a valid species, *Coelocephalapion atrirostre* (Fabricius, 1802), comb. n., and *C. luteirostre* (Gerstäcker, 1854) is a new synonym of it (syn. n.). The latter name cannot be declared a *nomen protectum* for want of enough citations in the literature.

Attelabus crotalariae Fabricius, 1802

Attelabus crotalariae Fabricius, 1802: 424

Location. Habitat in Crotalariae leguminibus Americae. Mus. D. Lund.

Comments. Schoenherr (1833: 251), based on specimens apparently from the type series in his collection, transferred this species to the genus *Apion* Herbst, 1797.

Zimsen (1964) reported two specimens in the "Copenhagen collection" and one in the "Kiel collection". I have studied the "Kiel collection" specimen and five specimens in the "Copenhagen collection", all belonging to the same species of *Piezotrachelus* and matching Fabricius' description. One of the males of the latter series carries a label: Essequibo / Isart[?] / Mus.: T. Lund / Attelabus / Crotalariae, and another label with an unpublished 1989 lectotype designation by M. Wanat. I have removed the latter label after consulting with Wanat and I added a white label with red margins and black writing reading: LECTOTYPUS / *Attelabus atrirostris* F. / Alonso-Zarazaga des. 2014,

to this male, which I here designate as the lectotype. The other syntype specimens have been provided with similar paralectotype labels.

Present status. A valid species, *Piezotrachelus crotalariae* (Fabricius, 1802), comb. n., whose origin is doubtful, as this genus is not known to occur in America, while it is very common in Africa, and I suspect that a mislabelling has happened.

Attelabus pisi Fabricius, 1802

Attelabus pisi Fabricius, 1802: 425

Location. Habitat in Austria. Dom. de Meyerle.

Comments. This is a well-known species, transferred to the genus *Apius* by Billberg (1820: 40), to *Apion* Herbst, 1797 by Schoenherr (1833a: 304) and to *Holotrichapion* by Alonso-Zarazaga (1990: 127).

According to Zimsen (1964) there is only a name label in the "Kiel collection". I have confirmed this situation, and consequently no type material is available in Fabricius' collection. Johann Carl Megerle von Mühlfeld's collection in Vienna should be checked, as it is possible that Fabricius returned this material to him.

Present status. A valid species, *Holotrichapion pisi* (Fabricius, 1802), very common and rather variable. For the moment, there is no need to designate a neotype.

Rhynchaenus granulatus Fabricius, 1802

Figs 4–6

Rhynchaenus granulatus Fabricius, 1802: 443

Location. Habitat in Amboina. D. Billardiere.

Comment. This species has not been mentioned in the taxonomic literature since description.

Zimsen (1964) reported the presence of a single specimen in the "Kiel collection". This specimen (ZMUC 00022539) is a male Cryptorhynchini with most of the metanapleural sutures absent, the metanepisterna being visible only as a small triangular piece corresponding to the apex, the metaventrite between middle and hind coxae as long as the mesocoxal diameter, the crypt hind wall weakly prominent, almost perpendicular to the mesoventrite, the elytra tuberculate, with two prominent tubercles on top of the declivity and two others at the apex, the femora edentate and not ventrally sulcate, not reaching the elytral apex, the scutellum minuscule, with a few punctures bearing small scales, the antennal clubs troncoconical, velvety, with slightly oblique sutures, the funicles comprising seven desmomeres, the first two very long, the second slightly shorter than the first, the seventh subannexed to the club and rather similarly velvety, and the intermetacoxal distance much larger than the metacoxal width. I select

this specimen as the lectotype and have added a red, handwritten label: LECTOTYPUS / Rhynchaenus / granulatus F./ Alonso-Z. 2008.

Present status. All these characters compel me to place this species in the genus *Poropterus* Schoenherr, 1844, as *Poropterus granulatus* (Fabricius, 1802), comb. n. This genus and its allies are in great need of revision. There is no other species of this genus recorded from Ambon or its adjacent islands, and the original locality could be incorrect, as the expedition commanded by Antoine Reymond Joseph Bruny d'Entrecasteaux (in which Jacques-Julien Houtou de La Billardière was enrolled as a naturalist) touched Australian lands at several places (La Billardière 1800) and a mislabelling could have happened.

***Rhynchaenus alienatus* Fabricius, 1802**

Rhynchaenus alienatus Fabricius, 1802: 471

Location. Habitat in Sumatra. D. Daldorff.

Comments. This species has not been treated since the original description.

Zimsen (1964) mentioned the presence of one specimen in the "Kiel collection". I have studied it (ZMUC 00022542), it is pinned with a short, rather thick pin in a piece of white plastic, which is in turn pinned with a longer pin. I designate this specimen as the lectotype and have added a red, handwritten label: LECTOTYPUS / Rhynchaenus / alienatus F. / Alonso-Z. 2008. It belongs to the genus *Camptorhinus* Schoenherr, 1825, showing a dark, wide sutural patch from the scutellum to middle of the elytra and it corresponds closely with specimens identified as *C. porcatus* Fåhraeus in the NHM collections, a nominal species currently in synonymy of *C. tibialis* (Sparrman). Even though the genus *Camptorhinus* needs a thorough revision, I propose a new synonymy here.

Present status. A synonym of *Camptorhinus tibialis* (Sparrmann, 1785), syn. n.

***Cossonus canaliculatus* Fabricius, 1802**

Cossonus canaliculatus Fabricius, 1802: 496

Location. Habitat in Sumatra. D. Daldorff.

Comments. This species was mentioned later by Schoenherr (1826: 331; 1838: 1022).

Zimsen (1964) reported one specimen in the "Kiel collection" and two in the "Copenhagen collection". I have seen all three, which are conspecific and belong to the genus *Cossonus* [Clairville], 1798 as usually understood. The "Kiel collection" specimen (ZMUC 00513702) is a male, pinned with a short, thick pin to a piece of plastic, which is pinned with a longer pin; the elytra are parted and it lacks the front left tibia and tarsus. The two "Copenhagen collection" specimens are males, glued to the apex of small triangular cards, but the elytra have big holes through which they were formerly

pinned, in a similar way as the "Kiel collection" specimen, which cannot therefore be ruled out as a syntype. Specimen ZMUC 00513703 carries a small, green, square label and a written label: Sumatra / Daldorff / Mus. S. & T. L. / Cossonus / canalicula / tus Fabr. It is here designated as the lectotype, and I have added a white label with red margins and black writing to its pin: LECTOTYPUS / *Cossonus canaliculatus* / F. / Alonso-Zarazaga des. / 2014. The other specimen (ZMUC 00513704) has just the small green label. I have designated it and the "Kiel collection" specimen as paralectotypes, and added labels similar to that of the lectotype to their pins.

Cossonus canaliculatus (Fabricius, 1792) is an American species described as *Curculio canaliculatus* Fabricius, 1792 (*l.c.*: 471), which is a primary homonym of *Curculio canaliculatus* Olivier, 1791. It was correctly placed by Schoenherr (1838: 1030) as a synonym of *Cossonus vulneratus* Illiger, 1805, the reason for which was not clear to Champion (1909: 68), who apparently was unaware of the homonymy. The catalogues of O'Brien and Wibmer (1982: 222) and Wibmer and O'Brien (1986: 358) also used the incorrect name *Cossonus canaliculatus* (Fabricius, 1792). The name of the species treated here, *Cossonus canaliculatus* Fabricius, 1802, is thus a secondary homonym (because both species are now in the genus *Cossonus*) and cannot be used either. Csiki (1936: 166) used as its valid name *Cossonus illigeri* Champion, 1909 (a replacement name for *Cossonus canaliculatus* Fabricius, 1802), but the synonym *Cossonus incisus* Pascoe, 1885 has priority. These are to be considered provisional placements, as the genus *Cossonus* needs a comprehensive revision to establish its limits and contents. The synonymy is as follows:

Cossonus incisus Pascoe, 1885, stat. res.
= *Cossonus canaliculatus* Fabricius, 1802 (secondary homonym)
= *Cossonus illigeri* Champion, 1909 (replacement name)
Cossonus vulneratus Illiger, 1805, stat. res.
= *Curculio canaliculatus* Fabricius, 1792 (non Olivier, 1791)

Present status. A synonym of *Cossonus incisus* Pascoe, as shown above.

Curculio innocuus Fabricius, 1802

Curculio innocuus Fabricius, 1802: 512

Location. Habitat in Mogador. D. Schousboe. Mus. D. de Sehestedt.

Comments. This species was transferred by Schoenherr (1833b: 525) to his genus *Cneorhinus*, as var. β of *Cneorhinus barcelonicus* (Herbst, 1797).

Zimsen (1964) mentioned the presence of three specimens in the "Copenhagen collection". I have examined them; each bears a small, green square and a red printed label with TYPE written on it. I have selected as lectotype the only female and added a red, handwritten label reading: LECTOTYPUS / Curculio / innocuus F. / Alonso-Z. 2008. This female carries a label reading: Mogador / Schousboe / Mus. Sehest. / Innocuus / Barcelonicus / Hbst. The two males are designated as paralectotypes and have been labelled accordingly. However, in the "Kiel collection" there is another specimen,

which differs only in the claws being slightly more unequal. I do not consider it to belong to the type series, as this was composed only of specimens in Sehestedt's collection.

Present status. A synonym of *Cneorhinus barcelonicus* (Herbst, 1797) as currently understood. The identity of this species nevertheless needs confirmation.

Curculio holosericeus Fabricius, 1802

Curculio holosericeus Fabricius, 1802: 526

Location. Habitat in Austria. D. Scheidler.

Comments. This nominal species was considered identical by Germar (1824: 405) with *Trachyphloeus ruficollis* (Fabricius, 1787) (a primary junior homonym) and was transferred by Schoenherr (1826: 192) to the genus *Omias* Germar, 1817. Schoenherr (1834b: 504) later synonymized it with *Omias rufipes* Boheman, 1834 (now in *Humeromima* Podlussány, 1998). However, Dalla Torre et al. (1937) placed it as a doubtful synonym of *Barypeithes indigens* (Boheman, 1834) (now in *Exomias* Bedel, 1883). It was treated as a valid species by Borovec (2013a: 84) replacing *Exomias indigens* (Boheman, 1834), but as a *nomen dubium* on the next page (*l.c.*: 85) and in the main text (*l.c.*: 382), showing that the poor original description does not allow a precise placement.

Zimsen (1964) mentioned a single specimen in the "Kiel collection". I have studied it and identified it as a specimen of *Exomias chevrolati* (Boheman, 1842) in poor condition, matching the original description, with the abdomen and part of the metasternum missing, all the legs lost, except the mid left one showing the characteristic femoral tooth, the rostrum also with a characteristic strong convexity, the elytra only with moderately erect setae in one row per interstria, no appressed scales or pubescence. I here designate it as the lectotype and have added a red, handwritten label reading: LECTOTYPUS / Curculio / holosericeus F. / Alonso-Z. 2008 and a white identification label.

Present status. This is a valid species, *Exomias holosericeus* (Fabricius, 1802), with *Exomias chevrolati* (Boheman, 1842) as its new synonym. This species, originally described from Austria, has nothing to do with *Exomias indigens*, which is a South-Western European species.

Curculio erinaceus Fabricius, 1802

Curculio erinaceus Fabricius, 1802: 527

Location. Habitat in Austria. Dom. de Meyerle.

Comments. Billberg (1820: 44) was the first to propose a relationship of this species with the tribe Trachyphloeini, transferring the species to the genus *Trachyphloeus*

Germar, 1817. Germar (1824: 412) suggested, with doubt, that it was a synonym of *Thylacites hirsutulus* (Fabricius, 1792), now in *Brachysomus* Schoenherr, 1823, whereas Schoenherr (1834b: 495) synonymized it with *Trachyphloeus horrens* Gyllenhal, 1834, also with doubt. Following the latter, Lona (1937) placed it in synonymy of *Cathormiocerus horrens* (Gyllenhal, 1834), with a question mark, and Borovec (2013b) recently placed it as a *nomen dubium* in the tribe Trachyphloeini.

Zimsen (1964) reported one specimen in the "Kiel collection" and two in the "Copenhagen collection". The first, matching the original description, belongs to *Brachysomus villosulus* (Germar, 1824), having the scapes with the characteristic apical club of this species. I here designate it as the lectotype and have added a red, handwritten label reading: LECTOTYPUS / Curculio / erinaceus F. / Alonso-Z. 2008 and a white identification label. The two other specimens do not match the original description; both are males of *Exomias holosericeus* (cf. above), one carrying a label: Austria / Megerle / Mus. S. & T. L. / Erinaceus F. They are not considered to belong to the type series.

Present status. A valid species, *Brachysomus erinaceus* (Fabricius, 1802), comb. n., and a senior synonym of *B. villosulus* (Germar, 1824), syn. n. I have been unable to fulfil the requirements of Art. 23.9.1.2 to declare the latter name a *nomen protectum*.

Curculio recurvus Fabricius, 1802

Figs 7–9

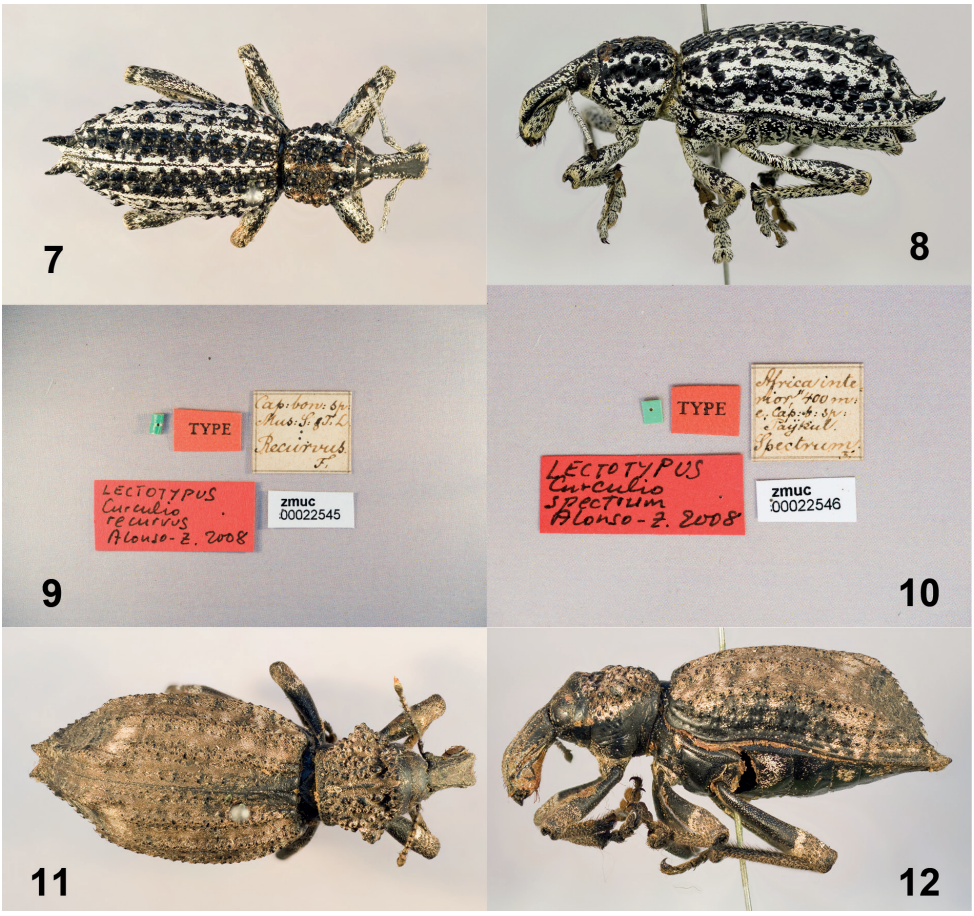
Curculio recurvus Fabricius, 1802: 535.

Location. Habitat ad Cap. Bon. Spei. Mus. D. Lund.

Comments. This nominal species was transferred to the genus *Hipporhinus* Schoenherr, 1823 by Schoenherr (1840a: 753).

Zimsen (1964) recorded one specimen in the "Copenhagen collection". I have studied it (ZMUC 00022545); it is pinned through the right elytron and carries a small, green, square label, a red printed label with TYPE and a whitish one reading: Cap : bon : sp: / Mus : S : & T. L. / Recurvus / F. It matches the original description, and I here designate it as the lectotype and have added the following red, handwritten label to ist pin: LECTOTYPUS / Curculio / recurvus F. / Alonso-Z. 2008. It is easily identifiable as *Bronchus nivosus* (Sparrman, 1785), comb. n., using Marshall's (1904) key, and fits the description of the latter almost exactly. It is evidently not the same species treated as *Hipporhinus recurvus* by Marshall (1904), whose valid name is *Bronchus sparrmani* (Gyllenhal, 1833), comb. n. I have compared this specimen with specimens of both species identified by Marshall in the collection of the NHM (London).

Present status. A new synonym of *Bronchus nivosus* (Sparrman, 1785) in Marshall's sense. The present problems with the identifications of the types of *Bronchus* make a modern revision of the genus necessary. Some of the characters used by Marshall in his keys, e.g., the "transverse basal furrow on the underside of rostrum" are



Figures 7–12. *Curculio recurvus* Fabricius lectotype **7** Dorsal view **8** Lateral view **9** Labels *Curculio spectrum* Fabricius lectotype **10** Labels **11** Dorsal view **12** Lateral view.

highly variable among individuals of the same species, difficult to see and subject to different interpretations of the degree of depth, while other characters, e.g., the kind and disposition of vestiture on legs, are underused. The genitalia of both sexes have not been described.

Primary homonyms

Curculio striatus Fabricius, 1787

Curculio striatus Fabricius, 1787a: 117.

Location. Habitat in Africa Dom. Vahl [restricted by Fabricius (1792) to Barbaria].

Comments. This nominal species has been treated subsequently by Olivier (1791: 539), Fabricius (1792: 470), Herbst (1795: 501) and Fabricius (1802: 528). It was recently treated as a *nomen dubium* in Curculionoidea by Alonso-Zarazaga (2013a). This name is a primary homonym of *Curculio striatus* O.F. Mueller, 1776, of *C. striatus* Strøm, 1783 and of *C. striatus* Herbst, 1783 and is consequently invalid.

According to Zimsen (1964), there is one specimen in the "Copenhagen collection", which I have studied. It is labelled: Vahl / Mus. S. & T. L. / "Striata". It is a specimen of *Brachypera* (*Brachypera*) *crinita* (Boheman, 1834). I have added a handwritten red label reading: LECTOTYPUS / Curculio / striatus / Alonso-Z. 2008, and an identification label to its pin, and I here designate this specimen as the lectotype.

Present status. A new synonym of *Brachypera* (*Brachypera*) *crinita* (Boheman, 1834).

Curculio planirostris Fabricius, 1787

Curculio planirostris Fabricius, 1787a: 119.

Location. Habitat Kiliae Dom. Daldorff.

Comments. This species was later treated by Fabricius (1792: 377), Manuel (1797: 606) (who transferred it to the genus *Macrocephalus* Olivier, 1789), Paykull (1800: 167) (who placed *Curculio fulvirostris* Fabricius, 1787 as its synonym) and Fabricius (1802: 410). It was transferred to the genus *Rhinosimus* Latreille, 1802 by Latreille (1802) and to the genus *Salpingus* Illiger, 1802 by Illiger (1802). Its name is a primary homonym of *Curculio planirostris* Piller & Mitterpacher, 1783, a junior synonym of *Tropideres albirostris* (Schaller, 1783) (Anthribidae).

Zimsen (1964) reported three specimens in the "Kiel collection", which I have studied and identified as *Salpingus planirostris* in the usual sense. I have not selected a lectotype from among the syntypes, preferring to leave this task for a specialist in the family Salpingidae.

Present status. A junior homonym, and consequently invalid, in use as *Salpingus planirostris* (Fabricius, 1787). Authors in Salpingidae seem to be unaware of this irregularity (e.g., Pollock and Löbl 2008). The correct name for this species is *Salpingus fulvirostris* (Fabricius, 1787) (see under this species above).

Curculio tuberculatus Fabricius, 1792

Curculio tuberculatus Fabricius, 1792: 480.

Location. Habitat in India orientali Dom. Prof. Abildgard.

Comments. This nominal species is a primary homonym of *Curculio tuberculatus* O.F. Müller, 1776 (a hitherto unidentified species) and of *C. tuberculatus* DeGeer, 1778 (a synonym of *Brachycerus obesus* Olivier, 1790). This species has been subsequently

treated by Herbst (1795: 510) and Fabricius (1802: 535) without adding any clue about its correct placement.

Zimsen (1964) mentioned the presence of one specimen in the "Kiel collection", which I have studied. It (ZMUC 00022543) is pinned with a thick, very short headless pin, which is pinned to a plastic piece, and this in turn is pinned with a long pin. The specimen is in relatively good state, despite suffering from a strong internal *Anthrenus* attack, but lacks the front left tarsus, the front right onychium and the right hind leg. The elytra are divaricate. I here designate this specimen as the lectotype and have added a red, handwritten label reading: LECTOTYPUS / Curculio / tuberculatus F. / Alonso-Z. 2008. This specimen belongs to the typical form of *Desmidophorus hebes* (Fabricius, 1781).

Present status. A new synonym of *Desmidophorus hebes* (Fabricius, 1781).

***Curculio spectrum* Fabricius, 1802**
Figs 10–12

Curculio spectrum Fabricius, 1802: 537.

Location. Habitat ad Cap.Bon.Spei. Mus. D. Lund.

Comments. This nominal species is a primary homonym of *Curculio spectrum* Fabricius, 1781 (now in *Gyllenhalia* Aurivillius, 1886). It was transferred to *Hipporhinus* by Schoenherr (1833b: 462) and subsequently treated by Marshall (1904) in his revision of the genus, now a synonym of *Bronchus*.

Zimsen (1964) mentioned one specimen in the "Copenhagen collection", which I have studied. It (ZMUC 00022546) is a female *Bronchus*, pinned through the right elytron, carrying a small, green, square label, a red printed label with TYPE, and a label reading: Africa inte / rior " 400 m / e Cap : b : Sp : / Paÿkul / Spectrum. It shows *inter alia* two teeth on each side of the hind margin of the 4th sternite and a laterally compressed declivity of the elytra, belonging therefore to *Bronchus abruptecostatus* (Gyllenhal, 1833). I here designate it as lectotype and have added a red, handwritten label reading: LECTOTYPUS / Curculio / spectrum F. / Alonso-Z. 2008. The unfortunate misidentification by Marshall leaves *Hipporhinus spectrum* sensu Marshall without a name, and I am describing it below as a new species.

Present status. A new synonym of *Bronchus abruptecostatus* (Gyllenhal, 1833).

New taxon

***Bronchus synthesys* Alonso-Zarazaga, sp. n.**
http://zoobank.org/60096CFF-4B29-484D-BE2C-8769466414A9

Type material. HOLOTYPE: 1 male, pinned, dissected, two legs absent, with the following labels: Marshall MS: Uitenhage / Cape Col. / Rev. J. ONeil; printed: G.A.K. Marshall / Coll. / B.M. 1950 – 255; Marshall MS: H. spectrum F. (Natural History

Museum, London). This is one of the specimens coming from Marshall's collection and mentioned in his 1904 revision. Despite its condition, it is considered to be the best specimen to meet the requirements for a holotype. A white label with red margins and black writing: HOLOTYPUS / *Bronchus synthesys* sp. n. / Alonso-Zarazaga des. / 2014 has been added to this specimen.

Description. See that of *Hipporhinus spectrum* in Marshall (1904, pp. 54–55).

Etymology. The specific epithet of this new species makes reference to the EC-funded project SYNTHESYS (http://www.synthesys.info) and is to be taken as a name in apposition. It pays homage to all the people who have made its operation possible.

Comments. This is the species misidentified by Marshall (1904) as *Hipporhinus spectrum*. As it is a misidentified nominal species, its name is unavailable but cannot be replaced, as replacement names can only be proposed for available taxa (homonyms). Marshall's species therefore is without a name, and I am describing it here.

Discussion

Reviews of the type specimens in old collections usually have taxonomic implications. This article was not going to be an exception. It is linked to an international initiative (the World Information Network on Weevils), which aims at making available all kinds of information about Curculionoidea for use by researchers, applied entomologists (in Agronomy, Forestry and Food Storage) and decision-makers. One of its results is WTaxa. During the building and checking of this database, many 'orphan species names' were found, and there is a need to know the true identity of these nominal species.

The original list of species for this article was much longer. The problems faced during the research and the writing suggested that a reduction was necessary. Even so, six years of careful examination of all the available data resulted in thirty-two nominal species being treated here, twenty-two having lecto- and sometimes paralectotypes designated, one having a neotype designated to remove uncertainty about its identity and four illustrated for the first time. The nomenclatural changes proposed do not severely affect the taxonomy of species, since most of them have seldom been treated. Nine new combinations, a new replacement name and fifteen new synonymies are proposed, and one new species is described. Many other such 'orphan species names' await study, and some of the species will merit a full redescription and placement in new, still undescribed genera. On the other hand, it is not unusual to find that some species that have been treated by later authors, and even monographed, cf. *Bronchus* (as *Hipporrhinus*) by Marshall (1904), do not match their types, and this article may serve as a warning to monographers failing to check old types.

It is surprising that some of these species described long ago have apparently been only rarely collected since. Some (such as the *Bronchus* or *Brachycerus* species) have their habitats and ranges severely affected by human action and urban settlement, whereas others, bearing incorrect label data, may have been described from the real range of the species under a different name (e.g., *Piezotrachelus crotalariae*, *Poropterus*

granulatus). In the end, this kind of study and its implications become archaeo-entomology, since we are studying a world that was but will never be again (cf. also Alonso-Zarazaga 2013b). It would be revealing to know how many of these species are already extinct. Let us hope not many of them.

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Notes on the genus *Xenocerogria* (Coleoptera, Tenebrionidae, Lagriini) from China

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Abstract

Three species of the genus *Xenocerogria* Merkl, 2007 have been recorded in China, *X. feai* (Borchmann, 1911), *X. ignota* (Borchmann, 1941) and *X. ruficollis* (Borchmann, 1912). *Xenocera xanthisma* Chen, 2002 is proposed as a junior synonym of *X. ruficollis*. Lectotype of *X. ignota* is designated, and the species is transferred to the genus *Lagria* Fabricius, 1775. New Chinese province records of *X. ruficollis* are provided.

Keywords

China, Lagriinae, lectotype designation, new synonym, redescription, Tenebrionidae, *Xenocerogria*

Introduction

Xenocerogria Merkl, 2007 is a small genus of Lagriini distributed in China, India and Southeast Asia. The generic name *Xenocerogria* was proposed by Merkl (2007) as a new replacement name for *Xenocera* Borchmann, 1936, preoccupied by *Xenocera* Broun, 1881 (Anobiidae).

* 19th contribution to the knowledge of Lagriini. 18th contribution: Masumushi. Special Publication of the Japanese Society of Scarabaeidology No. 1. Japanese Society of Scarabaeidology, Tokyo, pp. 301–312, 2011.

The genus *Xenocera* was established by Borchmann (1936) with *Lagriocera feai* Borchmann, 1911 from "Carin Chebà, Burma" designed as its type species ("Gattungstype" in the original description). Simultaneously, Borchmann (1936) assigned further six species to the genus all originally described in *Lagriocera* Fairmaire, 1896. One of them, *Xenocera ruficolli*s was recorded from China. Borchmann (1941) then added *Xenocera ignota,* described from China, Fujian. Merkl (2007) recorded *Xenocerogria feai* as a species new to China (Yunnan). Chen (2002) described *Xenocera xanthisma*, also from China (Fujian and Hunan). In the present study, we found that *Xenocera xanthisma* is the junior synonym of *Xenocerogria ruficollis*. Therefore, there are eight known species of the genus, of which three species have distribution records in China.

No modern revision has been published for the genus *Xenocerogria*, only a checklist was provided by Merkl (2007). In the present paper, all the three Chinese species are redescribed. Based on the male described hereunder, *Xenocera ignota* is removed from the genus, and transferred to the large collective genus *Lagria* Fabricius, 1775.

Material and methods

Photographs of the types of *Xenocera xanthisma* Chen, 2002 were taken by Leica M205A stereomicroscope; descriptions and measurements were performed under a stereomicroscope (Nikon SMZ1500), and photomicrographs of *Xenocerogria ruficollis* (Borchmann, 1912) were taken with a stereomicroscope (LEICA EZ4 HD) attached to a computer using Leica Application Suite version 2.1.0 software in Chongqing. Photographs of *Xenocerogria feai* and *X. ignota* were taken with Nikon Coolpix 4500 digital camera attached to Leica MZ 125 stereomicroscope in Budapest. Label text of type specimens is cited verbatim.

The following abbreviations are used for institutions where specimens are deposited (curators responsible for loans in parentheses):

CCLT Private collection of Chi-Feng Lee, Taipei, Taiwan;
CKMT Private collection of Kimio Masumoto, Tokyo, Japan;
CMNH Carnegie Museum of Natural History, Section of Invertebrate Zoology, Pittsburgh, PA, USA (R. Davidson);
CQNU Chongqing Normal University, Chongqing, P. R. China (Bin Chen);
CSBC Private collection of Stanislav Bečvář, České Budějovice, Czech Republic;
DEI Deutsches Entomologisches Institut, Müncheberg, Germany (Lothar Zerche);
HNHM Hungarian Natural History Museum, Budapest, Hungary (Ottó Merkl);
MHBU Museum of Hebei University, Baoding, P. R. China (Guo-Dong Ren);
MHNG Muséum d'histoire naturelle, Geneva, Switzerland (Giulio Cuccodoro);
MSNG Museo Civico di Storia Naturale "Giacomo Doria", Genova, Italy (Roberto Poggi);
NSMT National Science Museum (Natural History), Tokyo, Japan (Shuhei Nomura);
QCCC Private collection of Jian-Yue Qiu & Hao Xu, Chongqing, P. R. China;

SMNS Staatliches Museum für Naturkunde, Stuttgart, Germany (Wolfgang Schawaller);
SWU Southwest University, Chongqing, P. R. China (Li Chen);
ZFMK Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany (Michael Schmitt).

Taxonomy

Xenocerogria Merkl, 2007

Xenocera Borchmann, 1936: 116 (not Broun, 1881: 668). Type species: *Lagriocera feai* Borchmann, 1911, by original designation.
Xenocerogria Merkl, 2007: 269 (replacement name); Merkl 2008: 116.

Diagnosis. The genus was thoroughly described by Borchmann (1936), so only the most important characteristics are mentioned here. **Male:** Body small, body length 5–10 mm. Head slightly rounded. Apical maxillary palpomere securiform and thick. Labrum with anterior margin more or less emarginate. Clypeus with anterior margin emarginate, exposing labroclypeal membrane. Frontoclypeal suture deep and arcuate. Antennae strong, antennomeres 9 and 10 strongly transverse, with produced anterolateral corner, antennomere 11 strongly elongate, as long as combined length of at least 4 preceding antennomeres, concave ventrally. Pronotum slightly convex, slightly narrower than head. Elytra about 2× as wide as pronotum, slightly broadened toward apex, without striae, with irregular, but evenly scattered punctation. Legs simple, moderately robust; femur not clavate, tibia nearly straight, without modifications. **Female:** Similar to male but larger and broader. Antennomere 11 shorter than combined length of 3 preceding antennomeres. Pronotum slightly wider than head. Elytra more broadened toward apex.

Distribution. China, India, Burma, Java, Sumatra.

Remarks. This genus is distinguished from other lagriine genera on the basis of the male antenna: antennomeres 9 and 10 are strongly transverse; the terminal antennomere is concave in ventral surface, with length equal to at least combined length of 4 preceding antennomeres (female with shorter terminal antennomere). This antennal structure is combined with unmodified male tibiae. These characteristics make this genus vaguely defined, and it is possibly an artificial assemblage of species not closely related to each other. Even Borchmann (1936) himself emphasized that one of the species, *Xenocerogria andrewesi* (Borchmann, 1925) is somewhat different from other species: antennae are more slender, only antennomere 10 is transverse, and pronotum of female with a wide longitudinal impression.

The situation is similar in most genera of the tribe Lagriini. Most of the species of the subtribe Lagriina were originally described as members of the genus *Lagria* Fabricius, 1775. Later, species with unusual (apomorphic) characters were transferred from *Lagria* to separate genera established mainly by Borchmann, for instance *Aulonogria* Borchmann,

1929, *Cerogria* Borchmann, 1909, *Neogria* Borchmann, 1911 and *Schevodera* Borchmann, 1936, just to mention a few from East and Southeast Asia. However, these genera are defined mostly by modifications of male antennomeres and tibiae. Females not associated with males are difficult or virtually impossible to separate at generic level. The remaining species were retained in the genus *Lagria*. In fact, modifications can frequently be observed on the male antennae and legs of species of *Lagria*, although these are not as prominent as in *Cerogria*, for instance. The genus *Lagria* itself, used as a dumping ground for more "simple" species is therefore still quite diverse, and most of the Oriental species are rather different from the type species, the western Palaearctic *Lagria hirta* (Linnaeus, 1758).

Removal of species with unique characters, creating a hardly treatable mass of less distinctive species in a large genus is common throughout the Coleoptera, including the family Tenebrionidae (see comments by Campbell 2014 and Schawaller 2014). A natural classification of the subtribe Lagriina would be achieved by study of (often unavailable) types of all described species supported by extensive molecular studies, but this must be an enormous undertaking.

Key to Chinese species presently and formerly assigned to *Xenocerogria*

1(2) Elytral pubescence short and completely decumbent. Male: antennomere 3 much longer than 1 and 2 combined, antennomeres 9 and 10 not transverse, antennomere 11 unmodified, much shorter than 3 preceding combined; inner side of hind tibia finely denticulate...***Lagria ignota*** **(Borchmann, 1941)**

2(1) Elytral pubescence longer, semierect to erect. Male: antennomere 3 much shorter than 1 and 2 combined, antennomeres 9 and 10 transverse, antennomere 11 enlarged, concave, as long as at least 4 preceding combined; hind tibia without denticulation.

3(4) Elytral pubescence erect. Male: antennomere 11 as long as 4 preceding combined; antennomeres 5 to 10 with inner surface flattened, bordered with longitudinal carina. Female: length of elytra about 2× maximum width..... ..... ***Xenocerogria feai*** **(Borchmann, 1911)**

4(3) Elytral pubescence semierect. Male: antennomere 11 as long as 6 preceding combined; antennomeres 5 to 10 without flattened inner surface. Female: length of elytra about 3× maximum width ...***Xenocerogria ruficollis*** **(Borchmann, 1912)**

Xenocerogria ruficollis **(Borchmann, 1912)**
Figs 1–13

Lagriocera ruficollis Borchmann, 1912: 7 (type locality: China: Taiwan; type depository: DEI).

Lagriocera ruficollis: Borchmann 1916: 127 (China: Taiwan); Kôno 1929: 29 (China: Taiwan).

Xenocera ruficollis: Borchmann 1936: 117 (China: Taiwan); Sasaji 1986: 9 (China: Taiwan); Masumoto 1988: 46 (China: Taiwan); Chen 1997: 748 (China: Zhejiang, Fujian, Hubei, Chongqing, Taiwan).

Xenocerogria ruficollis: Merkl 2007: 270; Merkl 2008: 116 (China: Fujian, Taiwan).

Xenocera xanthisma Chen 2002: 178 (type locality: China: Fujian, Hunan; type depository: SWU). **syn. n.**

Redescription. Body length 5–8 mm. Body, including antennae and legs, black, except brownish red prothorax and scutellum. Teneral specimens may have elytra reddish brown or whole body tending to be paler.

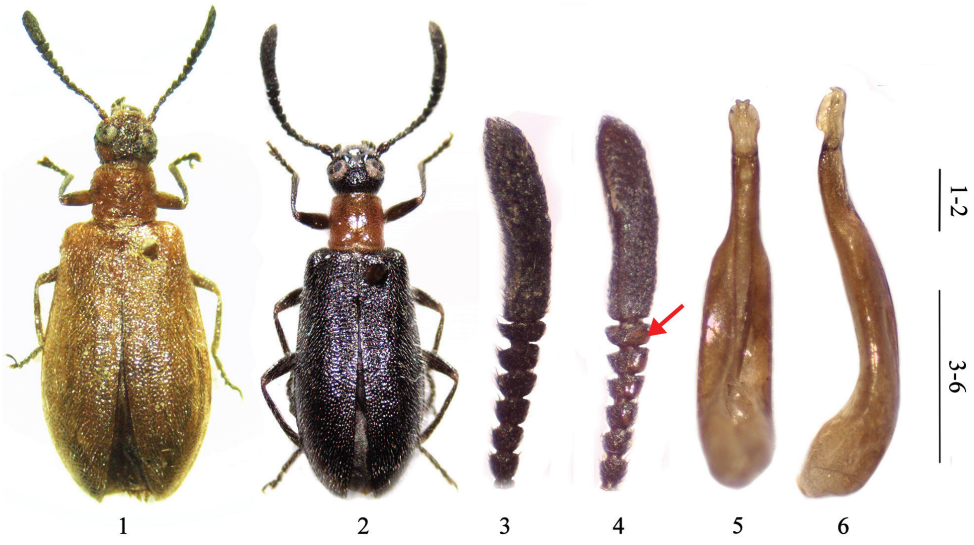
Male (Fig. 2). Head rounded, interocular distance 0.75× as wide as eye diameter; preorbital swelling elevated and glabrous; frons distinctly impressed, densely and coarsely punctate. Eyes reniform, slightly bulging, genal canthus encroaching to 0.7× eye width. Antennae (Figs 3–4) surpassing base of elytra when directed backwards, gradually broadening toward apex, antennomere 1 subglobular, 0.3× as long as distance between antennal insertions, antennomere 2 small, shorter than 1, antennomere 3 longer than 4 and 2, and about 2× longer than wide, antennomeres 5 to 9 short, trapezoidal, antennomere 10 strongly transverse, 2× as wide as long, antennomeres 5 to 10 glabrous in external side of ventral surface, antennomere 11 enormous, as long as combined length of 6 preceding antennomeres, as wide as antennomere 10, subparallel-sided, slightly curved, concave ventrally.

Pronotum subequal in length and width, barely constricted behind middle, maximum width just before middle; anterior and posterior angles rounded; disc with two indistinct oblique depressions before base; surface finely but densely punctate, punctures separated by interspaces of 0.3 to 0.5 puncture diameter on disc, tending to be subcontiguous toward lateral portions; disc with a small ill-defined impunctate spot at middle before base.

Elytra elongate, barely dilated posteriorly, widest at apical 1/3, about 4× as long as pronotum; punctation moderately dense, punctures separated by interspaces of 0.5 to 1 puncture diameter; interspaces slightly convex, forming short oblique or transverse wrinkles; dorsal pubescence consisting of short, semierect, sparsely set whitish hairs; humeral callosity separated from basal part of disc by indistinct impression; elytral margin visible in dorsal view except at humeral callosity; elytral epipleura densely punctate, parallel-sided from base to level of metacoxae, then gradually narrowing towards apex. Mesoventrite, mesepisternum, metepimeron, metepisternum finely and densely punctate; metaventrite very finely punctate, almost smooth, punctation becoming denser in lateral portions.

Legs narrow; apical 0.3 of middle and hind femora reaching beyond edge of elytra; fore and middle tibiae nearly straight, slightly shorter than femora, hind tibiae slightly curved, very weakly attenuated at middle, without visible denticulation. Tarsi simple.

Aedeagus with distal part of basale abruptly attenuating, much more slender than proximal part; apicale spoon-shaped, bifid at apex (Figs 5–6).



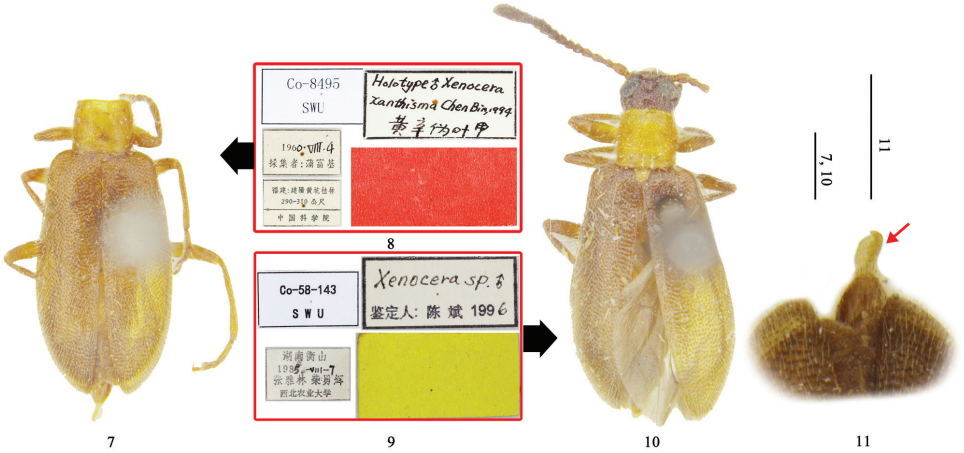
Figures 1–6. *Xenocerogria ruficollis* (Borchmann, 1912): **1** female, dorsal habitus **2** male, dorsal habitus **3** male antenna, dorsal view **4** male antenna, ventral view (arrow indicating glabrous part in external side) **5** aedeagus, ventral view **6** aedeagus, lateral view. Scale = 1 mm.

Female (Fig. 1). Larger than male. Head with interocular distance about 1.2× as wide as eye diameter. Preorbital swelling not developed. Antennomere 10 about 1.5× as wide as long; antennomere 11 shorter than combined length of 4 preceding antennomeres, but still broad. Elytra broader and more widening posteriorly. Legs shorter.

Type material examined. Holotype of *Xenocera xanthisma* Chen, 2002, male, (SWU, Fig. 7), pinned (head missing, but aedeagus visible (Fig. 11)), labelled (Fig. 8) as follows: 1) Co-8495 SWU [printed on white paper]; 2) 1960.VIII.4 collector: Fu-Ji Pu [first three numbers of year printed, last number of year, month and day handwritten, collector printed in Chinese on white paper]; 3) Fujian: Jianyang: Huangkeng: Guilin, 290–310 m, Chinese Academy of Science [printed on white label in Chinese]; 4) Holotype ♂ *Xenocera xanthisma* Chen Bin, 1994 [and the name of *Xenocera xanthisma* in Chinese, all handwritten on white paper]; 5) [red paper without text]. **Paratype** of *Xenocera xanthisma*, male (SWU, Fig. 10), labelled (Fig. 9) as follows:1) Co-58-143 SWU [printed on white paper]; 2) Hunan: Hengshan, 1985.VII.7, Ya-Lin Zhang, Yong-Hui Cai, Northwestern Agricultural University [the last number of year, month and day handwritten, others printed in Chinese on white paper]; 3) *Xenocera* sp. ♂ det.: Chen Bin 1996 ["*Xenocera* sp.", male symbol and the last number of year handwritten, others printed on white paper with black border]; 4) [yellow paper without text].

Other materials examined. China: Jiangsu: 6 ♂♂ (CQNU), 1 ♂ (QCCC): Mt. Zijinshan, Nanjing, 27.V.2012, Hao Xu and Jian-Yue Qiu leg. **Fujian:** 1 ♂, 1 ♀ (HNHM): Shaowu env., 13–16.VI.1991, Nikodým & Červenka leg. **Guangxi:** 1 ♂ (MHBU): Bapen, Fusui, 17–18.VIII.2004, Yang Yu and Yi-Bin Ba leg.; 1 ♂ (MHBU): Luocheng, 21.VII.2006, Fu-Ming Shi and Shao-Li Mao leg. **Guizhou:**

2 ♂♂ (MHBU): Sanchahe River, Libo, 29–31.VII.2010, Yong Zhou and Yi-Ping Niu leg.; 1 ♀ (CQNU): Maolan National Nature Reserve (Fig. 13), Libo County, 10.VIII.2012, Hao Xu and Jian-Yue Qiu leg.; 5 ♂♂ (CQNU), 1 ♂ (QCCC): Mt. Xiaogaoshan, Kaili (Fig. 12), 27.V.2013, Hao Xu and Jian-Yue Qiu leg. **Taiwan:** 1 (HNHM): Alishan, Chiayi Hsien, 22–25.VI.1974, M. Owada leg.; 4 (HNHM): Shanmei, Chiayi Hsien, 600 m, 2.V.1977, J. & S. Klapperich leg.; 2 (HNHM): Taihorinsho (= Talin), Chiayi Hsien, VIII.1909, H. Sauter leg.; 1 (HNHM): FuYuan Forest Recreation Area, Hualien Hsien, 28.V.1997, C. W. & L. B. O'Brien leg.; 1 (CCLT): Hohuenshan, Hualien Hsien, 13.VIII.2000, Chi-Feng Lee leg.; 1 (CCLT): Tienshiang, date and collector unknown; 3 (HNHM): Duona, Kaohsiung Hsien, 15.II.1993, M. L. Jeng leg.; 1 (HNHM): Liukuei, Kaohsiung Hsien, 29.IV.1970, Y. Kiyoyama leg.; 7 (HNHM): Kosempo (= Chiahsien), Kaohsiung Hsien, XI.1908 and IV.1909, H. Sauter leg.; 1 (CKMT): Paolai, Kaohsiung Hsien, 19.V.1975, K. Akiyama leg.; 2 (CKMT): Shanping, Kaohsiung Hsien, 27.IV.1981, S. Tsuyuki leg.; 1 (CKMT): Shanping, Kaohsiung Hsien, 1–2.V.1986, K. Masumoto leg.; 10 (HNHM): Shanping, Kaohsiung Hsien, 640 m, 23–31.III.1988, J. Rawlins & C. Young leg.; 33 (CMNH, HNHM): same locality and collectors, 1.IV.–20.V.1988; 3 (HNHM): Shanping Forest Recreation Area, near Liukuei, 22°58'16"N, 120°41'15"E, Kaohsiung Hsien, at light and swept & singled, 19–21. XI.2002, L. Ronkay & O. Merkl leg.; 17 (HNHM): Shanping LTER Site, near Liukuei, Kaohsiung Hsien, UV light trap, 1.IV.2003, L. Papp & M. Földvári leg.; 1 (HNHM): Shanping LTER Site, near Liukuei, along a creek, Kaohsiung Hsien, 1–2.IV.2003, L. Papp & M. Földvári leg.; 1 (HNHM): Takao (= Kaohsiung), Kaohsiung Hsien, 11.VII.1907, H. Sauter leg.; 1 (CMNH): Chungshin, Nantou Hsien, 30.V.1987, Chen Young leg.; 1 (HNHM): Fuhosho (= Wucheng), Nantou Hsien, VII.1909, H. Sauter leg.; 2 (NSMT): Hori, Nantou Hsien, 26.IV.1929, K. Sato leg.; 2 (HNHM): Hueisun, Nantou Hsien, 17.IV.1993, W. I. Chow leg.; 19 (HNHM): Huisun Forest Area, 15 km N of Puli, Nantou Hsien, 500 m, at light, 12–13. IV. 1997, G. Csorba & L. Ronkay leg.; 1 (HNHM): Huisun Forest Recreation Area, Nantou Hsien, 22. V. 1997, C. W. & L. B. O'Brien leg.; 1 (CKMT): Kuantoushan, Nantou Hsien, 18.VI.1993, Luo Chinchi leg; 1 (NSMT): Lienhuachi, Nantou Hsien, 750 m, 14–16.III.1980, T. Shimomura leg.; 1 (CKMT): same locality and collector, 16–17.III.1980; 2, Lienhuachi, Nantou Hsien, 27.VI.1998, K. Akiyama leg.; 1 (NSMT): Lushan, Nantou Hsien, 8.V.1975, K. Akiyama leg.; 2 (HNHM): 4 km above Lushan, Nantou Hsien, 18.V.1997, C. W. & L. B. O'Brien leg.; 21 (HNHM): Mong Gwu, 14 km E of Puli, 24°1.367'N, 121°5.063'E, Nantou Hsien, 850 m, swept from vegetation, 20.IV.2002, D. Anstine, Gy. Fábián & O. Merkl leg.; 3 (CKMT): Nanshanchi, Nantou Hsien, 16.V.1971, K. Sakai leg.; 1 (HNHM): Nanshanchi, Nantou Hsien, 27.VII.1972, K. Masumoto leg.; 6 (HNHM): same locality and collector, 23 to 29.IV.1974; 1 (CKMT): same locality and collector, 29.IV.1994; 1 (CKMT): Nanshanchi, Nantou Hsien, 30.III.1972, Y. Miyake leg.; 1 (CKMT): Nanshanchi, Nantou Hsien, 29.IV.1973, S. Tsuyuki leg.; 1 (CKMT): Nanshanchi, Nantou Hsien, 5.V.1979, K. Emoto leg.; 5 (CKMT): Nanshanchi,



Figures 7–11. Types of *Xenocera xanthisma* Chen, 2002: **7** holotype, dorsal view **8** holotype, labels **9** paratype, labels **10** paratype, dorsal view **11** holotype, arrow indicating apex of aedeagus. Scale = 1 mm.

Figures 12–13. Habitats of *Xenocerogria ruficollis* (Borchmann, 1912): **12** Mt. Xiaogaoshan, Kaili, Guizhou **13** Maolan, Guizhou.

Nantou Hsien, 3–15.IV.1986, M. Ohara leg.; 1 (CKMT): Nanshanchi, Nantou Hsien, 28.VII.1990, collector unknown; 1 (CKMT): Shizitou, Nantou Hsien, 7.V.1992, Luo Chinchi leg.; 4 (CCLT): Tungpu, Nantou Hsien, 13.IX.2001, C.-F. Lee; 1 (CKMT): Wushe, Nantou Hsien, 4.V.1979, S. Tsuyuki leg.; 1 (CKMT): Fenshuiling, Pingtung Hsien, 14.V.1996, S. Tsuyuki leg.; 1 (HNHM): Kenting National Park, Botanical Garden, Pingtung Hsien, 4–6.X.2000, L. Papp, L. Peregovits & L. Ronkay leg.; 2 (HNHM): Nanjensan, Pingtung Hsien, 13.III.1993, W. I. Chow leg.; 2 (HNHM): Taipei, Taipei City, 24.IX.2000, L. Papp, L. Peregovits & L. Ronkay leg.; 2 (NSMT): Yangmingshan, Taipei City, 25.V.1965, K. Morimoto leg.; 1 (HNHM): Yangmingsan, Taipei City, 15.IX.1970, Y. Kiyoyama leg.; 3 (HNHM): Guanyinshan, Taipei Hsien, 500 m, singled, 15.XI.2002, O. Merkl leg.; 1 (HNHM): Haeng-Lu Dyi, Taipei Hsien, swept, 2.IV.2002, Gy. Fábián & O. Merkl leg.; 15 (HNHM): same locality and collectors, around lights, 2–21.IV.2002;

1 (NSMT): Wulai, Taipei Hsien, 27.V.1965, K. Morimoto leg.; 2, Wulai, Taipei Hsien, 200 m, 3.IV.1977, J. & S. Klapperich leg.; 1 (CKMT): Chihpen, Taitung Hsien, 27.IV.1986, K. Masumoto leg.; 1 (HNHM): 5 km S Chinglun, Taitung Hsien, 30.V.1997, C. W. & L. B. O'Brien leg.; 3 (CKMT): Paling, Taoyuan Hsien, 28–29.IV.1979, S. Tsuyuki leg.; 1 (HNHM): Paling, Taoyuan Hsien, 25.IV.1982, N. Ohbayashi leg.; 1 (CCLT): Paling, Taoyuan Hsien, 23.V.1999, C.-F. Lee leg.; 1 (MHNG): Upper Paling, Taoyuan Hsien, 1200 m, 18.IV.1990, A. Smetana leg.

Distribution. China: Jiangsu (new record), Zhejiang, Fujian, Hubei, Guangxi (new record), Chongqing, Guizhou (new record), Taiwan. In Taiwan, *X. ruficollis* is one of the most common species of Lagriini, especially in the *Machilus-Castanopsis* zone, including disturbed places and secondary growth. Specimens are known from the lower *Quercus* zone, too. (The altitudinal vegetation zones of Taiwan see Su 1984).

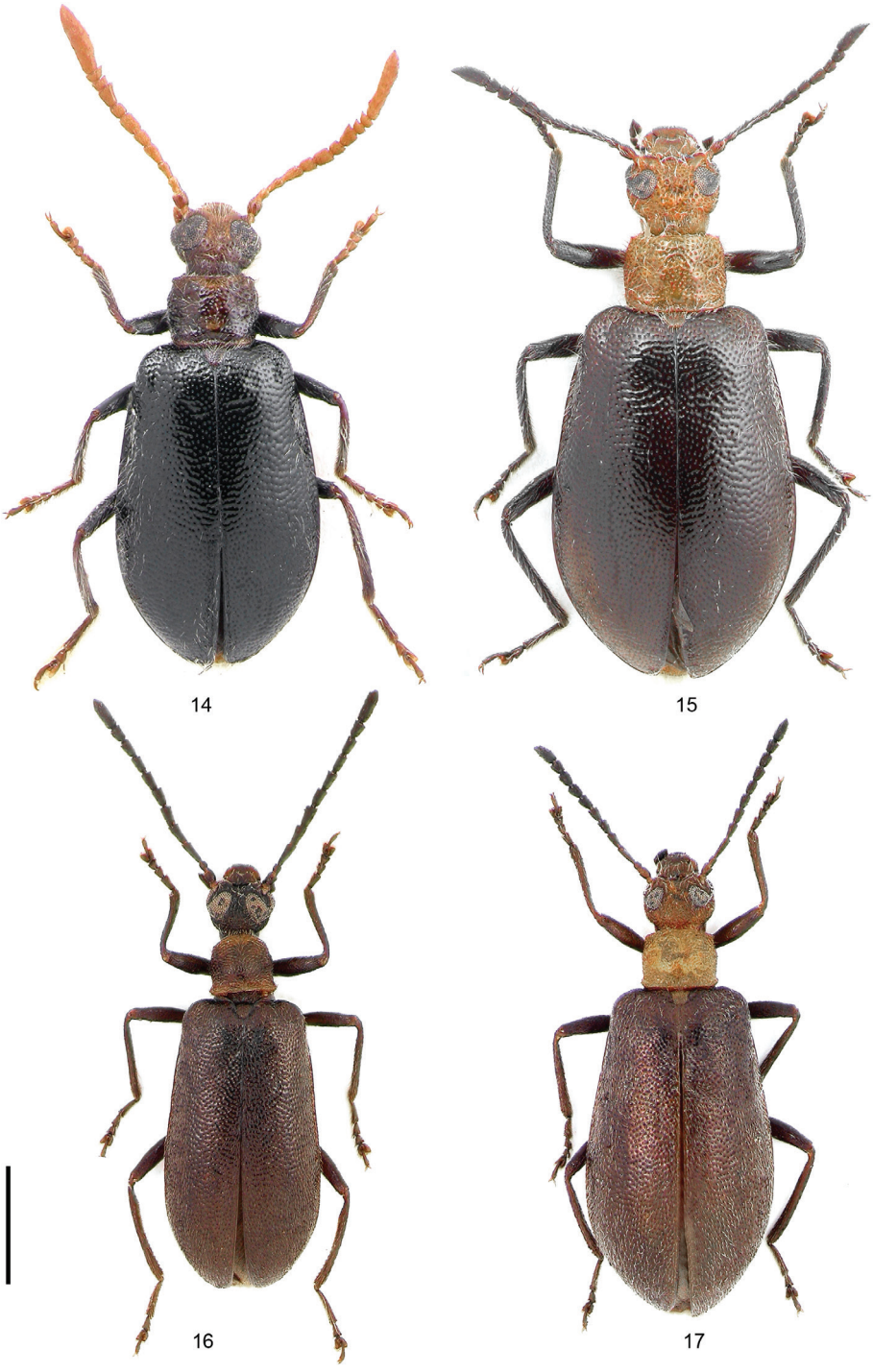
Remarks. *Xenocera xanthisma* was described by Chen (2002) on the basis of three males and one female from Fujian and Hunan. However, the original description was not mentioned in the Zoological Record, therefore this species was unknown to other coleopterists, and was not included in the Catalogue of Palaearctic Coleoptera (Merkl 2008). The type series was deposited in SWU, but at the moment only the male holotype and one male paratype are found there. The holotype is in bad condition with its head missing, but fortunately, the aedeagus is visible. Chen (2002) indicated "yellow color and curve[d] terminal antennomere" as diagnostic to *Xenocera xanthisma*. However, "curve[d] terminal antennomere" is also characteristic to *X. ruficollis*. "Yellow color" is typical to teneral individuals of *X. ruficollis* that are pale yellowish brown with head and antennae somewhat darker. Moreover, the aedeagus of the holotype is identical with that of *X. ruficollis*. Therefore we propose *Xenocera xanthisma* as a junior subjective synonym of *Xenocerogria ruficollis* (Borchmann, 1912).

***Xenocerogria feai* (Borchmann, 1911)**
Figs 14–15, 18–19, 22–23

Lagriocera feae Borchmann, 1909: 209 (type locality: Burma, Carin Chebà. type depository: MSNG).
Xenocera feai: Borchmann 1936: 117.
Xenocerogria feai: Merkl 2007: 270 (China: Yunnan; Burma); Merkl 2008: 116.

Redescription. Body length 5–10 mm. Body, including legs, black, except brownish red head, antennae, prothorax and scutellum. Brownish red parts sometimes darker brown to black.

Male (Fig. 14). Head rounded, interocular distance 0.75× as wide as eye diameter; preorbital swelling slightly convex and glabrous; frons distinctly impressed, sparsely and coarsely punctate. Eyes reniform, moderately bulging, genal canthus encroaching to 0.6× eye width. Antennae (Figs 18–19) surpassing base of elytra when directed backwards, gradually broadening toward apex, antennomere 1 subglobular, 0.3× as



Figures 14–17. Dorsal habitus: **14** *Xenocerogria feae*, male **15** *X. feae*, female **16** *Lagria ignota*, male **17** *L. ignota*, female. Scale = 2 mm.

long as distance between antennal insertions, antennomere 2 small, shorter than 1, antennomere 3 longer than 2 but shorter than 4, 5 subquadrate, shorter than 4, 6 longer and wider than 5 and 7, 7 slightly transverse, 8 twice longer than 7, anterior inner angle slightly produced, 9 and 10 strongly transverse, inner anterior angle almost dentiform; inner surface of antennomeres 5 to 10 flattened, smooth, glabrous, bordered with fine carinae; antennomere 11 as long as combined length of 4 preceding antennomeres, as wide as 10, subparallel-sided, strongly concave ventrally, inner margin of concavity bordered with carina forming sharp angulation in basal quarter.

Pronotum moderately transverse, maximum width at middle, anterior and posterior angles rounded; disc with four indistinct transverse lateral impressions; surface coarsely, sparsely and irregularly punctate, punctures separated by interspaces of 0.3 to 1.5 puncture diameter on disc, tending to be subcontiguous toward lateral portions; longitudinal midline with fine, obsolete carina.

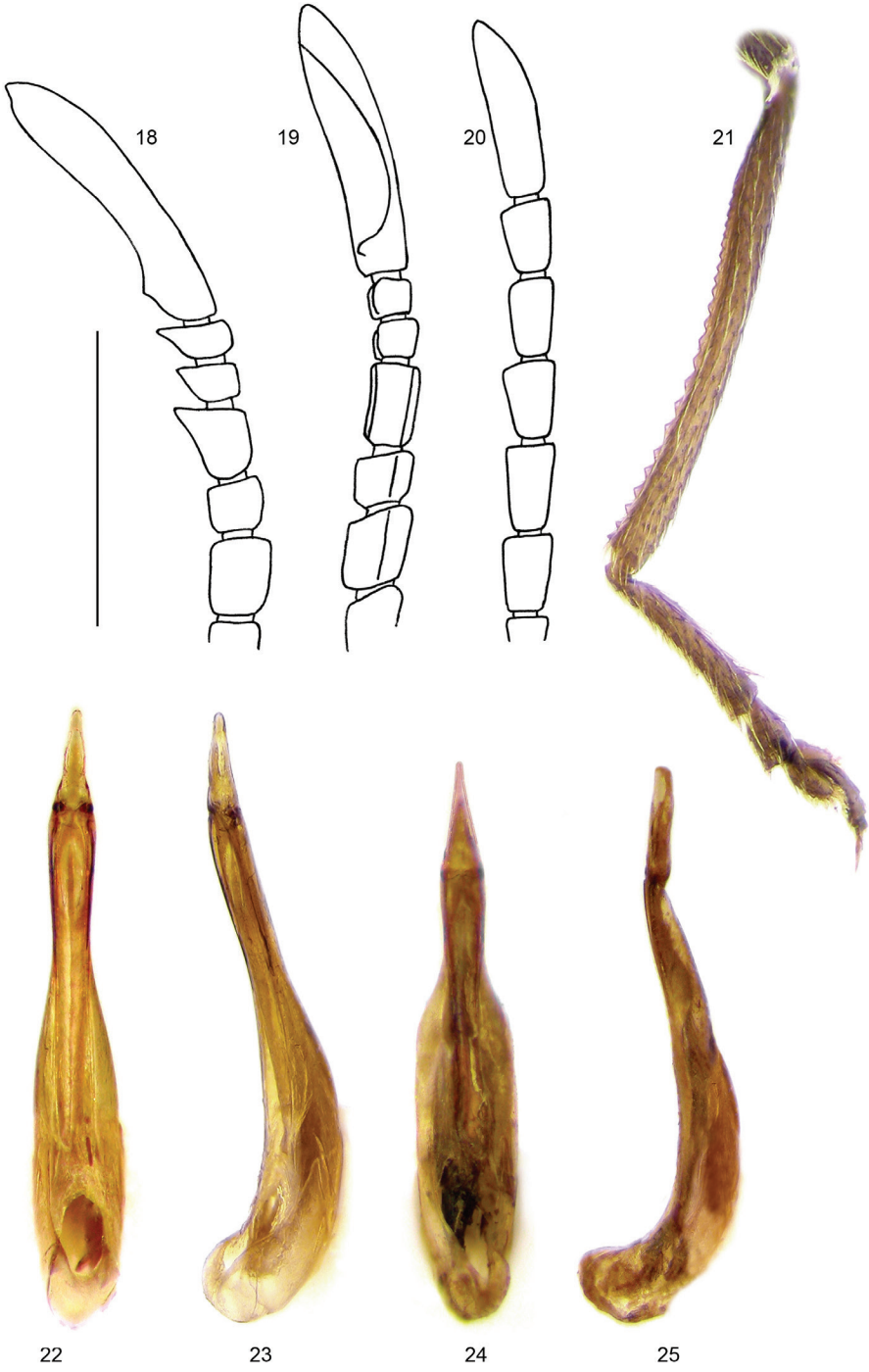
Elytra elongate, moderately dilated posteriorly, widest at apical 1/3, about 5× as long as pronotum; punctation moderately dense, punctures separated by interspaces of 0.5 to 1 puncture diameter; interspaces slightly convex, forming short oblique or transverse wrinkles; dorsal pubescence consisting of long, erect, sparsely set whitish hairs; humeral callosity separated from basal part of disc by distinct impression; elytral margin visible in dorsal view except at humeral callosity; elytral epipleura densely punctate, parallel-sided from base to level of metacoxae, then gradually narrowing towards apex. Mesoventrite, mesepisternum, metepimeron, metepisternum finely and sparsely punctate; metaventrite very finely punctate, almost smooth, punctation becoming denser in lateral portions.

Legs narrow; apical 0.3 of middle and hind femora reaching beyond edge of elytra; fore and middle tibiae nearly straight, slightly shorter than femora, hind tibiae straight, with sparse tuft of short and fine hairs before middle, without visible denticulation. Tarsi simple.

Aedeagus with distal part of basale narrow, gradually attenuating, apicale narrow, pointed (Figs 22–23).

Female (Fig. 15). Larger than male. Head with interocular distance about 1.2× as wide as eye diameter. Preorbital swelling not developed. Antennomeres not modified, 5 to 10 without flattened and smooth inner surface and produced inner anterior angle; antennomere 9 quadrate, 10 slightly transverse; antennomere 11 not concave, shorter than combined length of 2 preceding antennomeres. Elytra broader and more widening posteriorly. Legs shorter, hind tibiae without sparse tuft.

Type material examined. One syntype, female (MSNG, examined by O. Merkl in 1990), labelled as follows: 1) Carin Chebà 900–1100 m L. Fea V XII-88 [printed in black frame on white paper]; 2) Typus [printed with red in red frame on white paper]; 3) Feae Borch. [printed on white paper with black frame]; 4) Lagriocera Feae m. [Borchmann's handwriting on white paper in black frame]; 5) SYNTYPUS Lagriocera feae Borchmann 1911 (1909) [printed and handwritten on pink paper]; 6) Museo Civico di Genova [printed on white paper]. One syntype, male (MSNG, examined by O. Merkl in 1990), labelled as follows: 1) Carin Chebà 900–1100 m L. Fea V XII-88



Figures 18–25. Distal six male antennomeres: **18** *Xenocerogria feae*, lateral view **19** *X. feae*, ventrolateral view **20** *Lagria ignota*, lateral view **21** *L. ignota*, male right hind tibia **22–25** Aedeagus: **22** *X. feae*, ventral view **23** *X. feae*, lateral view **24** *L. ignota*, ventral view **25** *L. ignota*, lateral view. Scale = 1 mm.

[printed in black frame on white paper]; 2) SYNTYPUS Lagriocera feae Borchmann 1911 (1909) [printed and handwritten on pink paper]; 6) Museo Civico di Genova [printed on white paper].

Other materials examined. China: Yunnan: 1 ♂, 1 ♀ (CSBC), 5 ♂♂, 9 ♀♀ (HNHM): Jinghong, 10–14.VII.1990, S. Bečvář leg.

Distribution. China: Yunnan; Burma.

***Lagria ignota* (Borchmann, 1941), comb. n.**
Figs 16–17, 20–21, 24–25

Xenocera ignota Borchmann, 1941: 26 (type locality: China, Fujian, Kuatun. type depository: ZFMK).
Xenocerogria ignota: Merkl 2007: 270; Merkl 2008: 116 (China: Fujian; Oriental realm).

Redescription. Body length 5–8 mm. Body, including antennae and legs, black, except brownish red head, prothorax and scutellum. Brownish red parts sometimes darker brown to black.

Male (Fig. 16). Head rounded, interocular distance 0.5× as wide as eye diameter; preorbital swelling convex and glabrous; frons weakly impressed, densely and coarsely punctate. Eyes reniform, moderately bulging, genal canthus encroaching to 0.75× eye width. Antennae (Fig. 20) surpassing middle coxae when directed backwards, not broadening toward apex, antennomere 1 slightly longer than wide, 0.4× as long as distance between antennal insertions, antennomere 2 small, shorter than half of 1, antennomere 3 nearly 3× longer than 2 and 1.5× longer than 4 to 7 subequal in length, more than 2× longer than wide, 8 and 9 slightly narrower than preceding ones but still 2× longer than wide, 10 1.5× longer than wide, 11 about as long as 9 and 10 combined; none of apical antennomeres having modifications.

Pronotum moderately transverse, maximum width at middle, anterior and posterior angles rounded; disc with four indistinct transverse lateral impressions; surface coarsely, densely and irregularly punctate, punctures separated by interspaces of 0.3 to 0.5 puncture diameter on disc, tending to be subcontiguous mainly toward lateral portions; longitudinal midline with hardly discernible carina.

Elytra elongate, barely dilated posteriorly, widest at apical 1/5, about 5× as long as pronotum; punctation moderately dense, punctures separated by interspaces of 0.5 to 1 puncture diameter; interspaces slightly convex, forming short oblique or transverse wrinkles, mainly in basal 2/3; dorsal pubescence consisting of short, decumbent, sparsely set whitish hairs; humeral callosity separated from basal part of disc by vague impression; elytral margin visible in dorsal view except at humeral callosity; elytral epipleura sparsely punctate, parallel-sided from base to level of metacoxae, then gradually narrowing towards apex. Mesoventrite, mesepisternum, metepimeron, metepisternum finely and sparsely punctate; metaventrite very finely punctate, almost smooth, punctation becoming denser in lateral portions.

Legs narrow; apical half of middle and hind femora reaching beyond edge of elytra; fore and middle tibiae nearly straight, slightly shorter than femora, hind tibiae (Fig. 21) weakly curved, slightly dilated at basal ¼, inner margin between dilatation and apex with fine denticulation. Tarsi simple.

Aedeagus with distal part of basale broad, then abruptly attenuating, apicale narrow, pointed (Figs 24–25).

Female (Fig. 17). Larger than male. Head with interocular distance about 1.2× as wide as eye diameter. Genal canthus encroaching to 0.85× eye width. Preorbital swelling not developed. Antennomeres 11 shorter 9 and 10 combined length. Elytra broader and more widening posteriorly. Legs shorter, hind tibiae straight, without denticulation.

Type material examined. Lectotype, herewith designated, female (ZFMK), mounted on a card, left fore tarsus and middle right leg are missing, labelled as follows: 1) Kuatun (2300 m) 27,40n. Br. 117,40ö. L. J. Klapperich 4. 6. 1938 (Fukien) [printed on pale pink paper]; 2) Type [printed on dark pink paper with black frame]; 3) Xenocera ignota m. [Borchmann's handwriting on white paper]; 4) MUSEUM KOENIG BONN [printed on orange paper]; 5) Lectotypus ♀ *Xenocera ignota* Borchmann, 1941, des. Y. Zhou, O. Merkl & B. Chen, 2014 [printed on red paper].

Other materials examined. China: Fujian: 1 ♂ (ZFMK), 1 ♀ (HNHM): Kuatun [=Guadun, in Mt. Wuyishan], N 27°40', E 117°40', 11.IV.1938, L. J. Klapperich leg.; 1 ♂ (ZFMK): same locality and collector, 7.V.1938; 4 ♂♂, 1 ♀ (ZFMK), 3 ♂♂, 2 ♀♀ (HNHM): same locality and collector, 8.V.1938; 4 ♂♂, 1 ♀ (ZFMK), 2 ♂♂ (HNHM): same locality and collector, 11.V.1938; 7 ♂♂, 4 ♀♀ (ZFMK), 2 ♂ ♂ (HNHM): same locality and collector, 12.V.1938; 3 ♀♀ (ZFMK): same locality and collector, 13.V.1938; 2 ♂♂, 1 ♀ (ZFMK): same locality and collector, 19.V.1938; 1 ♀ (ZFMK): same locality and collector, 23.V.1938; 3 ♀♀ (ZFMK): same locality and collector, 24.V.1938; 1 ♂, 1 ♀ (ZFMK): same locality and collector, 26.V.1938; 1 ♀ (ZFMK): same locality and collector, 25.V.1938; 2 ♀♀ (ZFMK): same locality and collector, 30.V.1938; 1 ♀ (ZFMK): same locality and collector, 2.VI.1938; 1 ♂, 2 ♀♀ (ZFMK): same locality and collector, 4.VI.1938; 1 ♀ (ZFMK): same locality and collector, 6.VI.1938; 1 ♂ (ZFMK): same locality and collector, 8.VI.1938; 1 ♀ (ZFMK): same locality and collector, 14.VI.1938; 1 ♀ (ZFMK): same locality and collector, 15.VI.1938; 1 ♀ (ZFMK): same locality and collector, 20.VI.1938. **Vietnam: Vinh phu Province:** 1 (SMNS): 15–17.IV.1986, Tamdao, 80 km N of Hanoi, 900 m, collector unknown; 1 (SMNS): 19–21.IV.1986, same locality; 1 (SMNS), 1 (HNHM): 20.IV.1986, same locality; 1 (SMNS): 24–25.V.1985, same locality.

Distribution. China: Fujian; Vietnam.

Remarks. Borchmann (1941) described this species based on resemblance of the two female syntypes to *Xenocerogria ruficollis*. Male specimens were not available to him, although the long series collected by Klapperich in Fujian that included the two syntypes, contained several males as well. If he could have seen males, he would not have described the species in the genus *Xenocera*, because the male is unlike to that of the congeners: antennomeres 9 and 10 are not transverse, antennomere 11 is short and not concave ventrally, and the hind tibiae have distinctive serration. If it is accepted

that *Xenocerogria* is defined as having enlarged, concave antennomere 11 and unmodified tibiae of males, the only plausible approach is to remove *X. ignota* from this genus and transfer it to the composite genus of *Lagria*, for lack of a better place to put it.

Acknowledgements

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Phylogenetic relationships in the *Niviventer-Chiromyscus* complex (Rodentia, Muridae) inferred from molecular data, with description of a new species

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Abstract

Based on molecular data for mitochondrial (Cyt *b*, COI) and nuclear (IRBP, GHR) genes, and morphological examinations of museum specimens, we examined diversity, species boundaries, and relationships within and between the murine genera *Chiromyscus* and *Niviventer*. Phylogenetic patterns recovered demonstrate that *Niviventer* sensu lato is not monophyletic but instead includes *Chiromyscus chiropus*, the only previously recognized species of *Chiropus*. To maintain the genera *Niviventer* and *Chiropus* as monophyletic lineages, the scope and definition of the genus *Chiromyscus* is revised to include at least three distinct species: *Chiromyscus chiropus* (the type species of *Chiromyscus*), *C. langbianis* (previously regarded as a species of *Niviventer*), and a new species, described in this paper under the name *C. thomasi* **sp. n.**

Keywords

White-bellied rats, Fea's tree rat, Southeast Asia, Vietnam, molecular phylogeny, taxonomy, new species

Introduction

The genera *Niviventer* Marshall, 1976 and *Chiromyscus* Thomas, 1925 are members of the *Dacnomys* division of the tribe Rattini (Musser and Carleton 1993, 2005). The composition of the *Dacnomys* division was recently subjected to considerable taxonomic revision based on molecular data (Balakirev et al. 2012a,b, 2013) and now includes five other Indo-Sundaic and Philippine genera: *Dacnomys* Thomas, 1916; *Leopoldamys* Ellerman, 1947; *Saxatilomys* Musser et al., 2005; *Tonkinomys* Musser et al., 2006, and *Anonymomys* Musser, 1981 (in the case of the this last genus, based on morphological supposition, as indicated by Musser and Carleton (2005), given lack of genetic data for this species to date). *Niviventer* is the most speciose genus in the *Dacnomys* division. This division has been placed as a sister group to the *Rattus* division based on combined analysis of mitochondrial and nuclear genes (Lecompte et al. 2008). Both of these groups are included in the large phylogenetic clade of Murinae in Southern Asia corresponding to what has been variably called the "Southern-Asian group" [according to Watts and Baverstock (1995)], the *Rattus* sensu lato group [according to Verneau et al. (1998)], or the "*Rattus* group" [according to Steppan et al. (2005) and Jansa et al. (2006)] by various authors.

Taxonomic composition and preliminary views of relationships within the genus *Niviventer* were first established by Musser (1981) as part of a general revision of *Rattus* Fischer, 1803 sensu lato. Along with *Niviventer*, genera such as *Maxomys* Sody, 1936, *Leopoldamys*, *Lenothrix* Miller, 1903, *Dacnomys,* and *Chiromyscus* were separated from *Rattus* based on features of skull structure, such as the configuration of the lateral walls of the cranium above each pterygoid fossa, the details of the construction of the squamosal roots of the zygomatic arches, the position of the posterior margin of the palatal bridge against the third upper molars, the details of the construction of the mesopterygoid fossa, the proportions of the auditory bullae, and other specific skull structures. Initially, fifteen species were recognized by Musser (1981) within the genus, which has been subdivided into two groups/divisions. The "andersoni group" consisted of *N. andersoni* (Thomas, 1911) and *N. excelsior* (Thomas, 1911), and the "niviventer group" included *N. brahma* (Thomas, 1914), *N. eha* (Wroughton, 1916), *N. langbianis* (Robinson & Kloss, 1922), *N. hinpoon* (Marshall, 1976), and *N. cremoriventer* (Miller, 1900). The taxonomic status of a large series of forms, namely *N. niviventer* (Hodgson, 1836), *N. confucianus* (Milne-Edwards, 1871), *N. tenaster* (Thomas, 1916), *N. fulvescens* (Gray, 1847), *N. coninga* (Swinhoe, 1864), *N. rapit* (Bonhote, 1903), *N. lepturus* (Jentink, 1879) and *N. bukit* (Bonhote, 1903) was unclear, and this group was referred to as the "*niviventer* complex". Although actual species boundaries and taxonomical affiliation for some taxa and morpha have been debated for a long time, at the moment, the generic structure proposed by Musser (1981) is generally accepted by most recent authors (Nowak 1999, Wang 2003, Pavlinov 2005). As indicated in the most recent summary on the taxonomy of Muroidea (Musser and Carleton 2005), the genus comprises 17 species that are subdivided into the "andersoni" and "niviventer"

groups, with three additional species, *N. culturatus* (Thomas, 1916), *N. fraternus* (Robinson & Kloss, 1916) and *N. cameroni* (Chasen, 1940), recognized as distinct within the "niviventer group". *N. bukit* was not been given a specific status. At the same time, recent investigations using cytochrome *b* (Cyt *b*) gene sequences show that the intrageneric structure within the genus *Niviventer* is much more complex than is currently accepted. Three or four additional monophyletic groups can be separated within the genus (Balakirev and Rozhnov 2010, Balakirev et al. 2012a), namely, the "niviventer group", the "fulvescens group", the "langbianis group", and the "andersoni group", with the additional possibility of tracing other currently unrecognized groups, given that a number of species, especially in the Sundaic Islands, have yet to be investigated.

The monotypic genus *Chiromyscus* is most likely the closest relative to *Niviventer*. The only representative of this genus, *Chiromyscus chiropus* (Thomas, 1891), was first described as *Mus chiropus* from East Burma. This species is morphologically very similar to the Indochinese taxon *Niviventer langbianis* (Musser 1981, Musser and Carleton 1993, 2005, Musser et al. 2006) and the Sundaic taxon *N. cremoriventer* (Musser 1973) and may be confused with them, but it generally exhibits longer molar rows, higher supraorbital and temporal cranial ridges, a bicolored or mottled tail, a more expansive orange pattern on upperparts, and a nail-like claw on each hallux instead of a small claw. Unfortunately, this species is very rare in museum collections, so until recently little information was available about its natural history and only a few specimens were genetically characterized. In this paper, we investigate taxonomic diversity and reveal the genus composition and relationships between *Chiromyscus* and *Niviventer*.

Materials and methods

Newly collected museum specimens investigated here were obtained in Vietnam during a series of field expeditions of the Joint Russian-Vietnamese Tropical Research and Technological Centre between 2007 and 2013 and deposited at the Zoological Museum of Moscow State University (ZMMU, Moscow, Russia) and at the Zoological Institute of the Russian Academy of Sciences (ZIN, Saint Petersburg, Russia). Most specimens were collected by the authors (BAE, AAV). All animals were identified in the field based on external morphology according to field identification manuals (van Peenen et al. 1969, Musser 1981, Lunde and Nguyen Truong Son 2001, Francis 2008) and the specific traits of skulls that are described in Corbet and Hill (1992) and discussed in Balakirev et al. (2012a). All skulls were investigated later in the laboratory under a stereomicroscope for comparison with more detailed species descriptions (Musser 1973, 1981, Musser and Carleton 1993, 2005). We also studied specimens deposited in the Natural History Museum (BMNH, London, UK), the Museo Civico di Storia Naturale "Giacomo Doria" (MSNG, Genoa, Italy), and the National Museum of Natural History, Smithsonian Institution (USNM, Washington, USA). In total, 32 adult specimens (skulls and/or alcohol-preserved bodies) were examined.

DNA extraction, PCR amplification, and sequencing

Twenty eight specimens of *N. langbianis* and *Chiromyscus* from 6 localities in Vietnam were sampled for genetic analysis (See Suppl. material 1). Small quantities of liver and muscle tissue or fingertips and earclips were stored in 96% alcohol and used for DNA extraction. Total genomic DNA was extracted using a routine phenol/chloroform/proteinase K protocol (Kocher et al. 1989, Sambrook et al. 1989). The DNA was further purified either by double ethanol precipitation or by using a DNA Purification Kit (Fermentas, Latvia). We targeted four genes that proved to be useful for the phylogenetic analysis of various groups of the superfamily Muroidea generally (e.g., Serizawa et al. 2000, Jansa and Weksler 2004, Buzan et al. 2011) and for Asiatic murids specifically (Michaux et al. 2002, Suzuki et al. 2003, Jansa et al. 2006, Jing et al. 2007, Lecompte et al. 2008, Pages et al. 2010). These genes included a complete or substantial portion of the Cytochrome B gene (Cyt *b*; 950–1143 bp), a portion of the first exon of Interphotoreceptor Retinoid Binding Protein (IRBP; up to 1610 bp), and a portion of exon 10 of the Growth Hormone Receptor (GHR; 815 bp), all of which were amplified for further analysis. We also analyzed the 5'-proximal 680 bp portion of subunit I of the Cytochrome Oxidase gene (COI), which is generally used for species diagnoses and for DNA-barcoding (Hebert et al. 2003). The Cyt *b* was amplified using H15915R, CytbRglu (Kocher et al. 1989, Irwin et al. 1991), and CytbRCb9H (Robins et al. 2007) primers. The COI gene was amplified using the universal conservative primers BatL5310 and R6036R (Kocher et al. 1989, Irwin et al. 1991). The following universal PCR protocol was used to amplify both of the mtDNA fragments: initial denaturation for 1 min 30 sec at 95 °C, denaturation for 30 sec at 95 °C, annealing for 1 min at 52 °C, and elongation for 30 sec at 72 °C, followed by terminal elongation for 2 min at 72 °C. The PCR reaction was performed in a 30–50 ml volume. The final concentration of the PCR mixture in standard Taq PCR buffer with KCl (Fermentas, Latvia), was as follows: dNTPs – 0.2 mM; $MgCl_2$ concentration ranges of 2.0 ± 0.25 mM, 10–12 pmol of each primer, 20–50 ng of total DNA template and 1 unit of Taq DNA polymerase (Fermentas, Latvia) per tube. The reaction was performed using a Tercik (DNK-Tehnologia, Russia) thermocycler. The IRBP gene (1000–1610 bp in length) was amplified using the IRBP125f, IRBP1435r, IRBP1125r and IRBP1801r primers, according to the method of Stanhope et al. (1992). A nested PCR technique was applied to amplify the GHR gene, in accordance with Jansa et al. (2009). An approximately 1.0-kb portion of exon 10 from the GHR gene was amplified using the primers GHRF1 and GHRendAlt. This polymerase chain reaction product was re-amplified using the nested GHRF1 primer paired with GHR750R and the GHRF50 primer paired with GHRendAlt. The PCR products were purified using a DNA Purification Kit (Fermentas, Latvia).

The resulting double-stranded DNA products were directly sequenced in both directions using the Applied Biosystems 3130 Genetic Analyzer and the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit. All obtained sequences were deposited in GenBank (www.ncbi.nlm.nih.gov/genbank) under the accession

numbers KF154023–KF154052 and KF154054–KF154085, and certain COI gene sequences were also uploaded into the BOLD database (www.barcodinglife.org project "Indochinese Muridae", ICMBA).

We also analyzed 122 gene sequences of *Niviventer* (all "langbianis group" species sequences available, as well as some sequences from other species) and *Chiromyscus* that were available in the GenBank and BOLD databases as of 1 May 2013. Out of these 122 sequences, 35 were for Cyt *b*, 27 were for IRBP, 26 were for GHR, and 34 were for COI. The gene sequences from two outgroup species were used to root the phylogenetic tree [*Mus musculus* L., 1758 (V00711, complete mtDNA genome; AB033711, IRBP; NM001048147, GHR) and *Rattus rattus* L., 1758 (EU273707, complete mtDNA genome; AM408328, IRBP; DQ019074, GHR)].

Sequence editing and phylogenetic analyses

Sequences were aligned using BIOEDIT 3.0 (Hall 1999) and CLUSTAL W (incorporated into BIOEDIT and MEGA 5.05) software and were verified manually. Basic sequence parameter calculations (i.e., variable sites, parsimony-informative sites, base composition biases, nucleotide frequencies and nucleotide substitution tables), codon evolution model testing, and inter- and intra-population divergence evaluations were performed using MEGA 5.05 software (Tamura et al. 2011). All of the most frequently used algorithms, such as maximum parsimony (MP), maximum likelihood (ML), minimum evolution (ME), and neighbor-joining (NJ) were applied to the phylogenetic reconstructions and tree constructions using MEGA 5.05 software. Bayesian inference (BI) was performed using MRBAYES v.3.1 software (Huelsenbeck and Ronquist 2001). The best-fitting models of gene evolution out of 24 possible codon evolution models were determined using a model test module and implemented in MEGA 5.05 using the Maximum Likelihood value (lnL), the Bayesian Information Criterion (BIC) and the corrected Akaike Information Criterion (AICc). The TN93+G+I substitution model was applied for the Cyt *b* and COI genes, and for the combined Cyt *b* + COI data. The GTR+G substitution model was used for the IRBP gene, the GHR gene and for the combined four-gene data set. The calculated gamma shape parameters were 1.82, 1.7084, 0.3979, 0.165 and 0.1697 for the Cyt *b*, COI, IRBP, and GHR genes and for the combined data set, respectively. The robustness of the tree was assessed using a bootstrap procedure with 1000 replications. All of the trees were constructed and visualized directly with MEGA 5.05 or with TREEVIEW 1.6.6 software (Page 1996). We performed the Tajima's Relative Rate Test (Tajima 1993) to estimate the rate of molecular evolution between species-level branches. No differences between any species-level branches were detected. Intergroup/interspecies genetic divergences (*d*) were calculated under the Tamura 3-parameter (T3P) model using MEGA 5.05 software.

A phylogeny was first estimated for each gene independently, and subsequently for the concatenated dataset once the four genes were manually combined into a single data set in BIOEDIT 3.0 to produce combined samples. This restricted subset (12 var-

iables/taxa in total, see Suppl. material 2) was constructed based on species for which all four genes were available. The TREEROT v.3 program (Sorenson and Franzosa 2007) was used to examine Partitioned Branch Support values (PBS) in order to assess the contribution of each data partition to the combined analysis (Cyt *b*/COI/IRBP/GHR) (Baker and DeSalle 1997). This analysis was performed to test the sustainability of the primary internal nodes in the different genes studied.

Bayesian analysis for the combined data set was performed using four independent runs of 2×10^6 generations each. The most complex substitution model, GTR+G, was used for the combined data set to avoid multi-partition calculation procedures and relax computing process (even though the mitochondrial genes appeared to evolve under the more simple TN93+G+I substitution model). We used a flat Dirichlet prior distribution for the relative nucleotide frequencies and for the relative rate parameters, a discrete uniform prior distribution for the topologies and an exponential distribution for the gamma shape parameter and all branch lengths. A burn-in period of 500,000 generations was determined graphically using TRACER v1.4 (Rambaut and Drummond 2007) to ensure convergence and to ascertain that the runs were not trapped on local optima.

Results

Phylogenetic analyses

Single gene phylogenies revealed that relationships across the overall taxon sampling could not be reliably resolved for most basal nodes, irrespective of the phylogenetic approach (results not shown). The trees obtained from the different genes and methods differed mostly in the topology of the branches of species within the niviventer/fulvescens/langbianis groups and in the level of nodal supports. In Fig. 1, we present the ML tree obtained using the Cyt *b* gene and report the support values from ML, ME, NJ, MP and BI analyses. The Cyt *b* phylogeny revealed six multispecies groups in *Niviventer*, as mentioned previously (Balakirev and Rozhnov 2010); namely, "niviventer", "fulvescens", "langbianis" and "andersoni" groups, plus two more species level branches, one of which may correspond to *N. rapit* (see Balakirev et al. 2012a, for details), and one for Malayan species which we provisionally refer to here as *N.* cf. *cremoriventer*. We previously used the name *N. cremoriventer* (Balakirev and Rozhnov 2010, Balakirev et al. 2012a) for clade 2 within the "langbianis-chiropus" group. However, the samples from mainland Malaysia (named in GenBank as *N. cremoriventer*) constitute an independent specific sister clade to the "fulvescens" group. These samples could not be regarded as conspecific with any of the Vietnamese samples. Due to a lack of comparative morphological materials for Sunda Shelf *N. cremoriventer,* we are unable to discuss the proper attribution and taxonomic position for these samples here. According to Musser (1973), *N. cremoriventer* may represent not a single taxon but a set of vicarious species in the Sundaic region. It is also remarkable that, due to the high level of homoplasy, the "lang-

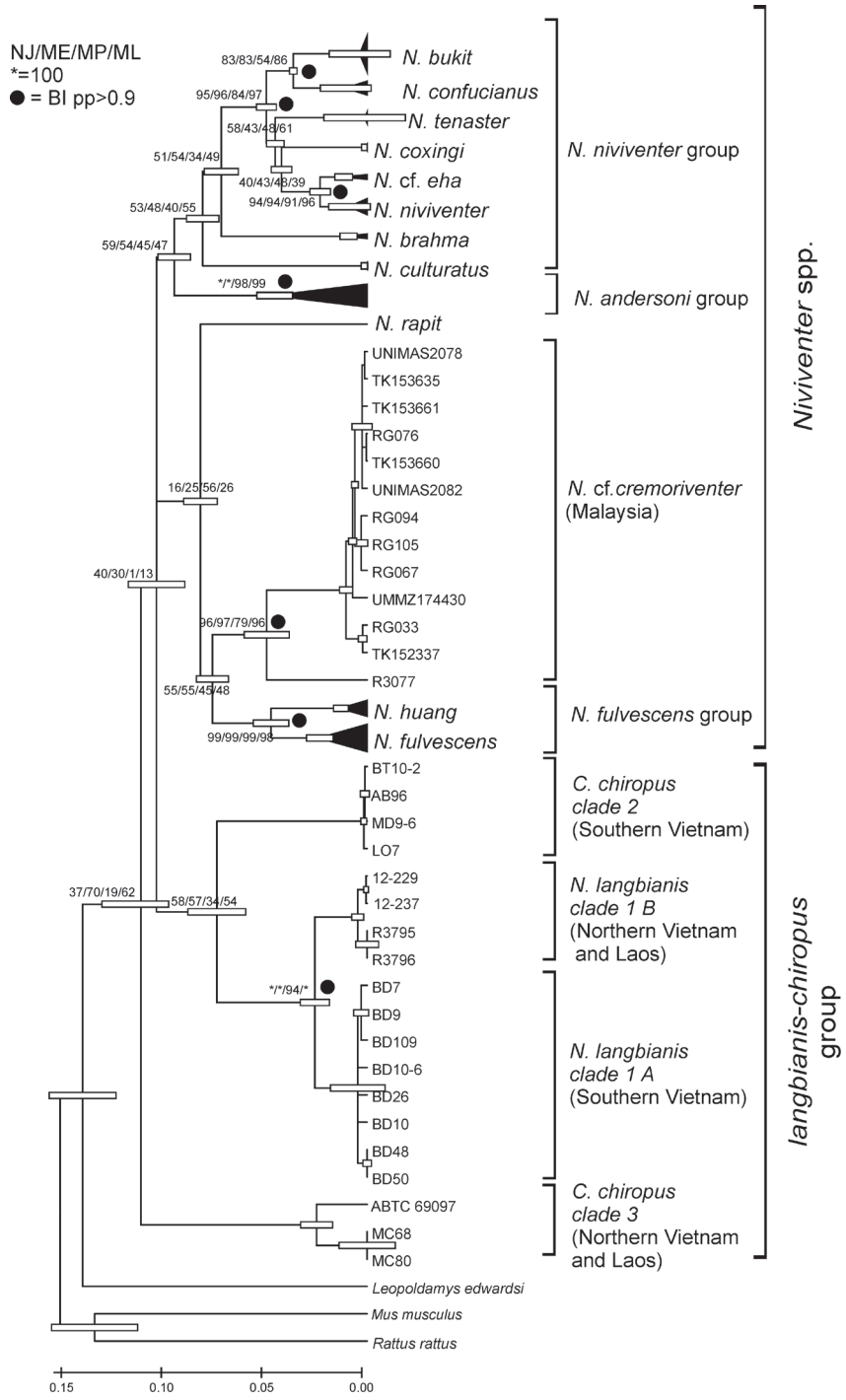

Figure 1. The ultrametric ML phylogenetic tree constructed based on complete Cyt *b* gene sequences (TN93+G+I; 1-2-3 pos. inc.) of the *Niviventer-Chiromyscus* complex. The scale bars at the bottom represent the level of divergence (*d*, T3P). The bars at the nodes represent the level of confidence of branch lengths.

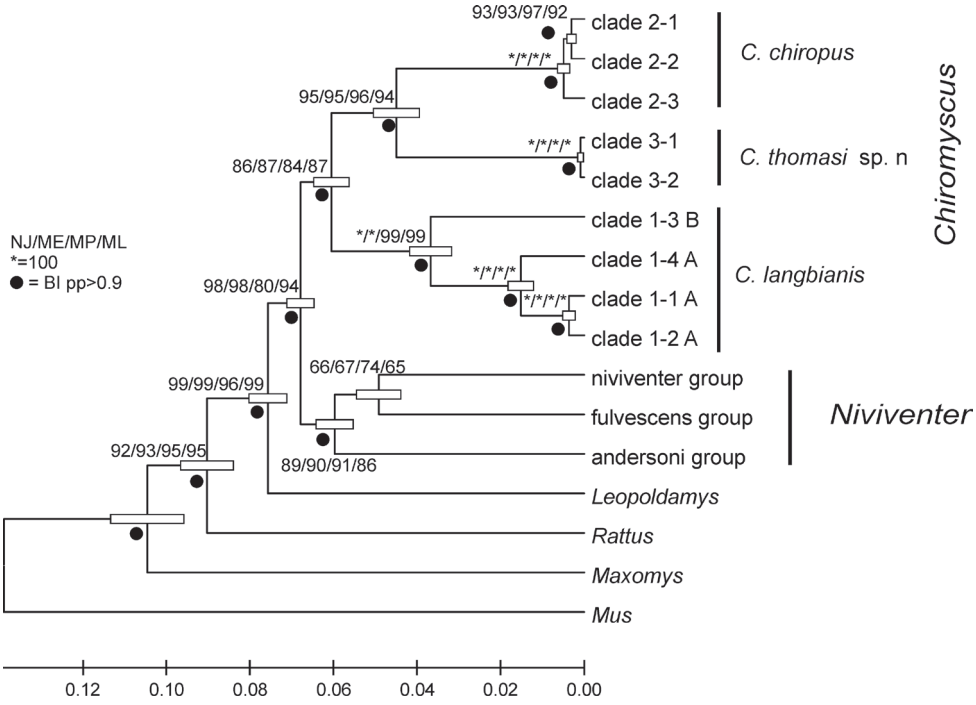

Figure 2. The ultrametric ML phylogenetic tree constructed based on the combined dataset Cytb+COI+IRBP+GHR gene sequences (GTR+G+I; 1-2-3 pos. inc.) of the *Niviventer-Chiromyscus* complex. The bars at the nodes represent the level of confidence of branch lengths.

bianis" group appears to be the most unsustainable group in *Niviventer*. Depending on what species or species-pair was chosen as its representative for comparison with other groups of species in *Niviventer,* and on what phylogenetic method was applied for any single gene and for the combined Cyt *b*+COI data set, the position of the "langbianis" group with respect to other species-groups in *Niviventer* varied considerably. In either case, the bootstrap levels for this branching node were low, preventing any reasonable conclusion about its proper relationships.

The combined analysis using all four genes on a reduced dataset resulted in a well-supported phylogeny (Fig. 2). Similarly to the individual gene phylogenies, the combined analysis revealed that *Niviventer* is not monophyletic, with *N. langbianis* (clade 1) recovered as sister to *C. chiropus* (clades 2) and *C.* sp. n. (clade 3). It is also remarkable that both specimens that undoubtedly belong to the genus *Chiromyscus* (Fig. 2) proved to be members of the "langbianis-chiropus" group. This clade appeared to be sister to the genus *Niviventer* and sufficiently genetically divergent to be regarded as a different genus. Thus, the current composition of the genus *Niviventer* did not demonstrate monophyly. Based on the phylogenetic patterns revealed and on the level of genetic subdivisions demonstrated between these three phyla comprising the "langbianis-chiropus" cluster (*d*, T3P =0.137–0.181 for Cyt *b*; *d*, T3P =0.074–0.147 for COI), the taxonomic structure and species composition of *Niviventer* and *Chiromyscus* should be revised.

Taxonomic implications

Given that *Niviventer* is shown not to be monophyletic, two taxonomic approaches to generic nomenclature could be undertaken. Either *Niviventer* could be regarded as a junior synonym of *Chiromyscus* based on taxonomic priority (ICZN 1999, article 23.1) or a revised concept of *Niviventer* could be employed, restricting application of this name to the group of species demonstrating monophyly with the type species of *Niviventer* (*N. niviventer* [Hodgson, 1836]), to the exclusion of those more closely related to the type species of *Chiromyscus* (*C. chiropus*), which could be maintained as a separate genus from *Niviventer*. It is a challenge to choose between these two possible decisions. On one hand, *Niviventer* is an established taxonomic name that has been widely and generally used since 1976 for this widespread and taxonomically complex group of rats (up to 17 species), while *Chiromyscus* is arguably a less familiar name in that it has usually been regarded as a rare and monotypic lineage. On the other hand, *Chiromyscus* has priority over *Niviventer*. We consider the principle of stability of nomenclature (ICZN 1999, article 48.1) and the fact that the type species of the genus *Niviventer* falls into the main cluster of species of the "niviventer" group that is paraphyletic with respect to *Chiromyscus*. We also note that the "langbianis-chiropus" species cluster is well differentiated from other *Niviventer* both genetically (*d*, T3P >0.15 for Cyt *b*; *d*, T3P >0.08 for COI), and morphologically (see the description below). The close relationship of *langbianis* to *Chiromyscus* was anticipated and earlier postulated by Musser et al. (2006: page 24 and table 3) in their description of *Tonkinomys*. Musser et al. (2006) pointed out that Musser's 1981 characterization of *Niviventer* was more of a taxonomic summary at the time and meant to be a working hypothesis, not a systematic revision. They also noted that in any future revision of *Niviventer* most species will remain in the genus, but *langbianis* and *chiropus* will likely be separated, and they listed some morphological traits shared by *langbianis* and *chiropus* (including the same number of roots anchoring first upper and lower molars), and noted that both were highly arboreal. Their paragraph closes with this observation: "A revisionary inquiry may either move *N. langbianis* to *Chiromyscus*, or *C. chiropus* will be subsumed within *Niviventer* (that name would then be a synonym of the older *Chiromyscus*)". Based on our field experience, these "langbianis-chiropus" species are indeed primarily arboreal (but may sometimes be trapped on the ground), a characteristic providing an additional ecomorphological basis for the generic identity of *Chiromyscus*. Based on the above considerations, we decided that the latter (restrictive) taxonomical approach would be more reasonable. Therefore, taking into consideration the principle of stability of nomenclature (ICZN 1999, article 48.1) we restrict the content of the genus *Niviventer* sensu stricto to exclude those species most closely related to *Chiromyscus chiropus.* At least three distinct species-level lineages (Fig. 1 and 2) can be allocated to the genus *Chiromyscus*. One of them corresponds to a morphologically easily-distinguished species usually referred to as *Niviventer langbianis* (Musser 1973, Musser and Carleton 1993, 2005, Musser et al. 2006, Balakirev et al. 2012a), whereas the other two include specimens usually attributed to *Chiromyscus chiropus*. However, as seen in Figs 1 and 2,

two distinct species can be distinguished among samples of "*Chiromyscus chiropus*" based not only on genetic comparisons but also on the distinguishing morphological features of these animals.

Morphological analysis and species attribution

Three morphologically distinct groups can be traced from the *N. langbianis*/*C. chiropus* complex (i.e., the redefined content of the genus *Chiromyscus*) that correspond to species-level phylogenetic clades revealed within the "langbianis-chiropus" cluster obtained from analyses of mitochondrial and nuclear genes (Figs 1 and 2).

Thirteen adult specimens identified here as *Chiromyscus langbianis* (Robinson & Kloss, 1922) were collected in the highlands of the Dalat Plateau, Lam Dong Province, southern Vietnam, close to the type locality of this taxon, and in the Huu Lien Nature Reserve, Lang Son Province, northern Vietnam. The corresponding samples formed two independent but closely related clusters, labeled as clade 1 with subclades A and B in Fig. 1. These rats are generally moderate in size (Fig. 3). Their fur is particularly dense, smooth and downy without any spines or guard hairs. The overall color of the dorsal pelage is generally dull and grayish with a touch of fulvous color. The pelage of the belly, as well as of the ventral side of the front legs, is white without any yellowish shade. Occasionally, fulvous or brown spots can be observed. The ventral coloration is sharply separated from the dorsal color. The tail is long and slender and is much longer than the body (135–155% of body length; 140% on average). It is uniformly tinged dark (chocolate) brown from the proximal part to the end. It is well covered with hair, but it lacks a terminal brush. The ears are relatively short, and the vibrissae are particularly long, extending backward well beyond the head. The dorsal sides of the fore and hind feet have a broad brown or chestnut stripe that extends straight beyond the middle part of the foot. The stripe becomes progressively narrower and disappears near the fingers so that the most distal third of the foot and the fingers are completely white. The claws are not so large (about 3.5 mm in length) but are sharp and curved, and adapted for climbing. The hallux bears a nail-like claw. The hallux is not as as perfectly opposable as for the other species of *Chiromyscus*, but is much more mobile in comparison with *Niviventer* species. Generally, *N. langbianis* may be reliably distinguished from the two species discussed below by its dull coloration, its contrasting stripe on the dorsal side of the hind feet and its appreciably narrower and darker tail (Fig. 3A, C). The skull is the most gracile of all species within the genus *Chiromyscus*, and its orbital ridges are not as highly developed as those of other species. Its cranial characters (summarized in Suppl. material 3) have been discussed in detail in Balakirev et al. (2012a). The general appearance of these specimens is consistent with the original description of *N. langbianis* (Robinson & Kloss, 1922). It should be noted that these animals referred to here as *N. langbianis* do not completely correspond to the description of that taxon as presented by Musser (1973, 1981). These animals lack some of the external characteristics, such as an olive hue

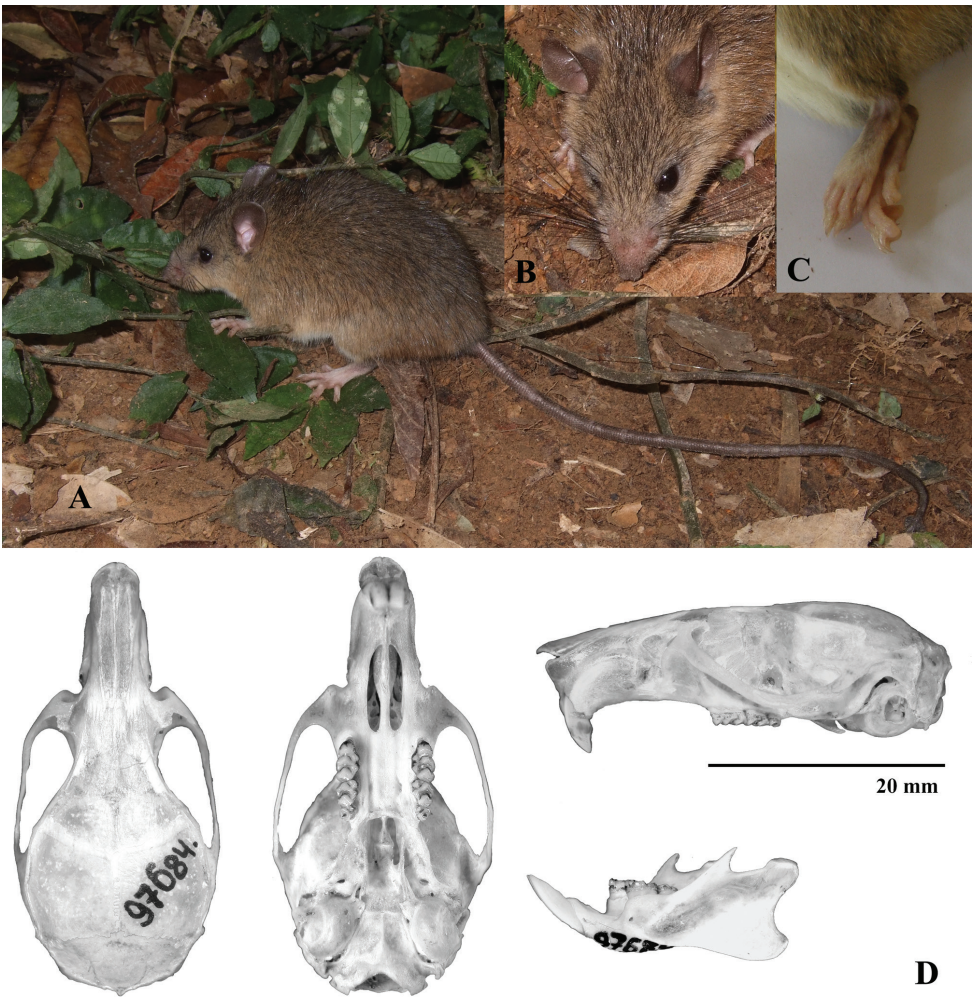

Figure 3. *Chiromyscus langbianis*, Bi Dup-Nui Ba Nature Reserve, Dalat Plateau, southern Vietnam. **A** General appearance (photo by Alexei V. Abramov), ZIN 96679, genetic voucher BD9 **B** Head, dorsal view **C** Hind foot, dorsal view **D** Skull, ZIN 97684.

in the coloration of their upper side or a creamy colored belly. Likewise, according to Musser's account, the incisive foramina in *N. langbianis* extend to the level of the first molars, but they did not exceed this limit in our specimens from the Dalat Plateau. Nevertheless, a comparative analysis with another *Niviventer* species inhabiting Vietnam (Balakirev and Rozhnov 2010, Balakirev et al. 2012a), namely *N. niviventer*, *N. fulvescens*, *N. huang*, *N. bukit*, *N. confucianus* and *N. tenaster* convinced us that these specimens should be attributed to *N. langbianis*. None of the other Vietnamese species may be attributed to *N. langbianis* based on the complex of specific features listed in original description (Robinson and Kloss 1922) and much more detailed descriptions made by Musser (1973, 1981).

Another thirteen adult animals we identified as *C. chiropus* (Fig. 4) were obtained from various locations in southern Vietnam, specifically, from the Dong Nai National Park (Ma Da Forest), Dong Nai Province; from the Binh Chau Nature Reserve, Ba Ria - Vung Tau Province; from the Lo Go Xa Mat Nature Reserve, Tai Ninh Province and from the Bao Loc Forestry, Lam Dong Province. In our previous publication we attributed these specimens to *N. cremoriventer* (Balakirev et al. 2012a) based on their close morphological similarity with that species and on the fact that these animals do not have the "dark mask" on their face, one of the most obvious morphological features noted as distinctive for *C. chiropus* (van Peenen et al. 1969, Corbet and Hill 1992, Lunde and Nguyen Truong Son 2001, Musser and Carleton 1993, 2005, Francis 2008, Lunde 2008) . The samples corresponding to this species are labeled as *C. chiropus* clade 2 on the phylogenetic tree (Fig. 1). The rats are rather small and brightly colored, clearly distinguishing them from the *C. langbianis* (as described above). The coloration of the upper side is a bright fulvous color with a pronounced orange hue, which is most prominent in the humeral area. Their fur is dense, smooth and downy with some blackish flexible guard hairs along a middle line of the back. A prominent buff-orange area separates the dorsal coloration from the creamy-yellowish belly. The sides are more brightly colored than the back. The cheeks, lateral surfaces of the neck and the front legs are a bright yellowish-orange, contrasting with the more dull coloration of the other parts of the body. The dorsal surfaces of both fore- and hindfeet are buffy-orange (Fig. 4H). The finger pads in the fore- and hindfeet are appreciably more developed than in *C. langbianis* (Fig. 4I). The claws are larger, and the thumbs of the hind feet bear a plain nail-like claw (Fig. 4H). The tail is long and slender and is much longer than the head-body (128–148% of head-body length; 138% on average). It is uniformly tinged brown from the proximal part to the end and is quite thick and covered with hair. The ears are large and dark-colored; the black vibrissae are long and oriented backward, extending well beyond the ears when laid flat against the head. Their skull morphology (measurements summarized in Suppl. material 3) has been described in detail in Balakirev et al. (2012a).

Chiromyscus chiropus was first described as *Mus chiropus* by Thomas (1891) in East Burma, Carin Hills, Thao (now Myanmar, Karen State, Tao, Karen Hills, also known as the Kayah-Karen Mountains, approximately 80 km NE of Toungoo, near 19°21'N 96°50'E). The holotypes stored in the Museo Civico di Storia Naturale "Giacomo Doria" are MSNG 18396 (skin) and MSNG 18397 (skull). The original description by Thomas (1891) was very general, with only one perceptible diagnostic trait indicated for species recognition, namely, an opposable hallux. The description was as follows: "*Mus chiropus*, sp. n. Similar in size and general appearance to *M. jerdoni*, Bly., but distinguished from that, as from every other member of the genus by the hallux being opposable as in *Chiropodomys*. Teeth strictly as in *Mus*. Head and body 125; tail 198; hind-foot 30." A much more explicit description delimiting the genus *Chiromyscus* was made by the same author at a later time (Thomas 1925). The genus was described based on two additional specimens originating from Bao Ha, Tonkin, 300 feet a.s.l. (now Vietnam, Lao Cai Province, Bao Ha, close to 22°10'N, 104°20'E). These two

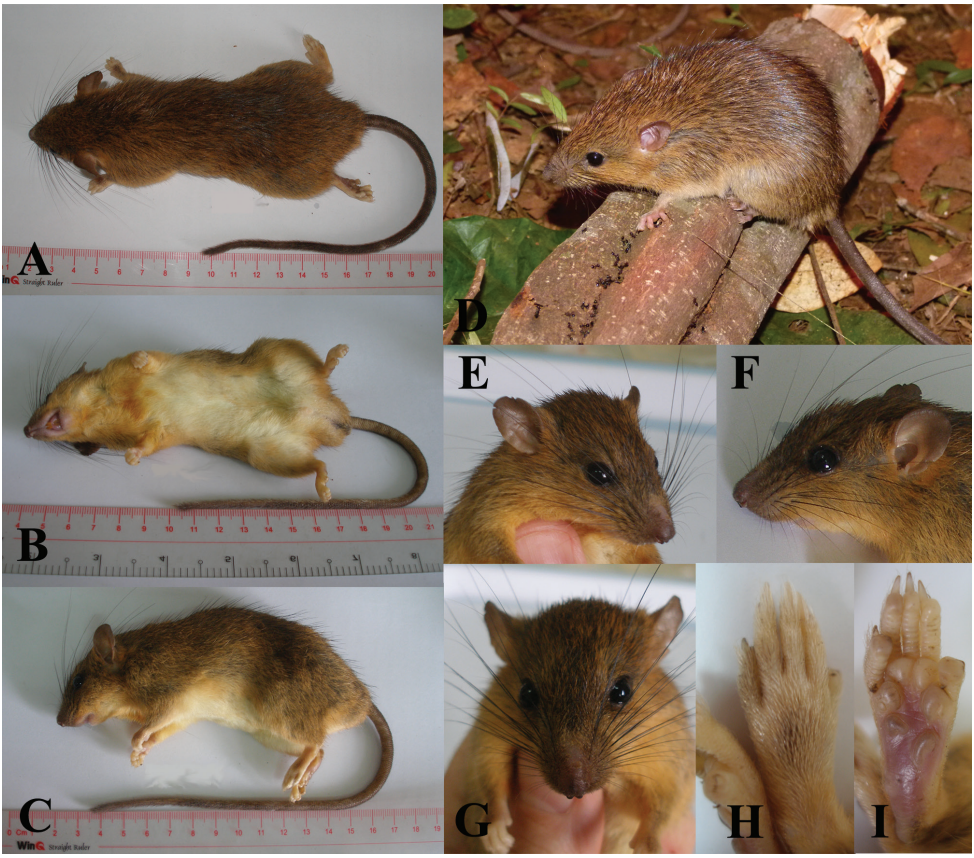

Figure 4. *Chiromyscus chiropus*, southern Vietnam. **A** Dorsal view **B** Ventral view **C** Lateral view **D** General appearance (photo by Alexander E. Balakirev) **E** Head, face-lateral view **F** Head, lateral view **G**Head, dorsal view **H** Hind foot, dorsal view **I** Hind foot, plantar surface. **A–C, E–I** specimen from the Binh Chau Nature Reserve, Ba Ria – Vung Tau Province, southern Vietnam, ZMMU S-191972, genetic voucher BT10-2 **D** specimen from the Bao Loc Forestry, Lam Dong Province, southern Vietnam, ZIN 100966, genetic voucher 12-068.

specimens were regarded by Thomas as "unquestionably of the same species" as the Burmese one (Thomas 1925: 504). He wrote as follows: "Now that a well-prepared skin is available, I am able to record that the colour and general appearance are much more striking then was evident on the typical spirit-specimen. For not only is the dorsal colour a warm lined buffy, with the ochraceous lateral band originally mentioned, but the whole side of the cheeks is bright ochraceous, the ochraceous area passing up beyond and behind the ears, forming a bright-coloured patch almost unique in Muridae, and reminiscent of some of the species of *Dremomys*. A rather darker ring around the orbit. Ears short-haired, flesh coloured. The rump, hips, and base of tail are also, like the ear-patches, rich ochraceous". Unfortunately, no body measurements are listed in the paper. Nevertheless, it should be stressed that the holotype of *C. chiropus* (Fig. 6)

is obviously outside the size limits (it is substantially smaller) when compared with the rats usually associated with the name *Chiromyscus* (van Peenen et al. 1969, Corbet and Hill 1992, Musser and Carleton 1993, 2005, Lunde and Nguyen Truong Son 2001, Francis 2008, Lunde 2008). It is remarkable that the most prominent features of *Chiromyscus*, a black "mask" or circumorbital "dark rings", were not mentioned in the original description of *C. chiropus* (see Thomas 1891). Thus, despite Thomas' assertion, it is rather doubtful that Fea's tree rat from Eastern Myanmar (*Chiromyscus chiropus* proper) and the "mask-bearing" Vietnamese samples are actually members of the same species. We studied images of the skin and skull of the holotype of *chiropus* (kindly provided by the Museo Civico di Storia Naturale). The specimen appeared to be in very good condition, with no perceptible traces of discoloration (Fig. 5). However, the most surprising finding was that two of the most prominent features usually attributed to *Chiromyscus chiropus* (Musser 1981, Corbet and Hill 1992, Francis 2008, Lunde 2008) do not characterize this specimen (Fig. 5D–E). Neither a dark "mask" around the eyes, nor a bicolored tail could be observed. Both the head and tail are unicolored (Fig. 5A–B). The holotype of *C. chiropus* is morphologically similar in general appearance, skull and teeth characteristics with our specimens from southern Vietnam, which were previously identified as Indochinese populations of *Niviventer cremoriventer* (Balakirev and Rozhnov 2010, Balakirev et al. 2012a). After analysis of the original description of *N. cremoriventer* (Miller 1900) and investigation of the holotype (USNM 86770, images kindly provided by USNM; Fig. 6) we concluded that the Vietnamese specimens are not correctly identified as *N. cremoriventer,* a Sundaic species. The skull of the holotype of *cremoriventer* is appreciably more gracile, with undeveloped supraorbital ridges, and the rostrum is considerably more narrow than in our samples from southern Vietnam. The clearest distinctions are in the construction of the pterygoid area, in the position and the shape of the foramina for cerebral nerves and arteries protruding from these bones. All GenBank samples originating from the Malay Peninsula and identified as *N. cremoriventer* constituted a deeply divergent, well supported branch (Fig. 1 and 2) that was closely related not to *Chiromyscus* but rather to the "fulvescens" group within *Niviventer*. Unfortunately, due to a lack of original samples from the Malayan and Sundaic regions, we cannot be completely certain as to the correct species affiliation of these genetic samples, but we suspect these may represent true *N. cremoriventer*.

Unfortunately, we did not have an opportunity to include the holotype of *C. chiropus* in our genetic comparisons. Nevertheless, based on apparent morphological similarity we attributed our southern Vietnamese specimens to *Chiromyscus chiropus* proper. Because of the scarcity of museum specimens and DNA-confirmed records for this species, it is difficult to estimate the true distributional range for this species. However, there are no substantial geographic barriers over the vast area stretching from the lowlands of southern Vietnam through Cambodia and central Thailand and west up to the hilly country of Peninsular Thailand and the eastern regions of Myanmar. Thus, there is every reason to believe the species may be distributed over substantial areas in Thailand and Cambodia, most likely scattered over patches of forested areas.

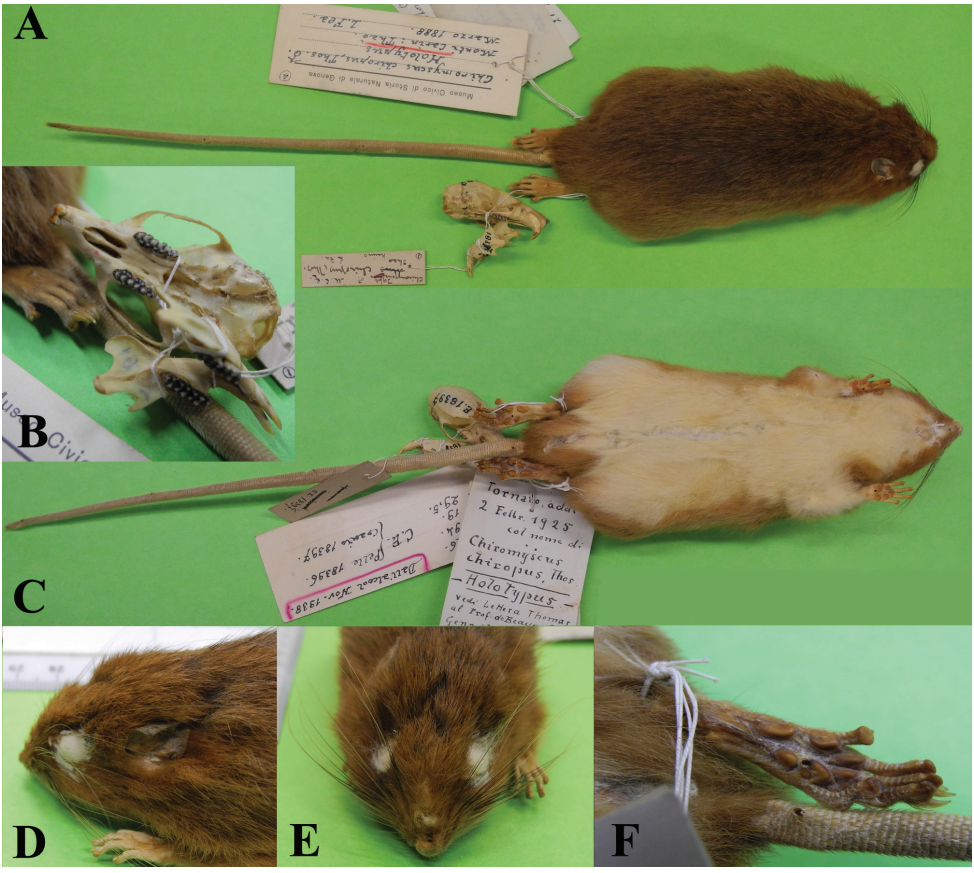

Figure 5. The holotype of *Chiromyscus chiropus*, stuffed skin (MSNG 18396) and skull (MSNG 18397). **A** Stuffed skin, dorsal view **B** Skull, ventral view; hind foot, dorsal view **C** Stuffed skin, ventral view **D** Head, lateral view **E** Head, face view **F** Hind foot, plantar surface. Images were kindly provided by the Museo Civico di Storia Naturale "Giacomo Doria", Genoa, Italy.

The third distinct species-level genetic lineage within *Chiromyscus* is labeled here as *C. chiropus* clade 3 (Fig. 1) and includes two specimens from Son La Province (Northern Vietnam) and one voucher sequence from Northern Laos obtained via GenBank. The general appearance of these Vietnamese specimens is completely consistent with the description of *C. chiropus* as detailed in the most recent guides (Francis 2008, Lunde 2008). This is a medium-sized, brightly colored rat (Fig. 7). The coloration of the upper side is a bright fulvous with a perceptible orange hue, which is most prominent in the humeral area. The fur is dense, smooth and downy. Ventrally, the belly, breast and throat are white without any colored patches. The sides are more brightly colored than the back. The cheek, lateral surface of the neck and the front legs are a bright yellowish-orange. A black strip, which is very prominent, passes over the eye, forming a remarkable face "mask" (Fig. 7D, E). The vibrissae are long, both black- and

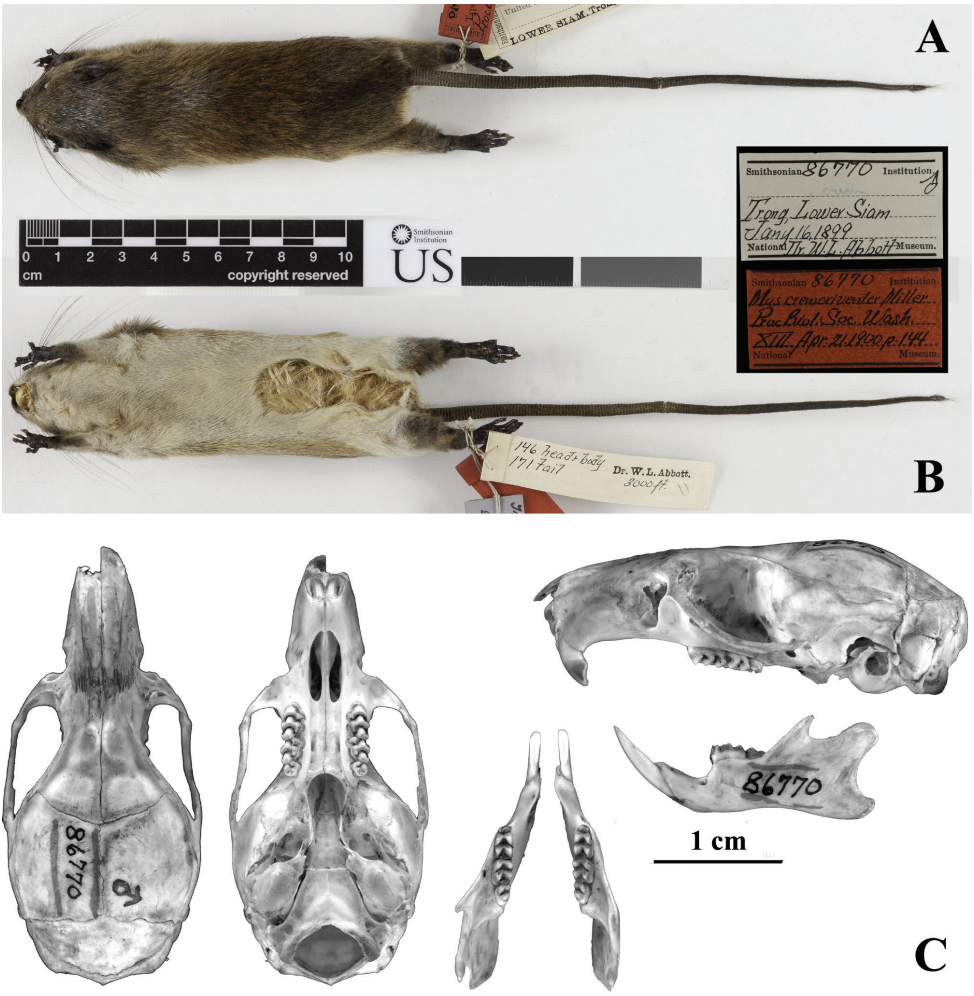

Figure 6. The holotype of *Niviventer cremoriventer*, stuffed skin and skull, USNM 86770. **A** Stuffed skin, dorsal view **B** Stuffed skin, ventral view; the natural coloration of front legs and feet is changed due to chemical treatment **C** Skull. Images were kindly provided by the National Museum of Natural History, Smithsonian Institution, Washington, USA.

white- colored, and the ears are small (18–20 mm) and rounded. The dorsal sides of both fore- and hindfeet are buffy-orange. The pads of the fore- and hindfeet are as well developed as in *N. cremoriventer*. The claws are large (4.2–5.0 mm in length) and the hallux bears a plain nail instead of a claw. The tail is very long, slender and hairy; it is much longer than the head-body (128–132% of head-body length). It is rather thick, almost uniformly tinged pale-brown from the proximal part to the tip. These specimens are morphologically very similar to specimens from northern Vietnam mentioned by Thomas (1925) in his description of the genus *Chiromyscus*. As discussed above, this taxon is morphologically and genetically different from *Chiromyscus chiropus* and rep-

resents a distinct species, which is described below. We can identify no previous taxonomic names applied to this taxon, but three synonyms (*indosinicus*, *vientianensis*, and *quangninhensis*) listed for *C. langbianis* in the recent taxonomic summary for mammals (Musser and Carleton 2005) deserve close review and consideration in this context.

Osgood (1932) described *Rattus indosinicus* from northern Vietnam (Sapa, Lao Cai Province). He noted it as being "similar to "*Chiromyscus chiropus* except in smaller size, in less prominent postorbital processes, and in more projecting infraorbital plate…" The coloration of the upper parts is "mixed dusky and ochraceous tawny", and no "mask" or similar feature is mentioned for this rat. Taking into consideration this description and the skull measurements provided (e.g. greatest length of skull < 38.1 mm for adults) we are convinced the name *indosinicus* is properly attributed to *C. langbianis.*

Bourret (1942) described a new rat from the Vientiane region of Laos as *vientianensis*, which [translation from French] "… is more closely related to *R. indosinicus* Osgood from Chapa, but has a tail clearly shorter, 99 to 125 per cent the length of the body (average = 111 per cent) instead of 127 to 145 per cent (average = 137 per cent) for the rat of Chapa… An adult male has the hair of the back deep grey at the base and ochre at the extremity… the pelage is fairly coarse; the underside is uniformly cream white, with hairs the same color throughout their length, paws white with a darker median band, not reaching the extremity". No dark "mask" or bright orange coloration was mentioned by the author. We follow here the opinion of Musser (1973) who ascribed *vientianensis* to the synonymy of *langbianis*.

Dao Van Tien (1970) described *Rattus cremoriventer quangninhensis* from Quang Ninh Province in central Vietnam. Dao Van Tien and Cao Vang Sung (1990) provided a detailed description of this form and made no mention of a face "mask" while noting a shorter tail, less than 125% of head and body length, and flat spines in the pelage. None of these features are characteristic for mask-bearing species of *Chiromyscus*, and we concur with Musser and Carleton (2005) in attributing this nominal taxon to the synonymy of *C. langbianis*.

***Chiromyscus thomasi* sp. n.**
http://zoobank.org/8127C488-5D01-4FFC-9556-0986A1198A26

Holotype. ZMMU S-191982, body in ethanol, skull extracted, genetic code MC68, adult male, collected 17 December 2011 by Alexander E. Balakirev. GenBank IDs: JQ755933, JQ755964, KF154025, KF154068.

Type locality. Vietnam, Son La Province, Muong Thai Village, near Lung Lo pass, 21°18'31"N, 104°41'34"E, elevation ~ 450 m above sea level.

Paratype. ZIN 101651, body in ethanol, skull extracted, genetic code MC80, adult female, collected 17 December 2011 by Alexander E. Balakirev from the type locality. GenBank IDs: JQ755934, JQ755965, KF154069).

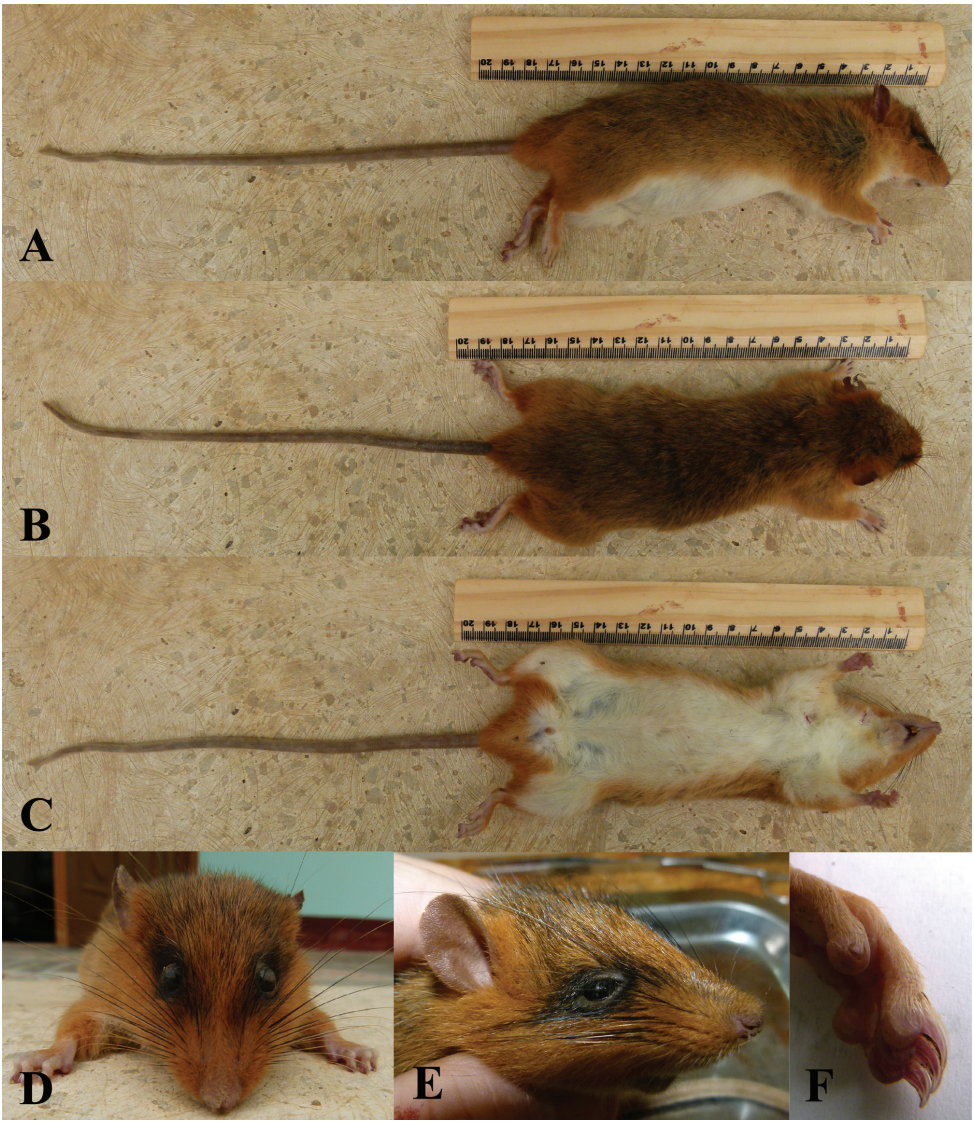

Figure 7. The paratype of *Chiromyscus thomasi* sp. n., Son La Province, northern Vietnam, specimen ZIN 101651, genetic voucher MC80. **A** Lateral view **B** Dorsal view **C** Ventral view **D** Head, face view **E** Head, lateral view **F** Hallux of the hind foot with the nail.

Referred material. BMNH 25.1.1.110, skin and skull, male, Bao Ha, Lao Cai Province, Vietnam; BMNH 26.10.4.167, skin and skull, female, Dak To, Kon Tum Province, Vietnam; BMNH 26.10.4.166, skin, male, Xieng Kuang, Laos.

Diagnosis. This species is set apart from all other described species within the genus *Chiromyscus* by the following combination of morphological traits: (1) Appreciably larger size. This species is the largest in size of any species of *Chiromyscus*. Head

and body length is 145–180 mm, tail length 200–231 mm, length of hind foot 27–29 mm, ear length 18–20 mm, greatest skull length 41.0–43.0 mm, upper molar lengths 7.0–8.0 mm; the supraorbital ridges are more developed than in other species, forming a distinct pointed triangle shelf at the point where the frontal and palatal bones come into contact. This shelf is very perceptible in the frontal view of the skull. (2) The upper parts are orange-brown. From the face to behind the ears, the pelage is bright orange, with a prominent darker ring around the eye forming a "mask" on the face. The under parts are pure white and sharply demarcated from the upper parts. The feet and toes are generally white with orange hairs on top. The tail is bicolored, dark on top and appreciably lighter below, where there is a pinkish hue. The hallux is shortened with rounded nails instead of pointed claws. The species is well differentiated genetically from other *Chiromyscus*. The DNA sequences that are deposited in GenBank under IDs JQ755933–JQ755934, JQ755964–JQ755965, KF154025 and KF154068–KF154069 may be used as genetic vouchers for this species.

Description. The fur is dense, smooth and downy. The coloration of the upper side is a bright fulvous with a perceptible orange hue, which is most prominent in the humeral area. On the underside, the belly is pure white without patches or creamy hues. The sides are more brightly colored than the back. The cheek, lateral surface of the neck and the front legs are a bright yellowish-orange. The rump, hips, and base of tail are also, like the cheek, a rich ochraceous color. A very prominent black strip passes over the eye, forming a characteristic "mask" on the face. The vibrissae are long (over 60 mm), both black- and white-colored, and the ears are small (18–20 mm), pale-brown colored and rounded. The dorsal sides of both the front and hind feet are completely buffy-orange. The pads both in the front and hind feet are very well developed. The claws are large (4.2–5.0 mm in length), curved and appreciably sharp. The hallux bears a plain nail instead of a claw. The tail is very long, slender and hairy; it is much longer than the body (128–132% of body length). It is rather thick and almost uniformly tinged pale-brown from the proximal part to the tip.

Comparisons. *C. thomasi* is a brightly colored species, a feature that obviously distinguishes it from *C. langbianis*, which is generally dull in coloration. With its bright fulvous or orange coloration *C. thomasi* is similar to *C. chiropus* but may be distinguished from it by its dorso-ventral coloration demarcation line. In *C. thomasi*, the white-colored belly replaces the bright orange ventral side coloration abruptly, without any intermediate zone, whereas a lighter-colored fulvous intermediate zone (0.5–1.0 cm in width) is perceptible on the back sides of *C. chiropus*. However, the most apparent distinguishing feature of *C. thomasi* is a dark "mask" on the face around the eyes, which may be used to visually separate it from any another *Chiromyscus* or *Niviventer* species. *Chiromyscus thomasi* is the largest species in the genus, appreciably bigger than *C. chiropus* and *C. langbianis*. Its skull well exceeds the known range of size variation for other *Chiromyscus* as well as for the majority of *Niviventer* species, with the exception of *N. tenaster* and the "andersoni" group, both of which are roughly equal in size to, or larger than, *C. thomasi*. In comparison with other *Chiromyscus* species, the skull of *C. thomasi* is also the most "heavily-built", with supraorbital ridges that are more

Figure 8. The holotype of *Chiromyscus thomasi* sp. n., Son La Province, northern Vietnam, skull ZMMU S-191982, genetic voucher MC68.

developed, forming prominent wide shelves. The skull of *C. langbianis* is much smaller and gracile, and the shelves are not so apparent, whereas in *C. chiropus* the skull has an obviously convex profile (when viewed from the side), in contrast with *C. thomasi*, which appears rather flattened when viewed from the side.

Etymology. The new species is named in honor of Oldfield Thomas (1858–1929), the British zoologist who named and described the genus *Chiromyscus* and the species *chiropus*.

Common name. Thomas' masked tree rat.

Distribution. Confirmed specimens of *Chiromyscus thomasi* have been recorded from the provinces of Son La and Lao Cai in northern Vietnam, the provinces of Kon Tum and Nhge An in central Vietnam, and the provinces of Xieng Khouang and Luang Prabang in northern Laos, based on published data and our (BAE) most recent and unpublished data. This species may have a wider distribution in central Vietnam (Dang Huy Huynh et al. 1994, Dang Ngoc Can et al. 2008) and in northern and central Laos (Aplin et al. 2008, Musser 1981, Corbet and Hill 1992) where similar "mask-bearing" specimens have been reported. It is also likely distributed in south-western China (see Wang 2003) and northern Thailand (see Marshall 1977) but clarifying comparisons are needed to rule out alternative identifications (*C. chiropus* and *C. langbianis*) before this wider potential geographic distribution is confirmed.

Conclusion

In spite of the close phylogenetic relationships evident within the *Niviventer-Chiromyscus* complex, the taxonomic composition within genera can be reliably resolved by

a combination of mitochondrial and nuclear gene analyses, which provide support to the traditional morphological segregation initially suggested for a *langbianis-Chiromyscus* cluster by Musser et al. (2006). The patterns revealed show that both *Niviventer* and *Chiromyscus* comprise multiple species and complex phylogenetic composition. Based on the divergence of genetic lineages, we suggest that the genus *Niviventer* sensu stricto can be subdivided into three major sections: the "andersoni" division comprising two species, the "niviventer" division consisting of at least 14 species and the "fulvescens" division comprising two or more species. The identity and position of the Malayan *N. cremoriventer,* which proved to also be related to the "fulvescens" division, remains to be established by additional study (see also Balakirev and Rozhnov 2010, Balakirev et al. 2012a). *Chiromyscus* contains at least three species: *C. chiropus* and *C. thomasi,* thought of as larger, "mask-bearing species," and *C. langbianis,* which comprises at least two genetic lineages – northern and southern. Taking into consideration the rarity of *Chiromyscus* specimens available and the scarcity of our knowledge about the natural range for the species belonging to this genus, there is reason to believe that the list of species presented remains incomplete.

Acknowledgments

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Supplementary material 1

Complete list of samples used for phylogenetic reconstructions

Authors: Alexander E. Balakirev, Alexei V. Abramov, Viatcheslav V. Rozhnov

Data type: species data

Supplementary material 2

The list of samples used for combined *Cyt* b+*COI*+*IRBP*+*GHR* analysis

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Data type: species data

Supplementary material 3

Cranial measurements, range, and standard deviation

Authors: Alexander E. Balakirev, Alexei V. Abramov, Viatcheslav V. Rozhnov

Data type: species measurements

Explanation note: Cranial measurements, including range and standard deviation (SD), for *Chiromyscus* species from Vietnam (intact, adult skulls of both sexes).