ZooKeys 81: 1–12 (2011)
doi: 10.3897/zookeys.81.816
www.zookeys.org

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

First Record of the Genus *Aprivesa* Melichar (Hemiptera, Fulgoromorpha) from South India, with Description of One New Species

Cui-Ping Bu†, Ai-Ping Liang‡

Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Road, Chaoyang District, Beijing 100101, P.R.China

† *urn:lsid:zoobank.org:author:A3EEB061-3A79-469B-8F6D-B17D94E6055D*
‡ *urn:lsid:zoobank.org:author:862BAA85-A5A9-44DE-A6E4-99D30C39B2C9*

Corresponding author: *Ai-Ping Liang* (liangap@ioz.ac.cn)

Academic editor: *Michael Wilson* | Received 5 August 2010 | Accepted 2 February 2011 | Published 18 February 2011

urn:lsid:zoobank.org:pub:7E07E64F-2EBD-4798-88B8-11FFCB43F6FB

Citation: Bu C-P, Liang A-P (2011) First Record of the Genus *Aprivesa* Melichar (Hemiptera, Fulgoromorpha) from South India, with Description of One New Species. ZooKeys 81: 1–12. doi: 10.3897/zookeys.81.816

Abstract

Aprivesa unimaculata **sp. n.** (Hemiptera: Fulgoromorpha: Ricaniidae) is described and illustrated from Coorg, south India. This represents the first record of the genus *Aprivesa* Melichar from India and the fourth known species of *Aprivesa*. The new taxon greatly extends the range of the genus *Aprivesa*, which was previously known as an endemic Australian genus. A checklist of all known species of the Ricaniidae from India and keys to all the known genera of the Ricaniidae from India and all species in the genus are provided.

Keywords

Ricaniidae, Fulgoroidea, taxonomy, biodiversity, distribution

Introduction

Ricaniidae is one of the larger families of the Fulgoroidea, comprising more than 450 described species in approximately 46 genera (Montrouzier 1861, Melichar 1898a,b, 1923, Metcalf 1955, Fennah 1968, 1969, 1971, Williams and Fennah 1980, Shcherbakov 2006, Fletcher 2008, Bu et al. 2010). Members of the group are distributed widely in the Afrotropical, Australian, Indo-Malayan and Oceania regions, and primarily around the tropics (Metcalf 1955, Miklos 1975). Most of the species are of little economic importance but a few are major agricultural pests, such as *Ricania speculum*, *Pochazia sublimate* and *Scolycopa australis* (Charles 1998, Fletcher 1979a,b, 2008, Luo 2003).

The ricaniid fauna of India remains inadequately studied and there is still much basic taxonomic work to be done on the group. To date, 28 species in 9 genera from the Ricaniidae are described or recorded from India (Distant 1906, 1909, 1916, Metcalf 1955, Ghauri 1973). The number of the described species likely represents only a small fraction of the actual diversity of the whole Indian ricaniid fauna considering the vast territory and various complex habitats of India.

The genus *Aprivesa* was established by Melichar (1923) for *Privesa exucta* Melichar, 1898 from Australia. Muir (1931) described the second species of the genus *Aprivesa varipennis* from Western Australia. More recently, Fletcher (2008) transferred *Privesa pronotalis* Walker, 1917 into the genus. Until now, *Aprivesa* contains 3 known species and they are all endemic to Australia.

While sorting and identifying the Ricaniidae from material in the Department of Entomology Insect Collection, North Carolina State University, Raleigh, NC, USA, we found a new species of *Aprivesa* from south India. The new species represents the first record of *Aprivesa* in India, and its discovery has broadened our knowledge of the morphology and biogeography of the genus. In this paper, we redescribe the genus *Aprivesa* and describe and illustrate the new species from south India. A key is given for the separation of the known species in *Aprivesa*. A checklist of all known species of the Ricaniidae from India and a key to all the known genera of the Ricaniidae from India are also provided.

Materials and methods

The specimens studied in the course of this work are deposited at the Department of Entomology Insect Collection, North Carolina State University, Raleigh, NC, USA (NCSU).

Specimens used for dissection were cleaned in 10% KOH at room temperature for ca. 12 hours, rinsed in distilled H_2O, stained by methylrosanilinium chloride (a clinical solution, comprising methyl violet, ethanol and purified water) to highlight the internal thin and transparent membranous parts, and then transferred to glycerol for examination. Morphological characters were observed with a Zeiss Stemi SV 11 optical stereomicroscope and were illustrated with the aid of a drawing tube attached to the microscope. Measurements were made with the aid of an eyepiece micrometer.

The following abbreviations are used in the text, BL: body length (from apex of cephalic process to tip of fore wing) and FWL: fore wing length.

The morphological terminology followed is that of Bu et al. (2010).

Taxonomy

Checklist of the Ricaniidae species from India

Ricaniidae Amyot & Serville, 1843

Apachnas Distant, 1909
  nobilis Distant, 1909. India (Madras)
Aprivesa Melichar, 1923
  unimaculata sp. n. India (Coorg)
Euricania Melichar, 1898
  ocellus (Walker, 1851). India (Assam, Sikkim)
Pochazia Amyot & Serville, 1843
  antica (Gray, 1832). India (Tamil Nadu)
  atkinsoni Distant, 1906. India (Sikkim)
  confusa Distant, 1906. India (Assam)
  guttifera Walker, 1851. India (Assam, Darjeeling, Sikkim)
  interrupta Walker, 1851. India (Assam, Malabar Coast)
  sinuata Stål, 1865. Northern India
  transversa Melichar, 1898. India (Darjeeling)
Ricania Germar, 1818
  apicalis (Walker, 1851). India (Assam, Sikkim)
  bicolorata Distant, 1906. India (Madras)
  coorgensis Distant, 1916. India (Coorg)
  fenestrata (Fabricius, 1775) India (Coorg, Kerala, Madras Travancore, Trivandrum)
  fumosa (Walker 1851). India (Assam)
  marginalis (Walker 1851). India (Assam, Bombay, Coorg)
  simulans (Walker, 1851). Northern India
  speculum (Walker 1851). India (Assam, Madras, Nilgiri Hills, Sikkim, Trivandrum)
  stupida (Walker, 1857). India (Assam)
  taeniata Stål, 1870. India
  zebra Distant 1906. India (Assam)
Ricanoides Zia, 1935
  flabellum (Noualhier, 1896). India (Assam)
Ricanoptera Melichar, 1898
  inculta Melichar, 1898. India (Assam, Nicobar Islands, Great Nicobar)
  polita Melichar, 1898. India (Nicobar Island, little Nicobar)
Ricanula Melichar, 1898
  pulverosa (Stål, 1865). India (Assam)
  stigma (Walker, 1851). India (Assam, Nicobar Islands)
Scolypopa Stål, 1859
  confinis (Distant, 1906). India (Bombay, Coorg, Madras)
  delecta (Melichar, 1898). India (Bombay)

Key to genera of Ricaniidae from India

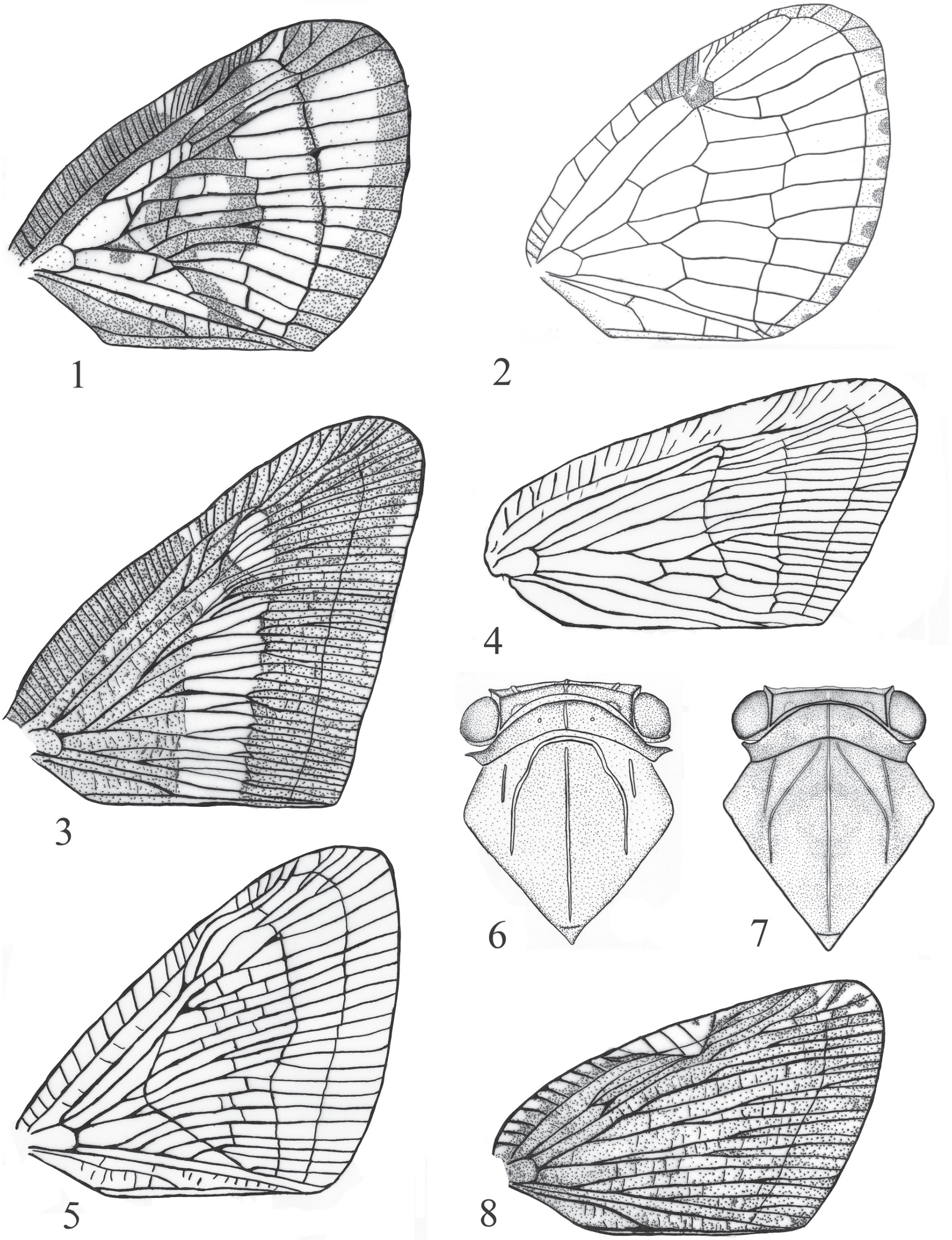
1 Forewing quadrate, with costal and sutural margins subparallel (Figs 9–10, 13) ...... ***Aprivesa*** **Melichar**
– Forewing more or less triangular (Figs 1–5, 8) **2**
2 Forewing with sparse longitudinal veins, costal cell without transverse veinlets (Figs 1–2) **3**
– Forewing with dense longitudinal veins, costal cell with transverse veinlets (Figs 3–5, 8, 13) **4**
3 Forewing with radial vein branched before pterostigma (Fig. 1) ...... ***Euricania*** **Melichar**
– Forewing with radial vein not branched before pterostigma (Fig. 2) ...... ***Apachnas*** **Distant**
4 Forewing large, with apical angle prominent, apical margin longer than claval suture (Fig. 3) ...... ***Pochazia*** **Amyot & Serville**
– Forewing relatively small, with apical angle rounded, apical margin nearly as long as claval suture **5**
5 Forewing with cells on basal portion with numerous anastomosed crossveins **6**
– Forewing with cells on basal portion without anastomosed crossveins **8**
6 Mesonotum with lateral carinae slightly, inwardly and anteriorly curved and bell-like (Fig. 6) ...... ***Ricanoides*** **Zia**
– Mesonotum with lateral carinae distinctly, inwardly and anteriorly curved and angle-like (Fig. 7) **7**
7 Forewing with precostal area with dense transverse veinlets, costal margin distinctly convex near base (see Yang 1989: 192, Fig. 11D) ...... ***Ricania*** **Germar**
– Forewing with precostal area with sparse transverse veinlets, costal margin slightly convex near base (Fig. 8) ...... ***Ricanula*** **Melichar**
8 Forewing narrower with costal margin/apical margin ratio>1.4 (Fig. 4) ...... ***Scolypopa*** **Stål**
– Forewing wider with costal margin/apical margin ratio<1.4 (Fig. 5) ...... ***Ricanoptera*** **Melichar**

Genus *Aprivesa* Melichar, 1923

Aprivesa Melichar 1923: 144.

Type species. *Privesa exuta* Melichar 1898b, designated by Melichar 1923: 144.

Redescription. General colour ochraceous or fuscous. Vertex and most part of frons usually pale brown or dark brown. Pronotum brown. Mesonotum usually fuscous. Legs pale yellow or brown. Forewing brown to fuscous. Hindwing hyaline, pale brown.



Figures 1–8. 1 *Euricania ocellus* (Walker, 1851) **2** *Apachnas nobilis* Distant, 1909 **3** *Pochazia confusa* Distant, 1906 **4** *Scolypopa delecta* (Melichar, 1898) **5** *Ricanoptera inculta* Melichar, 1898 **6** *Ricanoides flabellum* (Noualhier, 1896) **7** *Ricania speculum* (Walker, 1851) **8** *Ricanula pulverosa* (Stål, 1865)
Notes: Fig. 1 quoted from Xu et al. (2006), Figs 3, 5, 8 quoted from Melichar (1898a), Fig. 4 quoted from Distant (1906).

Head (Figs 9–12, see Fletcher 2008: 112, Figs 15–17) large. Vertex broad and narrow, nearly rectangular in outline; distinctly separated from the frons by a transverse carina, lateral margins ridged and nearly parallel, posterior margin archedly concave; shorter than pronotum at midline; disk planar with some faint wrinkles. Frons oblique, broader than long, with central, sublateral and lateral carinae; lateral margins carinate and strongly elevated, with a slight outward bulge at mid-length, converging below level of antennae to apex. Clypeus narrower than frons, convex medially, shallowly inserted, lateral marginal areas depressed, with central longitudinal carina. Rostrum with subapical segment just surpassing meso-trochanters, apical segment attaining post-trochanters. Eyes oval. Ocelli small, situated between eye and base of antennae. Antennae short, scape ring-liked; pedicel subglobose, about 2 times as long as scape; flagellum setaceous, basely expanded.

Pronotum (Figs 9–11) narrow, with median longitudinal carina, punctuated beside central carina; disk slightly sloping laterally, hind margin centrally distinctly arched anteriorly. Mesonotum (Figs 9–11) large, triangular and convex, with 3 carinae: central carina straight; lateral carinae inwardly and anteriorly curved, nearly parallel on anterior margin, each bifurcating outwardly near middle in a straight longitudinal carina. Forewing (Figs 9–10, 13, see Fletcher 2008: 110–112, Figs 13–15;) quadrate, with costal and sutural margins subparallel; apical margin convex, shorter than claval suture; precostal area at middle broader than costal cell, with transverse veinlets dense; three veins emanating from basal cell, R and Sc nearly parallel, the radial veins originating from a common point on the basal cell; M leaving basal cell as a single short stem but forking in more than length of basal cell; Cu1 with four or five branches just before the apical margin; subapical line complete; claval veins uniting near middle of clavus, common claval vein entering commissural margin, clavus with many transverse veinlets. Hindwing (Figs 10, 14, see Fletcher 2008: 108, Fig. 2) small, anterior margin strongly sinuate; Sc short, unforked, R with three or four branches, M with two or three branches, Cu1 with more than four branches; transverse veinlets including only R-M and M-Cu. Legs moderately long; hind tibiae with 2 lateral black-tipped spines.

Female and male genitalia. See description of *Aprivesa unimaculata* sp. n. below.

Biology. As with many ricaniid planthopper species, no biological data are currently available for species of *Aprivesa*, except that *A. exuta* was collected on *Melaleuca quinquenervia* (Fletcher 2008).

Distribution. Australia, India.

Remarks. *Aprivesa* is distinguished from other genera in Ricaniidae by the shape of frons and wing, the wing venation, and the minutiae of the male genitalia.

Species of *Aprivesa* are similar to those of *Privesa* Stål. But *Aprivesa* can be separated from *Privesa* by the lateral margins of the frons with a slight outward bulge below the antennae and the forewing with two radial veins originating from a common point on the basal cell (Fletcher 2008). In addition, the genus *Privesa* is distributed primarily in the Afrotropical region. Although the genus *Aprivesa* was an Australian endemic before, the finding of the new species in south India greatly extends the range of the genus *Aprivesa*. The similar distribution pattern is seen in another ricaniid genus *Sco-*

lypopa. Most of *Scolypopa* species are found in the Australian region; but three distinct species of *Scolypopa* are distributed in the Indo-Malayan region (Metcalf 1955, Fletcher 1979a,b, 2008).

Key to species of genus *Aprivesa*

1 Forewing pale dull ochraceous, with dark mottlings; precostal area at middle 1.6 times as broad as costal cell (see Fletcher 2008: 112, Fig. 15). BL: 4.5–5.5 mm (male) (Distant 1917). Australia (New South Wales) .... ***A. pronotalis*** **(Distant)**

– Forewing brown or fuscous, with hyaline mottlings; precostal area at middle 2.4 times as broad as costal cell **2**

2 Forewing with ratio of length to maximum width 1.8:1, MA relatively long (see Fletcher 2008: 111, Fig. 14). BL: 6.5 mm (male) (Muir 1931). Australia (Western Australia, South Australia, New South Wales) ....***A. varipennis*** **Muir**

– Forewing with ratio of length to maximum width 2.3:1, MA relatively short **3**

3 Forewing with three large hyaline spots, Cu forking about level of junction of claval veins (see Fletcher 2008: 110, Fig. 13); frons wider at widest part than long in middle line (1.5:1) (see Fletcher 2008: 112, Fig. 17). BL: 6.0 mm (male) (Melichar 1898a,b). Australia (Queensland, New South Wales) .... ***A. exuta*** **(Melichar)**

– Forewing with one large hyaline spot, Cu forking before junction of claval veins; frons wider at widest part than long in middle line (1.4:1) (Figs 9–13). BL: 6.0 mm (male). India (Coorg)............................***A. unimaculata*** **sp. n.**

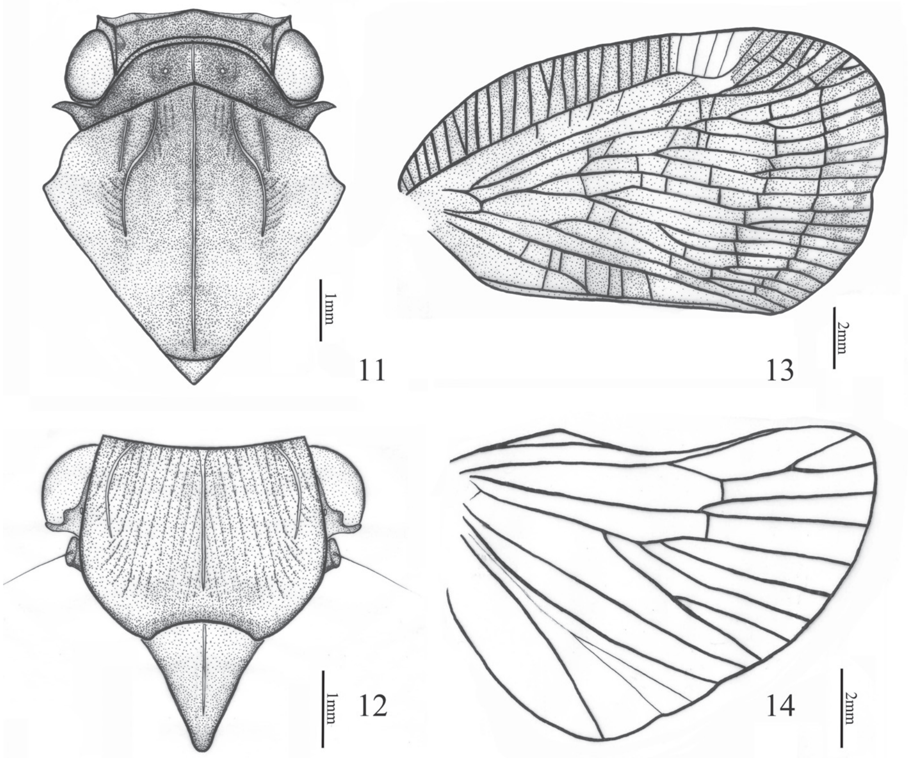
Aprivesa unimaculata sp. n.

urn:lsid:zoobank.org:act:446BD15B-E7E8-4132-A86E-B351EEEE07BC
Figs 9–23

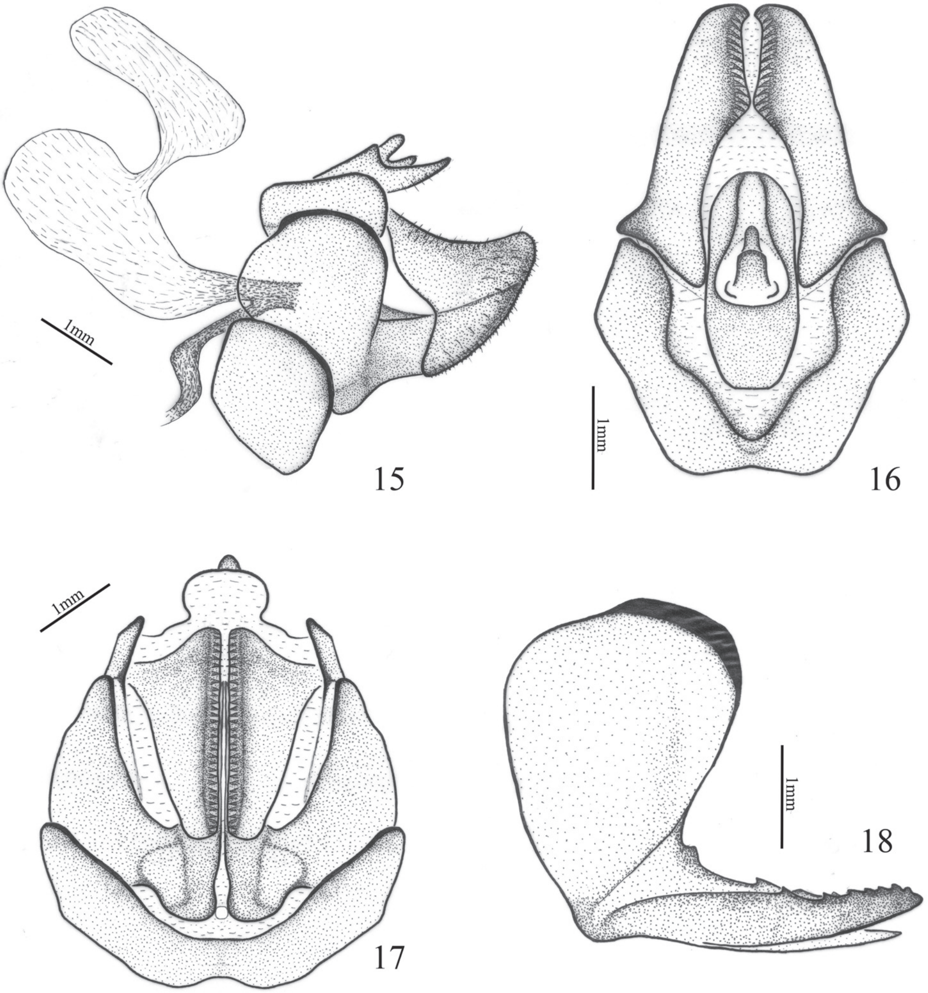
Description. ♂ (n=1), BL: 6.0 mm, FWL: 7.0 mm; ♀ (n=1), BL: 6.0 mm, FWL: 8.0 mm. General colour brown to fuscous. Vertex, frons and clypeus brown. Eye brown. Ocelli yellowish. Rostrum pallid. Pronotum and mesonotum fuscous. Thorax fuscous ventrally, marked with brown. Legs pale brown; tarsi and tips of tibiae fuscous. Abdomen fuscous ventrally, with pale brown transverse strips; pygofer fuscous. Forewing brown, with many pale brown areoles; stigma relatively large, white hyaline. Hindwing pale brown.

Head (including compound eyes) (Figs 9–12) slightly wider than pronotum. Vertex (Fig. 11) wider at anterior margin than long in middle line (5.8:1). Frons (Fig. 12) wider at widest part than long in middle line (1.4:1); disc tricarinate, with sublateral carinae shorter than central carina. Clypeus (Fig. 12) triangular, with central carina. Rostrum long, nearly reaching between hind coxae, with apical segment slightly shorter than basal segment.

Figures 9–10. Habitus of *Aprivesa unimaculata* sp. n. **9** ♂, south India, lateral view **10** ♀, south India, dorsal view.



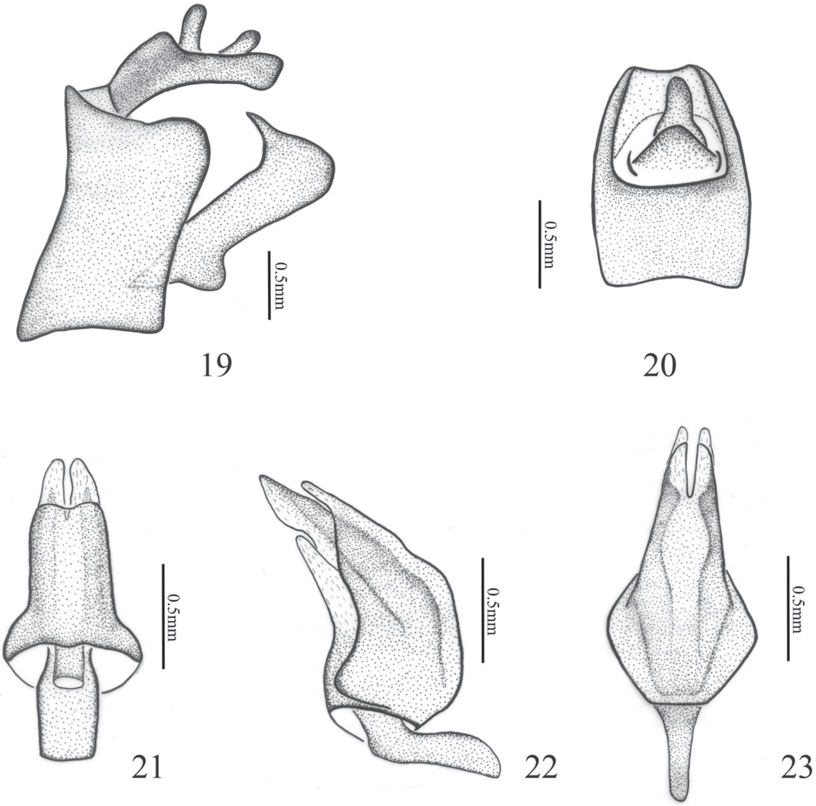
Figures 11–14. *Aprivesa unimaculata* sp. n. **11** head (♂), pronotum and mesonotum, dorsal view **12** head (♂), ventral view **13** fore wing (♂) **14** hind wing (♂).



Figures 15–18. *Aprivesa unimaculata* sp. n. **15** genitalia (♀), lateral view **16** anal tube (♀), dorsal view **17** genitalia (♀), ventral view **18** gonopophyses VIII (♀), lateral view.

Pronotum (Fig. 11) wider at widest part than long in middle line (7.1:1), punctuated beside central carina. Mesonotum (Fig. 11) large, longer than broad, tricarinate on disc, lateral carinae on each side diverging from the middle one, disunited on the anterior border. Wing venation as in Figs 13–14.

Female genitalia (Figs 15–18) symmetrical (Stroiński 2002; Liang 2003). Anal tube (Figs 15–16) relatively short and small, with apical margin rounded, nearly parallel-sided in dorsal view. Gonopophyses VIII (first valvulae) (Fig. 18) with two triangular lobes, the outer lobes saw-like, strongly sclerotised and having 7 blunt teeth on dorsal margin, and the inner lobes slightly sclerotised, with 3 blunt teeth on dorsal margin. Gonopophyses IX (second valvulae) small, degenerated. Gonoplacs (third valvulae)



Figures 19–23. *Aprivesa unimaculata* sp. n. **19** genitalia (♂), lateral view **20** anal tube (♂), dorsal view **21** aedeagus, dorsal view **22** aedeagus, lateral view **23** aedeagus, ventral view.

(Figs 15, 17) triangular with many teeth extending along ventral margin, directed mesad. Bursa copulatrix (Fig. 15) large, with two pouches, the first pouch connected to the second by a short and narrow duct, the opening directed into vagina. Genital opening singular (monotrysian), occurring between gonopophyses VIII.

Male genitalia with pygofer (Fig. 19) narrow and high, with dorsal posterior margin smoothly produced posteriorly in lateral view. Anal tube (Figs 19–20) moderately small, distinctly projected caudad, dorsally sulcate in distal half, trapezium in dorsal view, longer than wide at middle (1.3:1). Anal styles (Figs 19–20) relatively short and small. Genital styles (Fig. 19) relatively large and slender, with a long apical process, the base of inner margin curvedly produced, in profile longer than wide at middle (4.3:1). Aedeagus (Figs 21–23) cone-liked, nearly straight, partly sclerotised, symmetrical, having two pairs of caudad directed membranous processes at apex, with the inner pair longer and the outer pair slightly short.

Type material. Holotype ♂. Ammatti, S. Coorg, S. India, xi.1982, P S Nathan (NCSU). Paratype: 1♀, S. Coorg, S. India, Ammatti, 3100 ft., v.1951, P S Nathan (NCSU).

Etymology. This species is named for its forewing with one large hyaline spot.

Distribution. South India (Coorg).

Remarks. This species is externally similar to *Aprivesa exuta* (Melichar, 1898) from Australia, but can be distinguished from the latter by the characters given in the key.

Acknowledgments

We thank Dr. Murray J. Fletcher (Orange Agriculture Institute, NSW, Australia) for several discussions on the identification of the new species during the preparation of this manuscript. We are grateful to Mr. Robert L. Blinn and Dr. Lewis Deitz (NCSU), for the loan of specimens used in this study. The work on which this paper is based was supported by the National Natural Science Foundation of China (grant nos. 30770269, 30970400), a grant (No. O529YX5105) from the Key Laboratory of the Zoological Systematics and Evolution of the Chinese Academy of Sciences and the National Science Fund for Fostering Talents in Basic Research (Special subjects in animal taxonomy, NSFC-J0630964/J0109), all awarded to APL.

References

Bu C-P, Larivière M-C, Liang A-P (2010) *Parapiromis* nom. nov., a new name for *Piromis* Fennah (Hemiptera: Fulgoromorpha: Ricaniidae), with descriptions of three new species. Zootaxa 2400: 29–40.

Charles JG (1998) The settlement of fruit crop arthropod pests and their natural enemies in New Zealand: an historical guide to the future. Biocontrol News and Information 19: 47–58.

Distant WL (1906) The fauna of British India, including Ceylon and Burma, Rhynchota 3 (Heteroptera-Homoptera). Taylor & Francis, London, 503pp.

Distant WL (1909) Rhynchotal Notes xlix. Annals and Magazine of Natural History 8: 320–338.

Distant WL (1916) The fauna of British India, including Ceylon and Burma. Rhynchota 6 (Homoptera: Appendix). Taylor & Francis, London, 248pp.

Distant WL (1917) Descriptions of some Ethiopian and Australian Homoptera. Annals and Magazine of Natural History 8: 186–191.

Fennah RG (1968) A new genus and species of Ricaniidae from Palaeocene deposits in North Dakota. Journal of Natural History 2(1): 143–146.

Fennah RG (1969) Fulgoroidea (Homoptera) from New Caledonia and the Loyalty Islands. Pacific Insects Monograph 21: 1–116.

Fennah RG (1971) Homoptera: Fulgoroidea. Supplement. Insects of Micronesia 6(8): 563–609.

Fletcher MJ (1979a) The external morphology of *Scolypopa australis* (Walker) (Homoptera: Ricaniidae). Journal of the Australian Entomological Society 18: 157–168.

Fletcher MJ (1979b) Egg types and oviposition behaviour in some fulgoroid leafhoppers (Homoptera, Fulgoroidea). Australian Entomological Magazine 6: 13–18.

Fletcher MJ (2008) A key to the genera of Ricaniidae (Hemiptera: Fulgoromorpha) recorded in Australia with notes on the Australian fauna, including a new species of Epithalamium Kirkaldy. Australian Journal of Entomology 47: 107–120.

Ghauri MSK (1973) Taxonomic notes on a collection of Fulgoroidea from tea in southern India. Bulletin of Entomological Research 62(4): 541–544.

Liang A-P (2003) A new genus of Tropiduchidae (Hemiptera: Fulgoroidea) from China and Vietnam, with description of eggs. Florida Entomologist 86: 361–369.

Luo T-X (2003) Distinguishing pests of Ricanidae in the orchards. JiangXi Plant Protection 26(1): 14–15.

Melichar L (1898a) Monographie der Ricaniiden (Homoptera). Annalen des k.k. Naturhistorischen Hofmuseums. Wien 13: 197–359.

Melichar L (1898b) Vorläufige Beschreibungen veuer Ricaniiden. Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien 48: 384–400.

Melichar L (1923) Homoptera, fam. Acanaloniidae, Flatidae et Ricaniidae. In: Wytsman P (Ed) Genera Insectorum fasc.182. Tervuren, Belgium, 185 pp.

Metcalf ZP (1955) General catalogue of the Homoptera. Fasc. IV, Fulgoroidea. Part 16, Ricaniidae. Waverly Press, Baltimore, 199 pp.

Miklos DF (1975) A classification of the biogeographical provinces of the world. International Union for the Conservation of Nature and Natural Resources (IUCN) Occasional Paper No. 18. Morges, Switzerland, 48 pp.

Montrouzier X (1861) Essai sur la faune entomologique de la Nouvelle-Calédonie (Balade) et des iles des Pins, Art, Lifu, etc. Hémiptères. Annales de la Société Entomologique de France 4: 59–74.

Muir F (1931) Descriptions and records of Fulgoroidea from Australia and the South Pacific Islands, 1. Records of the Australian Museum 18: 63–83.

Shcherbakov DE (2006) The earliest find of Tropiduchidae (Homoptera: Auchenorrhyncha), representing a new tribe, from the Eocene of Green River, USA, with notes on the fossil record of higher Fulgoroidea. Russian Entomological Journal 15(3): 315–322.

Stroiński A (2002) Three new species of Miliprivesa Metclaf, 1952 (Hemiptera: Fulguromorpha: Ricaniidae). Annales Zoologici (Warszawa) 52(4): 587–591.

Williams JR, Fennah RG (1980) Ricaniidae (Hemiptera: Fulgoroidea) from Mauritius, with a description of Trysanor cicatricosus spec. nov., gen. nov. Journal of the Entomological Society of Southern Africa 43(1): 7–22.

Xu C-Q, Liang A-P, Jiang G-M (2006) The genus Euricania Melichar (Homoptera: Ricaniidae) from China. The Raffles Bulletin of Zoology, 54(1): 1–10.

Yang C-T (1989) Ricaniidae of Taiwan (Homoptera: Fulgoroidea). Taiwan Museum Special Publication Series, 8: 171–204.

ZooKeys 81: 13–25 (2011)
doi: 10.3897/zookeys.81.1122
www.zookeys.org

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Volatile compounds released by disturbed and undisturbed adults of *Anchomenus dorsalis* (Coleoptera, Carabidae, Platynini) and structure of the pygidial gland

Teresa Bonacci[1], Pietro Brandmayr[1], Tullia Zetto[1], Ida Daniela Perrotta[1], Salvatore Guarino[2], Ezio Peri[2], Stefano Colazza[2]

1 *Department of Ecology, Università degli Studi della Calabria – via P. Bucci s.n., 87036, Rende (CS), Italy*
2 *Department of Demetra Ed. 5, Università degli Studi di Palermo - Viale delle Scienze, 90128, Italy*

Corresponding author: *Teresa Bonacci* (t.bonacci@unical.it)

Academic editor: *Terry Erwin* | Received 20 September 2010 | Accepted 25 January 2011 | Published 18 February 2011

Citation: Bonacci T, Brandmayr P, Zetto T, Perrotta ID, Guarino S, Peri E, Colazza S (2011) Volatile compounds released by disturbed and undisturbed adults of *Anchomenus dorsalis* (Coleoptera, Carabidae, Platynini) and structure of the pygidial gland. ZooKeys 81: 13–25. doi: 10.3897/zookeys.81.1122

Abstract

Volatile compounds produced by adults of *Anchomenus dorsalis* under undisturbed and disturbed conditions were investigated with an all-glass aeration apparatus. GC-MS analysis of the crude extracts from undisturbed and disturbed adults highlighted four major volatile compounds, undecane, heneicosane, *Z*-9 tricosene and tricosane, of which significantly more undecane was released by disturbed adults compared to undisturbed beetles. The pygidial glands of adults of *A. dorsalis* were investigated using light and Transmission Electron Microscopy (TEM). Each gland showed dense aggregates of secretory cells organized into visually distinct lobes; a long collecting canal that drains the secretion towards the reservoir, a bean-shaped double lobed muscular reservoir in which secretion is stored and a short duct (efferent duct) through which the secretion is discharged. The function of the pygidial glands and the possible role played by undecane as a defensive allomone and/or chemical signalling molecule are discussed.

Keywords

undecane, GC/MS, chemical defences, gland morphology, predation avoidance mechanisms

Introduction

Carabid beetles are known to produce a large variety of defensive substances, and their chemical nature has been elucidated in more than 500 species (Schildknecht et al. 1964, 1968; Eisner et al. 1963; Eisner 1970; Eisner et al. 1977; Moore and Wallbank 1968; Moore 1979; Forsyth 1970, 1972; Kanehisa 1996; Scott et al. 1975; Dazzini-Valcurone and Pavan 1980; Will et al. 2000). The defensive compounds are produced by a pair of glands that open at the tip of the abdomen, known as the "pygidial glands" (Eisner 1958, 1970; Eisner and Aneshansley 1982, 1999; Eisner et al. 1992, 2000). The defensive substances are normally sprayed in the form of large droplets, but in the bombardier beetles the spray mechanism involves a two-chamber system by which benzoquinones are forced against attackers by way of an exothermic reaction (Schildknecht et al. 1968; Eisner et al. 2001). Previous chemical analytical studies on the defensive secretions of several Carabidae species revealed that these substances are blends, mainly characterized by polar organic compounds such as acids, phenols, aldehydes and quinones plus minor non-polar compounds such as ketones, esters and hydrocarbons (Dettner 1987). These chemicals are ejected or discharged mainly when a disturbance stimulus occurs, such as the attack of a predator (Schildknecht et al. 1968; Forsyth 1972; Thiele 1977; Rossini et al. 1997; Eisner et al. 2001). In this case, one or both glands can be discharged almost immediately and the secretion aimed towards the disturbance source (Forsyth 1972). To date, defensive compounds produced by these insects have been analyzed using whole body or droplet extracts but the differences in the quality and amounts of volatiles emitted from the undisturbed and disturbed carabid adults have been not yet been thoroughly investigated.

Anchomenus dorsalis (Pontoppidan, 1763) is a gregarious platynine carabid inhabiting muddy soils and fields across Europe. It is often found in association with species of *Brachinus* (Juliano 1985; Zaballos 1985; Lindroth 1986; Bonacci et al. 2004a, b; Mazzei et al. 2005; Zetto Brandmayr et al. 2006) and, like *Brachinus*, it has a bright bicoloured (green-blue and red-brown) coat body that contrasts with the background. These species usually aggregate under heavy stones in open lands with sparse vegetation, such as pasturelands, croplands or in humid, sun exposed soils (Bonacci et al. 2004b; Mazzei et al. 2005). Zetto Brandmayr et al (2006) described a peculiar "rubbing behavior" of *A. dorsalis* towards *B. sclopeta* (Fabricius, 1792) observed in laboratory conditions and in natural aggregations, where the individuals (conspecifics and no-conpecifics)live in strinct contact and in peaceful coexistence. Thiele (1977) defines the carabid aggregations as positive intraspecific relationships and as "indications of a type of social behavior", in which the members of the species are mutually beneficial. Aggregation in ground beetles seems to occur in only very few species and specially between conspicuous and chemically protected species (Thiele 1977; Bonacci et al. 2004b; Zetto Brandmayr et al. 2006). Laboratory investigations carried out by Bonacci et al. (2008) showed that *A. dorsalis* and *B. sclopeta* (which in natural habitats live gregariously), use aposematic colours and warning odours versus natural enemies (Bonacci et al. 2004a, 2006). The authors supposed that the combination of visual and olfactory signals, common in many insect groups (especially aposematic coloured insects, Rothschild and Moore 1987; Moore et al. 1990), can produce a multimodal warning display that, acting along many sensory channels (Rowe and Guilford 1999), increases the antipredatory strategies.

When disturbed, the ground beetles *A. dorsalis* releases a strong odour (perceived even by humans) (Bonacci, personal observation), and quickly retreat (with dispersal movement that produce a great confusion in the observer) into deeper soil crevices (like the *dilution effect*). Under laboratory conditions, Bonacci et al. (2004a, 2006, 2008) demonstrated that *A. dorsalis* and *B. sclopeta* are attacked less by predators, such as *Ocypus olens* (O. Muller, 1764) (Staphylinidae), *Crocidura leucodon* (Hermann, 1780) (Insectivora, Soricidae) and *Podarcis sicula* (Rafinesque, 1810) (Reptilia, Lacertidae) than other carabids used as preys (Juliano 1985; Zaballos 1985; Lindroth 1986; Bonacci et al. 2006, 2008; Zetto Brandmayr et al. 2006). Based on these behavioural studies and observations, experiments were conducted to characterize and quantify the volatile compounds produced by *A. dorsalis* upon disturbance. The putative organs producing such defensive compounds, the pygidial glands, were also investigated using light and electronic microscopy.

Materials and methods

Insects

Adults of *A. dorsalis* were collected by hand from different inter-specific aggregations (each composed by 100–130 individuals) of *B. sclopeta* and *A. dorsalis* found under stones or straw bales in Calabria (Crati Valley, province of Cosenza, latitude: 39°35'56"N; longitude: 16°15'48"E; elevation: 60 m a.s.l.). Following field collections, monospecific groups were placed in separate plastic cages (30 × 22 × 20 cm) with 4 cm of clay soil in a climatic chamber at 22 °C, photoperiod L/D of 18/6, and fed on veal meat and earthworm pieces (*Lumbricus terrestris* (Linnaeus, 1758)).

Air collection of adult volatiles

The collection of volatiles from *A. dorsalis* adults was conducted using a horizontal all-glass apparatus 1 l in volume. Humidified and charcoal filtered air was drawn through the apparatus at 0.5 l min^{-1} by a peristaltic pump for 2 h in a conditioned room at temperature of 22 ± 2 °C. The volatiles produced by experimental groups of 20 individuals of *A. dorsalis* adults of both sexes were trapped in glass collectors (6 mm ID) loaded with 600 mg of porapak Q, and held in place by glass wool plugs. Two experimental individual groups were considered: disturbed and undisturbed. Adults were considered undisturbed when they were gently transferred into the glass apparatus and disturbed when, before the start of the aeration, the glass chamber containing the adults was vigorously shaken for 10 seconds (Gomez et al. 2005). Preliminary observations showed that during this time the *A. dorsalis* adults released the odour. Five replicates were carried out for each groups: "disturbed" and "undisturbed". We proceeded in order to avoid any pseudo-replication and each individual was tested once. At the end of the aeration period, collectors were eluted with hexane (400 µl) and the extracts stored at -15 °C until used for GC-MS analysis. Blank aerations were also carried out with the empty apparatus using the same procedure.

Chemical Analyses

GC–MS analyses were performed using a Hewlett-Packard 5890 GC system interfaced with an HP 5973 quadrupole mass spectrometer detector. As a stationary phase an HP5–MS capillary column (5% diphenyl-95% dimethylpolysiloxane 30 m - 0.2 mm, 0.25 µm film thickness, J&W Scientific, USA) was used. Injector and detector temperatures were 250 °C and 270 °C respectively. Helium was used as the carrier gas. The GC oven temperature program was 60 °C for 5 min, than increased by 10 °C/ min to 280 °C. Electron impact ionization spectra were obtained at 70 eV, recording mass spectra from 42 to 550 uma. Compound analysis and identification was carried out using a commercial NIST 2005 mass spectra library search and by comparison with standard analytical grade compounds purchased from Sigma-Aldrich (U.S.A.). Quantitative analysis was carried out for 4 compound identified by GC-MS analysis: undecane, heneicosane, *(Z)*-9 - tricosene and tricosane.

For this analysis the elutes were diluted in 1 ml of hexane using a volumetric flask. Six point calibration curves, using analytical standards undecane, heneicosane, *(Z)*-9 - tricosene and tricosane, in the 0.2–100 ng μl^{-1} range, were used in order to evaluate the chromatographic response. The mean amount ± SE of each of these compounds was calculated dividing the amount of the compound obtained per replicate per the number of individuals used in each replicate.

Gland anatomy

For anatomical study by optical microscopy, adult beetles were killed at -15 °C and their abdomens were treated with 10% potassium hydroxide for 4 days before examination of the chitinous structures. The glands were mounted on clean glass slides and observed by optical microscopy equipped with Nomarsky interference contrast and photographed with a Coolpix 4500 camera (Nikon).

For light and transmission electron microscopy (TEM), samples were fixed in 3% glutaraldehyde solution in 0.1 M phosphate buffer (pH. 7.4) for 2 h at 4 °C and post fixed with 3% osmium tetroxide for 2 h. The specimens were then washed in phosphate buffer, dehydrated through graded acetone solutions and embedded in Araldite

(Fluka, Buchs, Switzerland). Semithin sections (1 µm) were obtained with a Leica Ultracut UCT ultramicrotome by using glass knives, mounted on clean glass slide and stained with 1% toluidine blue. They were then photographed with the Zeiss Axioskop microscope. For transmission electron microscopy, ultrathin sections (600–900 Å) were prepared using a diamond knife and collected on copper grids (G 300 Cu), contrasted using both lead citrate and uranyl acetate and then examined with a "Zeiss EM 900" electron microscope (TEM). Gland structure terminology follows Forsyth (1972) and Eisner et al. (2001).

Statistical Analysis

The quantitative analysis to determine differences in the amount of undecane, heneicosane, *(Z)*-9 - tricosene and tricosane recovered from *A. dorsalis* adults were compared by t-test (Sokal and Rohlf 1995). The statistical analysis was performed using Statistica for Windows 6.0 (Stat Soft Italia 1997).

Results

Chemical analysis of adult volatiles

GC-MS analysis of volatile collections showed that disturbed and undisturbed adults of *A. dorsalis* released the same four major volatile compounds: undecane, heneicosane, *(Z)*-9 - tricosene and tricosane (Table 1; Fig. 1). A significant difference between disturbed and undisturbed adults was observed only in the released amount of undecane. An amount of 22.37 ± 8.48 ng (Mean ± SE) were collected from each disturbed adult vs. 0.94 ± 0.29 ng collected from a undisturbed adult (t = 2,52; df = 8; *p* = 0.035). Other chemicals (heneicosane, *(Z)*-9 - tricosene and tricosane) tend to be released more when *A. dorsalis* individuals are disturbed but their amount is not statistically significant (Table 1).

Table 1. Volatile compounds from *A. dorsalis* adults obtained from air collections carried out for 2 hours at 0.5 l min^{-1}. **RT**: retention time at the GC-MS analysis; **df**: degree of freedom.

Compound	**R.T. (min.)**	**Amount (ng) /adult (mean ± SE)**		***t*- value**	**df**	**p**
		Disturbed	**Undisturbed**			
Undecane	9.82	22.37 ± 8.48	0.94 ± 0.29	2.52	8	0.035
Heneicosane	32.92	1.09 ± 0.96	0.44 ± 0.31	0.63	8	NS
(*Z*)- 9 - Tricosene	35.96	2.19 ± 2.10	0.78 ± 0.70	0.63	8	NS
Tricosane	36.98	0.93 ± 0.86	0.38 ± 0.28	0.60	8	NS

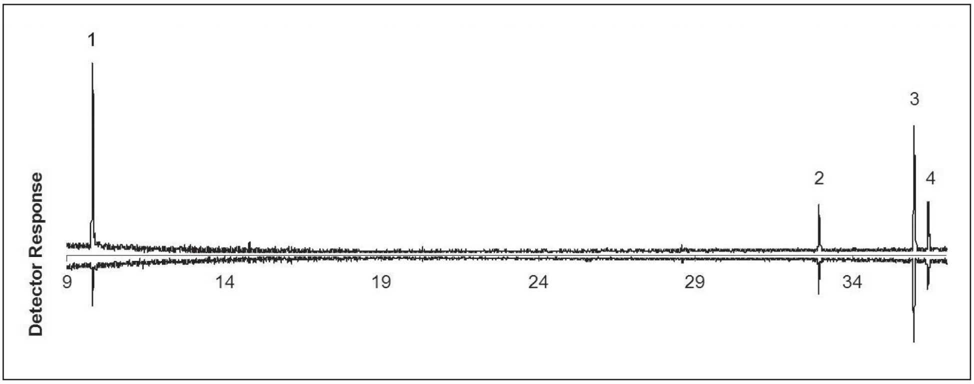


Figure 1. Gas chromatograms of volatile compounds collected from disturbed (up) and undisturbed (down) adults of *A. dorsalis*. **1** undecane **2** heneicosane **3** (*Z*)-9 - tricosene **4** tricosane. On the x axis is reported the retention time (minutes). As a stationary phase an HP5–MS capillary column was used. The GC oven temperature program was 60 °C for 5 min, than increased by 10 °C/min to 280 °C.

Gland structure

The pygidial glands of *A. dorsalis* are cuticular invaginations of the body wall that open outside immediately behind the eighth abdominal tergite. Each gland consists of a aggregate of secretory cells, an collecting canal, a reservoir and an efferent duct through which the secretion is discharged. The efferent duct open near the abdominal tip to the sides of anus. Each lobe is essentially a ball of cells (fig. 2 A) aligned radially around a central collecting lumen (sensu Forsyth 1970) that carry the secretion towards collecting canal. The overall structure of the secretory lobe and collecting canal resemble a cluster of grapes (fig. 2 A). Each cluster of cells converges to form a long efferent duct that drains the chemical product into a bean-shaped sac (reservoir) in which it is stored (fig. 2 C). The reservoirs extend forward, one along each side of the hindgut. This reservoir or "storage sac" (sensu Rossini et al. 1997) in *A. dorsalis* has a smooth constriction at about one third from its hind end, where both the collecting canal as well the efferent duct (sensu Forsyth 1970) converge (fig. 2 C and D). The collecting canal (Forsyth 1970) is a cylindrical tube twice the length of the body of the carabid (fig. 2 B and D), its lumen occupying about one third of the diameter. It carries the secretion from the secretory lobes to the reservoir and shows a continuous and regular spiral ridge along the whole length of its outer surface (fig. 2 D). Each efferent canal (treated with potassium hydroxide) show evident apical ramifications (fig. 2 E) extending towards the center of the secretory lobe. These structures, as observed by Eisner et al. 2001 in *Crepidogaster* Boheman, 1848 genus and defined by these authors also microtubules (intracellular organelle sensu Rossini et al. 1997), are grouped into convergent clusters, to form tiny individual "floret" that carry the secretion from the secretory lumen (fig. 2 E) to the main collecting canal. The tubules and florets are cuticular and could be isolated readily by potassium hydroxide treatment of the glands.

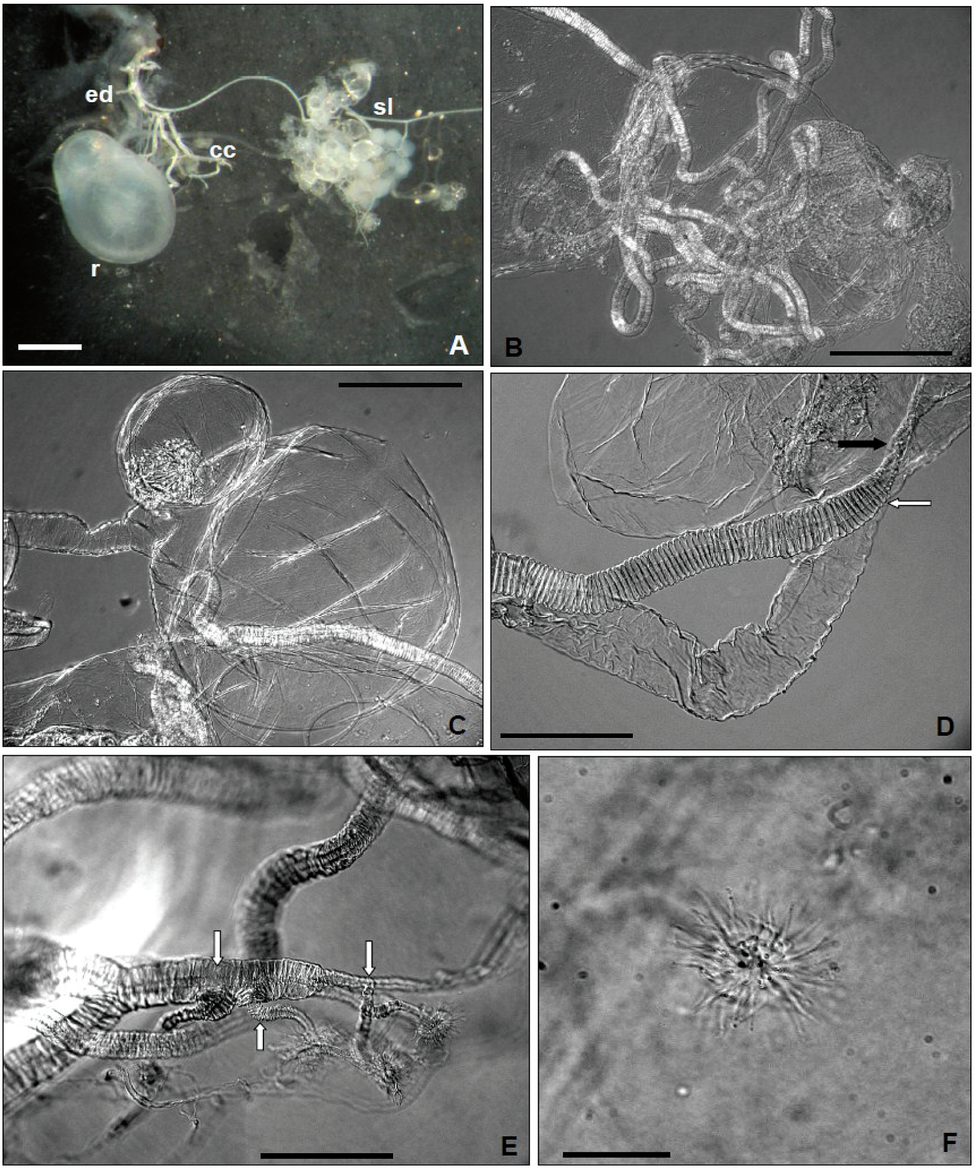


Figure 2. Light microscope: **A** Dorsal aspects of pygidial gland; **ed**, efferent duct; **r**, reservoir; **cc**, collecting canal; **sl**, secretory lobe (Scale bar = 0.5 mm) (not treated with potassium hydroxide) **B** collecting canal (Scale bar = 0.125 mm) **C** reservoir with smooth constriction at about one third from its hind end (Scale bar = 0.125 mm) **D** insertion of collecting canal (black arrow) and efferent duct (white arrow) in the reservoir (Scale bar = 0.05 mm) **E** collecting canal with apical ramifications (white arrows) (Scale bar = 0.05 mm) **F** “floret” (sensu Eisner et al. 2001) (Scale bar = 0.015 mm) (treated with potassium hydroxide).

Examined by TEM, the wall of the collecting canal is lined by epidermal part (fig. 3 A) that consist of cells connected to each other by micro-canals projecting into the collecting canal lumen. The lumen of collecting canal contain a heterogeneous secretion (fig. 3 A).

Each secretory lobe consist of secretory cells arranged radially around the central lumen. Each secretory cell has an secretory vesicle which is almost as long as the cell itself (fig. 3 B) with a coated membrane and bear many microvilli projecting into the cavity (fig. 3 C). Between the secretory cells are evident (fig. 3 B) the vesicular ducts that carry the secretion in the collecting lumen (fig. 3 B). Each microvillus (fig. 3 D) is formed by three structures: one with a typical spiral shape and other two structures, similar in shape and size. All the structures are enveloped by a thin lamina (fig. 3 D). The inner wall of the reservoir is composed of a thick chitinous layer (basal lamina) (fig. 3 E). A thick muscle layer was found surrounding the reservoir. Likely, the muscles play an important role for the expulsion of the secretory products through the efferent duct. This is composed of muscle bundles that pass spirally around it.

Discussion and conclusions

The chemical analyses of disturbed and undisturbed *A. dorsalis* adults showed that undecane was produced significantly in larger amounts in disturbed individuals. This suggests that this compound (which can be perceived even by humans) could play a prominent role in the chemical defence of the species. The role of undecane as a defensive substance has been widely reported in the Insecta: *Acanthomyops claviger* (Roger, 1862) (Regnier and Wilson 1968), *Formica polyctena* Foerster, 1850 (Löfqvist and Bergström 1980), *Paratrechina longicornis* (Latreille, 1802) (Morgan et al. 2005) and in Oxytelinae rove beetles (*Bledius spectabilis* Kraatz 1857, *Platystethus arenarius* (Fourcroy, 1785), and *Oxytelus piceus* L.) (Dettner and Schwinger 1982). Although in other carabids, hydrocarbons are generally produced in lesser amounts than polar compounds such as acids, phenols, aldehydes or quinones (Dettner 1987), but the occurrence of undecane in pygidial glands has been described in Pterostichini (*Abaris aenea* Dejean, 1831, *Pseudabarys* Chaudoir, 1873, sp), Loxandrini (*Loxandrus* LeConte, 1852, spp), Morionini (*Morion simplex* Dejean, 1831, *Moriosomus seticollis* Straneo, 1985), Catapieseini (*Catapiesis* Solier, 1835, spp), Perigonini (*Diploharpus laevissimus* Chaudoir, 1850) and Odacanthini (*Colliuris pensylvanica* Linnaeus, 1758) (Will et al. 2000). Furthermore, Peschke and Eisner (1987) reported that hydrocarbons produced by carabid species are potentially defensive.

Undecane is an optimal chemical signalling molecule, its molecular weight and polarity combining moderate olfactory efficiency with a sufficiently high vapour pressure to broadcast in the centimetre range when present in microgram quantities or less (Regnier and Wilson 1968). As with a number of carabids (Eisner 1958; Eisner et al. 1963; Will et al. 2000), a defensive spray constituted mainly of undecane appears to be an effective deterrent of predators (Bonacci et al. 2004a, 2008). Moreover, the efficacy of these chemicals is improved in that carabid species that are able to direct their ejections directly against the head or the eyes of the predator (Eisner 1958; Peschke and Eisner 1987).

The laboratory observations of the pygidial glands of *A. dorsalis* show that they resemble those of other Carabidae in their structure (Forsyth 1970, 1972), and the form

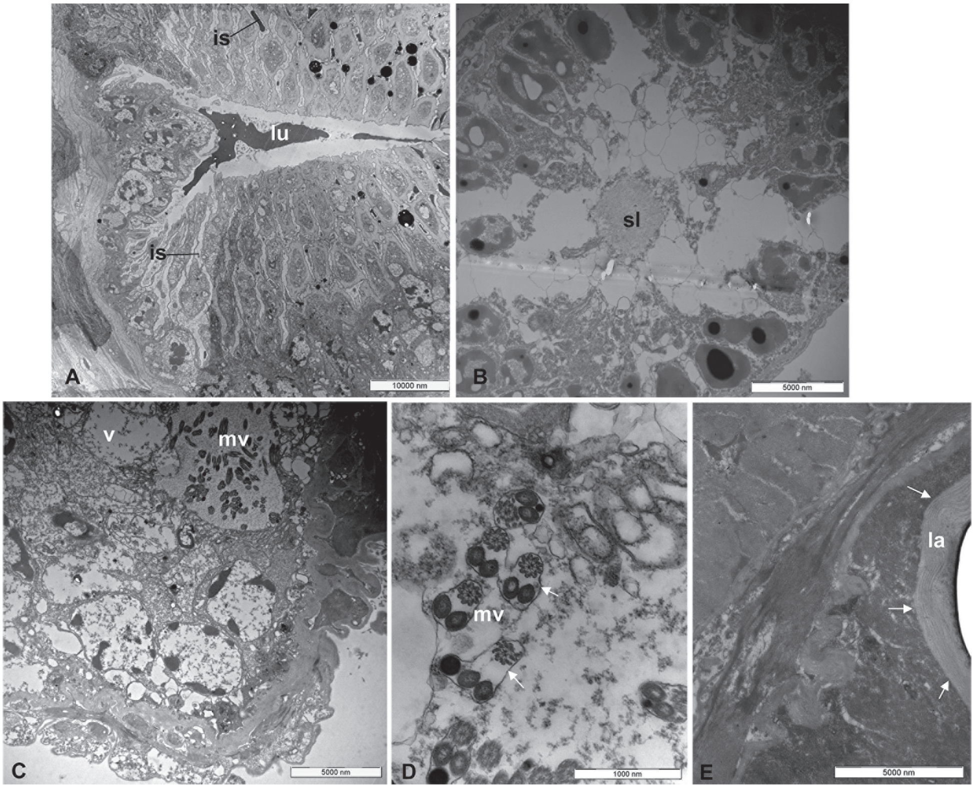


Figure 3. Transmission electron microscope (TEM): **A**, collecting canal with lumen (**lu**); the black arrows show interstitial spaces (**is**) **B** secretory lobe with secretory lumen (**sl**) **C** vesicle (**v**) with microvilli (**mv**) **D** microvilli structure (**mv**) at highest enlargement (the white arrows show the thin lamina) and **E** Inner wall of the reservoir with chitinous basal lamina (**la**) (black arrows) and massive muscle layer around.

of the reservoir, clearly double lobed, resembles the apparatus of *Anthia* and *Harpalines* described by Forsyth (1972). The secretory “florets” show a very small lumen, compared to other tribes of carabid beetles, (Forsyth 1972; Rossini et al. 1997; Eisner et al. 2001), but very little investigations have been done on Platynine (Agoninae) ground beetles. The inner wall of reservoir of pygidial glands in *A. dorsalis* shows a thin coat of chitinous tissue which probably preserves the cells by the toxic mixture of chemicals and requires that this secretion must be efficiently isolated from the rest of the body. Another feature of the glands of *A. dorsalis* is the extreme length of the collecting canal. This tube seems to be much longer than necessary for the transfer of chemical secretion. Very long collecting canals were found by Forsyth (1970) in *Pterostichus madidus* (Fabricius) and the author proposed that this feature serves to abridge the back-pressure from the reservoir.

As mentioned above, the defense glands in carabid beetles produce chemical compounds primarily to provide protection against putative predators (Thiele 1977; Will et al. 2000). Nevertheless, in *A. dorsalis*, volatile compounds ejected after disturbance could have a double function: repellent function to predators and chemical signalling function for conspecifics. In fact, previous studies showed that the defensive compounds released by a number of disturbed *A. dorsalis* individuals is not only a repellent towards natural enemies, but also elicited dispersal behavior in conspecifics (Bonacci et al. 2004a,b, 2006, 2008). Such an intra-specific dispersal function is supported by the gregarious nature of this species; gregariousness and high population density allowing rapid intra-specific communication is generally thought to be necessary for evolution of chemical signalling molecule (Nault and Phelan 1984). It is reasonable to believe that in *A. dorsalis,* undecane emission (characterized by strong smell, which can be perceived even by humans) by the pygidial glands causes dispersal movement of individuals inside the aggregation (authors' personal observations). If the assumption of Blum (1985) is correct (deterrence against predators and intraspecific alarm function of the same compounds being coupled), the chemicals of this carabids evoke an alarm reaction in conspecifics and avoidance behaviour in natural enemies, as showed by Bonacci et al. (2004a, 2006, 2008). Usually *A. dorsalis* occurs in dense aggregations of many individuals with other carabids belonging to the genus *Brachinus.* It can be expected that in species occurring in such masses an adequate defense mechanism has evolved towards potential predators. *B. sclopeta* producing several defensive chemicals (Zetto Brandmayr et al. 2006) and *A. dorsalis* producing a putative chemical signalling molecule, undecane, from the pygidial glands. Assemblages of mixed species that share common predators may experience benefits that are similar to or exceed those of monospecific groups. These benefits may be particularly pronounced if individuals of one species can recognize the alarm signals produced by individuals of other species in the assemblage (in Mathis and Smith 1993).

In summary, undecane and the pygidial glands appear to play a role in the defence mechanism of *A. dorsalis*. Further studies will carry on to investigate if undecane emission is able to elicit dispersal and retreating movements both in co-specific and interspecific groups.

References

Bonacci T, Aloise G, Brandmayr P, Zetto Brandmayr T (2004a) Risposte comportamentali di *Crocidura leucodon* (Hermann, 1780) (Insectivora, Soricidae) ai meccanismi antipredatori di alcuni Artropodi. Hystrix, Italian Journal of Mammalogy (Nova Series, Pavia, Italy) 15(1): 73–76.

Bonacci T, Mazzei A, Zetto Brandmayr T, Brandmayr P (2004b) Aposematic aggregtion of carabid beetles (Coleoptera Carabidae): preliminary data. Redia LXXXVII: 243– 245.

Bonacci T, Massolo A, Brandmayr P, Zetto Brandmayr T (2006) Predatory behaviour on ground beetles (Coleoptera: Carabidae) by *Ocypus olens* (Müller) (Coleoptera: Staphylinidae) under laboratory conditions. Entomological News 117(5): 545–551.

Bonacci T, Aloise G, Brandmayr P, Zetto Brandmayr T, Capula M (2008) Testing the predatory behaviour of *Podarcis sicula* (Reptilia: Lacertidae) towards aposematic and non-aposematic preys. Amphibia-Reptilia 29: 449–453.

Blum MS (1985) Alarm pheromones. In: Kerkut GA, Gilbert LI (Eds) Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon Press, Oxford, 194–224.

Dazzini Valcurone M , Pavan M (1980) Glandole pigidiali e secrezioni difensive dei Carabidae (Insecta Coleoptera). Publicazioni dell'Istituto di Entomologia dell'Università di Pavia, 12: 1–36.

Dettner K, Schwinger G (1982) Defensive secretions of three oxytelinae rove beetles (Coleoptera: Staphylinidae). Journal of Chemical Ecology, 8(11): 1411–1420.

Dettner K (1987) Chemosystematics and evolution of beetle chemical defense. Annual Review of Entomology 32: 17–48.

Eisner T (1958) The protective role of the spray mechanism of the bombardier beetle, *Brachynus ballistarius* Lec. Journal of Insect Physiology 2: 215–220.

Eisner T, Hurst JJ, Meinwald J (1963) Defense Mecanisms of Arthropods. XI. The Structure, Function, and Phenolic Secretions of the Glands of a Chordeumoid Millipede and a Carabid Beetle. Psyche 70: 94–116.

Eisner T (1970) Chemical defense against predation in arthropods. In: Sondheimer E, Simeone JB(Eds) Chemical Ecology. Academic Press, New York, 235–280.

Eisner T, Jones TH, Aneshansley DJ, Tschinkel WR, Silbergleid RE, Meinwald J (1977) Chemistry of defensive secretions of Bombardier Beetles (Brachinini, Metriini, Ozaenini, Paussini). Journal of Insect Physiology 23: 1383–1386.

Eisner T, Aneshansley DJ (1982) Spray aiming in bombardier beetles: jet deflection by the Coanda effect. Science 215: 83–85.

Eisner T, Attygalle AB, Eisner M, Aneshansley DJ, Meinwald J (1992) Chemical defense of a primitive Australian bombardier beetle: *Mystropomus regularis*. Chemoecology 2: 29–34.

Eisner T, Aneshansley DJ (1999) Spray aiming in the bombardier beetle: photographic evidence. Proceeding of National Academy of Science, USA 96: 9705–9709.

Eisner T, Aneshansley DJ, Eisner M, Attygalle AB, Alsop DW, Meinwald J (2000) Spray mechanism of the most primitive bombardier beetle (*Metrius contractus*). The Journal of Experimental Biology 203: 1265–1275.

Eisner T, Aneshansley DJ, Yack J, Attygalle AB, Eisner M (2001) Spray mechanism of crepidogastrine bombardier beetles (Carabidae; Crepidogastrini). Chemoecology 11:209–219.

Forsyth DJ (1970) The ultrastructure of the pygidial defence glands of the *Pterostichus madidus* F. Journal of Morphology 131: 397–416.

Forsyth DJ (1972) The structure of the pygidial defence glands of Carabidae (Coleoptera). Transactions of the Zoological Society of London 32: 249–309.

Gómez J, Barrera JF, Rojas J, Macias-Samano J, Liedo JP, Cruz-Lopez L, Badii MH (2005) Volatile compounds released by disturbed females of *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae): a parasitoid of coffee berry borer *Hypotenemus hampei* (Coleoptera: Scolytidae). Florida Entomologist 88(2): 180–187.

Juliano SA (1985) Habitat associations, resources, and predators of an assemblage of *Brachinus* (Coleoptera: Carabidae) from southeastern Arizona. Canadian Journal of Zoology 63: 1683–1691.

Kanehisa K (1996) Secretion of defensive substance by carabidae and Brachinidae. Bulletin Resesearch Institute Bioresources, Okayama Univ. 4: 9–23. [in Japanese]

Lindroth CH (1986) The Carabidae (Coleoptera) of Fennoscandia and Denmark Fauna. Entomologica Scandinavica, 15, part 2. Scandinavian Science Press, Copenhagen, 223–497.

Löfqvist J, Bergström G (1980) Volatile communication substances in Dufour's gland of virgin females and old queens of the ant *Formica polyctena*. Journal of Chemical Ecology 6(2): 309–320.

Mazzei A, Bonacci T, Zetto Brandmayr T, Brandmayr P (2005) Capacità di aggregazione di Coleotteri geoadefagi, in ambiente ipolitico di suoli argillosi del bioclima mediterraneo arido. Proceedings of XV Congress of the Italian Society of Ecology. Torino, Italy. 12–14 September 2005. 1–4.

Mathis A, Smith RJF (1993) Intraspecific and cross-superorder responses to chemical alarm signals by brook stickleback. Ecology, 74(8): 2395–2404.

Moore BP (1979) Chemical defense in carabids and its bearing on phylogeny. Carabids beetles: their evolution, natural history, and classification. In Erwin TL, Ball GE, Whitehead DR, Halpern AL (Eds) The Ilague, Junk, 193–203.

Moore BP, Wallbank BE (1968) Chemical composition of the defensive secretion in carabid beetles and its importance as a taxonomic character. Proceeding of Royal Entomological Society of London B 37: 62–72.

Moore BP, Vance Brown W, Rothschild M (1990) Methylalkylpyrazines in aposematic insects, their hostplants and mimics. Chemoecology 1: 43–51.

Morgan ED, Jackson BD, Billen J (2005) Chemical secretions of the "crazy ant" *Paratrechina longicornis* (Hymenopetra: Formicidae). Sociobiology 46 (2): 299–304.

Nault LR, Phelan PL (1984) Alarm pheromones and sociality in pre-social insects. Chemical ecology of Insects. In Bell WJ, Cardè RT(Eds) Chapman and Hall, United Kingdom, 237–256.

Peschke K, Eisner T (1987) Defensive secretion of the tenebrionid beetle, *Blaps mucronata*: Physical and chemical determinants of effectiveness. Journal of Comparative Physiology A 161: 377–388.

Regnier FE, Wilson EO (1968) The alarm-defense system of the ant *Acanthomyops claviger*. Journal of Insect Physiology 14: 955–970.

Rossini C, Attygalle AB, González A, Smedley SR, Eisner M, Meinwald J, Eisner T (1997) Defensive production of acid formic (80%) by a carabid beetle (*Galerita lecontei*). Proceeding of National Academy of Science, USA 94: 6792–6797.

Rothschild M, Moore B (1987) Pyrazines as alerting signals in toxic plants and insects. In: Laberie V, Fabres G, Lachaise D (Eds) Insects-Plants. Dordrecht, Holland, 97–107.

Rowe C, Guilford T (1999) The evolution of multimodal warning displays. Evolutionary Ecology 13: 655–671.

Schildknecht HK, Holoubek KH, Weiss H, Kramer H (1964) Defensive substances of the Arthropods, their isolation and identification. Angewandte Chemie International Edition 3: 73–82.

Schildknecht H, Maschwitz E, Maschwitz U (1968) Die Explosionschemie der Bombardierkäfer (Coleoptera, Carabidae) III. Mitteilung: Isolierung und Charakterisierung der Explosionskatalysatoren. Zeitschrift für Naturforschung. 23b: 1213–1218.

Scott PD, Hepburn HR, Crewe RM (1975) Pygidial defensive secretions of some carabid beetles. Insect Biochemistry 5 (6): 805–811.

Sokal RR, Rohlf FJ (1995) Biometry. 3rd ed. W.H. Freeman, New York, 887 pp.

Statsoft Italia (1997) Statistica per Windows. User's Manual. Statsoft Italia, Vigonza.

Thiele HU (1977) Carabid beetles in their environments. A study on habitat selection by adaptations in physiology and behavior. Springer-Verlag, Berlin, Heidelberg, New York, 369 pp.

Will KW, Attygalle AB, Herath K (2000) New defensive chemical data for ground beetles (Coleoptera: Carabidae): interpretations in a phylogenetic framework. Biological Journal of the Linnean Society 71: 459–481.

Zaballos P (1985) Paralelismo fenológico en *Brachinus variventris* Schaufuss, 1862 y *Anchomenus dorsalis* (Pontoppidan, 1963). (Coleoptera Carabidae). Actas do II Congresso Ibérico de Entomologia, Lisboa, Ispagna.

Zetto Brandmayr T, Bonacci T, Massolo A, Brandmayr P (2006) What is going on between aposematic carabid beetles? The case of *Anchomenus dorsalis* (Pontoppidan 1763) and *Brachinus sclopeta* (Fabricius 1792) (Coleoptera Carabidae). Ethology Ecology and Evolution 18: 335–348.

ZooKeys 81: 27–37 (2011)
doi: 10.3897/zookeys.81.940
www.zookeys.org

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

The first *Strigocis* Dury (Coleoptera, Ciidae) from the southern Neotropical region and a provisional key to world species

Cristiano Lopes-Andrade

Departamento de Biologia Animal, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais, Brazil

urn:lsid:zoobank.org:author:6A3F4101-8599-44D9-9210-BCD5DD8564E9

Corresponding author: *Cristiano Lopes-Andrade* (ciidae@gmail.com)

Academic editor: *Wolfgang Schawaller* | Received 24 January 2011 | Accepted 1 February 2011 | Published 18 February 2011

urn:lsid:zoobank.org:pub:57DEF403-5471-478B-943A-F4231CE8C8FE

Citation: Lopes-Andrade C (2011) The first *Strigocis* Dury (Coleoptera, Ciidae) from the southern Neotropical region and a provisional key to world species. ZooKeys 81: 27–37. doi: 10.3897/zookeys.81.940

Abstract

Strigocis vicosensis Lopes-Andrade, **sp. n.** is described based on specimens from a single locality (type locality: Viçosa, state of Minas Gerais, southeastern Brazil), being the southernmost record of a *Strigocis* Dury species. Diagnostic to this new species are the dorsal vestiture consisting of stout yellowish bristles, pronotal punctures separated from each other by at least 0.75× puncture-width and sutural flange of elytra not diverging near apex. Males have both frontoclypeal ridge and anterior pronotal margin produced forward and emarginated at middle forming two small subtriangular plates, and a small abdominal sex patch. Among the New World *Strigocis*, it most resembles *S. bilimeki* (Reitter), of which images of male terminalia, as far as ventral and dorsal SEM images of males are also provided. The morphological limits of *Strigocis* are briefly discussed and redefined, and a provisional key to the world species is provided.

Keywords

Minute tree-fungus beetles, Ciinae, new species, southeastern Brazil

Introduction

Strigocis Dury (Coleoptera: Ciidae: Ciinae) comprises five described species (sensu Lawrence 1971): *S. bicornis* (Mellié) from central and southern Europe, *S. bilimeki* (Reitter) from Mexico and probably occurring in southern Arizona, *S. opacicollis* Dury and *S. opalescens* (Casey) from eastern North America and Mexico, and *S. tokunagai* (Nobuchi) from Hokkaido, Japan. Therefore, the known species occur in the Nearctic, northern Neotropical and Palearctic regions.

In the latter decade, small series of a new *Strigocis* were collect at Viçosa, in the state of Minas Gerais, southeastern Brazil. In spite of the great collection effort in several states of Brazil, the species was not found in any other locality. Here, I describe it as *Strigocis vicosensis* sp. n., compare to the other described *Strigocis* and provide a provisional key to the world species of the genus.

Material and methods

Measurements, final comparisons and description of general external morphology were made under a Zeiss Stemi 2000 stereomicroscope with a scale ocular. Digital photographs of the holotype were taken with a Canon S70 adapted to a Leica MZ16 stereomicroscope, and final images (Figs 1–3) were generated by combining 20 to 40 photographs in different focus using the image stacking freeware CombineZM (Hadley 2006). Scanning Electron Microscope (SEM) images of male paratypes of *S. vicosensis* sp. n. and males of *S. bilimeki* were obtained using a LEO 1430VP. Specimens were analyzed under variable pressure (SEM-VP) using a backscattered electrons detector, without prior dehydration or gold covering. The holotype (Figs 1–3) was not dissected. Three male paratypes of the new species and five males *S. bilimeki* were dissected for slide preparation and photographing of terminalia (one of each species shown in Figs 7 and 10, after dissection). Besides these, males of *S. bicornis*, *S. opacicollis* and *S. opalescens* were also dissected for examining their terminalia. Permanent slide preparations were made using a water-soluble mounting media called "Downs' gel" (polyvinyl lactophenol), prepared by mixing 56% saturated aqueous solution of polyvinil alcohol, 22% phenolic acid and 22% lactic acid. Detailed information on its preparation and use are provided by Downs (1943) and Salmon (1947). Examination and photography of slide preparations were made under a Zeiss Axioskop 40 compound microscope equipped with a Canon A640 digital camera.

Terms for external morphology and male terminalia of ciids are explained and discussed by Lopes-Andrade and Lawrence (2005) and Lopes-Andrade (2008). The new species described here was compared to named specimens of all the described *Strigocis*, except for *S. tokunagai*. In the latter case, however, a drawing of an adult male was provided in the original description (Nobuchi 1960).

Ten males (including the holotype) and ten females were measured. Range, mean and standard deviation are given for measurements and ratios. Measurements of antennomeres were taken from the holotype. The following abbreviations are used for measurements and ratios: CL, length of the antennal club; EL, elytral length (median length from base of scutellum to elytral apex); EW, greatest elytral width; FL, length of the antennal funicle; GD, greatest depth of the body; PL, pronotal length along midline; PW, greatest pronotal width; TL, total length (EL+PL; head not included).

The ratio GD/EW was taken as an indication of degree of convexity; TL/EW indicates degree of body elongation. The description is based on the holotype, which is a fully pigmented male. Differences among paratypes are given in the section on "Variation", together with standard measurements and ratios of the type series.

The following acronyms are used in this paper:

LAPC	Cristiano Lopes-Andrade Private Collection (Viçosa, MG, Brazil)
MZSP	Museu de Zoologia da Universidade de São Paulo (São Paulo, SP, Brazil)

Taxonomy

Strigocis vicosensis Lopes-Andrade, sp. n.

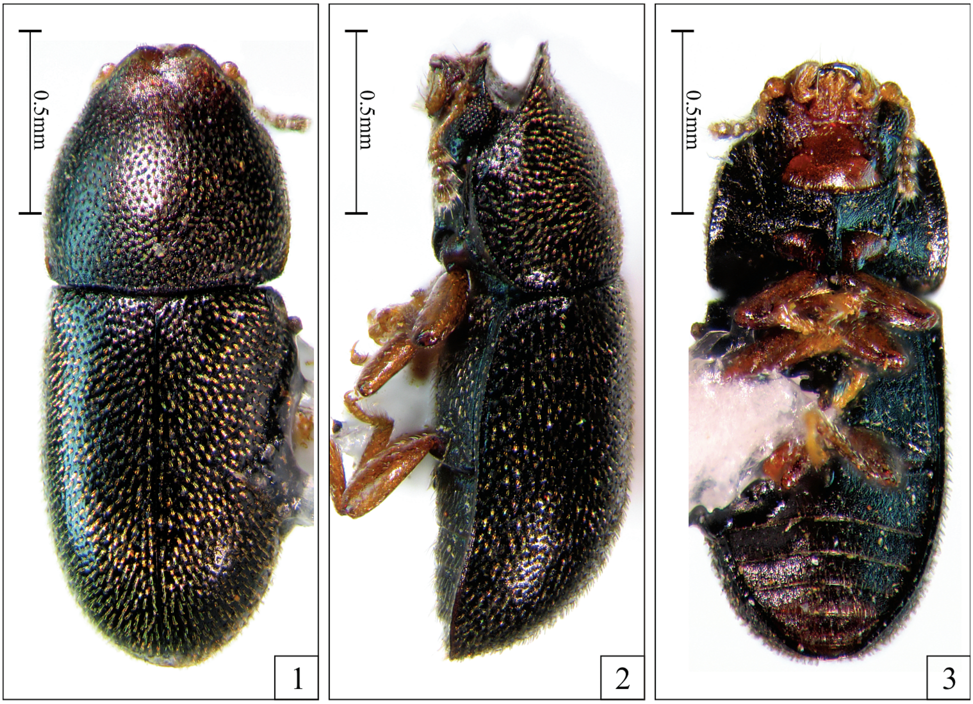
urn:lsid:zoobank.org:act:34BA5411-44C2-4D28-B020-0C9F63DB1828
Figs 1–7

Type-locality. Viçosa, in the state of Minas Gerais, southeastern Brazil (20°45'S, 42°53'W).

Etymology. The specific epithet is an adjective referring to the *terra typica* of the species.

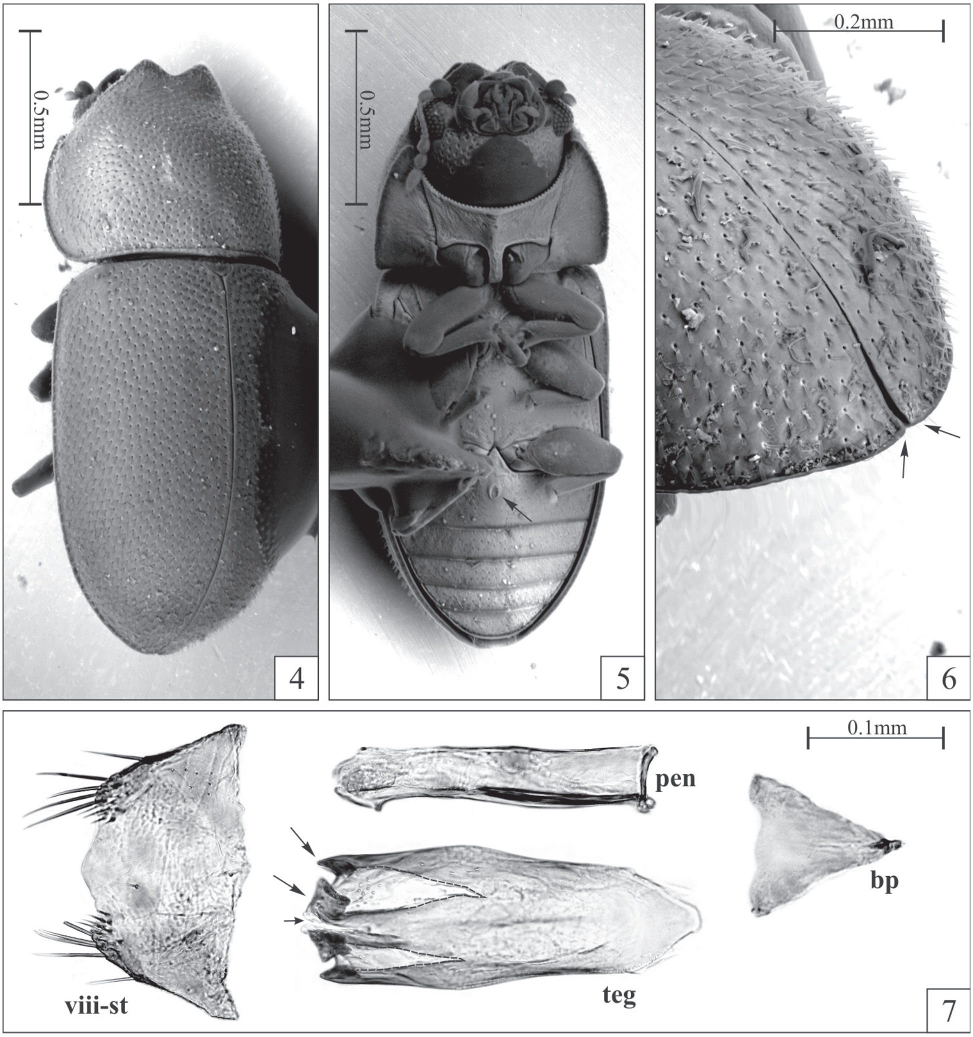
Diagnosis. Dorsal vestiture consisting of stout yellowish bristles, pronotal punctures coarse and separated from each other by 0.75× to 1× puncture-width, and sutural flange of elytra not diverging near apex. Males have both frontoclypeal ridge and anterior pronotal margin produced forward and emarginated at middle forming two small subtriangular plates (Figs 1, 2, 4, 5). Additionally, the abdominal sex patch of males is small, with around one-fifth the length of the first abdominal ventrite at the longitudinal midline.

Description. Male holotype (Figs 1–3), measurements in mm: TL 1.70; PL 0.55; PW 0.68; EL 1.15; EW 0.73; GD 0.55. Ratios: PL/PW 0.81; EL/EW 1.59; EL/PL 2.09; GD/EW 0.76; TL/EW 2.34. Body elongate, moderately convex; dorsal and ventral surfaces mostly unicolored, almost black, with reddish brown appendages; dorsal vestiture consisting of stout suberect or decumbent yellowish bristles; ventral vestiture consisting of slender decumbent yellowish setae. Head barely seen from above; dorsal surface concave, glabrous; punctation sparse, consisting of course shallow punctures; frontoclypeal ridge slightly raised and produced, its anterior margin with an emargination at middle forming two short subtriangular plates with relatively rounded apices. Eyes coarsely facetted, each one bearing more than 60 ommattidia; greatest eye width 0.10 mm. Antenna (left antenna measured; FL 0.10 mm; CL 0.16 mm; CL/FL 1.63) with length of antennomeres (in mm) as follows: 0.06; 0.05; 0.04; 0.02; 0.01; 0.01; 0.01; 0.05; 0.05; 0.06; sensillifers of the antennal club whitish, conspicuous (seen in magnification of 50×). Pronotum densely punctate, the punctures being coarse and separated from each other by 0.75× to one puncture-width; in between punctures somewhat microreticulate; the stout bristles



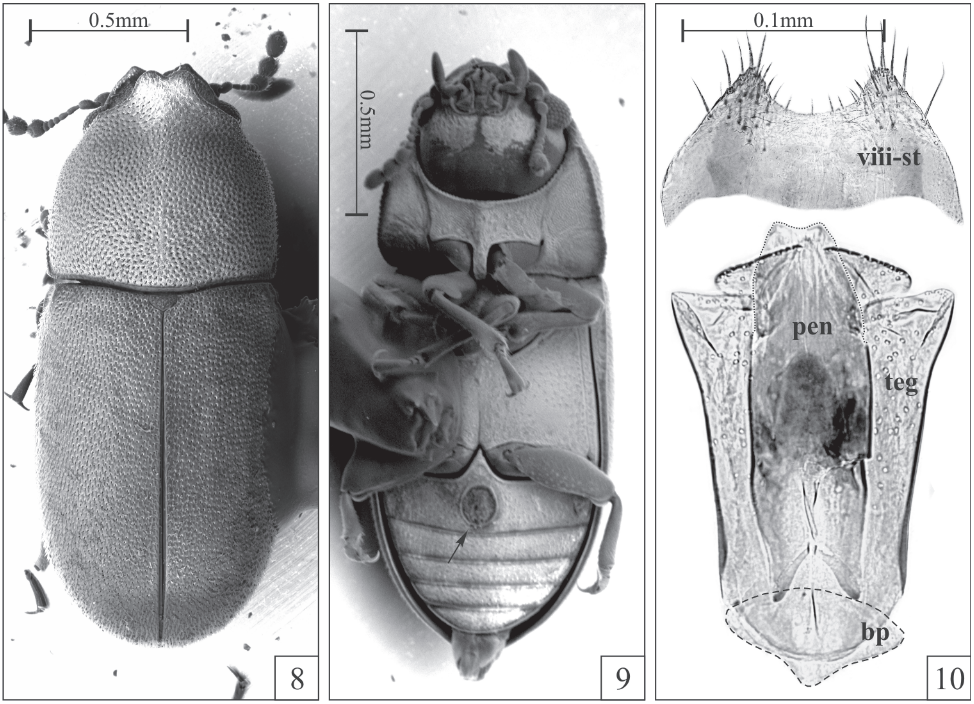
Figures 1–3. *Strigocis vicosensis* Lopes-Andrade, sp. n., adult male holotype. **1** Dorsal view **2** Lateral view **3** Ventral view.

of the pronotal disc are decumbent, so attached to the surface that they resemble small scales and are more easily visible in lateral view; anterior portion produced forward and almost concealing the head when seen from above; anterior margin bearing an emargination at middle, forming two small subtriangular plates that are slightly larger than those of the head (Figs 1–2, 4–5); anterolateral angles (corners) produced forward and rounded; lateral margins slightly crenulate, almost straight, barely visible from above except for their posterior portions. Scutellum very small, so close to the elytra that makes it barely discernible; posterior margin broadly rounded, so that the entire structure resembles a half-circle; surface bearing small punctures very close to each other, giving a creasy appearance to its surface; basal width 0.07 mm. Hind wings fully developed (macropterous species). Elytra with lateral margins subparallel at basal half, then gradually converging to the apex; punctation single and confused, the punctures being coarser than those on pronotum and closer to each other; bristles similar to those on pronotum, but suberect and a little bit bigger; in between punctures smooth and shiny; sutural flange not diverging near apex (slightly divergent when examined in SEM; see Fig. 6, arrows). Ventral sclerites with most of their surfaces finely granulate. Hypomera unpunctate and bearing a few sparse slender setae. Prosternum biconcave and distinctly tumid at the longitudinal midline, carinate. Each protibial with its outer apical angle expanded forming a small acute tooth; apex bearing a row of spines closest to the inner apical angle. Metaventrite with a



Figures 4–7. *Strigocis vicosensis* Lopes-Andrade, sp. n., SEM-VP of adult male paratypes (4–6) and slide preparation of a male terminalia (7). **4** Slightly oblique dorsal view **5** Ventral view showing the sex patch at the first abdominal ventrite (arrow) **6** Dorsal view of the posterior portion of the elytra in an oblique position, showing the barely discernible diverging sutural flange (arrows) **7** Male terminalia showing the eighth sternite (pregenital sclerite) and dissected aedeagus with conspicuous subtriangular basal piece, subcylindrical membranous penis and tegmen. Note the emargination at the middle of the tegmen's apex (small arrow), the two V-shaped concavities (dashed lines), and the two sclerotized tips of each lateral lobe (large arrows). Abbreviations: Eighth sternite (viii-st), basal piece (bp), tegmen (teg) and penis (pen).

few very shallow and coarse punctures, barely discernible; discrimen with one-third the length of the ventrite at the longitudinal midline. Abdominal ventrites bearing several slender setae; first abdominal ventrite twice as long as the second, bearing a small oval margined sex patch, with near one-fifth the length of the ventrite at the longitudinal midline.



Figures 8–10. *Strigocis bilimeki* (Reitter) from Cualac (Mexico, Guerrero), SEM-VP of adult males (8–9) and male terminalia (10). **8** Dorsal view **9** Ventral view showing the sex patch at the first abdominal ventrite (arrow) **10** Slide preparation of a male terminalia showing aedeagus and eighth sternite slightly squashed to emphasize their morphology. Note the membranous penis (dotted line) and the small basal piece (dashed line). Abbreviations: Eighth sternite (viii-st), basal piece (bp), tegmen (teg) and penis (pen).

Male terminalia in paratypes (Fig. 7). Eighth sternite (Fig. 7) with its posterior margin membranous (tending to collapse during slide preparation), sinuous; corners bearing long setae. Aedeagus (Fig. 7) with near 0.3 mm of length; basal piece large, conspicuous, with two-fifths the length of tegmen; penis subcylindrical, membranous, 0.8× the length of tegmen; tegmen with its posterior portion bearing a narrow emargination at middle (Fig. 7, small arrow) delimiting two lateral lobes, each lobe with a somewhat V-shaped concavity (dashed lines) ending in two sclerotized tips (large arrows).

Females. Head with frontoclypeal ridge just slightly sinuous. Anterior margin of pronotum broadly rounded. Vertex of head with sparse suberect stout bristles. First abdominal ventrite devoid of sex patch.

Variation. Males, measurements in mm (n = 10, including the holotype): TL 1.33–1.75 (1.59 ± 0.14); PL 0.50–0.70 (0.60 ± 0.07); PW 0.55–0.75 (0.66 ± 0.06); EL 0.80–1.15 (0.98 ± 0.10); EW 0.63–0.78 (0.70 ± 0.05); GD 0.50–0.60 (0.54 ± 0.04). Ratios: PL/PW 0.81–1.00 (0.92 ± 0.06); EL/EW 1.27–1.59 (1.40 ± 0.09); EL/PL 1.43–2.09 (1.64 ± 0.21); GD/EW 0.73–0.83 (0.77 ± 0.03); TL/EW 2.12–2.34 (2.26 ± 0.08). A few males were almost devoid of secondary sexual characters on frontoclypeal ridge and anterior margin of pronotum. However, the small abdominal sex patch was observed in all available males.

Females, measurements in mm (n = 10): TL 1.30–1.65 (1.52 ± 0.13); PL 0.43–0.63 (0.54 ± 0.06); PW 0.53–0.70 (0.62 ± 0.06); EL 0.83–1.10 (0.99 ± 0.08); EW 0.60–0.75 (0.69 ± 0.06); GD 0.45–0.58 (0.54 ± 0.05). Ratios: PL/PW 0.75–0.96 (0.86 ± 0.06); EL/EW 1.38–1.54 (1.44 ± 0.06); EL/PL 1.60–2.18 (1.85 ± 0.17); GD/EW 0.75–0.81 (0.78 ± 0.02); TL/EW 2.14–2.30 (2.22 ± 0.05).

Type series. Male holotype (MZSP) “BRASIL: MG Viçosa; Campus UFV 18.vi.2006 C.B. Oliveira leg. ex *Phellinus* sp.” “*Strigocis vicosensis* Lopes-Andrade HOLOTYPUS” [printed on red paper]. Paratypes: one male (LAPC), same data as holotype; six females, three males (LAPC) “BRASIL: MG Viçosa; 2° Represa UFV; 20.ii.2006 leg. CB Oliveira”; one female (LAPC) “BRASIL: MG Viçosa 16.ii.2002 leg. C. Lopes-Andrade”; 13 females, five males (LAPC) “BRASIL: MG; Viçosa Campus UFV, 3° represa, próx. supermercado 18.vii.2007; colônia II leg C. B. Oliveira”. All paratypes distinguished labeled “*Strigocis vicosensis* Lopes-Andrade PARATYPUS” [printed on yellow paper].

Host fungus. Possibly a single species of an undetermined *Phellinus* Quél. (Hymenochaetaceae). It’s important to emphasize that only adult ciids were found and they were not observed breeding in the collected basidiomes.

Natural history. All the known specimens were collected in open areas of the Federal University of Viçosa’s campus. It was not found in either forested or urban areas of Viçosa, although ciids were continuously collected there from September 1998 until recently. Adults could not be maintained in laboratory for much longer, which suggests that it colonizes and consumes the basidiomes just after sporulation and before basidiomes’ decaying. Another possibility is that the *Phellinus* sp. is not a host for *S. vicosensis* sp. n., and adults were incidentally collected in the fungus. The latter explanation is supported by the fact that all the other *Strigocis* species are known to feed on fungi in the *Trametes* host-use group and were never found in association with fungi of the *Phellinus* host-use group (Orledge and Reynolds 2005). *Xylographus gibbus* Mellié were found in basidiomes possibly conspecific to the ones inhabited by *S. vicosensis* sp. n., in the same open areas. However, these two ciid species have not been collected together.

Comparative notes. Among the examined *Strigocis*, the most similar to *S. vicosensis* sp. n. is the Mexican *S. bilimeki*. The latter species has pronotal and elytral punctation comparatively denser, subtriangular plates of the frontoclypeal ridge in males usually with acute apices and anterior pronotal margin with a short and relatively narrow lamina bearing a small emargination at middle (Fig. 8). Additionally, in *S. bilimeki* the male sex patch in the first abdominal ventrite is very large (Fig. 9, arrow), its diameter being at least 0.55× the length of the sclerite at its longitudinal midline. Male terminalia of both species are similar in form, mainly in the subcylindrical membranous penis (Figs 7, 10) and the shape of the posterior portion of tegmen. However, the whole aedeagus of *S. bilimeki* (Fig. 10) has around 0.8× the length of the one of *S. vicosensis* sp. n., a proportionally smaller basal piece (Fig. 10, dashed lines) and is less sclerotized. Moreover, the posterior margin of the eighth sternite has a broad U-shape emargination (Fig. 10) similar to that of *S. bicornis*, *S. opacicollis* and *S. opalescens*.

Strigocis opalescens has a vestiture of minute setae barely visible even in high magnifications (50×), sparse pronotal and elytral punctation, and broad lateral margins of pronotum forming a raised lip. The tegmen of its male terminalia is subquadrate, with a deep U-shaped emargination at middle delimiting two lateral lobes, each lobe bearing a small emargination at apex. *Strigocis bicornis* is a small blackish species with shallow pronotal and elytral punctation, irregular elytral surface with in between punctures finely granulate, and dorsal vestiture of fine setae. Its tegmen is elongate, with a rounded posterior margin bearing a very narrow V-shape emargination at middle. It is similar to the examined named specimens of *S. opacicollis*, in which the elytral margins are not so parallel and elytral punctation is coarser and denser. Additionally, the tegmen in *S. opacicollis* is quite different, with an almost straight posterior margin bearing a small U-shaped emargination at middle. I could not examine any named specimen of *S. tokunagai*, but data and drawing provided by Nobuchi (1960) show that it is similar to *S. vicosensis* sp. n. in the vestiture of yellowish bristles, pronotal and elytral punctation. However, the abdominal sex patch of *S. tokunagai* is described as being large.

Provisional key to the species of *Strigocis* Dury

1 Vestiture dense, especially on elytra, and consisting of short stout bristles ... **2**

1’ Vestiture of somewhat sparse fine setae, conspicuous or not **4**

2(1) Pronotal punctation very dense, punctures separated by less than 0.75× puncture-width; in between punctures granulate, giving a dull appearance to the pronotal surface. Prosternum tumid but not carinate. Mexico and probably southern Arizona .... ***S. bilimeki* (Reitter)**

2’ Pronotal punctures separated by a distance of at least 0.75×; in between punctures microreticulate. Prosternum carinate **3**

3(2’) Male pronotum with apices of the subtriangular plates as separated as the ones of the frontoclypeal ridge. Male with small abdominal sex patch, with around 0.2× the length of the ventrite at the longitudinal midline. Known only from Viçosa (southeastern Brazil) .... ***S. vicosensis* Lopes-Andrade, sp. n.**

3’ Male pronotum with the apices of the subtriangular plates closer than those of the frontoclypeal ridge. Male with large abdominal sex patch. Japan: Hokkaido .... ***S. tokunagai* (Nobuchi)**

4(1’) Body suboval. Lateral margins of pronotum relatively broad and with a raised lip. Elytral vestiture of barely visible (magnification of 50×) minute setae. Eastern North America .... ***S. opalescens* (Casey)**

4’ Boby subparallel-sided. Lateral margins of pronotum narrow and devoid of raised lip. Elytral vestiture of small but distinct setae.................................. **5**

5(4’) Elytral punctation coarse; punctures separated by a distance of one puncture-width or less. Eastern North America............................ ***S. opacicollis* Dury**

5’ Elytral punctation fine; punctures separated by a distance of 2× puncture-widths or more. Central and southern Europe .... ***S. bicornis* (Mellié)**

Discussion

The debate on the morphological limits, and consequently on the species that belong or not to *Strigocis*, is far from being concluded. In its original description, Dury (1917) has not included any other species besides the type-species, *S. opacicollis*. *Strigocis bicornis* and *S. bilimeki* were originally assigned to *Cis* Latreille, *S. opalescens* to *Xestocis* Casey, and *S. tokunagai* to *Ropalodontus* Mellié in their original descriptions. Lawrence (1965) tentatively included *S. bicornis* and *S. tokunagai* in *Sulcacis* Dury possibly due to their spinose protibial apex. Finally, Lawrence (1971) transferred all the four species to *Strigocis* and provided a table of characters for distinguishing it from *Sulcacis*. However, some European taxonomists have considered *S. bicornis* as belonging to *Sulcacis* until recently (see Orledge and Booth 2006 for a brief discussion). The greatest taxonomic problem of the genus, which possibly has led to such instability in literature, is the absence of exclusive characters for defining the taxon.

Lawrence (1971) proposed the spinose protibal apex and the diverging sutural flange of elytral apex as diagnostic to *Strigocis*. At that time, only species of *Strigocis* and *Orthocis* Casey were known to have the latter feature. Diagnostic to *Orthocis* were the rounded protibial apex, devoid of spines, and absence of secondary sexual modifications besides the male sex patch of several species. However, the diverging sutural flange of elytral apex was not observed in *S. vicosensis* sp. n., and it was already observed in *Ennearthron pruinosulus* (Perris), *Wagaicis wagae* (Wankowicz) and *Odontocis denticollis* Nakane and Nobuchi (Lopes-Andrade pers. obs.). The spinose protibial apex is also not exclusive to *Strigocis*, and it may vary among species in a single genus, like in *Cis*. Several species of *Cis* have pronotal and frontoclypeal modifications and body form similar to those of *Strigocis*, as well as spinose protibial apex in which the spines are concealed by long setae. These are the cases for *Cis graecus* Schilsky and species of the *Cis bilamellatus* group (sensu Lopes-Andrade et al. 2009), for instance. In the case of the abovementioned *Cis* species, they are distinguishable from *Strigocis* by their dual elytral punctation.

It is early to take the decision of synonymizing *Strigocis* to *Cis* before a careful taxonomic revision of both groups. Moreover, *Cis* is certainly not a clade (Buder et al. 2008) and remains as the most speciose and morphologically diversified genus of Ciidae, with around 375 described species. Although the matter on the limits of *Strigocis* remains far from being satisfactorily resolved, I prefer to re-evaluate the diagnostic characters of the genus, as follows: (i) protibial apex bearing a row of spines, sometimes concealed by adjacent long setae; (ii) prosternum always biconcave, slightly tumid to carinate; (iii) elytra with both vestiture and punctation single; (iv) frontoclypeal ridge and anterior margin of pronotum in males usually emarginated at middle; (v) sutural flange of the elytral apex diverging near apex in most species (except in *S. vicosensis* sp. n.); (vi) ovipositor with gonostyli well-developed. The latter feature separates *Strigocis* from *Sulcacis*, in which the gonostyli, as far as the whole terminalia, are reduced (Lawrence and Lopes-Andrade 2010).

Acknowledgements

I'm grateful to John F. Lawrence for his comments and suggestions, to Caio Antunes de Carvalho for finding measurement errors in the final version of the manuscript and to Fernando Z. Vaz de Mello for the *S. bilimeki* he collected in Mexico. Electron microscopy facilities were provided by "Núcleo de Microscopia e Microanálise" (NMM, UFV), with equipments financed by FINEP/FAPEMIG and CNPq. I made images of the holotype at "Laboratório de Orthopterologia" (DBG/UFV) during my post-doctoral research (CNPq post-doctoral fellowship nº 151800/2007-3, from November 2007 to October 2008). Images of male terminalia were made at "Laboratório de Acarologia" (DBA/UFV) by Vivian E. Sandoval Gómez. Financial supports were provided by FAPEMIG (Edital PPP 21/2008 2008, APQ-00049-09; Edital PPM 03/2010, PPM-00017-10), CNPq (PROTAX 52/2010 nº 562229/2010-8) and the Graduation Program in Entomology of the Federal University of Viçosa.

References

Buder G, Grossmann C, Hundsdoerfer A, Klass K-D (2008) A contribution to the phylogeny of the Ciidae and its relationships with other cucujoid and tenebrionoid beetles (Coleoptera: Cucujiformia). Arthropod Systematics & Phylogeny 66 (2): 165–190.

Downs WG (1943) Polyvinyl alcohol: a medium for mounting and clearing biological specimens. Science (N.S.) 97(2528): 539–540.

Dury C (1917) Synopsis of the coleopterous family Cisidae (Cioidae) of America north of Mexico. Journal of the Cincinnati Society of Natural History 22 (2): 2–28.

Hadley A (2006) CombineZM public domain image processing software. Available from: http://www.hadleyweb.pwp.blueyonder.co.uk/CZM/combinezm.htm [accessed 10/March/2007]

Lawrence JF (1965) Comments on some recent changes in the classification of the Ciidae (Coloptera). Bulletin of the Museum of Comparative Zoology 133 (5): 273–293.

Lawrence JF (1971) Revision of the North American Ciidae (Coleoptera). Bulletin of the Museum of Comparative Zoology 142: 419–522.

Lawrence JF, Lopes-Andrade C (2010) Ciidae Leach in Samouelle, 1819. In: Leschen RAB, Beutel RG, Lawrence JF (Eds) Handbuch der Zoologie/Handbook of Zoology. Band/Volume IV Arthropoda: Insecta. Teilband/Part 38. Coleoptera, Beetles. Volume 2. Morphology and Systematics (Polyphaga partim). Walter de Gruyter, Berlin, 504–514.

Lopes-Andrade C (2008) An essay on the tribe Xylographellini (Coleoptera: Tenebrionoidea: Ciidae). Zootaxa 1832: 1–110.

Lopes-Andrade C, Lawrence JF (2005) *Phellinocis*, a new genus of Neotropical Ciidae (Coleoptera: Tenebrionoidea). Zootaxa 1034: 43–60.

Lopes-Andrade C, Matushkina N, Buder G, Klass K-D (2009) *Cis pickeri* sp. n. (Coleoptera: Ciidae) from Southern Africa. Zootaxa 2117: 56–64.

Nobuchi A (1960) Ciidae from Hokkaido (Coleoptera). Entomological Review of Japan 11 (2): 37–41.

Orledge GM, Booth RG (2006) An annotated checklist of British and Irish Ciidae with revisionary notes. The Coleopterist 15 (1): 1–16.

Orledge GM, Reynolds SE (2005) Fungivore host-use groups from cluster analysis: patterns of utilisation of fungal fruiting bodies by ciid beetles. Ecological Entomology 30: 620–641.

Salmon JT (1947) New methods in microscopy for the study of small insects and arthropods. Transactions of the Royal Society of New Zealand 77: 250–253.

ZooKeys 81: 39–50 (2011)
doi: 10.3897/zookeys.81.833
www.zookeys.org

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Review of the genus *Fibuloides* Kuznetsov in China (Lepidoptera, Tortricidae, Olethreutinae)

Aihuan Zhang[1,2,†], Houhun Li[1,‡]

1 *College of Life Sciences, Nankai University, Tianjin 300071, P. R. China* **2** *Beijing Key Laboratory of New Technology in Agricultural Application, College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, P. R. China*

† *urn:lsid:zoobank.org:author:40700691-16E7-4B32-ABD2-D4D1F53CB37D*
‡ *urn:lsid:zoobank.org:author:F6AAB0C0-F312-4035-A08B-3DFE03D02F62*

Corresponding author: *Houhun Li* (lihouhun@nankai.edu.cn)

Academic editor: *E. van Nieukerken* | Received 26 December 2010 | Accepted 8 February 2011 | Published 18 February 2011

urn:lsid:zoobank.org:pub:4C4CC5D8-11E2-4DAF-BD38-8D9485147AE0

Citation: Zhang A, Li H (2011) Review of the genus *Fibuloides* Kuznetsov in China (Lepidoptera, Tortricidae, Olethreutinae). ZooKeys 81: 39–50. doi: 10.3897/zookeys.81.833

Abstract

Species of the genus *Fibuloides* Kuznetsov that occur in China are reviewed. *Fibuloides trapezoidea*, **sp. n.** is described as new; *F. levatana* (Kuznetsov) and *F. modificana* Kuznetsov are newly recorded for China; *Acroclita nigrovenana* Kuznetsov, **syn. n.** is considered as a synonym of *F. corinthia* (Meyrick); and *Eucoenogenes elongata* Zhang & Li and *E. wuyiensis* Zhang & Li are transferred to *Fibuloides*, resulting in two new combinations. A key to the nine Chinese species of *Fibuloides* is given.

Keywords

Lepidoptera, Tortricidae, *Fibuloides*, new species, new combination, synonym, China

Introduction

Fibuloides was proposed by Kuznetsov (1997b) with *F. modificana* Kuznetsov, 1997 from South Vietnam as the type species. The characters of *Fibuloides* given by Kuznetsov are as follows: the costal fold narrow in male; the forewing with R_4 and

R_5 stalked, R_3 with base close to this stem; CuA_1 strongly curved and originating from near base of M_3; hindwing with M_3 and CuA_1 stalked; and the valva extremely modified, with an unusually long, sclerotized process originating from the apex of the sacculus. Brown (2005) included one species, *F. modificana*, in the world catalogue of Tortricidae.

Horak (2006) described *F. phycitipalpia* Horak, 2006 and *F. minuta* Horak, 2006 from Queensland and New South Wales, Australia, and transferred seventeen species to the genus. She pointed out that males of *Fibuloides* usually have a notch at the base of the flagellum, bear modified fringe scales along the anal margin of the hindwing or a pencil of long hairscales from its base, have transverse bands of modified scaling dorsally on the abdomen, and the weak lateral arms of the gnathos from below middle of the tegumen end in two sclerotized vertically rising bands. Pinkaew (2008) described *F. khaonanensis* from Thailand and transferred *Eucoenogenes bicucullus* Pinkaew, 2005 and *E. vaneeae* Pinkaew, 2005 to *Fibuloides*.

Currently the genus includes 23 species distributed in the Palaearctic, Oriental and Australian regions (Brown 2005; Horak 2006; Pinkaew 2008; Baixeras et al. 2009). Prior to this study, five species were recorded from China (Kawabe et al. 1992; Liu and Li 2002; Zhang and Li 2005). In the present paper, we describe one new species, record two species new for the Chinese fauna, transfer two species from *Eucoenogenes* to *Fibuloides*, and propose *Acroclita nigrovenana* Kuznetsov, 1988 from North Vietnam as a synonym of *F. corinthia* (Meyrick, 1912) described from Sri Lanka. A key to the Chinese species is provided based on the male genitalia of the examined specimens.

Material and methods

This study is based on the examination of specimens collected using light traps in the forests and mountains, mainly from the southern part of China. The terminology for the forewing pattern follows Brown and Powell (1991) as refined by Baixeras (2002). Methods of genitalia dissection follow Li (2002). Unless indicated otherwise, all the specimens examined, including the types, are deposited in the Insects Collection, College of Life Sciences, Nankai University, Tianjin, China.

Abbreviations

TL	Type locality;
BMNH	The Natural History Museum, London, UK;
ZMAS	Zoological Institute, Russian Academy of Sciences, St. Petersburg (Leningrad), Russia;
NKUM	Insects Collections, College of Life Sciences, Nankai University, Tianjin, China;
USNM	National Museum of Natural History, Washington, D. C., USA.

Taxonomic accounts

Genus *Fibuloides* Kuznetsov, 1997

Fibuloides Kuznetsov, 1997: 810. Type species: *F. modificana* Kuznetsov, 1997: 810, by original designation.

Key to Chinese species of *Fibuloides* based on male genitalia

1 Uncus blunt apically **2**

– Uncus pointed apically........ **4**

2 Sacculus with a digitate process, neck of valva with two enlarged, flattened bristles .... ***F. japonica***

– Sacculus without digitate process, neck of valva with one or more than three enlarged, flattened bristles........ **3**

3 Uncus drooping, projected outward, socius upturned; neck of valva with five enlarged, flattened bristles on left side and four on right side, cucullus somewhat elliptic .... ***F. elongata***

– Uncus not drooping, socius drooping, neck of valva with one enlarged, flattened bristle, cucullus somewhat trapezoidal........ ***F. modificana***

4 Neck of valva without enlarged, flattened bristles........ **5**

– Neck of valva with one or more enlarged, flattened bristles........ **7**

5 Cucullus bipartite, nearly ovate, or nearly trapezoidal **6**

– Cucullus very long and narrow, with long point on end........ ***F. levatana***

6 Cucullus nearly trapezoidal, uncus with tips straight and parallel........ ***F. trapezoidea***

– Cucullus bipartite, nearly ovate, uncus with tips bent outward ....***F. corinthia***

7 Socius triangular, angle of sacculus indistinct........ ***F. wuyiensis***

– Socius lobe-shaped, angle of sacculus obtuse........ **8**

8 Cucullus nearly triangular; socius broad and short, about twice as long as wide........ ***F. aestuosa***

– Cucullus ovate; socius slender, about three times as long as wide ....***F. cyanopsis***

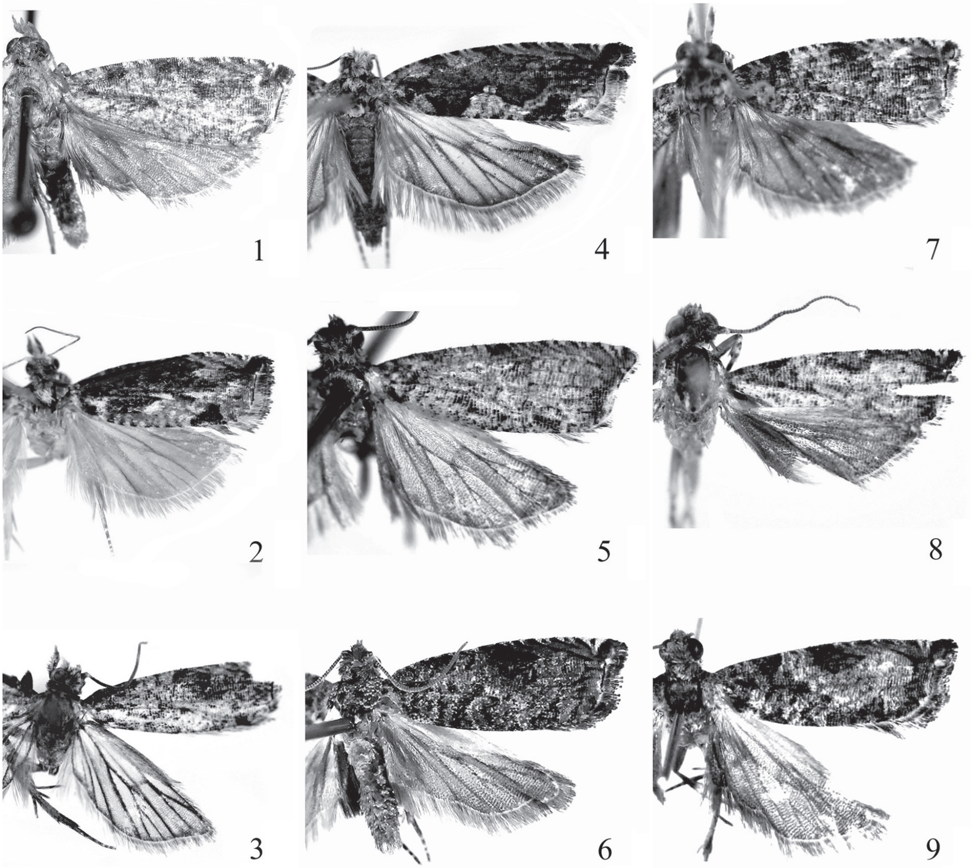
Fibuloides trapezoidea Zhang & Li, sp. n.

urn:lsid:zoobank.org:act:EA201525-B594-47F4-A928-6C254A1B37C6

Figs 1, 10

Type material. Holotype ♂ – **China, Guizhou Province:** Chishui (28°34'N, 105°42'E), 390 m, 30.V.2000, coll. Yanli Du, genitalia slide no. YHL04481; Paratype – 1 ♂, 27.V.2000, other same data as for holotype, genitalia slide No. ZAH10019.

Diagnosis. This species is similar to *F. cyanopsis* (Meyrick, 1912) in the shape of uncus and socius, but can be distinguished by the trapezioidal cucullus and the absence



Figures 1–9. Adults of *Fibuloides* spp. **1** *F. trapezoidea* Zhang & Li, sp. n., ♂ **2** *F. aestuosa* (Meyrick), ♂ **3** *F. corinthia* (Meyrick), ♂ **4** *F. cyanopsis* (Meyrick), ♀ **5** *F. elongata* (Zhang & Li), ♂ **6** *F. japonica* (Kawabe), ♂ **7** *F. levatana* (Kuznetsov), ♂ **8** *F. modificana* Kuznetsov, ♂ **9** *F. wuyiensis* (Zhang & Li), ♂.

of the enlarged, flattened bristles on the neck of valva. In *F. cyanopsis* the cucullus is ovate and the neck of valva has two or more short enlarged, flattened bristles on its ventral side.

Description. Adult (Fig. 1). Forewing length 6.5 mm. **Head:** Vertex with gray scales; frons white. Antenna light brown. Labial palpus slender, gray intermixed with brown, third segment porrect. **Thorax:** Thorax and tegula gray intermixed with light brown. Forewing elongate triangular, with ground color dark gray; basal patch extending from costal 1/4 to 1/3 of dorsum, protrudent in middle on outer side; median fascia short and broad, extending from costal half, terminated at end of cell; ocellus nearly quadrate, containing some short brown striae; apex brown, protrudent; termen slightly concave below apex, bordered by brown scales; costa with nine pairs of strigulae from base to apex, each pair of strigulae with a short brown stria extending obliquely; first to fourth pairs between base of wing and the point where Sc meets costa, broad brown patch lying between second and third pairs; fifth and sixth pairs between Sc and R_1; distal three pairs distributed between pairs of veins

R_1–R_2, R_2–R_3, R_3–R_4 respectively, separated from each other by dark brown scales; cilia gray mixed with brown. Hindwing and cilia gray. Legs gray, tarsi with brown rings.

Abdomen: Male genitalia (Fig. 10). Uncus bifurcated from near base, produced into two slender, parallel and widely separated tips. Socius broad and short, about twice as long as wide, drooping, hairy, with round end. Valva broad at base; neck distinct, without enlarged, flattened bristles; sacculus about twice size of cucullus, with a line of flattened long bristles; cucullus trapezoidal, hairy, with marginal spines. Anellus closely surrounding basal 1/3 of aedeagus; aedeagus long tubular, cornuti consisting of a bunch of curved spines.

Female. Unknown.

Distribution. China (Guizhou).

Etymology. The specific name is derived from Latin *trapezoideus* (= trapeziform), referring to the shape of the cucullus.

***Fibuloides aestuosa* (Meyrick, 1912)**

Figs 2, 11, 19

Spilonota aestuosa Meyrick, 1912: 854. Holotype ♂, TL: India, deposited in BMNH.
Acroclita ligyropis Meyrick, 1937: 176; Clarke 1958: 267.
Eucoenogenes aestuosa (Meyrick, 1912): Kuznetsov 1976: 12; Kawabe 1982, 1: 120, 2: 173; Razowski 1989: 256; Byun *et al.* 1998: 160; Razowski 1999: 446; Kuznetsov 2001: 402; Liu and Li 2002: 319.
Fibuloides aestuosa (Meyrick, 1912): Horak 2006: 330.

Material examined. China, Sichuan Province: 1 ♀, Baoxing County, 1600 m, 3.VIII.2004, coll. Yingdang Ren. **Zhejiang Province:** 2 ♂♂, 3 ♀♀, Mt. Tianmu, 350–1500 m, 18-20.VIII.1999, coll. Houhun Li *et al.* **Hubei Province:** 2 ♂♂, 3 ♀♀, Hefeng County, 1260 m, 15–16.VII.1999, coll. Houhun Li *et al.* **Henan Province:** 1 ♀, Song County, 1580 m, 23.VII.2002, coll. Xinpu Wang.

Host plants. Fagaceae: *Castanea mollissima* Blume and *C. cranata* Sieb. *et* Zucc. (Kuzenetsov 2001; Liu and Li 2002).

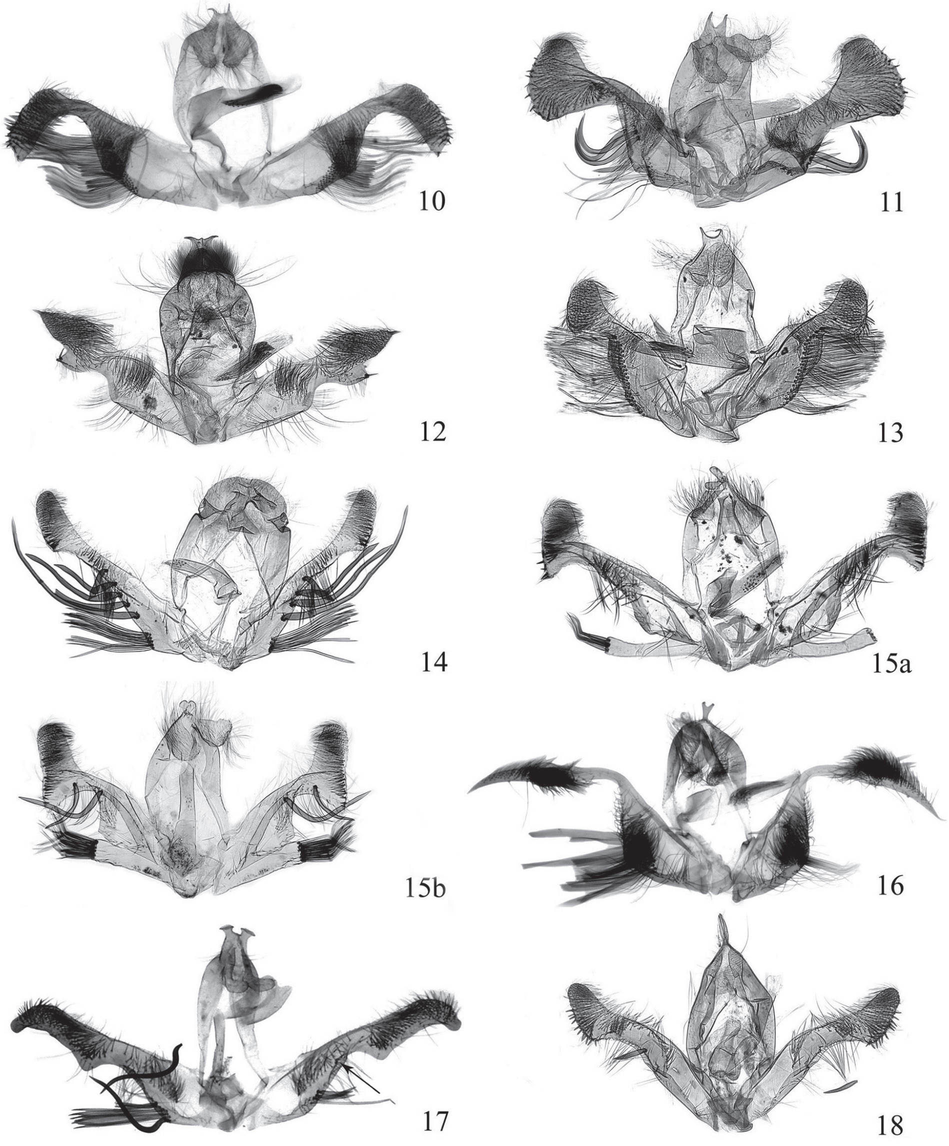
Distribution. China (Anhui, Henan, Hubei, Guangxi, Liaoning, Sichuan, Yunnan, Zhejiang), Korea, Japan, India, Bengal.

Remarks. The number of thick flattened bristles below the neck of the valva in the male genitalia is variable (two or more).

***Fibuloides corinthia* (Meyrick, 1912)**

Figs 3, 12

Acroclita corinthia Meyrick, 1912: 858; Diakonoff 1950: 277; Clarke 1958: 271; Kawabe *et al.* 1992: 108. Lectotype ♂, TL: Sri Lanka, deposited in BMNH.
Fibuloides corinthia (Meyrick, 1912): Horak 2006: 330.



Figures 10–18. Male genitalia of *Fibuloides* spp. **10** *F. trapezoidea* Zhang & Li, sp. n., slide No. YHL04481 **11** *F. aestuosa* (Meyrick), slide No. ZAH03603 **12** *F. corinthia* (Meyrick), slide No. ZAH04036 **13** *F. cyanopsis* (Meyrick), slide No. ZAH03758 **14** *F. elongata* (Zhang & Li), slide No. ZAH03725 **15a–15b** *F. japonica* (Kawabe), slide Nos. **a**: ZAH03173, **b**: ZAH04296 **16** *F. levatana* (Kuznetsov), slide No. ZAH04299 **17** *F. modificana* Kuznetsov, slide No. LJM04401 **18** *F. wuyiensis* (Zhang & Li), slide No. ZAH04215.

Acroclita nigrovenana Kuznetsov, 1988: 88; Nasu 1993: 216; Kuznetsov 2001: 408.
Holotype ♂, TL: Vietnam, deposited in ZMAS. **syn. nov.**
Fibuloides nigrovenana (Kuznetsov, 1988): Horak 2006: 330.

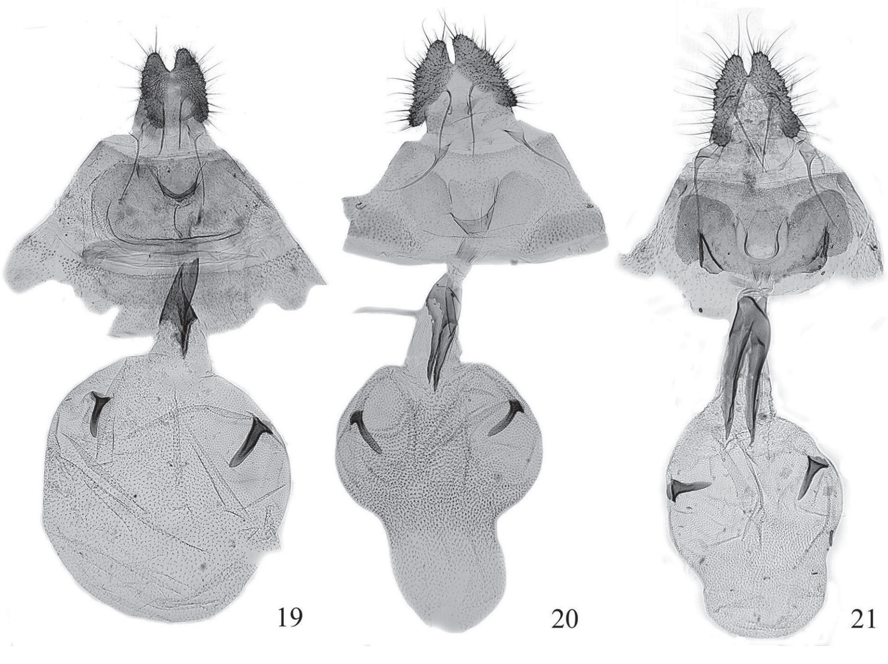
Material examined. China, Yunnan Province: 1 ♂, Yuanjiang County, 710 m, 28.IV.1995, coll. Guangyun Yan.

Host plant. Sapindaceae: *Litchi chinensis* Sonn. (Kuznetsov 2001).

Distribution. China (Yunnan, Taiwan); Sri Lanka; India.

Remarks. The male uncus is produced into two sharp, outwardly bent tips, and the cucullus is nearly elliptic, ending in a spine, with a ventral process bearing a short distal spine.

Diakonoff (1950) designated the male lectotype and Clarke (1958) provided photographs of the adult and male genitalia. Horak (2006) transferred *A. corinthia* and *A. nigrovenana* to *Fibuloides*. We synonymize *F. nigrovenana* with *corinthia* based on the the study of the adult and the male genitalia. Though we were unable to locate the type of *F. corintha* in BMNH, the two species appear to be conspecific based on a comparison of the photo in Clarke (1958) and the illustration in Rose and Pooni (2005) of *Acroclita corinthia* with the photo of *A. nigrovenana* from Japan in Nasu (1993) as well as with the adult and male genitalia of the Chinese specimen.



Figures 19–21. Female genitalia of *Fibuloides* spp. **19** *F. aestuosa* (Meyrick), slide No. ZAH03604 **20** *F. cyanopsis* (Meyrick), slide No. ZAH03757 **21** *F. japonica* (Kawabe), slide No. ZAH03645.

Fibuloides cyanopsis **(Meyrick, 1912)**

Figs 4, 13, 20

Eucosoma cyanopsis Meyrick, 1912: 866; Clarke 1958: 360. Holotype ♂, TL: India, deposited in BMNH.

Eucoenogenes cyanopsis (Meyrick, 1912): Kuznetsov 1988: 81; Kawabe 1989: 52; Razowski 1999: 446; Kuznetsov 2001: 402.

Fibuloides cyanopsis (Meyrick, 1912): Horak 2006: 330.

Eucosma melanochlaena Meyrick, 1936: 611. Holotype ♂, TL: Indonesia, deposited in BMNH.

Material examined. China, Guangxi Zhuangzu Autonomous Region: 1 ♂, 3 ♀♀, Shangsi County, 260–770 m, 1–3.IV.2002, coll. Shulian Hao and Huaijun Xue; 1 ♂, 3 ♀♀, Mt. Maoer, 550–1100 m, 19–20.IV.2002, coll. Shulian Hao and Huaijun Xue. **Guangdong Province:** 1 ♀, Lianzhou, 650 m, 23.VI.2004, coll. Dandan Zhang. **Guizhou Province:** 1 ♀, Jiangkou County, 1700 m, 29.VII.2001, coll. Houhun Li and Xinpu Wang.

Distribution. China (Guangdong, Guangxi, Guizhou), Japan, Vietnam, Indonesia, India.

Remarks. The cucullus of this species is ovate, and the neck of valva has two or three short enlarged, flattened bristles.

Fibuloides elongata **(Zhang & Li, 2005) comb. n.**

Figs 5, 14

Eucoenogenes elongata Zhang & Li, 2005: 126. Holotype ♂, TL: China, deposited in NKUM.

Material examined. Holotype ♂, **China, Yunnan Province:** Weishan County, 2200 m, 20.VII.2001, coll. Houhun Li and Xinpu Wang, genitalia slide no. ZAH03725.

Distribution. China (Yunnan).

Remarks. This species can be easily distinguished from its congeners by the drooping uncus with a blunt apex, and the narrow valva with the neck bearing five long, flattened flagellate bristles on the left side and four on the right side.

Fibuloides japonica **(Kawabe, 1978)**

Figs 6, 15a–15b, 21

Eucoenogenes japonica Kawabe, 1978: 185; Kawabe 1982, 1: 120, 2: 173; Byun *et al.* 1998: 160; Razowski 1999: 446; Kuznetosv 2001: 402; Liu and Li 2002: 319. Holotype ♂, TL: Japan, deposited in USNM.

Fibuloides japonica (Kawabe, 1978): Horak 2006: 330.

Material examined. China, Shaanxi Province: 2 ♂♂, Ningshan County, 880 m, 17.VI.1987, coll. Houhun Li; 9 ♂♂, 3 ♀♀, Yangxian County, 600–680 m, 17–18. IV.1995, coll. Hongjian Wang. **Henan Province:** 3 ♂♂, Luoshan County, 350 m, 2.V.2000, coll. Haili Yu; 2 ♂♂, Xinyang City, 700 m, 11.VII.1997, coll. Houhun Li; 1 ♂, Tongbai County, 300 m, 11.IX.2001, coll. Houhun Li and Ole Karsholt; 2 ♂♂, Chishui County, Guizhou Province, 240 m, 21–22.IX.2000, coll. Haili Yu. **Guizhou Province:** 1 ♂, Xishui County, 500 m, 25.IX.2000, coll. Haili Yu; 5 ♂♂, 1 ♀, Daozhen County, 1300 m, 20.VIII.2004, coll. Yunli Xiao. **Zhejiang Province:** 1 ♂, Mt. Tianmu, 650 m, 20.VIII.1999, coll. Houhun Li. **Hunan Province:** 1 ♀, Zhangjiajie, 650 m, 7.VIII.2001, coll. Houhun Li and Xinpu Wang. **Fujian Province:** 1 ♂, Nanping, 850 m, 23.IX.2002, coll. Xinpu Wang; 1 ♂, Jianning County, 350 m, 25.IX.2002, coll. Xinpu Wang. **Hubei Province:** 1 ♂, Hefeng County, 1260 m, 16.VII.1999, coll. Houhun Li.

Distribution. China (Zhejiang, Anhui, Fujian, Henan, Hubei, Hunan, Sichuan, Guizhou, Shaanxi, Taiwan), Korea, Japan.

Remarks. This species is distinguished by the digitate process on the sacculus in the male genitalia bearing either dense tufted bristles or five enlarged, flattened bristles distally.

Discussion. In the examined specimens, the appearance of the adults and the female geniatlia are identical, but the male genitalia have two types (Figs 15a and 15b): in figure 15a, the relatively elongate uncus looks like a pair of long ears of a rabbit, and the slender digitate process of the sacculus bears five enlarged, flattened bristles distally; in figure 15b, the short uncus is emarginated posteriorly and somewhat heart-shaped, and the digitate process of the sacculus is relatively broad and bears dense tufted bristles distally. The two types of male genitlia might represent two different species, but in this paper we treat these differences as individual variations. We may confirm whether they are two species or just one species after a geographic analysis, which can be done when more specimens are available.

Fibuloides levatana **(Kuznetsov, 1997)**

Figs 7, 16

Eucoenogenes levatana Kuznetsov, 1997: 197. Holotype ♂, TL: Vietnam, deposited in ZMAS.

Fibuloides levatana (Kuznetsov, 1997): Horak 2006: 330.

Material examined. China, Zhejiang Province: 1 ♂, Mt. Tianmu, 350 m, 20.VIII.1999, coll. Houhun Li *et al.* **Fujian Province:** 1 ♂, Yongtai County, 550 m, 18.IX.2002, coll. Xinpu Wang.

Distribution. China (Zhejiang, Fujian), Vietnam.

Remarks. This species can be easily distinguished by its Y-shaped uncus and the slender distally attenuate cucullus. It is new for China.

Fibuloides modificana **Kuznetsov, 1997**

Fig. 8, 17

Fibuloides modificana Kuznetsov, 1997: 810. Holotype ♂, TL: Vietnam, deposited in ZMAS.

Material examined. China, Guangxi Zhuangzu Autonomous Region: 1 ♂, Leye County, 665 m, 24.VII.2004, coll. Jiasheng Xu.

Distribution. China (Guangxi), Vietnam.

Remarks. The uncus of this species is broad and distally bifurcate, the valva has a long, sinuate, flattened bristle on the neck, and the cucullus is elongate subrectangular and distally downcurved. In figure 17 the long flattened bristle is off the inserted hole which is located on the ventral side of the neck instead of on the angle of the sacculus (see arrow in fig. 17). It is new for China.

Fibuloides wuyiensis **(Zhang & Li, 2005) comb. n.**

Figs 9, 18

Eucoenogenes wuyiensis Zhang & Li, 2005: 127. Holotype ♂, TL: China, deposited in NKUM.

Material examined. Holotype ♂, **China**, **Fujian Province:** Mt. Wuyi, 1000 m, 26.V.2004, coll. Haili Yu, genitalia slide no. ZAH04215; Paratype: 1 ♂, same data as for holotype.

Distribution. China (Fujian).

Remarks. This species is distinguishable from its congeners by the following characters: the uncus tips are slender and closely parallel; the socius is laterally triangular; and the neck of the valva has a short flattened bristle.

Acknowledgements

The authors are grateful to Dr. N. Pinkaew, Department of Entomology, Kasetsart University, Thailand and Dr. Y. Nasu, Osaka Prefectural Agricultural, Forestry Research Center, Japan for providing us with references and good advice; to Dr. John W. Brown (Systematic Entomology Lab, National Museum of Natural History, Washington, DC) and Dr. Marianne Horak (CSIRO Ecosystem Sciences, Canberra) for reviewing the manuscript and giving valuable suggestions. The research was supported by the National Natural Science Foundation of China (No. J0930005) and Beijing Nova Program (No. 2008B23).

References

Baixeras J (2002) An overview of genus-level taxonomic problems surrounding *Argyroploce* Hübner (Lepidoptera: Tortricidae), with description of a new species. Annals of the Entomological Society of America 95(4): 422–431.

Baixeras J, Brown JW, Gilligan TM (2009) T@RTS: Online World Catalogue of the Tortricidae (Version 1.3.1). Available from http://www.tortricidae.com/catalogue.asp. [accessed 23 June 2010]

Brown JW (2005) Tortricidae (Lepidoptera). World Catalogue of Insects 5: 741 pp.

Brown RL, Powell J (1991) Description of a new species of *Epiblema* (Lepidoptera: Tortricidae: Olethreutinae) from coastal redwood forests in California with an analysis of the forewing pattern. Pan-Pacific Entomologist 67(2): 107–114.

Byun BK, Bae YS, Park KT (1998) Illustrated Catalogue of Tortricidae in Korea (Lepidoptera). Korea Research Institute of Bioscience and Biotechnology & Center for Insect Systematics: 96–317.

Clarke JFG (1958) Catalogue of the Type Specimens of Microlepidoptera in the British Museum (Natural History) described by Edward Meyrick. 3: 1–600, Trustees of the British Museum.

Diakonoff A (1950) The type specimens of certain oriental Eucosmidae and Carposinidae (Microlepidoptera) described by Edward Meyrick together with descriptions of new Eucosmidae and Carposinidae in the British Museum (Natural History). Bulletin of the British Museum (Natural History) (Entomology) 1: 275–300.

Horak M (2006) Olethreutine moths of Australia (Lepidoptera: Tortricidae). Monographs on Australian Lepidoptera 10: 1–522.

Kawabe A (1978) Descriptions of three new genera and fourteen new species of the subfamily Olethreutinae from Japan (Lepidoptera, Tortricidae). Tinea 10(19): 173–191.

Kawabe A (1982) Tortricidae. In: Inoue H, Sugi S, Kuroko H, Kawabe A (Eds) Moths of Japan 1: 1–955; 2: 1–552.

Kawabe A (1989) Records and descriptions of the subfamily Olethreutinae (Lepidoptera: Tortricidae) from Thailand. Microlepidoptera of Thailand 2: 23–82.

Kawabe A, Komai F and Razowski J (1992) Tortricoidea. In: Heppner JB, Inoue H (Eds) Lepidoptera of Taiwan 1(2) Checklist: 103–109.

Kuznetsov VI (1976) New species and subspecies of the leafrollers (Lepidoptera, Tortricidae) of the fauna of the Palaearctic. Trudy Zoologicheskogo Instituta, Leningrad 64: 3–33.

Kuznetsov VI (1988) New and little-known leaf-rollers of the subfamily Olethreutinae (Lepidoptera, Tortricidae) of the fauna of North Vietnam. Trudy Zoologicheskogo Instituta, Leningrad 176: 72–97.

Kuznetsov VI (1997a) Little known and new species of tortricid moths (Lepidoptera, Totricidae) of the fauna of Vietnam. Entomologicheskoe Obozrenie 76(1): 186–202, 236.

Kuznetsov VI (1997b) New species of the subfamily Olethreutinae (Lepidoptera, Tortricidae) from the south of Vietnam. Entomologicheskoe Obozrenie 76(4): 797–812, 953.

Kuznetsov VI (2001) Tortricoidea. In: Ler PA (Ed) Key to the insects of Russian Far East. Vol. V. Trichoptera and Lepidoptera. Pt. 3. Vladivostok Dal'nauka, 621 pp.

Li HH (2002) The Gelechiidae of China (I) (Lepidoptera: Gelechioidea). 538 pp. Nankai University Press, Tianjin.

Liu YQ, Li GW (2002) Fauna Sinica, Insecta, Vol. 27, Lepidoptera, Tortricidae. 463 pp. Chinese Science Press, Beijing.

Meyrick E (1912) Descriptions of Indian Microlepidoptera. Journal of the Bombay Natural History Society 21: 852–877.

Meyrick E (1936) Exotic Microlepidoptera 4: 609–642.

Meyrick E (1937) Exotic Microlepidoptera 5: 65–160.

Nasu Y (1993) New record of *Acroclita nigrovenana* (Lepidoptera, Tortricidae) from Japan. Japanese Journal of Entomology 61(2): 216.

Pinkaew N (2008) A new species and two new combinations in the genus *Fibuloides* Kuznetsov (Lepidoptera: Tortricidae) from Thailand. Zootaxa 1688, 61–65.

Pinkaew N, Chandraptya A, Brown RL (2005) Two new species and a new record of *Eucoenogenes* (Meyrick) (Lepidoptera: Tortricidae) from Thailand with a discussion of characters defining the genus. Proceedings of the Entomological Society of Washington 107(4): 869–882.

Razowski J (1989) The genera of Tortricidae (Lepidoptera). Part II: Palaearctic Olethreutinae. Acta Zoologica Cracoviensia 32(7): 107–328.

Razowski J (1999) Catalogue of the species of Tortricidae. Part V: Palaearctic Eucosmina and Enarmoniina (Insecta: Lepidoptera). SHILAP Revista de lepidopterologia 27(108): 437–506.

Rose HS, Pooni HS (2005) Taxonomic studies on the family Tortricidae (Tortricoidea: Lepidoptera) from Northwestern India–Tribe Eucosmini (Olethreutinae). Zoos' Print Journal 20(2): 1751–1765.

Zhang AH, Li HH (2005) A Study on the Genus *Eucoenogenes* from China (Lepidoptera: Tortricidae: Olethreutinae). Entomotaxonomia 27(2): 125–130.

ZooKeys 81: 51–71 (2011)
doi: 10.3897/zookeys.81.1111
www.zookeys.org

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

A new *Gephyromantis* (*Phylacomantis*) frog species from the pinnacle karst of Bemaraha, western Madagascar

Angelica Crottini[1,2,†], Frank Glaw[3,‡], Maurizio Casiraghi[4,§], Richard K.B. Jenkins[5,6,|], Vincenzo Mercurio[7,¶], J. Christian Randrianantoandro[5,#], Jasmin E. Randrianirina[8,††], Franco Andreone[9,‡‡]

1 *Division of Evolutionary Biology, Zoological Institute, Technical University of Braunschweig, Spielmannstr. 8, 38106, Braunschweig, Germany* **2** *Sezione di Zoologia e Citologia, Dipartimento di Biologia, Universita´ degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy* **3** *Zoologische Staatssammlung München, Münchhausenstraße 21, 81247 München, Germany* **4** *ZooPlantLab, Universitá degli Studi di Milano-Bicocca, Dipartimento di Biotecnologie e Bioscienze, Piazza della Scienza 2, 20126 Milano, Italy* **5** *Madagasikara Voakajy, BP 5181, Antananarivo 101, Madagascar* **6** *Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent, Canterbury, CT2 7NR, UK* **7** *Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung an der Humboldt-Universität zu Berlin, Invalidenstraße 43, D-10115 Berlin, Germany* **8** *Parc Botanique et Zoologique de Tsimbazaza, BP 4096, Antananarivo 101, Madagascar* **9** *Museo Regionale di Scienze Naturali, Via G. Giolitti 36, 10123 Torino, Italy*

† *urn:lsid:zoobank.org:author:83EAA6D8-7A0D-438A-B364-400DA13AB2E7*
‡ *urn:lsid:zoobank.org:author:764DC666-B65D-40C1-BE19-0F5A7294E452*
§ *urn:lsid:zoobank.org:author:2F4BFDF6-70D8-4379-ACAA-F8E0351C43D0*
| *urn:lsid:zoobank.org:author:FE413BBC-EDF8-4184-BF90-E164961087C2*
¶ *urn:lsid:zoobank.org:author:63555F61-BCE9-435E-95CA-18B03A10FC85*
# *urn:lsid:zoobank.org:author:0A7D813A-8E77-405E-866E-D6BF67EDDF1C*
†† *urn:lsid:zoobank.org:author:964423BF-E878-469F-A560-88ED01A89EC5*
‡‡ *urn:lsid:zoobank.org:author:CB6E4DE1-2069-4DDE-92EC-F3037669A7D1*

Corresponding author: *Franco Andreone* (franco.andreone@regione.piemonte.it)

Academic editor: *Natalia Ananjeva* | Received 25 August 2010 | Accepted 17 January 2011 | Published 18 February 2011

urn:lsid:zoobank.org:pub:067DDF53-DB9E-4B69-AFD3-EA87B3A45800

Citation: Crottini A, Glaw F, Casiraghi M, Jenkins RKB, Mercurio V, Randrianantoandro JC, Randrianirina JE, Andreone F (2011) A new *Gephyromantis* (*Phylacomantis*) frog species from the pinnacle karst of Bemaraha, western Madagascar. ZooKeys 81: 51–71. doi: 10.3897/zookeys.81.1111

Abstract
We describe a new mantellid frog of the subfamily Mantellinae from the karstic Bemaraha Plateau, western Madagascar. The new species belongs to the genus *Gephyromantis,* subgenus *Phylacomantis*, which previously included *G. azzurrae, G. corvus* and *G. pseudoasper. Gephyromantis atsingy* **sp. n.** has a snout-vent length of 35–43 mm and is a scansorial frog living among the Tsingy de Bemaraha pinnacles and inside the caves present in the area. A morphological analysis and biomolecular comparison revealed the degree of differentiation between these four species of the *Phylacomantis* subgenus. The new species seems to be endemic to Tsingy de Bemaraha.

Keywords
Amphibia, *Gephyromantis atsingy* sp. n., Madagascar, Tsingy de Bemaraha

Introduction

The intense herpetological activity carried out in Madagascar during the last decades, together with the wider use of integrative taxonomic tools has led to the description of an astonishingly high number of new amphibians species (Köhler et al. 2005, Vences et al. 2008, Glaw et al. 2010) and to the identification of numerous still undescribed candidate species (Vieites et al. 2009).

Although, the highest species richness of amphibians is typically found along the eastern rainforest belt (Andreone et al. 2005, Glaw and Vences 2007), an increasing number of peculiar species are known from the arid western part of Madagascar (Glaw et al. 1998, 2006, Glos et al. 2005, Mercurio and Andreone 2007, Bora et al. 2010). At these sites the research effort has been gradually increased in recent years, and systematic surveys have recently taken place (e.g. Mercurio et al. 2008, Raselimanana 2008, Bora et al. 2010).

While the species already described from the arid West mostly belong to radiations of explosive breeders reproducing in ephemeral ponds, a special attention has been given to species ascribed to clades that are more typical of humid habitats and rainforest biomes. This was the case, for example, with the recent discovery of two new mantellines at the Isalo Massif (Mercurio and Andreone 2007), the peculiar *Tsingymantis antitra* at Ankarana (Glaw et al. 2006), some *Boophis* and some cophyline microhylids (Köhler et al. 2007, Glaw et al. 2007) in the huge karstic massif of Tsingy de Bemaraha, and four large-bodied cave-dwelling species of *Stumpffia* from karstic regions in the North (Köhler et al. 2010).

During recent herpetofaunal inventories we discovered a further new species of a rather inconspicuous *Gephyromantis* frog inhabiting the deciduous forest of the karstic Bemaraha Plateau.

Thirty-six described species are currently ascribed to *Gephyromantis* that is currently divided in five subgenera, including *Phylacomantis.* Four species are currently ascribed to this subgenus: *G. corvus* Glaw & Vences, *G. pseudoasper* Guibé, *G. azzurrae* Mercurio & Andreone and the new species described in the present paper. With the exception of *G. pseudoasper*, that mostly inhabits the rainforests of the North, the other species are found only in xeric habitats in the south-western (*G. corvus* and *G. azzurrae*) and western Madagascar (the new species described herein) (Fig. 1).

Due to morphological and external similarities, the new frog was formerly believed to be related to *G. corvus*, a frog endemic of the Isalo Massif.

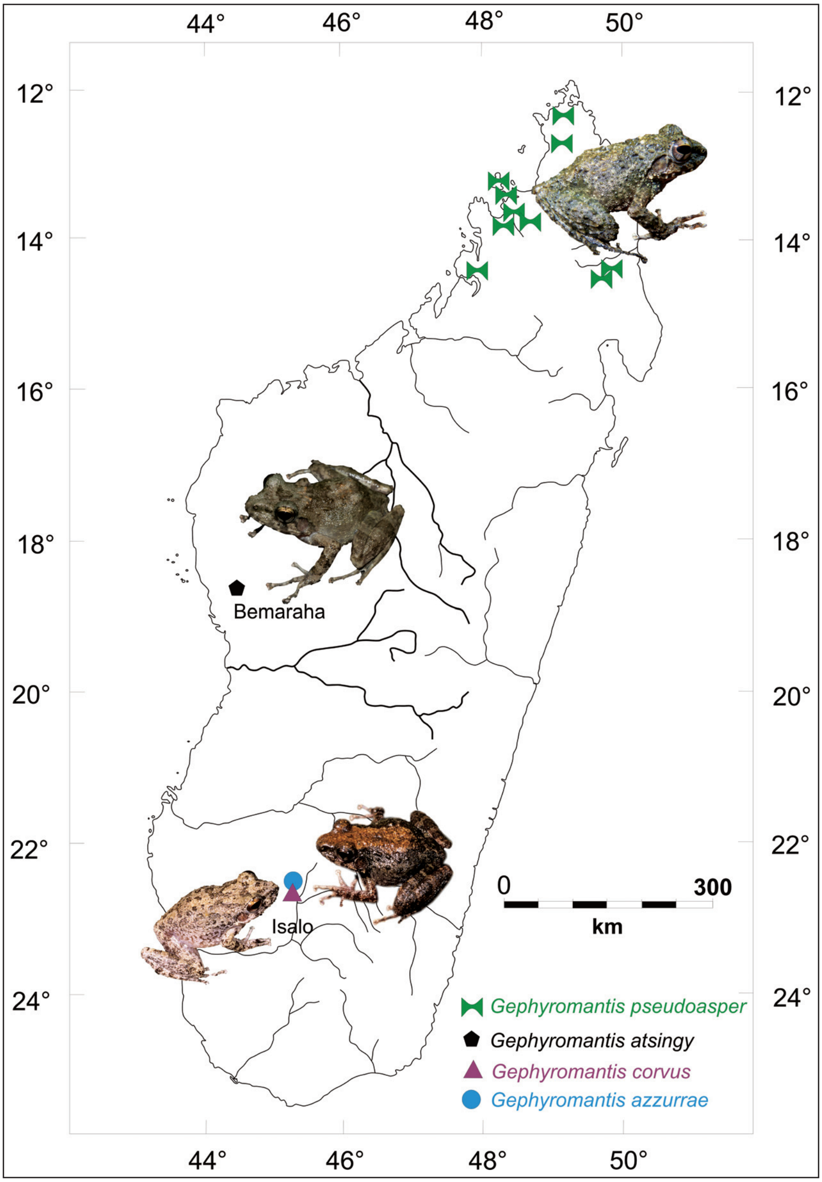


Figure 1. Schematic map of Madagascar with images and distribution of the four described species of the genus *Gephyromantis*, subgenus *Phylacomantis*.

Unfortunately, the secretive life of this new species prevented us from obtaining much biological informations and we still lack information about its acoustic repertoire, breeding behaviour and larval morphology. Notwithstanding these challenges, the ongoing collaborative effort generated by the ACSAM (A Conservation Strategy for the Amphibians of Madagascar, Andreone and Randriamahazo 2008) allowed us to integrate the data and photographs obtained by three independent survey teams.

We present here the formal description of this new *Gephyromantis* species of the subgenus *Phylacomantis*, which differs from the other *Phylacomantis* species by a combination of morphological traits colouration and by a high divergence in mitochondrial DNA sequences.

Methods

Study site

The Tsingy de Bemaraha is a karstic plateau in the Melaky Region, five to 15 km wide and about 100 km long, located in western Madagascar. Numerous long, sharp pinnacles of rock, that may reach 45 meters in height, outcrop along the plateau and form the characteristic landscape (the so-called "tsingy" or "atsingy" in Malagasy language). Dry, deciduous forest is the most common vegetation type but humid areas occur within some of the larger canyons. An extensive area of forest and rock outcrop is included within two adjacent protected areas (Parc National Tsingy de Bemaraha and Réserve Naturelle Intégrale du Tsingy de Bemaraha). Savanna grasslands surround the plateau and there are numerous marshy depressions, caves and gorges associated with the main outcrop. This area has been object of some herpetological surveys that led to the discovery and description of several new species of amphibians (Vences et al. 2000, Glos et al. 2005, Köhler et al. 2007, Glaw et al. 2007, Andreone and Randrianirina 2008) and reptiles (Schimmenti and Jesu 1996, Nussbaum and Raxworthy 2000, Glaw et al. 2009a, b).

Sampling methods

We searched for frogs at night with the aid of hand torches and headlamps. Geographic coordinates were taken using a GPS device. Toponyms often follow the indications by local people, and must be therefore seen as largely unofficial names. Frogs were collected by hand and euthanised by immersion in chlorobutanol solution, fixed in 5% formalin or in 90% ethanol and finally stored in 75% ethanol solution. Voucher specimens (Tab. 1) are currently housed at the Museo Regionale di Scienze Naturali di Torino (MRSN), Zoologische Staatssammlung München (ZSM), and Université d'Antananarivo, Département de Biologie Animale (UADBA). Original field numbers are FN and FAZC (Franco Andreone Zoological Collection), FGZC (Frank Glaw Zoological Collection), BMR (Jasmin E. Randrianirina), and RBJ (Richard K. B. Jenkins). A few individuals do not bear any field number (no field number = NFN). The specimens of the type series were compared with the specimens of the other known species of the *Phylacomantis* subgenus (Tab. 1): six specimens of *G. azzurrae* from Isalo, five specimens of *G. corvus* from Isalo, and three specimens of *G. pseudoasper* from Nosy Be (see Tab. 1). The comparative specimens of *G. pseudoasper* were most probably temporary stored in denaturating solutions, and the sequencing was therefore not successful. For this reason, the three specimens here analyzed were only compared morphologically (Tab. 1), and the required sequences were retrieved from GenBank (DQ987513, DQ987515, DQ987517, DQ987518; DQ926890; AY848422-AY848424). Morphological information on *G. azzurrae* specimens were taken from Mercurio and Andreone (2007) and sequences were retrieved from GenBank (EF222300- EF222305).

Morphological measurements

Morphological measurements were made with a digital calliper to the nearest 0.1 mm. The following biometric measurements were taken (according to Mercurio and Andreone 2007): SVL (snout-vent length), HW (head width), HL (head length), ED (horizontal eye diameter), END (eye-nostril distance), NSD (nostril-snout tip distance), NND (nostril-nostril distance), TD (horizontal tympanum diameter), HAL (hand length), FORL (forelimb length), HIL (hind-limb length), FOL (foot length), FOTL (foot length including tarsus), IMTL (length of inner metatarsal tubercle), IMTH (height of inner metatarsal tubercle), FGL (length of the femoral macrogland cluster), FGW (width of femoral macrogland cluster). Webbing formulae follow Blommers-Schlösser and Blanc (1991), and femoral glands definition follows Glaw et al. (2000). For a few individuals we also counted the number (NG) and mean diameter (GD) of granules composing the right femoral gland. Granules were counted after having opened and flipped the gland.

DNA analysis

A fingertip, or part of the muscle of the tongue, was cut from each collected individual and stored in 99% ethanol. Total genomic DNA was extracted from the tissue samples using proteinase K digestion (10 mg/ml concentration) following Bandi et al. (1994) protocol. To sequence a fragment of ca. 550bp of the mitochondrial 16S rRNA gene, which has proven to be suitable in anuran species identification (Vences et al. 2005) we used the primers 16SA-L 5'-CGCCTGTTTATCAAAAACAT-3' and 16SB-H 5'-CCGGTCTGAACTCAGATCACGT-3', modified from Kocher et al. (1989) and Palumbi et al. (1991). PCR reactions were performed using standard cycling protocols (Vences et al. 2003) and the light strands were sequenced using an ABI3730XL by Macrogen Inc. Sequences were blasted in GenBank, checked by eye, edited, aligned using the BioEdit sequence alignment editor (version 7.0.5.3; Hall 1999). The alignment of all the processed samples required the inclusion of gaps to account for indels in only a few cases in one hypervariable region. All newly determined sequences have been deposited in GenBank (HQ640413-HQ640426). Mean genetic distances matrix (uncorrected *p*-distance transformed into percent) between and within individuals belonging to the type series of *G. atsingy* (holotype and 7 paratypes) and of other species of the subgenus *Phylacomantis* (*Gephyromantis corvus, G. pseudoasper* and *G. azzurrae*) were computed.

Results

***Gephyromantis* (*Phylacomantis*) *atsingy* sp. n.**

urn:lsid:zoobank.org:act:94995B02-B47C-4275-A6BA-DD4134B51203
Figures 2, A–L

Etymology. The specific noun "*atsingy*" (pronounced: "a-tseen-jě") is a Malagasy word. The terms "atsingy" or "tsingy" are the common names used to refer to the pointed and sharp calcareous lime stone formations and pinnacles originated through rainfall erosion. Although present in several other localities in western Madagascar (e.g.: Ankarana), the outcrops of Bemaraha are typical of this area and the specific name is therefore associated with the locality of provenience of the types.

Remark. This species has been referred to as *Gephyromantis* sp. aff. *corvus* "Bemaraha" by Glaw and Vences (2007), as *Gephyromantis* sp. 10 "Bemaraha" by Vieites et al. (2009), and *Gephyromantis* sp. aff. *corvus* by Bora et al. (2010).

Holotype. MRSN A5487 (NFN), subadult male, collected at Tsingy de Bemahara National Park, western Madagascar, Andamozavaky (Bekopaka commune, Antsalova district, Melaky region, Mahajanga province), 19°01.86'S, 44°46.80'E; 122 m a.s.l., collected by J. E. Randrianirina on 23 May 2003.

Paratypes. MRSN A5486 (BMR 001), subadult male without evident femoral glands, MRSN A5484 (NFN), adult female, MRSN A5482 (BMR 008), MRSN A5483 (BMR 031), MRSN A5485 (BMR 002), three juveniles (sex unknown) sampled from the same locality, collector and date of the holotype (tissue sample taken for genetical analysis for all individuals); ZSM 23/2006 (FGZC 0715), adult female, from Grotte Crystal, close to Andranopasazy, Tsingy de Bemaraha National Park (Antsalova commune, Antsalova district, Melaky region, Mahajanga province), 18°42'31"S, 44°43'08" E, 146 m a.s.l., collected by F. Glaw, J. Köhler, P. Bora and H. Enting on 19 March 2006, fixed in ethanol (tissue sample taken for genetical analysis), individual found at night on limestone cliffs, close to the entrance of the cave; ZSM 37/2006 (FGZC 0746), juvenile (unknown sex) from Grotte Anjohimbazimba, Tsingy de Bemaraha National Park (Antsalova commune, Antsalova district, Melaky region, Maha-

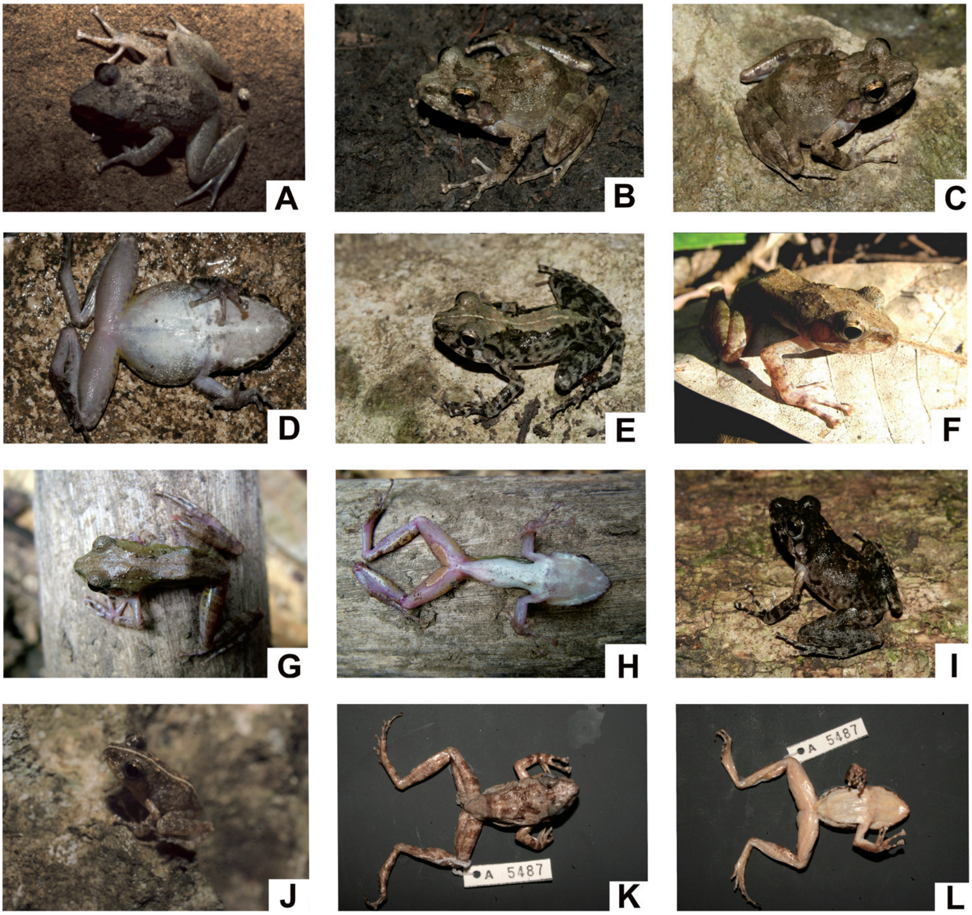


Figure 2. Images of *Gephyromantis atsingy* sp. n. **A** MRSN A5487 (NFN), subadult male (holotype) from Andamozavaky, dorsal view (photo by J. E. Randrianirina) **B–C** ZSM 23/2006 (FGZC 0715), adult female (paratype) from Grotte Crystal, close to Andranopasazy, dorsolateral views (photos by F. Glaw) **D** ZSM 23/2006 (FGZC 0715), adult female (paratype) from Grotte Crystal, close to Andranopasazy, ventral view (photo by F. Glaw) **E** ZSM 37/2006 (FGZC 0746), juvenile (paratype) from Grotte Anjohimbazimba (photo by F. Glaw) **F–G** UADBA 39099 (RBJ 627), adult male (paratype) from Andranopasazy, dorsolateral and dorsal views (photos by C. Randrianantoandro) **H** UADBA 39099 (RBJ 627), adult male (paratype) from Andranopasazy, ventral view, with evident and developed femoral glands of "Type 2" (photo by C. Randrianantoandro) **I** ZSM 107/2006 (FGZC 0886), juvenile (paratype) from Bendrao Forest ("Camp 3"), dorsolateral view (photo by F. Glaw) **J** MRSN A5483 (BMR 031), juvenile (paratype) from Andamozavaky, dorsolateral view (photo by J. E. Randrianirina) **K–L** MRSN A5487 (NFN), subadult male (holotype) from Andamozavaky, dorsal and ventral views of the preserved specimen.

janga province), 18°41'34"S, 44°42'36"E, 160 m a.s.l., collected by F. Glaw, J. Köhler, P. Bora and H. Enting on 20 March 2006 (tissue sample taken for genetical analysis), individual found in the cave; ZSM 107/2006 (FGZC 0886), juvenile (sex unknown) from Bendrao Forest ("Camp 3"), Tsingy de Bemaraha National Park (Antsa-

Table 1. Morphometric measurements (in mm) of specimens of *Gephyromantis atsingy*, *G. corvus*, *G. azzurrae* and *G. pseudoasper*. **HT** (holotype), **PT** (paratype). **M** (male). **F** (female). **J** (juvenile), **SMF** (Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt, Germany). Other abbreviations are given in the text.

Catalogue number	Field number	Species	Locality	GenBank	Rank	SEX	SVL	HW	HL
MRSN A5487	NFN	*G. atsingy*	Andamozavaky	HQ640419	HT	M	34.8	14.1	14.5
MRSN A5486	BMR 001	*G. atsingy*	Andamozavaky	HQ640421	PT	M	31.3	12.4	13.5
MRSN A5484	NFN	*G. atsingy*	Andamozavaky	HQ640418	PT	F	43.4	15.9	17.3
ZSM 23/2006	FGZC 0715	*G. atsingy*	Grotte Crystal, close to Andranopasazy	HQ640414	PT	F	38.5	14.3	15.2
MRSN A5482	BMR 008	*G. atsingy*	Andamozavaky	HQ640420	PT	J	22.0	8.4	9.6
MRSN A5483	BMR 031	*G. atsingy*	Andamozavaky	HQ640417	PT	J	19.6	7.3	8.3
MRSN A5485	BMR 002	*G. atsingy*	Andamozavaky	HQ640415	PT	J	23.6	8.9	10.5
ZSM 107/2006	FGZC 0886	*G. atsingy*	Bendrao Forest	HQ640416	PT	J	22.5	7.8	9.5
ZSM 37/2006	FGZC 0746	*G. atsingy*	Grotte Anjohimbazimba	-	PT	J	24.6	8.2	10.1
UADBA 39099	RBJ 627	*G. atsingy*	Andranopasazy	HQ640413	PT	M	36.6	11.2	18.6
UADBA 39081	RBJ 609	*G. atsingy*	Andranopasazy	-	PT	F	38.4	14.8	16.8
UADBA 28120	RBJ 791	*G. atsingy*	Ankilogoa	-	PT	F	35.9	11.1	17.1
UADBA 39100	RBJ 658	*G. atsingy*	Anjaha	-	PT	F	35.1	12.1	17.8
UADBA 28127	RBJ 718	*G. atsingy*	Ranotsara	-	PT	F	39.1	12.7	17.9
UADBA 39082	RBJ 630	*G. atsingy*	Andranopasazy	-	PT	F	38.1	12.6	16.9
UADBA 28112	RBJ 708	*G. atsingy*	Ranotsara	-	PT	F	38.4	11.8	16.8
UADBA 39057	RBJ 660	*G. atsingy*	Anjaha	-	PT	F	35.3	11.4	17.4
UADBA 28116	RBJ 792	*G. atsingy*	Ankilogoa	-	PT	F	33.9	11.0	16.3
MRSN A5373	FAZC 12859	*G. corvus*	Isalo, Tsiombivositra	HQ640423	-	M	39.8	16.1	15.3
MRSN A5325	FAZC 13000	*G. corvus*	Isalo, Ambovo	-	-	F	40.0	15.2	16.2
MRSN A5323	FAZC 12661	*G. corvus*	Isalo, Malaso	HQ640422	-	F	39.0	15.0	15.7

Catalogue number	Field number	Species	Locality	GenBank	Rank	SEX	SVL	HW	HL
MRSN A5324	FAZC 12758	*G. corvus*	Isalo, Zahavola	HQ640424	-	F	40.1	15.0	16.3
MRSN A2786	FAZC 11964	*G. corvus*	Isalo, Andranomena	HQ640425	-	F	40.8	15.0	15.8
MRSN A5310	FAZC 12568	*G. azzurrae*	Isalo, Andriamanero	EF222301	HT	M	41.1	16.9	13.4
MRSN A5309	FAZC 12567	*G. azzurrae*	Isalo, Andriamanero	EF222300	PT	M	38.5	15.3	12.8
MRSN A5311	FAZC 12569	*G. azzurrae*	Isalo, Andriamanero	EF222302	PT	M	40.2	15.8	14.1
MRSN A5312	FAZC 12910	*G. azzurrae*	Isalo, Iambahatsy	EF222304	PT	M	23.3	8.8	8.8
SMF 85859	NFN	*G. azzurrae*	Isalo, Sakamalio	EF222305	PT	M	42.7	16.4	14.3
SMF 85860	NFN	*G. azzurrae*	Isalo, Sakamalio	EF222303	PT	M	43.7	16.4	13.5
MRSN A3415	NFN	*G. pseudoasper*	Nosy Be	-	-	M	33.3	12.1	13
MRSN A3416	FN 6696	*G. pseudoasper*	Nosy Be	-	-	M	37.4	12.9	15
MRSN A3417	FN 6423	*G. pseudoasper*	Nosy Be	-	-	F	33.1	11.9	14

Table 1. (Continued)

Catalogue number	ED	END	NSD	NND	TD	HAL	HIL	FORL	FOTL	FOL	IMTL	IMTH	FGL	FGW	NG	GD
MRSN A5487	5.7	3.8	2.6	3.6	3.3	11.6	37.5	18.6	24.8	17.7	1.1	1.1	-	-	5	0.7
MRSN A5486	5.3	3.9	1.9	3.1	2.8	10.6	33.1	16.2	23.6	15.5	1.7	1.2	-	-	-	-
MRSN A5484	6.4	4.5	3.1	3.9	3.6	13.3	43.2	20.1	30.9	20.4	2.2	1.0	-	-	-	-
ZSM 23/2006	6.1	4.3	2.2	3.6	2.7	11.7	39.9	18.0	26.0	16.7	1.6	0.9	-	-	-	-
MRSN A5482	3.8	2.6	1.6	2.5	2.2	7.4	23.7	11.1	17.1	9.9	1.1	0.6	-	-	-	-
MRSN A5483	3.5	2.2	1.1	2.1	1.7	6.3	17.9	9.4	13.3	8.1	0.4	0.2	-	-	-	-
MRSN A5485	4.1	2.9	1.4	2.6	2.3	8.1	25.5	13.2	18.4	11.6	0.6	0.2	-	-	-	-
ZSM 107/2006	3.1	2.4	0.9	2.1	1.8	6.5	22.4	10.9	16.5	10.0	1.0	0.3	-	-	-	-
ZSM 37/2006	3.2	2.7	1.5	2.3	1.9	6.9	24.2	11.1	17.1	10.8	1.1	0.5	-	-	-	-
UADBA 39099	4.2	3.6	1.9	2.9	3.4	11.5	62.1	16.4	25.5	17.5	1.1	0.8	7.5	3.1	70	0.5
UADBA 39081	4.3	3.6	1.9	3.4	3.3	11.9	63.5	17.4	27.3	19.3	1.4	0.8	-	-	-	-
UADBA 28120	4.5	3.3	1.6	2.6	3.2	11.1	64.0	16.9	27.1	18.1	1.1	0.5	-	-	-	-
UADBA 39100	4.4	3.9	1.7	2.7	3.3	11.0	62.2	17.3	26.8	17.3	1.3	0.7	-	-	-	-
UADBA 28127	4.6	3.7	1.6	3.2	3.3	12.1	64.0	18.4	28.4	18.7	1.4	0.6	-	-	-	-
UADBA 39082	3.9	4.1	1.6	2.9	2.9	11.6	63.9	17.8	27.4	17.6	1.5	0.5	-	-	-	-
UADBA 28112	4.2	4.0	1.1	2.5	3.0	11.4	61.9	18	26.3	16.4	1.2	0.5	-	-	-	-
UADBA 39057	4.3	3.6	1.4	2.9	2.8	11.6	63.9	16.6	27.5	18.4	0.9	0.5	-	-	-	-
UADBA 28116	3.4	3.7	1.2	2.2	2.5	11.0	58.6	16.4	25.0	15.9	1.0	0.5	-	-	-	-
MRSN A5373	5.8	4.8	2.9	4.3	3.7	11.8	38.7	16.3	28.6	19.6	2.4	1.4	9.1	4.1	96	0.6
MRSN A5325	6.4	4.3	2.6	4.1	3.2	10.7	40.3	18.2	30.1	20.0	1.6	0.6	-	-	-	-
MRSN A5323	6.1	4.4	2.8	4.2	3.6	11.9	39.3	17.6	29.7	20.3	1.6	0.8	-	-	-	-

Catalogue number	**ED**	**END**	**NSD**	**NND**	**TD**	**HAL**	**HIL**	**FORL**	**FOTL**	**FOL**	**IMTL**	**IMTH**	**FGL**	**FGW**	**NG**	**GD**
MRSN A5324	6.1	4.4	2.8	4.2	3.3	10.8	37.2	16.9	28.0	19.8	1.3	0.7	-	-	-	-
MRSN A2786	6.3	4.7	2.7	4.3	3.6	11.1	40.5	17.7	30.2	20.4	1.9	0.7	-	-	-	-
MRSN A5310	6.1	3.9	2.5	4.0	4.0	12.1	41.1	20.0	30.0	18.8	2.0	1.1	6.3	2.0	45	0.5
MRSN A5309	5.2	4.3	2.2	3.7	3.7	11.1	41.1	19.9	26.7	18.9	1.9	1.3	6.5	2.7	38	0.5
MRSN A5311	6.0	4.0	2.7	4.0	4.1	11.2	41.0	19.9	27.7	19.9	2.1	1.1	6.7	2.7	40	0.6
MRSN A5312	4.1	2.8	1.4	2.2	2.5	8.8	24.5	11.1	17.7	12.1	1.1	0.5	_	_	_	_
SMF 85859	5.4	3.7	2.6	3.9	3.5	13.4	41.1	21.0	29.9	20.0	1.9	1.1	7.0	3.0	42	0.6
SMF 85860	5.7	4.0	2.5	3.8	4.0	12.2	42.3	21.1	27.7	20.0	1.9	1.0	7.5	2.7	42	0.5
MRSN A3415	4.9	3.8	2.6	2.8	3.1	10.4	52.4	15.7	23	17.5	2.1	1.1	6.1	2.5	43	0.3
MRSN A3416	4.9	4.4	3	3.8	4.4	10.3	54.2	16.4	25	15.8	2.4	1.1	7.2	2.9	39	0.4
MRSN A3417	5.3	3.7	2.7	2.9	3	9.9	56.5	16.5	24	18.2	1.9	1	-	-	-	-

lova commune, Antsalova district, Melaky region, Mahajanga province), 18°47'04"S, 44°51'37"E, 427 m a.s.l., collected by F. Glaw, J. Köhler, P. Bora and H. Enting on 26–27 March 2006; (tissue sample taken for genetical analysis). All these specimens were fixed in 90% ethanol and preserved in 70% ethanol. UADBA 28112 (RBJ 708), female from Ranotsara (Bekopaka commune Antsalova district, Melaky region, Mahajanga province), 19°02'08"S, 44°46'29"E, 65 m a.s.l., collected by R. Andriantsimanarilafy on 18 November 2006; UADBA 28116 (RBJ 792), female from Ankilogoa (Bekopaka commune, Antsalova district, Melaky region, Mahajanga province), 19°07'52"S, 44°48'32"E, 57 m a.s.l., collected by R. Randrianavelona on 13 December 2006; UADBA 28120 (RBJ 791), female from Ankilogoa (Bekopaka commune, Antsalova district, Melaky region, Mahajanga province), 19°07'52"S, 44°48'32"E, 57 m a.s.l., collected by R. Randrianavelona on 13 December 2006; UADBA 28127 (RBJ 718), female from Ranotsara (Bekopaka commune, Antsalova district, Melaky region, Mahajanga province), 19°02'08"S, 44°46'29"E, 65 m a.s.l., collected by R. Randrianavelona on 19 November 2006; UADBA 39057 (RBJ 660), female from Anjaha (Antsalova commune, Antsalova district, Melaky region, Mahajanga province), 18°39'43"S, 44°49'33"E, 403 m a.s.l., collected by J.C. Randrianantoandro, R. Randrianavelona, R.K.B. Jenkins, R.R. Andriantsimanarilafy and E.F. Hantalalaina and Madagascar National Parks personnel on 15–24 February 2006; UADBA 39081 (RBJ 609), female from Andranopasazy (Melaky region, Mahajanga province), 18°42'31"S, 44°43'02"E, 146 m a.s.l. collected by J.C. Randrianantoandro, R. Randrianavelona, R.K.B. Jenkins, R.R. Andriantsimanarilafy and E.F. Hantalalaina and Madagascar National Parks personnel on 13–30 January 2006; UADBA 39082 (RBJ 630), female from Andranopasazy (Antsalova commune, Antsalova district, Melaky region, Mahajanga province), 18°42'31"S, 44°43'02"E, 146 m a.s.l. collected by J.C. Randrianantoandro, R. Randrianavelona, R.K.B. Jenkins, R.R. Andriantsimanarilafy and E.F. Hantalalaina and Madagascar National Parks personnel on 13–30 January 2006; UADBA 39099 (RBJ 627), adult male (with developed glands) from Andranopasazy (Antsalova commune, Antsalova district, Melaky region, Mahajanga province), 18°42'31”S, 44°43'02”E, 146 m a.s.l. collected by J.C. Randrianantoandro, R. Randrianavelona, R.K.B. Jenkins, R.R. Andriantsimanarilafy and E.F. Hantalalaina and Madagascar National Parks personnel on 13–30 January 2006; UADBA 39100 (RBJ 658), female from Anjaha (Antsalova commune, Antsalova district, Melaky region, Mahajanga province), 18°39'43"S, 44°49'33"E, 403 m a.s.l., collected by J.C. Randrianantoandro, R. Randrianavelona, R.K.B. Jenkins, R.R. Andriantsimanarilafy and E.F. Hantalalaina and Madagascar National Parks personnel on 15–24 February 2006.

Diagnosis. A medium sized frog species (adult SVL 35–43 mm), assigned to the genus *Gephyromantis* (sensu Glaw and Vences 2006), subgenus *Phylacomantis*, according to genetic, phenetic and morphological similarities to the other known species (*G. azzurrae, G. corvus,* and *G. pseudoasper*), and recognizable by the presence of the following characters: (a) femoral glands of “Type 2” (sensu Glaw et al. 2000), (b) webbing between toes present, (c) inner and outer metatarsal tubercles present, (d) tongue bifid, (e) lateral metatarsalia partly connected, (f) enlarged triangular finger tips, (g) not evident paired subgular vocal sacs, (h) crepuscular/nocturnal activity, (i) occurrence in limestone caves and deciduous forest habitat of dry western Madagascar.

Description of the holotype. Subadult male in mediocre state of preservation, with the belly opened for gonadal inspection and part of the ventral surface of thighs cut and opened to check the glands. SVL 34.8 mm; for other measurements see Tab. 1. Body slender; head longer than wide, in line with the body; snout slightly pointed in dorsal view, rather rounded in lateral view; nostrils directed laterally, much nearer to tip of snout than to eye; canthus rostralis well defined; tympanum distinct, rounded, its horizontal diameter about 50% of eye diameter; supratympanic fold well distinct, regularly curved; tongue distinctly bifid posteriorly. Arms slender; subarticular tubercles single; outer and inner metacarpal tubercles paired; fingers without webbing; finger disks triangular distinctly enlarged; nuptial pads absent. Hind limbs slender; tibiotarsal articulation reaching the nostril when hindlimbs are adpressed along body; lateral metatarsalia partly connected; inner metatarsal tubercle distinct, outer metatarsal tubercle small but recognizable; webbing of foot 1(1), 2i(1), 2e(1), 3i(2), 3e(1), 4i(2), 4e(2), 5(1). Skin slightly granular on dorsum and belly, ventral skin smooth on throat and chest. Femoral glands cluster ("Type 2", according to Glaw et al. 2000) hardly recognizable from external view, but with an overall granular structure and with 4–6 single whitish granular glands of ca. 1 mm diameter scattered on thighs. The vocal sacs in the male holotype are indistinct. The live colouration, based upon the photograph taken by J.E. Randrianirina is light brownish with darker dots and marbling (Fig. 2; A). The finger and toe tips are lighter than the remnant parts of fore- and hindlegs. After about seven years of preservation in ethanol the holotype still conserves the original marbled-brownish colour patterns, although it showed a slight loss of colour (Fig. 2; K–L). In particular, the belly became much whitish and inconspicuous. A rather characteristic and darker X-shaped marking is visible on the shoulder region, as well as a diffuse marbling darker pattern on the back and head. The tympanum is whitish. Limbs are brownish, with dark brown cross-bands: 3 on femur, 3 on tibia, 5–6 on tarsus and foot, 4 on lower arm and hand. On the flanks, the dorsal colour fades into the whitish ventral colour. The ventral side is uniformly cream-whitish on forelimbs and belly, while the throat is very lightly pigmented.

Variation. We based the current description of variability upon some specimens (paratypes and complementary individuals), part of which (ZSM 23/2006, 37/2006, 107/2006, MRSN A5486 and MRSN A5483) were also photographed in nature, and thus provided more diagnostic characters. The female ZSM 23/2006 (Fig. 2; C–E), shows a back with sparse larger warts. Its colouration appears light brown with greyish shadings, darker dots and transversal bands on the back and legs. These are more evident in the preserved individual, where a pattern of darker spots is visible on the back, suggesting the presence of a darker X-shaped drawing. These spots are visible in two other individuals, MRSN A5484 (a female) and in the holotype MRSN A5487 (Fig. 2; A, K), although for the former specimen we do not have photographs taken in life. The tympanum is uniformly brownish, and the iris is yellowish with darker reticulations. The belly is comparatively smooth, with fewer warts on its lateral parts.

The throat is quite smooth. The central part of the belly is lighter than the flanks and the ventral sides of thighs, whitish on breast and thorax, with sparse darker spots. The inguinal part appears yellowish. The throat is darker than the belly, with a median lighter (although not so contrasted) line. The lateral borders of the lower jaw bear darker spots. After preservation, the colouration appears substantially similar, although faded. The juvenile ZSM 37/2006 (Fig. 2; E) presents a rather smooth back and flanks with sparse and barely evident warts. The colouration is brownish shading to the grey on the flanks and lateral parts of the back, with darker spots, extending around the flanks. The central part of the back is crossed by a longitudinal light (almost beige) band which enlarges on the head to cover the upper eyelids. The posterior part of such a band narrows to shade almost totally at the level of the vent. A thin, almost continuous whitish longitudinal line runs from the tip of the snout until the groin. The juvenile ZSM 107/2006 (Fig. 2; I) also shows a rather smooth back. The colouration is much darker, and the markings and spots are less visible. The tympanum is lighter than the surrounding areas, and the upper ridge is entoured by black pigment. Both these juveniles after about four years of preservation present a similar pattern of colouration as in life. In ZSM 107/2006 the central part of the back appears quite lighter than the surrounding areas, with a sort of arrow pattern. An interesting comparison is with the only mature available male (SVL 36.6 mm) photographed in life, the individual labelled UADBA 39099 (Fig. 2; F–H). This male appears quite slim in the photographs (either in dorsal or ventral view), with rather uniform light brown shading to greenish in life, and a moderately glandular skin texture (Fig. 2; F–G). The belly appears rather smooth in life, with the whole venter and thorax whitish (Fig. 2; H). The throat is darker with a rather indistinct central whitish band and vocal sacs are not recognizable. Lower parts of arms and thighs are pinkish, while tibiae are more whitish pigmented. The plantar surfaces are also reddish-pink. In this male, the glands are well visible and yellowish, and appear similar to those observed in *G. azzurrae, G. corvus* and *G. pseudoasper.* In particular, they clearly belong to the gland "Type 2", sensu Glaw et al. (2000), with 70 granules counted from the inner side of the right gland itself (whose external measure is 7.5*3.1 mm). In MRSN A5484 (a female) we notice a dark bar between the eyes, and an X-shaped darker spot at mid-dorsum; quite large and isolated dark spots are visible in the posterior part of the back. The belly is uniformly whitish and smooth. The three juveniles MRSN A5483, MRSN A5482, and MRSN A5485, are similar in colouration (excepting for MRSN A5483 exhibiting a light mid-dorsal line), with dark back with sparse lighter spots and shading, and almost whitish bellies. Of MRSN A5483 we also dispose of a photo taken in life, where the longitudinal light line is evident (Fig. 2; J).

Natural history. According to our observations, the species lives in habitats that retain some humidity, such as rock cavities and along the walls of the canyon-like formations. One important notation comes from the fact that several of the collectors, independently (JER, FG, JCR) found this species within the caves which are typical of the area. We suspect that the species uses caves because these sites presumably have a higher humidity than the surrounding areas. In such a sense it behaves similarly to

G. corvus at Isalo, which is known to frequent narrow canyons and cave-like canyons (Mercurio et al. 2008). Apparently, the new species (both adults and juveniles) is not confined to the proximity of water, and it has been observed jumping among the tsingy pinnacles also far from water bodies. All the individuals were active at night on tsingy rocks or during the day in caves. No data are available about mating behaviour, advertisement calls and tadpole morphology.

Distribution. Only known from the localities of the type specimens within the Tsingy de Bemaraha National Park.

Comparison with other species. *Gephyromantis atsingy* sp. n. differs from *G. pseudoasper*, *G. azzurrae* and *G. corvus* by the lack of paired blackish skin folds (vocal sacs) along the lower jaws in adult males, and from *G. azzurrae* also by details of colouration (see below). Following our measurements, adult males of *G. atsingy* can also be differentiated among each other by the number of granules in the femoral glands: 70 granules in *G. atsingy*; 96 granules in *G. corvus*; 38–45 granules in *G. azzurrae* and 39–43 granules in *G. pseudoasper*. In addition, the new species differs from all three species by substantial genetic differentiation (see below).

All the described species of *Gephyromantis,* subgenus *Phylacomantis,* show similarities with *G. atsingy* (Tab. 2). The dorsal pattern is similar in all species, showing an assemblage of darker spots and reticulations on the lighter background, and barred legs and arms. The dorsal colouration in *G. atsingy* is usually light brown-beige, with a somehow greenish shading, while in *G. corvus* it is uniformly grey or dark grey with sparse darker (uniformly-sized) warts and dots. Notwithstanding, the examined specimens of *G. atsingy* have a much more contrasted X-shaped dark spot on the back. This is less evident in *G. corvus,* where the dark-light pattern is more confuse and irregular. We observed a longitudinal repetition of lighter elements, a longitudinal light band or a middorsal light line only in *G. atsingy.* The belly in both species is light, but in *G. atsingy* we detected more frequently the darker drawing with a lighter central area on the throat and chest. According to the original description and subsequent papers (Mercurio and Andreone 2007, Mercurio et al. 2008), *G. azzurrae* has a quite variable dorsal colouration. The holotype of the species, as depicted by Glaw and Vences (2007), has a wide lighter dorsal band upon a darker dorsal colouration, and the belly is reddish. Other examined specimens of *G. azzurrae* present more uniform dorsal colouration. In both species the dorsal skin is featured by the presence of similar larger warts. In comparison to *G. atsingy,* the *G. pseudoasper* specimens are smaller and have a more warty back. The colouration in *G. pseudoasper* is much darker and the belly is much more pigmented: the throat, the thorax and the anterior part of the belly are heavily spotted in dark, with a clear median light line on the throat. The posterior parts of the belly and parts of the ventral side of the legs in *G. pseudoasper* are often orange. The external vocal sacs are evident and well developed, while these are not visible in *G. atsingy.*

Mitochondrial variation and differentiation. The molecular data confirm the attribution of *G. atsingy* to the subgenus *Phylacomantis* (Glaw and Vences 2006, Vieites et al. 2009). The analyzed specimens of *G. atsingy*, *G. azzurrae, G. corvus* and *G.*

pseudoasper appear genetically very uniform and show an intraspecific uncorrected divergence of 0.5%, 0.4%, 0.1% and 0.1% respectively, in the 16S rRNA gene sequences. The genetic distance between *G. atsingy* and the three other *Phylacomantis* species ranges between 10.2% (comparison between *G. atsingy* and both *G. corvus* and *G. pseudoasper*) and 11.2% (comparison between *G. atsingy* and *G. azzurrae*). Among the genus *Gephyromantis* the smallest genetic distance is observed between *G. corvus* and *G. azzurrae* (9.1%) and the highest uncorrected divergence between *G. azzurrae* and *G. pseudoasper* (13.1%). *Gephyromantis corvus* and *G. pseudoasper* have a genetic distance of 12%. These divergences are comparatively high among mantelline species (see Vences et al. 2005, Vieites et al. 2009), and corroborate the species status of *G. atsingy.* The phylogenetic relationships between the species of the *Phylacomantis* subgenus have been resolved recently (N. Kaffenberger et al., in preparation). These analyses confirm the monophyly of the subgenus, provide evidence for the basal position of *G. pseudoasper* and uncover the sister relationship between *Gephyromantis atsingy* and the complex made of *G. corvus* and *G. azzurrae*.

Conservation. This species appears to be restricted to the Bemaraha Plateau, where it has been found in seven localities within the Tsingy de Bemaraha National Park. It may also occur in the Réserve Naturelle Intégrale, which forms the northerly limit of the Bemaraha Plateau, but survey data are lacking from this site. Within the national park, some areas of forest are damaged by conversion to agriculture and charcoal production, but the humid canyons where G. atsingy occur are generally well protected. We therefore recommend assigning a category of Near Threatened because the species nearly qualifies for listing as Vulnerable under D2: the species is confined to a single site, the Bemaraha Massif (1,577 km^2), with a plausible threat that could impact the species in

Table 2. Distribution, habitats and diagnostic characters of the nominal species in the genus *Gephyromantis*, subgenus *Phylacomantis*.

Species	Distribution	Habitat	SVL	Vocal Sacs	Dorsal colouration	Ventral colouration	Dorsal texture
Gephyromantis atsingy	Tsingy de Bemaraha	Karst pinnacles and caves	35–43 mm	Non-evident	Light brownish with greenish shadings	Whitish	Slightly warty
Gephyromantis azzurrae	Isalo Massif	Open canyons and permanent rivers	23–44 mm	Double and brownish	Brownish, sometimes with wide light band	Whitish, with reddish shadings	Warty with heterogeneous warts
Gephyromantis corvus	Isalo Massif	Close canyons and cave-like canyons	39–41 mm	Double and blackish	Greyish with darker spots	Whitish	Warty
Gephyromantis pseudoasper	Sambirano, N, NW and NE	Rainforests, karstic areas	33–37 mm	Double and blackish	Brownish	Whitish	Slightly warty

the near future. If the threat became operational, the species would be eligible for listing as Endangered since its extent of occurrence is well within the 5,000km^2 threshold under the B criterion and it would occur at a single location (where the threat is habitat loss from agricultural activities and charcoal production) and there would be a continuing decline in the quality and area of habitat, qualifying the species for the criteria B1ab(iii).

Discussion

The Bemaraha plateau is one of the most peculiar areas of western Madagascar in terms of amphibian richness and endemicity (Raselimanana 2008, Bora et al. 2010). The new *Gephyromantis* species described here adds one more taxon to a list of endemics, which includes *Heterixalus carbonei, Plethodontohyla fonetana, Rhombophryne* sp., *Stumpffia* sp. aff. *helenae*, *Boophis tampoka* (Vences et al. 2000, Köhler et al. 2007, Glaw et al. 2007, Andreone and Randrianirina 2008, Bora et al. 2010), although some of these species might also be present at other sites of the West. The description of *G. atsingy*, and the previously mentioned works underline how little we still know about the amphibian fauna of this part of Madagascar and stresses the importance of further systematic surveys in these isolated areas.

One powerful tool is the application of an integrated taxonomy approach, which includes aspects of direct field surveys, behavioural assessment, molecular screening and bioacustic analysis. This is what allowed Vieites et al. (2009) to identify a high number of candidate species boosting a descriptive process of a large number of poorly differentiated species in an astonishing short lapse of time (Mercurio and Andreone 2007, Cramer et al. 2008, Köhler et al. 2008, 2010, Wollenberg et al. 2008, Andreone et al. 2010, D'Cruze et al. 2010, Glaw et al. 2010, Vallan et al. 2010, Vences et al. 2010a, b).

As already stressed by Glaw and Vences (2007) and Bora et al. (2010), Isalo and Bemaraha were probably in contact until relatively recently and were covered by humid vegetation, which allowed the existence of typical rainforest species at Isalo (e.g., *Boophis luteus, Mantidactylus femoralis* and *M. lugubris*), and rainforest-derived species at Bemaraha (e.g., *Boophis tampoka, Plethodontohyla fonetana* and *Rhombophryne* sp.). This hypothesis is also supported by the shared presence of typical rainforest elements, and by the presence of other species, like *Mantella* sp. aff. *expectata* and *Blommersia* sp. aff. *wittei*.

We expect that other forest blocks in western Madagascar may host further undescribed species of *Gephyromantis* and we highlight the need of conservation actions in Madagascar's dry forests due to the increasing deforestation rate and changing climatic scenarios.

The accelerated species discovery in Malagasy amphibians points to the importance of taxonomic surveys (see www.sahonagasy.org), and we like to consider *G. atsingy* as another "forceps delivered" species, according to the attractive definition given by Vallan et al. (2010) for *Boophis calcaratus*. In fact, we knew about the existence of *G. atsingy* for several years, but did not have enough data to describe it, since *Gephyromantis atsingy* and *G. corvus* appear to be rather similar to each other and share several life history traits. We here described this new species, recognizing that a species without a formal description and an attached name is simply an "invisible" species, hard to be protected and classified within the IUCN Red List (IUCN 2010).

Acknowledgements

Many friends and colleagues helped us providing samples that have been used in this study. Thanks to S. Federici and M. Barbuto for their help in the lab. Special thanks to M. Vences, P. Bora, J. Köhler and H. Enting for the help in the field and for providing information on the herpetofauna of Bemaraha. We thank N. Kaffenberger for sharing unpublished information on molecular relationships, and A. Angulo and M. Hoffmann for their precious help on analysing the conservation status of this newly described species. This work has been carried out in the framework of collaboration agreements of the authors' institutions with UADBA. The Malagasy authorities kindly granted research and export permits. A. Crottini was supported by a Dote Ricercatore of Regione Lombardia fellowship. Fieldwork by RKBJ and JCR was funded by the Darwin Initiative.

References

Andreone F, Cadle JE, Glaw F, Nussbaum RA, Raxworthy CJ, Vallan D, Vences M (2005) Species review of amphibian extinction risks in Madagascar: conclusions from the Global Amphibian Assessment. Conservation Biology 19: 1790–1802.

Andreone F, Cox NA, Glaw F, Köhler J, Rabibisoa NHC, Randriamahazo H, Randrianasolo H, Raxworthy CJ, Stuart SN, Vallan D, Vences M (2008) Update of the Global Amphibian Assessment for Madagascar in light of species discoveries, nomenclature changes, and new field information. In: Andreone F (Ed) A Conservation Strategy for the Amphibians of Madagascar. Monografie del Museo Regionale di Scienze Naturali di Torino 45: 419–438.

Andreone F, Randriamahazo H (2008) Sahonagasy Action Plan. Conservation Programs for the Amphibians of Madagascar / Programmes de Conservation pour les Amphibiens de Madagascar. Museo Regionale di Scienze Naturali, Conservation International, IUCN, Amphibian Specialist Group, 96 pp.

Andreone F, Randrianirina JE (2008) An unexpected *Rhombophryne* record at Tsingy de Bemaraha confirms the presence of cophyline frogs in western Madagascar. Zootaxa 1812: 46–48.

Andreone F, Rosa GM, Noël J, Crottini A, Vences M, Raxworthy CJ (2010) Living within fallen palm leaves: the discovery of an unknown *Blommersia* (Mantellidae: Anura) reveals a new reproductive strategy in the amphibians of Madagascar. Naturwissenschaften 97: 525–543.

Bandi C, Damiani G, Magrassi L, Grigolo A, Fani R, Sacchi L (1994) Flavobacteria as intracellular symbionts in cockroaches. Proceeding of the Royal Society of London B, Biological Sciences 257: 43–48.

Blommers-Schlösser RMA, Blanc CP (1991) Amphibiens (première partie). Faune de Madagascar 75(1): 1–379.

Bora P, Randrianantoandro JC, Randrianavelona R, Hantalalaina EF, Andriantsimanarilafy RR, Rakotondravony D, Ramilijaona OR, Vences M, Jenkins RKB, Glaw F, Köhler J (2010) Amphibians and reptiles of the Tsingy de Bemaraha Plateau, Western Madagascar: Checklist, biogeography and conservation. Herpetological Conservation and Biology 5: 111–125.

Cramer A, Rabibisoa N, Raxworthy CJ (2008) Descriptions of two new *Spinomantis* frogs from Madagascar (Amphibia: Mantellidae), with new morphological data for two other poorly known species: *S. brunae* and *S. massorum*. American Museum Novitates 3618: 1–22.

D'Cruze N, Köhler J, Vences M, Glaw F (2010) A new fat fossorial frog (Microhylidae: Cophylinae: *Rhombophryne*) from the rainforest of the Forêt d'Ambre Special Reserve, northern Madagascar. Herpetologica 66: 182–191.

Glaw F, Hoegg S, Vences M (2006) Discovery of a new basal relict lineage of Madagascan frogs and its implications for mantellid evolution. Zootaxa 1334: 27–43.

Glaw F, Köhler J, Bora P, Rabibisoa NHC, Ramilijaona O, Vences M (2007) Discovery of the genus *Plethodontohyla* (Anura: Microhylidae) in dry western Madagascar: description of a new species and biogeographic implications. Zootaxa 1577: 61–68.

Glaw F, Köhler J, de la Riva I, Vieites DR, Vences M (2010) Integrative taxonomy of Malagasy treefrogs: combination of molecular genetics, bioacoustics and comparative morphology reveals twelve additional species of *Boophis*. Zootaxa 2383: 1–82.

Glaw F, Köhler J, Vences M (2009a) A new species of cryptically coloured day gecko (*Phelsuma*) from the Tsingy de Bemaraha National Park in western Madagascar. Zootaxa 2195: 61–68.

Glaw F, Nagy ZT, Köhler J, Franzen M, Vences M (2009b) Phylogenetic relationships of a new species of pseudoxyrhophiine snake (Reptilia: Lamprophiidae: *Thamnosophis*) suggest a biogeographical link between western and northern Madagascar. Organisms Diversity & Evolution 9: 13–22.

Glaw F, Vences M (2006) Phylogeny and genus-level classification of mantellid frogs. Organisms Diversity and Evolution 6: 236–253.

Glaw F, Vences M (2007) A Field Guide to the Amphibians and Reptiles of Madagascar. Third Edition. Vences & Glaw Verlags GbR, Köln, 496 pp.

Glaw F, Vences M, Böhme W (1998) Systematic revision of the genus *Aglyptodactylus* Boulenger, 1919 (Amphibia: Ranidae), and analysis of its phylogenetic relationships to other Madagascan ranid genera (*Tomopterna, Boophis, Mantidactylus,* and *Mantella*). Journal of Zoological Systematics and Evolutionary Research 36: 17–37.

Glaw F, Vences M, Gossmann V (2000) A new species of *Mantidactylus* (subgenus *Guibemantis*) from Madagascar, with a comparative survey of internal femoral gland structure in the genus (Amphibia: Ranidae: Mantellinae). Journal of Natural History 34: 1135–1154.

Glos J, Glaw F, Vences M (2005) A new species of *Scaphiophryne* from western Madagascar. Copeia 2005: 252–261.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

IUCN (2010). IUCN Red List of Threatened Species. Version 2010.2. http://www.iucnredlist.org. [accessed on 29 June 2010].

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences of the United States of America 86: 6196–6200.

Köhler J, Glaw F, Vences M (2007) A new green treefrog, genus *Boophis* Tschudi 1838 (Anura Mantellidae), from arid western Madagascar: phylogenetic relationships and biogeographic implications. Tropical Zoology 20: 215–227.

Köhler J, Glaw F, Vences M (2008) Two additional treefrogs of the *Boophis ulftunni* species group (Anura: Mantellidae) discovered in rainforests of northern and south-eastern Madagascar. Zootaxa 1814: 37–48.

Köhler J, Vences M, D'Cruze N, Glaw F (2010) Giant dwarfs: discovery of a radiation of large-bodied 'stump-toed frogs' from karstic cave environments of northern Madagascar. Journal of Zoology 282: 21–38.

Köhler J, Vieites DR, Bonett RM, Hita Garcia F, Glaw F, Steinke D, Vences M (2005) New amphibians and global conservation: a boost in species discoveries in a highly endangered vertebrate group. Bioscience 55: 693–696.

Mercurio V, Andreone F (2007) Two new canyon-dwelling frogs from the arid sandstone Isalo Massif, central-southern Madagascar (Mantellidae, Mantellinae). Zootaxa 1574: 31–47.

Mercurio V, Aprea G, Crottini A, Mattioli F, Randrianirina JE, Razafindrabe TJ, Andreone, F (2008) The amphibians of Isalo Massif, southern-central Madagascar: high frog diversity in an apparently hostile dry habitat. In: Andreone F (Eds) A Conservation Strategy for the Amphibians of Madagascar. Monografie del Museo Regionale di Scienze Naturali di Torino 45: 143–196.

Nussbaum RA, Raxworthy CJ (2000) Systematic revision of the genus *Paroedura* Günther (Reptilia: Squamata: Gekkonidae), with description of five new species. Miscellaneous Publications, Museum of Zoology, University of Michigan 189: 1–26.

Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The Simple Fools Guide to PCR. Version 2.0, Dept. Zool. and Kewalo Marine Lab., Univ. of Hawai'i.

Raselimanana AP (2008) Herpétofaune des forets sèches malgaches. Malagasy Nature 1: 46–75.

Schimmenti G, Jesu R (1996) *Brookesia exarmata* sp. n. (Reptilia, Chamaeleonidae): a new dwarf chameleon from the limestone outcrops of western Madagascar. Italian Journal of Zoology 63: 193–197.

Vallan D, Vences M, Glaw F (2010) Forceps delivery of a new treefrog species of the genus *Boophis* from eastern Madagascar (Amphibia: Mantellidae). Amphibia-Reptilia 31: 1–8.

Vences M, Jovanovic O, Glaw F (2008) Historical analysis of amphibian studies in Madagascar: an example for increasing research intensity and international collaboration. In: Andreone F (Ed) A Conservation Strategy for the Amphibians of Madagascar. Monografie del Museo Regionale di Scienze Naturali di Torino 45: 47–58.

Vences M, Glaw F, Jesu R, Schimmenti G (2000) A new species of *Heterixalus* (Amphibia: Hyperoliidae) from western Madagascar. African Zoology 35: 269–276.

Vences M, Köhler J, Crottini A, Glaw F (2010a) High mitochondrial sequence divergence meets morphological and bioacustic conservatism: Boophis quasiboehmei sp. n., a new cryptic treefrog species from south-eastern Madagascar. Bonn zoological Bulletin 57: 241–255.

Vences M, Glaw F, Köhler J, Wollenberg KC (2010b) Molecular phylogeny, morphology and bioacoustics reveal five additional species of arboreal microhylids of the genus *Anodonthyla* from Madagascar. Contributions to Zoology 79: 1–32.

Vences M, Thomas M, Bonett RM, Vieites DR (2005) Deciphering amphibian diversity through DNA barcoding: chances and challenges. Philosophical Transactions of the Royal Society London, Series B 360: 1859–1868.

Vences M, Vieites DR, Glaw F, Brinkmann H, Kosuch J, Veith M, Meyer A (2003) Multiple overseas dispersal in amphibians. Proceeding of the Royal Society of London B, Biological Sciences 270: 2435–2442.

Vieites DR, Wollenberg KC, Andreone F, Köhler J, Glaw F, Vences M (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. Proceedings of the National Academy of Sciences of the United States of America 106: 8267–8272.

Wollenberg KC, Andreone F, Glaw F, Vences M (2008) Pretty in pink: a new treefrog species of the genus *Boophis* from north-eastern Madagascar. Zootaxa 1684: 58–68.