RESEARCH ARTICLE

ZooKeys 353: 1–24 (2013) doi: 10.3897/zookeys.353.5991 www.zookeys.org



Molecular and microscopic analysis of the gut contents of abundant rove beetle species (Coleoptera, Staphylinidae) in the boreal balsam fir forest of Quebec, Canada

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Academic editor: Michael Ivie | Received 18 July 2013 | Accepted 15 October 2013 | Published 20 November 2013

Citation: Klimaszewski J, Morency M-J, Labrie P, Séguin A, Langor D, Work T, Bourdon C, Thiffault E, Paré D, Newton AF, Thayer MK (2013) Molecular and microscopic analysis of the gut contents of abundant rove beetle species (Coleoptera, Staphylinidae) in the boreal balsam fir forest of Quebec, Canada. ZooKeys 353: 1–24. doi: 10.3897/ zookeys.353.5991

Abstract

Experimental research on beetle responses to removal of logging residues following clearcut harvesting in the boreal balsam fir forest of Quebec revealed several abundant rove beetle (Staphylinidae) species potentially important for long-term monitoring. To understand the trophic affiliations of these species in forest ecosystems, it was necessary to analyze their gut contents. We used microscopic and molecular (DNA) methods to identify the gut contents of the following rove beetles: *Atheta capsularis* Klimaszewski, *Atheta klagesi* Bernhauer, *Oxypoda grandipennis* (Casey), *Bryophacis smetanai* Campbell, *Ischnosoma longicorne* (Mäklin), *Mycetoporus montanus* Luze, *Tachinus frigidus* Erichson, *Tachinus fumipennis* (Say), *Tachinus quebecensis* Robert, and *Pseudopsis subulata* Herman. We found no apparent arthropod fragments within the guts; however, a number of fungi were identified by DNA sequences, including filamentous fungi and budding yeasts [Ascomycota: *Candida derodonti* Suh & Blackwell (accession number FJ623605), *Candida mesenterica* (Geiger) Diddens & Lodder (accession number FM178362), *Candida railenensis* Ramirez and Gonzáles (accession number JX455763), *Candida sophie-reginae* Ramirez & González (accession number HQ652073), *Candida*

Copyright Her Majesty the Queen in Right of Canada. This is an open access article distributed under the terms of the Creative Commons Attribution License 3.0 (CC-BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. sp. (accession number AY498864), *Pichia delftensis* Beech (accession number AY923246), *Pichia membranifaciens* Hansen (accession number JQ26345), *Pichia misumaiensis* Y. Sasaki and Tak. Yoshida ex Kurtzman 2000 (accession number U73581), *Pichia* sp. (accession number AM261630), *Cladosporium* sp. (accession number KF367501), *Acremonium psammosporum* W. Gams (accession number GU566287), *Alternaria* sp. (accession number GU584946), *Aspergillus versicolor* Bubak (accession number AJ937750), and *Aspergillus amstelodami* (L. Mangin) Thom and Church (accession number HQ728257)]. In addition, two species of bacteria [*Bradyrhizobium japonicum* (Kirchner) Jordan (accession number BA000040) and *Serratia marcescens* Bizio accession number CP003942] were found in the guts. These results not only provide evidence of the consumer-resource relations of these beetles but also clarify the relationship between rove beetles, woody debris and fungi. Predominance of yeast-feeding by abundant rove beetles suggests that it may play an important role in their dietary requirements.

Keywords

Rove beetles, Staphylinidae, Coleoptera, diet, fungivory, mycophagy, gut analysis, trophic relationship, saproxylic, boreal forest, Canada, Ascomycota, Basidiomycota, bacteria

Introduction

Rove beetles (Coleoptera: Staphylinidae) have proven to be useful indicators of forest disturbance and recovery because they are sensitive to environmental perturbations, diverse in species and trophic roles, easily sampled, and at least in central Europe and Canada, mostly readily identified using a wealth of available taxonomic tools (Boháč 1990, 1999, Pohl et al. 2008). Many staphylinid species show distinct response patterns following forest disturbances (e.g., Pohl et al. 2007, 2008, Klimaszewski et al. 2008, Work et al. 2013). For example, in a recent study of rove beetles following removal of logging residues by whole-tree harvesting in boreal balsam fir forests of Quebec, three Atheta species, Tachinus fumipennis (Say) and Tachinus frigidus Erichson were negatively affected by the removal of forest biomass, while Gabrius brevipennis (Horn), Pseudopsis subulata Herman and Quedius labradorensis Smetana were not and their catch increased (Work et al. 2013). While studies comparing species assemblages can quantify the overall effects of harvest treatments or other forest disturbances, they are often not designed to identify specific underlying mechanisms for individual species' responses. Study of trophic roles may provide useful insights into these response patterns by assessing factors such as individual predator-prey (or consumer-resource) relationships, the degree of diet specialization, and possible associations of beetles with specific microhabitats that may serve as habitat or substrate for their food resources.

Rove beetles are a diverse group exhibiting a wide variety of trophic relationships and occupying numerous microhabitats in forest ecosystems. Many Aleocharinae and Staphylininae, e.g., species of *Aleochara, Philonthus, Platydracus*, and *Staphylinus*, are voracious predators of other arthropods such as fly larvae (Klimaszewski 1984, Smetana 1995). At least some species of Scaphidiinae, Osoriinae, Tachyporinae, and Aleocharinae (Gyrophaenina) eat the flesh or spores of fungal sporocarps (Seevers 1951, Ashe 1984, Newton 1984). Oxytelinae are generally detritivores and feed on decaying plant ma-

terial and algae (Thayer 2005, Makranczy 2006). A few Omaliinae, e.g. Eusphalerum and some other genera, are pollen-feeders (Thayer 1987, 2005). A few species, such as the aleocharine Himalusa thailandensis Pace, Klim. & Cent., feed on live plant tissue (Klimaszewski et al. 2010). Most information on food sources of rove beetles has been obtained through observation of individual beetles in the field or laboratory, or inference from habitat associations of species. For example, some groups (e.g., Aleochara, Philonthus) that are collected in decaying mushrooms are predators of dipteran larvae that co-occur within these fungi (Klimaszewski 1984, Smetana 1995). While direct observations of feeding provide compelling evidence of dietary preferences, inferences based on habitat preferences are not definitive evidence of consumer-resource relationships. Other methods have been used to more definitively establish feeding habits of beetles including microscopical examination of gut contents (Newton 1984, Thayer 1987) and immunological methods (Dennison and Hodkinson 1983). The use of molecular techniques to investigate dietary preferences and trophic links in rove beetles is presented here for the first time, but similar techniques were used in the past to investigate invertebrate predators for multiple prey using DNA targets (Harper et al. 2005). Increasingly large databases of DNA sequences in repositories such as GenBank and MycoBank will make these techniques more and more useful for examining relationships between beetles and cryptic food items such as fungi and bacteria (Crous et al. 2004, Robert et al. 2005). For beetles that feed on organisms with relatively strict habitat requirements, such as fungal species that require lignocellulose, molecular gut analyses may lead to inferences on the importance of habitat elements such as downed deadwood (Suh and Blackwell 2005a, b).

In this study we use both microscopic examination and molecular analysis of gut contents to more precisely characterize the feeding habits and trophic role of 10 rove beetle species common in the boreal forest of Quebec. There are few published data on gut contents, of these species and little is known of their food affiliations, except for some general statements on habitat preferences of *Tachinus* species (Campbell 1973) and limited observations on hosts and gut contents of some *Tachinus* and *Pseudopsis* species (Newton 1984).

Material and methods

Sampling sites and rove beetle species

Rove beetles were collected as part of a large field experiment examining the impacts of biomass harvesting on forest ecosystem functioning (Thiffault et al. 2011, Venier et al. 2012) within the Montmorency Teaching and Research Forest (ranges of latitude and longitude: 47°13' to 47°22'N, and 71°05' to 71°11'W) approximately 70 km north of Quebec City, Quebec, Canada. This site is part of a 60-year-old boreal balsam firwhite birch dominated forest in the Laurentian Mountains. The site and experimental layout were described in detail by Work et al. (2013). All beetles were collected using pitfall traps deployed between June and August 2012. Beetles were collected from both harvested and unharvested stands in 75% ethanol with some vinegar, and later cleaned

with 75 % ethanol and mounted on cards (Aleocharinae) or points (e.g., Tachyporinae, Pseudopsinae). We used the 10 most abundant rove beetle species for this study, together constituting 78% of all rove beetles collected (85–1785 specimens per species): Aleocharinae: Atheta capsularis Klimaszewski, Atheta klagesi Bernhauer, Oxypoda grandipennis (Casey); Tachyporinae: Bryophacis smetanai Campbell, Ischnosoma longicorne (Mäklin), Mycetoporus montanus Luze, Tachinus fumipennis (Say), Tachinus frigidus Erichson, Tachinus quebecensis Robert; and Pseudopsinae: Pseudopsis subulata Herman (Figs 2–11).

Gut extraction for microscopical analysis

Six dried and mounted specimens of each species were selected from samples collected in 2012. Individual specimens were softened in distilled water and ammonia solution for about 15 minutes and their guts were dissected in distilled water under a stereoscopic microscope. The colon and rectum of the hindgut were transferred directly to absolute alcohol, placed on a glass slide with Canada balsam, and pressed by dissecting needles to liberate gut contents and then covered with a cover slip. Slides were studied under a compound microscope (Reichert, Vienna, Austria) and photographs were taken using an Olympus DP73 digital camera. The following publications were consulted for fungal spore illustrations: Hanlin (1990, 1998).

Gut DNA extraction

DNA from gut contents was extracted from 10 individuals of each species of rove beetle using the QIAamp DNA Micro kit from Qiagen, according to the manufacturer's specifications. Gut contents from the 10 individuals were pooled for DNA extraction. DNA samples were eluted from the columns in 100 μ L of PCR grade nuclease-free water and the concentration was determined spectrophotometrically by reading absorbance at 260 nm and 280 nm with the Synergy Mx microplate reader (BioTek).

PCR amplifications, cloning and sequence analysis

PCR amplifications were performed using three primers universal to the internal transcribed spacer (ITS) regions of the nuclear ribosomal repeat and used in the following combinations







Figures 2–7. Body images of rove beetles in dorsal view: 2 *Atheta capsularis* Klimaszewski 3 *Atheta klagesi* Bernhauer 4 *Oxypoda grandipennis* (Casey) 5 *Bryophacis smetanai* Campbell 6 *Ischnosoma longicorne* (Mäklin) [previously cited as synonymous *I. fimbriatum* Campbell] 7 *Mycetoporus montanus* Luze [previously cited as synonymous *M. rugosus* Hatch].

(ITS9mun+ITS4 or ITS5+ITS4). The detailed sequences of the primers are given in Table 1; they specifically amplify a DNA fragment covering the ITS1 region, the 5.8S rRNA gene, and the ITS2 region between the 18S and 28S rRNA genes (Fig. 1).

Primer name	Primer sequence, 5'-3'	Primer source study
ITS9mun	TGTACACCGCCCGTCG	Egger (1995)
ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)
ITS4	TCCTCCGCTTATTGATATGC	White et al. (1990)

Table 1. Primers used in this study.







8. Tachinus frigidus

9. Tachinus fumipennis

10. Tachinus quebecensis



 Figures 8–11. Body images of rove beetles in dorsal view: 8 Tachinus frigidus Erichson 9 Tachinus fumipennis

(Say) **10** Tachinus quebecensis Robert **11** Pseudopsis subulata Herman.

D 1 1 1	Spore Type									
Rove beetle species	Yeast	1	2	3	4	5	6	7		
Atheta capsularis (A)	×									
Atheta klagesi (A)	×									
Oxypoda grandipennis (A)	×									
Bryophacis smetanai (T)	×	×								
Ischnosoma longicorne (T)	×		×							
Mycetoporus montanus (T)	×			×						
Tachinus frigidus (T)	×				×	×	×			
Tachinus fumipennis (T)	×			×	×	×				
Tachinus quebecensis (T)	×							×		
Pseudopsis subulata (P)	×									

Table 2. Distribution of yeast and spores in different rove beetle species from microscopical observation. Subfamilies are: A, Aleocharinae; P, Pseudopsinae; T, Tachyporinae.

The PCR reactions contained 30 ng of DNA, 2X HotStarTaq Plus Master Mix from Qiagen, which contains one unit of HotStarTaq Plus DNA Polymerase, PCR Buffer with 1.5 mM MgCl₂, 200 μ M of each dNTP and 0.3 μ M of each primer in a 30 μ L final reaction. PCR amplification was carried out using an initial denaturation step at 95°C for 15 min, followed by 35 cycles: 15s at 95°C, 30s at 52°C, 30s at 72°C, and a final extension for 10 min at 72°C. Cycling was performed on a PTC200 Peltier Thermal Cycler (MJ Research). Amplified fragments were inserted directly in the TA cloning vector (Invitrogen) and transformed into *E. coli* strain DH10B. Plasmids were isolated using the Qiacube with the Qiagen miniprep columns (Qiagen) and sequenced with an ABI 3730xl Data Analyzer (Applied Biosystems). After removing the DNA cloning vector segments, the remaining sequences were compared with reference sequences contained in the GenBank nucleotide sequence database using the BLAST algorithm (Altschul et al. 1990) and in the MycoBank database search engine (Robert et al. 2005) to find the closest matching sequences. A total of 228 clones were sequenced in this study.

Results

Microscopic observations

We observed no cuticle characteristic of arthropods in the guts of any dissected individuals. The only identifiable material was yeasts and fungal spores. Through microscopic observation of spore morphology, we were unable to discriminate among the yeast species, so these were recorded simply as "yeasts" (Figs 12–18, 20, 30, 32, 34, 35). However, at least seven different spore types could be discriminated using microscopic techniques and available taxonomic resources, although they could not be identified with certainty (Figs



Figures 12–15. Images of hindgut content of the following rove beetle species: **12–13** *Atheta capsularis* Klimaszewski **14–15** *Atheta klagesi* Bernhauer.



Figures 16–19. Images of hindgut content of the following rove beetle species: **16** Oxypoda grandipennis (Casey) **17–18** Bryophacis smetanai Campbell **19** Ischnosoma longicorne (Mäklin).



23. Tachinus frigidus

Figures 20-23. Images of hindgut content of the following rove beetle species: 20 Ischnosoma longicorne (Mäklin) 21–22 Mycetoporus montanus Luze 23 Tachinus frigidus Erichson.



26. T. frigidus

27. Tachinus fumipennis

Figures 24–27. Images of hindgut content of the following rove beetle species: **24–26** *Tachinus frigidus* Erichson **27** *Tachinus fumipennis* (Say).





31. Tachinus quebecensis

Figures 28–31. Images of hindgut content of the following rove beetle species: **28–30** *Tachinus fumipennis* (Say) **31** *Tachinus quebecensis* Robert.





34. Pseudopsis subulata



33. T. quebecensis



35. P. subulata

Figures 32–35. Images of hindgut content of the following rove beetle species: 32–33 *Tachinus quebecensis* Robert 34–35 *Pseudopsis subulata* Herman.

17 [spore type 1]; 19 [spore type 2]; 21, 22, 27, 29? [spore type 3]; 23, 24 [spore type 4]; 25, 26, 28 [spore types 5 and/or 6]; and 31, 33 [spore type 7]). Some of these spores are of the following morphology: (long arthrospore fragment, Fig. 17); immature ascomycete cleistothecia or pycnidia, (Fig. 19, spore # 2); and dark walled spores (Figs 31, 33, spore # 7; dark coloured spores, Figs 23, 24, spore # 4). All 10 rove beetle species had yeasts in their hindgut, but spores were found only in the six tachyporine species and were missing in Aleocharinae and Pseudopsinae (Table 2). Yeasts were densely packed in Aleocharinae and Pseudopsinae and less so in remaining species. Spore types 1, 2, 6, and 7 were each found in a single species, while types 3, 4, and 5 were found in *Tachinus fumipennis* and either *T. frigidus* or *Mycetoporus montanus* (Table 2). These two species of *Tachinus* had the most diverse spore diets (three types each).

Molecular analyses

In total, we obtained 186 fungal and bacterial sequences from the 10 species of rove beetles, ranging from 19–33 sequences per species (Table 3). Of these, 134 (72%) could be identified to genus, species, or unnamed clones with high certainty (>90% sequence match) by comparison to sequences in the GenBank and MycoBank databases. Twenty-nine sequences (2 fungal and all 27 bacterial) showed lower levels of sequence similarity (78–90%). We could not match 23 sequences (a range of 0 to 24% unmatched sequences per species, see Table 3).

In all, we identified 17 fungal taxa in the phyla Ascomycota and Basidiomycota and two bacterial taxa in the phylum Proteobacteria through molecular analysis (Table 3). The number of taxa distinguished from each staphylinid species varied from one in *O. grandipennis* and *B. smetanai* to eight in *T. quebecensis*, and averaged 3.3 per species (Table 3). We found yeasts in all of the 10 beetle species studied, with *Candida mesenterica* (Geiger) Diddens & Lodder accounting for 92 sequences and occurring in 9 of the 10 beetle species. The next most commonly identified taxon was the bacterial species *Serratia marcescens* Bizio, which accounted for 24 sequences found in five beetle species. The vast majority of taxa in beetle guts were found in just one (13 taxa) or two (1 taxon) sequences.

Discussion

Both dissection and molecular analysis of guts strongly suggest that rove beetles in this study may feed primarily on yeasts. Yeasts are ubiquitous (in soil, on decaying plant material including deadwood, and on berries) and they are an important part of the diet of at least some fungivorous beetle species (Suh and Blackwell 2005b). Some species of *Candida* yeasts have close associations with saproxylic insects and are capable of transforming d-xylose and other important components of lignocellulose to ethanol (Wang et al. 2005). However, yeasts within the *C. mesenterica* clade are associated with

Specific taxon	A. capsularis	A. klagesi	O. grandipennis	B. smetanai	I. longicorne	M. montanus	T. frigidus	T. fumipennis	T. quebecensis	P. subulata	Total
Fungi											
Acremonium psammosporum (GU566287)					2ª						2ª
Alternaria sp. (GU584946)	1										1
Aspergillus amstelodami (HQ728257)	1										1
Aspergillus versicolor (AJ937750)										1	1
Candida cretensis (HF558653)	1					1	16				18
Candida mesenterica (FM178362)	12	8	12	14	5	9	4	20	8		92
Candida sophiae-reginae (HQ652073)									1		1
Candida railenensis (JX455763)						1					1
<i>Candida</i> sp. (AY498864)							1				1
Cladosporium tassiana (AF393706)										1	1
Cryptococcus (uncultured) (KC753404)		1			2				1		4
Hypocreales sp. TR114 (HQ608125)					1						1
Penicillium spinulosum (GU566252)					1						1
Pichia delftensis (AY923246)								1			1
Pichia misumaiensis (U73581)									1		1
Pichia membranifaciens (JQ26345)									1		1
Rhodotorula mucilaginosa (HQ702343)									1		1
Uncultured fungus clone 50-p12-A5 (HQ267068)		2							5		7
Insects											
Ten species from this study		6	8	1	5	10				7	37
Bacteria											
Bradyrhizobium japonicum (BA000040)				1 ^b	2 ^b						3 ^b
Serratia marcescens (CP003942)				4 ^b			1 ^b	1 ^b	2 ^b	16 ^b	24 ^b
Unmatched sequences	4	3	1	2	1	1	3	0	0	8	23
Total	19	20	21	22	19	22	25	22	20	33	223

Table 3. Number and identity of genetic sequences extracted from the gut contents of 10 species of Staphylinidae. Accession numbers in brackets follow species name in the first column.

^a Sequences with 86 to 90% sequence similarity.

^b Sequences with 78 to 85% sequence similarity.

many insect groups and are likely indicative of habitat associations rather than being highly specific gut symbionts (Suh and Blackwell 2005a). Yeasts in the *C. mesenterica* clade, particularly species in its subclade A, are known to be associated with fungal basidiocarps and have previously been isolated from the digestive tracts and body surfaces of six families of basidiocarp-inhabiting beetles, including one unidentified species of Staphylinidae (Suh and Blackwell 2005a).

With the exception of the relatively broad consumption of *C. mesenterica* yeasts by most species, finer patterns in feeding preferences among rove beetles were difficult to assess. This was partly a result of the limited number of matched sequences for some species, which in turn probably reflects in part the limits of available reference sequences. The limited numbers of sequences obtained in our study could be related to degradation of DNA as the result of suboptimal preservation medium.

The prevalence of other fungi in addition to yeasts and the presence of spores in rove beetle guts was not unexpected, as many rove beetle species are associated with fungi (Campbell 1973, Newton 1984, Newton et al. 2000, Thayer 2005). Spores of at least seven species of fungi were found in the guts of six of the rove beetle species, al-though it is not known whether the beetles derive nutrition from spores. Many spores have tough walls that enable them to pass through digestive tracts; however, others are certainly digested and some are cracked by the mouthparts to provide nutrition for beetles specializing in spore feeding (Lawrence 1989, Betz et al. 2003). It cannot be determined from available data whether the ingestion of spores by these rove beetles is incidental or intentional. However, the absence of spores in four species, including all three species of Aleocharinae, is notable and raises the question of whether some species do not ingest spores, either because they are unable to, or because they have difficulty finding them in a particular microhabitat.

Although we isolated bacteria less commonly than fungi, we did find them in six beetle species. The second most commonly detected sequence, in fact, was from the bacterium *S. marcescens*, which is associated with soils; it may sometimes be pathogenic to insects (Flyg et al. 1980). This species of bacteria is probably not an important food source for rove beetles. It may simply be so common in the soil that incidental ingestion is frequent. The other species of bacterium we isolated, *B. japonicum*, is a soil-dwelling, nitrogen-fixing species associated with legume plants (Rivas et al. 2009), so seems unlikely to be a food source for rove beetles.

It is notable that no arthropod cuticle or evidence of animal DNA sequences were found in the guts of any of these species despite the fact that predation on arthropods (especially mites, springtails, and smaller insects) is common in the family (Newton et al. 2000, Thayer 2005). Two possible interpretations are that: (1) the adults of these species are entirely fungivorous, or (2) they are predaceous and use preoral digestion, as many staphylinids do (e.g., Evans 1965, Dennison and Hodkinson 1983, Thayer 2005) and specifically as has been hypothesized for *Pseudopsis* (Pseudopsinae) and the entire group of subfamilies to which it belongs (Grebennikov and Newton 2009). The fact that these 10 species represent seven genera and three subfamilies suggests that analysis of gut contents of many more species is needed to provide a better sampling of rove beetle diets. Identification of the many presently unmatched DNA sequences, which could include animal DNA, and ruling out of preoral digestion are also required before carnivory can be excluded with certainty for the species studied here.

In work conducted to characterize rove beetle responses to removal of logging residues following clearcut harvesting in boreal balsam fir forests of Quebec (Work et al. 2013; Klimaszewski, unpublished data), several response patterns were shown by different species. Seven species (*A. capsularis, A. klagesi, B. smetanai, O. grandipennis, T. frigidus, T. fumipennis* and *T. quebecensis*) were found predominantly or exclusively in uncut forest rather than forest subjected to harvesting treatments. Except for *T. quebecensis*, all of these may feed partly or wholly on basidiocarps, as the predominance of basidiocarpassociated *Candida* spp. in their guts suggests. These species may not persist well in harvested stands because their drier, disturbed conditions are generally far less favourable to mushroom and other sporocarp production (Langor, personal observation). *Tachinuss quebecensis* was found only in uncut stands, and had *Candida sophiae-reginae* and *C. mesenterica* isolated from guts in the present study. However, this beetle species had the highest diversity of ingested fungal species, five of them unique to it, and possibly one or more of these represent important food sources that are absent (or rare) in harvested stands, although it is also possible that it is predaceous and all fungi are incidental.

Pseudopsis subulata was the most common species to show a strong affinity for disturbed stands, specifically stands subjected to whole tree harvesting, although it was also found in uncut stands and in stands subjected to harvesting with debris left behind (Work et al. 2013). Interestingly, this is the only rove beetle species that did not have *Candida* spp. in its gut; however, its gut was typically packed with other yeasts that could not be identified. Perhaps these yeasts have a strong association with disturbed and open habitats.

Mycetoporus montanus was not collected during the first year of the study (Work et al. 2013), but it was common during the second year, when it was collected almost exclusively in harvested treatments (Klimaszewski, unpublished data). It appears that this species moved into disturbed stands and multiplied rapidly, taking advantage of food or breeding sites that became more available in such stands. Although *M. montanus* had *C. mesenterica* in its gut, it also had a large variety of other organisms that could not be identified, some of which may be the primary food source for this species.

Ischnosoma longicorne was commonly found in both uncut and disturbed stands (Work et al. 2013). This species had a high diversity of species in its gut (six fungal and one bacterium species), which may indicate a broad diet and, therefore, a capacity to succeed in many habitat types.

Feeding associations between rove beetles and yeasts provide some insight into potential mechanisms by which biomass harvesting may impact rove beetles. Our results may suggest that dominant rove beetles are feeding on yeasts and other fungi that may or may not be directly associated with sporocarps growing on deadwood substrates. It is important to understand the complexity of factors linking the studied beetles to biomass removal treatments. The removal of additional forest biomass may be affecting beetles not only via potential food linkages, but also by other non-trophic mechanisms such as changes in physical conditions following the removal of the forest overstory (Work et al. 2013).

In addition to characterizing food sources for some abundant species of rove beetles, many of which are good ecological indicators, our work provides some possible explanations for beetle response patterns in the wake of forest disturbance. The relatively easy application of DNA sequencing to gut contents and the steadily increasing wealth of sequence data available to serve as an identification resource means that these techniques can now be readily applied in disturbance ecology research to investigate species response patterns and habitat preferences. We encourage broader use of this approach to support future work.

Acknowledgements

We are grateful to the following individuals for contributing to our research: M. Blais, G. Laflamme, P. DesRochers, and J. Bérubé (Laurentian Forestry Centre - LFC) for useful advice and reference recommendations; S. Dagnault and J. Morissette (LFC) for their help with site preparation, and casual employees R. Batista (Montreal) and A. Gilbert (Québec) for helping with site preparation and collecting and processing insect samples at the Montmorency Experimental Forest. Julie Bouliane and Patrick Pineault of Université Laval, Quebec, helped us with the logistics, site preparation and helpful advice. Pamela Cheers and Isabelle Lamarre (LFC) edited the manuscript and prepared it for publication.

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Appendix A

Biological notes about some taxa found in staphylinid guts (Fungal classification below is from Mycoses Study Group (2007))

Kingdom FUNGI Phylum ASCOMYCOTA

Ascomycota, also known as sac fungi, is a sister group of the Basidomycota. This group contains the majority of fungi, including yeasts.

Saccharomycetales: Saccharomycetaceae: Candida

Candida is a yeast and the most common cause of opportunistic mycoses worldwide. It is also a frequent colonizer of human skin and mucous membranes. It is also a pathogen and a colonizer, found on leaves, flowers, in water, and in soil. While most *Candida* species are mitosporic, some have a known teleomorphic state and produce sexual spores.

Saccharomycetales: Saccharomycetaceae: Cladosporium

Cladosporium is a dematiaceous (pigmented) mould widely distributed in the air and rotten organic material, and frequently isolated as a contaminant in food. Some species are predominant in tropical and subtropical regions. Some *Cladosporium* species are isolated from fish and are associated with infections.

Saccharomycetales: Endomycetaceae: Pichia

Pichia is a teleomorph that produces ascospores. The anamorphs of the *Pichia* species are various *Candida* species. The connection of *Candida* species with their corresponding *Pichia* teleomorphs is based on observation of the ascospores produced by the *Candida* isolate or, more specifically, on the 28S gene sequence data. *Pichia ohmeri* was initially isolated from cucumber brine and is commonly used in the food industry for fermentation in pickles, rinds, and fruits. Clinically, *Pichia* is generally considered to be a contaminant. However, some *Pichia* species are now recognized as clinically significant opportunistic pathogens.

Eurotiales: Trichocomaceae: Aspergillus

Aspergillus is a filamentous, cosmopolitan and ubiquitous fungus found in nature. It is commonly isolated from soil, plant debris, and indoor air environment. While a teleomorphic state has been described for only some of the *Aspergillus* species, others are accepted to be mitosporic, without any known sexual spore production.

Pleosporales: Pleosporaceae: Alternaria

Alternaria is a cosmopolitan dematiaceous (pigmented) fungus commonly isolated from plants, soil, food, and indoor air environment. The production of melaninlike pigmentation is one of its major characteristics. Its teleomorphic genera are *Clathrospora* and *Leptosphaeria*.

Hypocreales: Hypocreaceae: Acremonium

Acremonium contains, cosmopolitan filamentous fungi commonly isolated from plant debris and soil. The sexual state of Acremonium is not well-defined. Thus, it is classified among the deuteromycetes group of fungi by some authorities. Others prefer to include it in the phylum Ascomycota, since its structural properties are similar to those of this group.

Phylum BASIDIOMYCOTA

Basidiomycota contains a wide variety of organisms. It is estimated that there are about 30,000 species in this group. While it is best known for fruiting bodies such as mushrooms, puffballs, and bracket fungi, it also contains microscopical fungi. These include rust and smut fungi, which are both parasites. Basidiomycota fungi are considered to be the most evolutionarily derived of all fungal phyla. Like Ascomycota, the Basidiomycota also contains some forms of yeast. Therefore, the organisms within this classification can be either unicellular or multicellular. There are three major groups within this classification: Urediniomycetes, which includes rusts and other taxa; Ustilaginomycetes, which are largely composed of smuts; and Hymenomycetes, which are composed of mushrooms and jelly fungi.

Sporidiales: Sporidiobolaceae: Cryptococcus

Cryptococcus is an encapsulated yeast. Following its first identification in nature from peach juice samples, the major environmental sources of *Cryptococcus neoformans* have been shown to be either soil contaminated with pigeon droppings (*Cryptococcus neoformans* var. *neoformans*) or eucalyptus trees and decaying wood forming hollows in living trees (*Cryptococcus neoformans* var. *gattii*). *Cryptococcus neoformans* var. *gattii* was also isolated from goats with pulmonary disease.

Sporidiales: Sporidiobolaceae: Rhodotorula

Rhodotorula is a yeast found in air, soil, lakes and ocean water, and dairy products. It may colonize plants.

Kingdom EUBACTERIA Phylum: PROTEOBACTERIA

Enterobacteriales: Enterobacteriaceae: Serratia

Serratia marcescens is a motile, short, rod-shaped, gram-negative, facultative anaerobe bacterium classified as an opportunistic pathogen. *Serratia marcescens* was first thought to be harmless (non-pathogenic). Optimally, *S. marcescens* grows at 37°C, but it can grow at temperatures that range from 5 to 40°C. It grows at pH levels that range from 5 to 9. *Serratia marcescens* is well known for the red pigmentation it produces, called prodigiosin.

Rhizobiales: Bradyrhizobiaceae: Bradyrhizobium

Members of this genus, including *Bradyrhizobium japonicum*, are gram-negative soil bacteria that fix nitrogen and are commonly associated with legume plants.

Appendix **B**

	NCBI Taxonomy Browser	MycoBank
FUNGI, Ascom	vcota	,
Saccharomyceta	les	
Candida cretensis	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?id=268492	http://www.mycobank.org/BioloMICS.aspx? Table=Mycobank&Rec=432161&Fields=All
Candida mesenterica	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?mode=Info&id=45568& lvl=3&lin=f&keep=1&srchmode=1&unlock	http://www.mycobank.org/BioloMICS.aspx? Table=Mycobank&Rec=106492&Fields=All
Candida sophiae-reginae	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?mode=Info&id=45593& lvl=3&lin=f&keep=1&srchmode=1&unlock	http://www.mycobank.org/BioloMICS.aspx? Table=Mycobank&Rec=105324&Fields=All
Davidiella tassiana	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?mode=Info&id=29918& lvl=3&lin=f&keep=1&srchmode=1&unlock	http://www.mycobank.org/BioloMICS. aspx?Table=Mycobank&Rec=411246&- Fields=All
<i>Pichia</i> sp.	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?mode=Info&id=4925&l vl=3&lin=f&keep=1&srchmode=1&unlock	http://www.mycobank.org/BioloMICS.aspx ?Table=Mycobank&Rec=97263&Fields=All
Pichia delftensis	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?mode=Info&id=3247 39&lvl=3&lin=f&keep=1&srchmode=1&u nlock	http://www.mycobank.org/BioloMICS.aspx? Table=Mycobank&Rec=106343&Fields=All
Pichia misumaiensis	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?id=131113	http://www.mycobank.org/BioloMICS. aspx?Table=Mycobank&Rec=105848&- Fields=All
Pichia membranifaciens	http://www.ncbi.nlm.nih.gov/Taxo- nomy/Browser/wwwtax.cgi?mode=In- fo&id=4926&lvl=3&lin=f&keep=1&srchmo- de=1&unlock	http://www.mycobank.org/BioloMICS.aspx? Table=Mycobank&Rec=108031&Fields=All
Eurotiales		
Aspergillus amstelodami	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?id=5054	http://www.mycobank.org/BioloMICS.aspx ?Table=Mycobank&Rec=9994&Fields=All
Aspergillus versicolor	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?mode=Info&id=46472& lvl=3&lin=f&keep=1&srchmode=1&unlock	http://www.mycobank.org/BioloMICS.aspx ?Table=Mycobank&Rec=2780&Fields=All
Penicillium spinulosum	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?id=63822	http://www.mycobank.org/BioloMICS.aspx ?Table=Mycobank&Rec=19325&Fields=All
Pleosporales		
<i>Alternaria</i> sp.	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?mode=Info&id=13202 40&lvl=3&lin=f&keep=1&srchmode=1&u nlock	http://www.mycobank.org/BioloMICS.aspx ?Table=Mycobank&Rec=31788&Fields=All

Hyperlinks for microorganism identification.

	NCBI Taxonomy Browser	MycoBank
Hypocreales	· · · · ·	
<i>Hypocreales</i> sp. TR114	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?id=929379	
Acremonium psammosporum	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?mode=Info&id=7455 71&lvl=3&lin=f&keep=1&srchmode=1&u nlock	http://www.mycobank.org/BioloMICS.as- px?Table=Mycobank&Rec=485&Fields=All
FUNGI, Basidie	omycota	
Cryptococcus (uncultured)	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?id=526442	http://www.mycobank.org/BioloMICS.aspx ?Table=Mycobank&Rec=54225&Fields=All
Rhodotorula mucilaginosa	http://www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?id=5537	http://www.mycobank.org/BioloMICS.aspx? Table=Mycobank&Rec=107159&Fields=All
BACTERIA, Pr	otobacteria	
Enterobacterial	es	
Bradyrhizobium	http://www.ncbi.nlm.nih.gov/Taxonomy/	
japonicum	Browser/wwwtax.cgi?mode=Info&id=375&lvl =3&lin=f&keep=1&srchmode=1&unlock	
Serratia	http://www.ncbi.nlm.nih.gov/Taxonomy/	
marcescens	Browser/wwwtax.cgi?mode=Info&id=615&lvl =3&lin=f&keep=1&srchmode=1&unlock	

RESEARCH ARTICLE



A revision of the Neotropical genus Paraberismyia Woodley (Diptera, Stratiomyidae, Beridinae) with three new species

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Academic editor: <i>M. Hauser</i>	Received 24 September 2013 Accepted 12 November 2013 Published 20 November 2013

Citation: Woodley NE (2013) A revision of the Neotropical genus *Paraberismyia* Woodley (Diptera, Stratiomyidae, Beridinae) with three new species. ZooKeys 353: 25–45. doi: 10.3897/zookeys.353.6301

Abstract

The Neotropical genus *Paraberismyia* Woodley, 1995, is revised. Three new species, *P. chiapas* sp. n., *P. mathisi* sp. n., and *P. triunfo* sp. n. are described, all having type localities in Chiapas, Mexico. A key to the four known species is provided.

Keywords

Beridinae, Mexico, Neotropical Region, new species, taxonomy

Introduction

Woodley (1995) described the genus *Paraberismyia* based on a single new species, *P. tzontehuitza* Woodley, in the stratiomyid subfamily Beridinae. At the time that the genus was described, a few specimens representing several new species were known. It was hoped that additional material would become available, but since then no new specimens have been collected. The purpose of this revision is to describe the new species that are currently known.

Paraberismyia is known only from the state of Chiapas in southern Mexico and Totonicapán Department in southwestern Guatemala. Specimens with associated altitudinal data have been collected at a minimum of 1300 meters and range to more than 2800 meters. Despite some recent collecting in areas further south in Central America, particularly several extensive surveys at all elevations in Costa Rica, *Paraberismyia* has not been found south of Guatemala.

Methods

Specimens have been utilized from two institutions for which acronyms are given that are used in the specimen data citations:

- **CNC** Canadian National Collection of Insects, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada
- **USNM** Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

Specimens were examined with a Zeiss Stemi SV 11 stereomicroscope. Male terminalia were dissected from specimens relaxed in a humidity chamber for about 24 hours, cleared in hot KOH, neutralized with weak acetic acid, and rinsed with water. The terminalia are preserved in a microvial on the specimen pin. Morphological terminology follows that of McAlpine (1981) as modified by Cumming and Wood (2009). Body lengths are given exclusive of antennae.

Taxonomy

Paraberismyia Woodley, 1995

http://species-id.net/wiki/Paraberismyia

Paraberismyia Woodley 1995: 134. Type species, *Paraberismyia tzontehuitza* Woodley, by original designation.

Diagnosis. Woodley (1995) fully diagnosed and described *Paraberismyia* within the context of the world fauna of Beridinae. It may be separated from all other world genera of beridines except *Allognosta* Osten Sacken and *Berismyia* Giglio-Tos by having distinct hair-like setae around the apical margin of the first antennal flagellomere. *Paraberismyia* may be separated from *Allognosta* because it possesses denticles on the posterior margin of the scutellum, which *Allognosta* lacks. *Allognosta* is also not known to occur in Mexico or Central America. Species of *Berismyia* overlap in distribution with *Paraberismyia*. *Berismyia* are drab colored flies with a brownish to black mesonotum with at most faint metallic reflections and a dark brownish to black, unicolorous abdomen, whereas *Paraberismyia* are more brightly colored with a distinctly metallic mesonotum and an abdo-

men that is bicolored. Also, all known species of *Berismyia* (including several that are undescribed) have the genital capsule with a very long, narrow, posteromedial process on the synsternite that is sharply acute apically (Woodley 1995: 191, fig. 126), while in *Paraberismyia* this process is shorter, broader, and has a rounded apex (Figs 11, 20, 29).

Remarks. The species of *Paraberismyia* are very similar in general structure, differing mainly in characters of coloration and the male terminalia. The coloration differences are very consistent and therefore can be used to accurately identify the species.

Key to species of Paraberismyia

1	Males (eyes contiguous on frons)2
_	Females (eyes widely separated on frons)4
2(1)	Wing cell cup completely covered with microtrichia; at least distal half of
	hind basitarsus dark brown
_	Wing cell <i>cup</i> not completely covered with microtrichia, extensively bare in
	basal half; hind basitarsus completely pale colored, at most with vague dark-
	ening of extreme apex
3(2)	Dorsal half of an pisternum with bare, shiny area without tomentum; hind
. ,	basitarsus completely dark brown, only vaguely pale at extreme base (Fig. 27);
	wing cell r, infuscated, essentially concolorous with cell r,
	<i>P. tzontebuitza</i> Woodlev
_	Dorsal half of anepisternum completely covered with tomentum; hind basi-
	tarsus with basal one-third to one-half pale whitish vellow (Fig. 9); wing cell
	r. weakly infuscated, obviously paler than cell r
4(1)	Wing cell <i>cup</i> completely covered with microtrichia: pleura vellowish to or-
-(-)	angish, not obviously marked with darker coloration (Figs 10, 28)
_	Wing cell <i>cup</i> not completely covered with microtrichia, extensively bare
	in basal half: pleura with obvious dark markings or nearly completely dark
	(Figs 2, 19)
5(4)	Lateral margins of upper frons bare, without tomentum (Fig. 6): dorsal part
2(1)	of occiput (postocular orbit) only narrowly visible in profile with brownish
	grav tomentum that is not conspicuous (Fig. 28): anepisternum on dorsal
	half with hare, shiny area P. tzontehuitza Woodley
_	Lateral margins of upper frons with bands of gravish tomentum along eve
	margins (Fig. 4): dorsal part of occiput (postocular orbit) wide, easily visible
	in profile with conspicuous silvery grav tomentum (Fig. 10): apenisternum
	on dorsal half completely covered with tomentum (1.6, 1.6), antipisterinan
6(4)	Postalar callus postpronotal lobe and laterotergite vellowish orange: abdom-
0(1)	inal sternites 2–5 completely vellowish orange <i>P. chiapas</i> sp. n .
_	Postalar callus and postpronotal lobe with at least some dark brownish col-
	oration, laterotervite black with bluish reflections: abdominal sternites 2–5
	with distinct dark lateral markings
	with distinct dark lateral markings

Paraberismyia chiapas sp. n.

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http://zoobank.org/9426518C-4A9D-4772-B395-4A1E763C2318
http://species-id.net/wiki/Paraberismyia_chiapas
Figs 1–3
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Diagnosis. *Paraberismyia chiapas* can be distinguished from other species in the genus by the combination of having cell *cup* only partially covered with microtrichia and all of the abdominal sternites completely yellowish orange. The other species with cell *cup* only partly covered with microtrichia, *P. triunfo*, has distinct dark markings on the sternites.

Description. Male. Unknown.

Female (Figs 1, 2). *Head:* Black, without metallic reflections except upper frons has very faint greenish reflections (Figs 1, 3); upper frons 0.25 width of head at anterior ocellus; upper frons very finely punctate; lower frons and face densely grayish white tomentose, lower frons with medial, rounded bare area which extends from upper frons, occiput also tomentose except for median occipital sclerite, but tomentum is darker, brownish gray; upper frons with short, sparse pale hairs about one-half length of scape; face with pale hairs about two-thirds length of scape; gena with pale yellowish hairs a little longer than those of face, occiput with pale hairs becoming progressively shorter above gena; eye densely pilose, hairs pale, less than half length of scape; antenna 1.10 times length of head; first two antennal segments with stiff black hairs, longer hairs on flagellum black; palpus yellow, with numerous long hairs, most of which are pale yellowish, a few dark hairs present at apex of second segment; proboscis yellow.

Thorax: Scutum and scutellum dark metallic green (Fig. 1), postpronotal lobe and postalar callus dark yellow; pleura yellowish orange with ventral two-thirds of anepisternum, entire katepisternum and anterior two-thirds of anepisternum, and posterior half of meron brown to brownish black (Fig. 2) and subscutellum and mediotergite similarly colored; scutum and scutellum finely, densely punctate; thorax with inconspicuous pale tomentum present on prothorax, anepimeron, meron, subscutellum and mediotergite, difficult to observe; anepisternum on dorsal half bare and shiny medially; mostly pilose with more or less erect pale hairs, those on scutum and scutellum slightly appressed, about length of pedicel on dorsum, ranging to length of scape + pedicel on pleura with middle of anepisternum, entire katepimeron, meron, mediotergite, and subscutellum bare; legs (Fig. 2) yellowish, but hind femur with brownish area on extreme dorsoapical region, front tibia vaguely suffused with brownish color, hind tibia dark brownish except of extreme base and indistinct area ventrally on proximal half, front and middle tarsi are brownish-black except for basal two-thirds of basitarsi, although yellowish coloration is somewhat obscured by dark pilosity, hind tarsus brownish black but basitarsus is wholly yellow; legs short pilose, posterior surfaces of middle and hind femora with sparse, scattered longer hairs, posteroventral surface of hind tibia with a few longer hairs, coloration of pilosity generally similar to cuticular ground color, except basitarsi have some darker hairs on pale regions; wing hyaline with moderate brownish infuscation on anterior one-third and apex, veins brownish, yellowish



Figures 1–2. Holotype female of *Paraberismyia chiapas* Woodley. I Dorsal view 2 Left lateral view.



Figures 3–6. Frontal view of female heads of *Paraberismyia* species. 3 *Paraberismyia chiapas* Woodley (holotype) 4 *Paraberismyia mathisi* Woodley (paratype) 5 *Paraberismyia triunfo* Woodley (paratype) 6 *Paraberismyia tzontehuitza* Woodley (paratype).

at extreme base of wing; wing entirely covered with microtrichia except cell *cu*p is bare except at base and apex; halter yellowish, knob vaguely suffused with brownish.

Abdomen: Tergites brownish, except first tergite and central two-thirds of tergites 2–6 dark yellow with narrow extensions of yellow reaching lateral margin at anterior corners of tergites 3–5 (Fig. 1); tergite 7 similar but less distinctly marked, tergal grooves on tergites 2–5 brownish black; sternites entirely dark yellow except sternite 8 vaguely infuscated with brown; tergites vaguely, almost imperceptibly tomentose, quite shiny; pilosity of tergites mostly brownish and very short, some pale hairs on tergites 1 and 2, pilosity longer on lateral margins and wholly pale on tergite 1 and intermixed on tergites 2–4; sternites with short, yellowish hairs, a few dark hairs laterally on sternite 6, sternite 7 with pilosity completely brownish; cerci dark yellow, second segment ovoid and slightly shorter than first, with pale yellow hairs on both segments but dark hairs present at apex of second segment.

Length: 7.8 mm.

Distribution. Known only from the state of Chiapas, Mexico.

Type material. Holotype female (USNM), **MEXICO:** Chiapas, El Triunfo (49 km S of Jaltenango, 15°39.4'N, 92°48.5'W), 2000 meters, 13–15.v.1985, Amnon Freidberg. The holotype is missing the right antennal flagellum and the left wing, but is otherwise in good condition.

Etymology. The species epithet, *chiapas*, is a noun in apposition based on the state of Chiapas, Mexico, where the type locality is located.

Remarks. Although general coloration is difficult to characterize, in appearance this species has a more greenish mesonotum, and the yellow coloration is a little more orangish than in the other species.

Paraberismyia mathisi sp. n.

http://zoobank.org/41597C79-F222-4FD8-9EB1-6D2A422158BA http://species-id.net/wiki/Paraberismyia_mathisi Figs 4, 7–15

Diagnosis. *Paraberismyia mathisi* can be distinguished from other species in the genus by having the dorsal half of the anepisternum completely covered with fine tomentum, without a bare, shiny medial area. No other species has this character state. Females have tomentose spots on the lateral margins of the upper frons (Fig. 4) and a relatively broad strip of the occiput (postocular orbit) visible behind the eye in profile covered in conspicuous grayish tomentum (Fig. 10). These two character states are also unique within the genus.

Description. Male (Figs 7, 9). *Head:* Black, without metallic reflections; lower frons and face densely silvery gray tomentose, occiput also tomentose except for median occipital sclerite, but tomentum is sparser and dark, not strongly contrasting with background coloration, median area of upper frons very sparsely tomentose; upper frons with sparse dark hairs, lower frons just above antennae with mostly pale pilosity that is shorter than the first antennal segment; face pilose with mostly dark, longer hairs, with a few yellowish hairs intermixed; gena with pale yellowish hairs a little longer than those of face, occiput with scattered long pale hairs; eye densely pilose, hairs brownish black, about half length of first antennal segment; antenna 0.75–0.85 length of head, first two segments and first flagellomere yellowish, following two flagellomeres yellow internally, apical flagellomeres brownish black; first two antennal segments with stiff black hairs, longer hairs on flagellum black; palpus yellow, with numerous long hairs, those on first segment pale yellowish, second segment with hairs mostly black; proboscis yellow.

Thorax: Scutum and scutellum metallic bluish green (Fig. 7), postpronotal lobe and postalar callus brownish; pleura blackish brown, anepisternum and katepisternum with metallic reflections similar to coloration of mesonotum, posterior margin of anepimeron slightly yellowish; mesonotum finely, densely punctate; thorax with grayish tomentum present over most of prothorax, anterior two-thirds of anepisternum, entire



Figures 7-8. Dorsal views of Paraberismyia mathisi Woodley. 7 Male (paratype) 8 Female (paratype).

katepimeron and meron, anatergite, mediotergite, and subscutellum, most conspicuous on an episternum, which has the dorsal half completely tomentose medially; mostly pilose with long, erect pale hairs, a little longer than first two antennal segments combined, intermixed with short, pale, semi-appressed hairs on scutum and scutellum, with small strip on anterior part of anepisternum, entire katepimeron, meron, anatergite, mediotergite, and subscutellum bare; hind tarsus with tarsomeres 1-3 moderately inflated; legs (Fig. 9) yellowish, except hind femur brown on apical one-fifth, front tibia brownish anteriorly and dorsally, middle tibia with irregular pale brown infuscation, hind tibia entirely brownish black, and all tarsi are brownish-black except middle basitarsus is paler on basal half, and hind basitarsus is yellowish on basal one-fourth to one-half; legs short pilose, posterior surfaces of all femora with longer, erect pale hairs, posteroventral surface of hind tibia with scattered longer, erect hairs, coloration of pilosity similar to cuticular ground color, except narrow apices of femora have blackish hairs; wing hyaline with brownish infuscation anteriorly and apically, but noticeably hyaline in cells c and $r_{2,3}$, cell r_1 brown, veins brownish, yellowish at extreme base of wing; cell *cup* with entire surface covered with microtrichia; halter yellowish, knob slightly darker than stem.

Abdomen: Tergites (Fig. 7) dark brownish with extensive translucent pale area on tergites 2–5, the pale area not discreetly defined, tergal grooves brown, tergite 6 and beyond brown; sternite 1 brown except for wide posterior margin pale yellowish, sternites 2–5 pale yellowish, 4 and 5 with vague lateral brown areas, sternite 6 and beyond brown; tergites vaguely, almost imperceptibly tomentose, quite shiny, with short, blackish pilosity, lateral margins with a fringe of pale hairs longer than antennal flagellum, tergites 5 and beyond with some dark hairs intermixed; sternites with short, yellowish hairs, becoming dark on dark-colored posterior segments.



Figures 9–10. Left lateral views of *Paraberismyia mathisi* Woodley. 9 Male (paratype) 10 Female (paratype).



Figures 11–15. Male terminalia of *Paraberismyia mathisi* Woodley. **11** Genital capsule, dorsal view **12** Gonostylus, lateral view **13** Phallic complex, dorsal view **14** Phallic complex, left lateral view **15** Epandrium and postgenital segments. Abbreviations: *c*, circus; *ep*, epandrium; *ga*, gonocoxal apodeme; *gc*, gonocoxite; *gs*, gonostylus; *pps*, posteromedial process of synsternite.

Terminalia: Gonocoxites (Fig. 11) with lateral margins tapering anteriorly, slightly arcuate, with low, broadly rounded process ventral to gonostylus; gonocoxal apodemes short, not reaching anterior margin of genital capsule; synsternite of genital capsule with triangular-shaped process that is broadly rounded at apex (Fig. 11); gonostylus (Figs 11, 12) slightly arcuate, shorter than in *P. tzontehuitza*, with internal triangular process near apex of ventral margin that is proportionately larger than in *P. tzontehuitza*; phallic complex (Figs 13, 14) trifid, moderately arcuate in lateral view, lateral

lobes nearly parallel, medial lobe only slightly shorter than lateral lobes; epandrium (Fig. 15) narrow, posterior margin evenly rounded; cercus of moderate width, apex moderately rounded.

Length: 5.7-6.1 mm.

Female (Figs 8, 10). Differs from male as follows: *Head:* Frons 0.25–0.28 width of head at anterior ocellus; upper frons (Fig. 4) black with greenish metallic reflections, very finely punctate, with lateral silvery gray tomentose spots, lower frons with inverted triangular bare area which extends from upper frons; occiput (posterior orbit) (Fig. 10) visible in profile, slightly wider than length of scape, densely silvery gray tomentose; pilosity of head shorter than in male, at most one-half length of scape, except on gena; upper frons evenly, sparsely pilose with pale hairs; antenna longer than in male, 0.96–1.04 length of head, flagellomeres 1–3 yellow; palpus more robust, entirely pale pilose except for a few dark hairs at apex.

Thorax: Scutum and scutellum metallic bluish green (Fig. 8), but postpronotal lobe yellow, postalar callus pale brownish, and entire remainder of thorax yellow (Fig. 10), except subscutellum and mediotergite, which are brownish; tomentum as in male, but more whitish on pale cuticular areas; pilosity generally shorter, on scutum at most as long as first antennal segment and without longer, erect hairs; hind tarsus without inflated tarsomeres; leg coloration as in male, but hind femur with a brownish black blotch near apex which is less extensive than in male, front tibia light brownish anteriorly, middle tibiae wholly yellow, hind tibia yellowish to brownish, not as dark as in male, middle basitarsus yellowish on basal half, hind basitarsus yellow except at apex; pilosity of legs similar to that of male, but posterior hairs on femora are shorter; halter pale yellowish.

Abdomen: Tergites (Fig. 8) with yellowish areas not especially translucent, this coloration confined to medial third of tergites 2–5; sternites entirely yellow; cerci small but robust, second segment shorter than first, first segment yellow, second slightly infused with brownish color, hairs yellowish except some on second segment dark.

Length: 5.4–5.6 mm.

Distribution. Known only from the state of Chiapas, Mexico.

Type material. Holotype male (USNM), **MEXICO:** Chiapas, El Triunfo (49 km S of Jaltenango, 15°39.4'N, 92°48.5'W), 1300–2000 meters, 13–15.v.1985, W.N. Mathis. The holotype is in excellent condition. Paratypes (all in USNM): 1 male, 2 females, same data as holotype; 2 males, same data as holotype except collected by A. Freidberg; 2 females, same data as holotype except elevation 1500 meters and collected by A. Freidberg; 1 male, same data as holotype except elevation 2000 meters and collected by A. Freidberg.

Etymology. The species epithet, *mathisi*, is a patronym honoring Wayne N. Mathis of the Smithsonian Institution, who collected part of the type series. Wayne has been a colleague and friend for nearly 40 years. He has collected many interesting Stratiomyidae in the course of his extensive field work.

Remarks. This distinctive species is the smallest in the genus, and has several character states not found in other species of the genus as noted in the diagnosis.

Paraberismyia triunfo sp. n.

http://zoobank.org/1E28150D-6BED-4C78-A7F0-389E016C4A6E http://species-id.net/wiki/Paraberismyia_triunfo Figs 5, 16–24

Diagnosis. *Paraberismyia triunfo* can be distinguished from other species in the genus by having the combination of wing cell *cup* not completely covered with microtrichia and abdominal sternites 2–5 with distinct dark lateral markings. It is also the only species in which the pleura of the female (Fig. 19) is completely dark, without yellow coloration.

Description. Male (Figs 16, 18). *Head:* Black, without metallic reflections; lower frons and face densely silvery gray tomentose, lower frons usually with a very narrow bare median line, occiput also tomentose except for median occipital sclerite, but tomentum is sparser and dark, not strongly contrasting with background coloration, median area of upper frons very sparsely tomentose; upper frons with scattered dark pilosity, lower frons just above antennae with brownish pilosity that is shorter than scape; face pilose with dark, longer hairs about length of scape, sometimes a few pale hairs intermixed; gena with pale yellowish hairs a little longer than those of face, occiput with scattered long pale hairs; eye densely pilose, hairs brownish black, about half length of scape; antenna 0.97–1.07 length of head, first two segments and first two flagellomeres yellowish, third and sometimes fourth flagellomeres yellow internally, apical flagellomeres brownish black; first two antennal segments with stiff black hairs, longer hairs on flagellum black; palpus dark yellow, with numerous long hairs, those on first segment pale yellowish, second segment with hairs mostly black; proboscis yellow.

Thorax: Scutum and scutellum metallic bluish (Fig. 16), but medial portion of scutum postsuturally and sometimes lateral margins of scutum bronzy black, postpronotal lobe and postalar callus brownish; pleura blackish brown, anepisternum and katepisternum with some dull greenish metallic reflections, posterior margin of anepimeron usually brownish; mesonotum finely, densely punctate; thorax with grayish tomentum present over most of prothorax, narrow anterior margin of anepisternum, entire katepimeron and meron, anatergite, much sparser on mediotergite, and dense and conspicuous on subscutellum; anepisternum on dorsal half bare and shiny medially; mostly pilose with long, erect pale hairs, a little longer than first two antennal segments combined, intermixed with short, pale, semi-appressed hairs on scutum and scutellum, with anteromedial part of anepisternum, entire katepimeron, meron, anatergite, mediotergite, and subscutellum without pilosity; hind tarsus with tarsomeres 1-3 weakly inflated; legs (Fig. 18) yellowish, except fore and mid femora can be weakly browned apically, hind femur brown on apical one-fourth to one-half, front tibia usually partly suffused with brownish coloration, hind tibia entirely brownish black, and all tarsi are brownish-black except middle basitarsus is paler on basal three-fourths, and hind basitarsus is yellowish, sometimes vaguely brownish at extreme apex dorsally; legs short pilose, posterior surfaces of all femora with longer, erect pale hairs, posteroventral surface of hind tibia with scattered longer, erect hairs, coloration of pilosity similar to cuticular ground color, except hind basitarsus, fore and mid tibiae, and apices of femora


Figures 16–17. Dorsal views of Paraberismyia triunfo Woodley. 16 Male (paratype) 17 Female (paratype).

have blackish hairs; wing hyaline, evenly infuscate anteriorly and apically, weakly so on posterior part of wing, cell r_1 brown, veins brownish, yellowish at extreme base of wing; cell *cu*p with with microtrichia but a large area medially is bare; halter with stem yellowish, knob dark brown.

Abdomen: Tergites (Fig. 16) dark brownish with extensive medial, translucent pale area on tergites 1–5, wider anteriorly on tergites 3–5, tergal grooves brown, tergite 6 and beyond brown; sternites 1–5 pale yellowish, sternites 2–5 with distinct lateral triangular brown markings, sternite 6 and beyond brown; tergites vaguely, almost imperceptibly tomentose, quite shiny, with short, blackish pilosity, lateral margins with a fringe of dark hairs longer than antennal flagellum; sternites with short, yellowish hairs, mostly dark on lateral markings and becoming dark on dark-colored posterior segments.

Terminalia: Gonocoxites (Fig. 20) with lateral margins tapering anteriorly, arcuate, with low, broadly rounded process ventral to gonostylus; gonocoxal apodemes short, not reaching anterior margin of genital capsule; synsternite of genital capsule with triangular-shaped process that is moderately rounded at apex (Fig. 20); gonostylus (Figs 20, 21) slightly arcuate, shorter than in *P. tzontehuitza*, with internal ventral triangular process on ventral margin that is more medial and proportionately smaller



Figures 18–19. Left lateral views of *Paraberismyia triunfo* Woodley. 18 Male (paratype) 19 Female (paratype).



Figures 20–24. Male terminalia of *Paraberismyia triunfo* Woodley. 20 Genital capsule, dorsal view 21 Gonostylus, lateral view 22 Phallic complex, dorsal view 23 Phallic complex, left lateral view 24 Epandrium and postgenital segments.

than in *P. tzontehuitza*; phallic complex (Figs 22, 23) trifid, strongly arcuate in lateral view, lobes slender, lateral lobes very slightly arcuate medially, medial lobe shorter than lateral lobes; epandrium (Fig. 24) narrow, posterior margin evenly rounded but with angular corners; cercus of moderate width, apex moderately rounded.

Length: 7.2-7.5 mm.

Female (Figs 17, 19). Differs from male as follows: *Head:* Frons 0.20–0.23 width of head at anterior ocellus; upper frons (Fig. 5) black, without metallic reflections, very finely punctate; lower frons with inverted triangular bare area which extends from upper frons that has curved lateral margins; pilosity of head shorter than in male, at most one-half length of scape, except on gena; upper frons evenly, sparsely pilose with pale

hairs; pilosity of face mostly to entirely pale; antenna longer than in male, 1.16–1.30 length of head; palpus more robust.

Thorax: Scutum and scutellum (Fig. 17) dark metallic greenish blue, without prescutellar bronzy area, but postpronotal lobe dark yellow around margins; pilosity generally shorter, on scutum at most as long as first antennal segment and without noticeable longer, erect hairs; hind tarsus (Fig. 19) without inflated tarsomeres; pilosity of legs similar to that of male, but posterior hairs on femora are shorter.

Abdomen: Tergites (Fig. 17) with yellowish areas not especially translucent; sternite 6 variably yellow along anterior margin and/or medially; cerci small but robust, second segment shorter than first, both segments brownish, pilosity dark.

Length: 6.3–7.3 mm.

Distribution. Known only from the state of Chiapas, Mexico and Totonicapán Department in southwestern Guatemala.

Type material. Holotype male (USNM), **MEXICO:** Chiapas, El Triunfo (49 km S of Jaltenango, 15°39.4'N, 92°48.5'W), 1300–2000 meters, 13–15.v.1985, W.N. Mathis. The holotype is in excellent condition. Paratypes (all in USNM): 5 males, 13 females, same data as holotype; 2 males, 2 females, same data as holotype except elevation 1500 meters and collected by A. Freidberg; 5 females, same data as holotype except elevation 2000 meters and collected by A. Freidberg; 3 males, **GUATEMALA:** Totonicapán Department, 14°55'N, 91°22'W, July 1902, Dr. Eisen.

Etymology. The species epithet, *triunfo*, is a noun in apposition based on the name of the type locality.

Remarks. The specimens from Guatemala were labeled "Totonicapán", so it is uncertain if this refers to the department or the city within the department. I have included general geographic coordinates for the department in the specimen data citations above. The Guatemala specimens have a slightly paler overall appearance, but this is likely due to the age of the specimens.

Paraberismyia tzontehuitza Woodley, 1995

http://species-id.net/wiki/Paraberismyia_tzontehuitza Figs 6, 25–33

Paraberismyia tzontehuitza Woodley, 1995: 136.

Diagnosis. *Paraberismyia tzontehuitza* can be distinguished from other species in the genus by having the combination of cell *cup* completely covered with microtrichia and the anepisternum with a bare, shiny medial area. The other species with cell *cup* completely covered with microtrichia, *P. mathisi*, has the anepisternum completely covered with tomentum. Also, the female of *P. tzontehuitza* lacks the tomentose spots along the lateral margins of the upper frons (Fig. 6) that are present in *P. mathisi*.

Redescription. Male (Figs 25, 27). *Head:* Black, without metallic reflections; lower frons and face densely grayish white tomentose, occiput also tomentose except



Figures 25–26. Dorsal views of *Paraberismyia tzontehuitza* Woodley. 25 Male (holotype) 26 Female (paratype).

for median occipital sclerite, but tomentum is dark, not strongly contrasting with background coloration, median area of upper frons very sparsely tomentose; upper frons and lower frons just above antennae with blackish pilosity which is shorter than the first antennal segment; face similarly pilose with somewhat longer hairs, with a few yellowish hairs intermixed; gena with pale yellowish hairs a little longer than those of face, occiput with some long pale hairs and more numerous short, inconspicuous blackish hairs; eye densely pilose, hairs brownish black, about half length of first antennal segment; antenna 1.0 times length of head, antenna with first two segments and first flagellomere yellowish, apical flagellomeres brownish black; first two antennal segments with stiff black hairs, longer hairs on flagellum black; palpus yellow, with numerous long hairs, most of which are pale yellowish, a few dark hairs present toward apex of second segment; proboscis yellow.

Thorax: Scutum and scutellum metallic bluish green (Fig. 25), with vague purplish reflections, lateral portion of postpronotal lobe yellowish, remainder of this and postalar callus brownish; pleura blackish brown with faint metallic reflections, but anepisternum and katepisternum more strongly metallic, not much different in coloration from mesonotum, some sutural regions below wing yellowish; mesonotum rather finely, densely



Figures 27–28. Left lateral views of *Paraberismyia tzontehuitza* Woodley. **27** Male (holotype) **28** Female (paratype).



Figures 29–33. Male terminalia of *Paraberismyia tzontehuitza* Woodley. 29 Genital capsule, dorsal view 30 Gonostylus, lateral view 31 Phallic complex, dorsal view 32 Phallic complex, left lateral view 33 Epandrium and postgenital segments.

punctate; thorax with grayish white tomentum present over most of prothorax, anterior margin of anepisternum, entire katepimeron and meron, anatergite, mediotergite, and subscutellum; anepisternum on dorsal half bare and shiny medially; mostly pilose with long, erect pale hairs, a little longer than first two antennal segments combined, intermixed with short pale hairs on scutum, with middle of anepisternum, entire katepimeron, meron, anatergite, mediotergite, and subscutellum bare; hind tarsus with first three tarsomeres slightly inflated; legs (Fig. 27) yellowish, except front and middle tibiae are brownish beyond basal third, especially distinct on front leg and a broad ring near apex of hind femur, hind tibia except extreme base, and all tarsi are brownish-black, with basitarsi slightly paler near bases; legs short pilose, posterior surfaces of all femora with longer hairs,

posteroventral surface of hind tibia with a few longer hairs, coloration of pilosity similar to cuticular ground color, except apices of femora have blackish hairs; wing hyaline with vague brownish infuscation, especially noticeable in cells br, r_{2+3} , r_4 , r_5 , and widely along veins, cell r_1 brown, veins brownish, yellowish at extreme base of wing; cell *cup* with entire surface covered with microtrichia; halter yellowish, vaguely suffused with brownish.

Abdomen: Tergites (Fig. 25) dark brownish, except medial third of each pale, translucent yellowish, but brownish on each segment beyond tergal groove, the pale area slightly wider anteriorly on segments 2–5, with very narrow extension toward lateral margin, reaching lateral margin only between first and second segments; sternites more extensively yellowish, with small, vague brownish spots on segments 2–5, more brownish beyond segment 5; tergites vaguely, almost imperceptibly tomentose, quite shiny, with short, rather sparse blackish hairs medially, becoming quite long laterally, with a few pale hairs laterally which are about length of antennal flagellum; sternites with short, yellowish hairs.

Terminalia: Gonocoxites (Fig. 29) with lateral margins tapering anteriorly, slightly arcuate, with low, broadly rounded process ventral to gonostylus; gonocoxal apodemes short, not reaching anterior margin of genital capsule; synsternite of genital capsule with triangular-shaped process that is narrowly rounded at apex (Fig. 29); gonostylus (Figs 29, 30) slightly arcuate, with internal triangular process near apex of ventral margin; phallic complex trifid (Figs 31, 32), strongly arcuate in lateral view, lateral lobes slightly arcuate medially, medial lobe distinctly shorter than lateral lobes; epandrium (Fig. 33) narrow, posterior margin weakly rounded; cercus narrow, very slightly arcuate laterally, apex narrowly rounded.

Length: 8.1 mm.

Female (Figs 26, 28). Differs from male as follows: *Head:* Frons 0.22–0.25 width of head at anterior ocellus; upper frons (Fig. 6) black, without metallic reflections, very finely punctate; tomentum similar to that of male, but occiput slightly paler, and lower frons has a medial, rounded bare area which extends from upper frons; pilosity of head shorter, at most one-half length of first antennal segment, except on gena; upper frons evenly, sparsely pilose with brownish black hairs; antenna longer than in male, 1.3–1.5 times length of head, flagellomeres less compact, second and internal surface of third flagellomeres yellowish; palpus more robust.

Thorax: Scutum and scutellum metallic bluish green (Fig. 26), but postpronotal lobe, postalar callus, and entire remainder of thorax yellowish (Fig. 28), except subscutellum and mediotergite, which are brownish; tomentum as in male, but more whitish on pale cuticular areas; pilosity generally shorter, on scutum at most as long as first antennal segment; hind tarsus without inflated tarsomeres; leg coloration as in male (Fig. 28), but front and middle tibiae are wholly yellow, hind femur with a brownish black blotch near apex which is less extensive than in male, hind tibia brownish, not as dark as in male, front and middle basitarsi yellowish on basal halves, hind basitarsus yellow except at apex; pilosity of legs similar to that of male, but posterior hairs on femora are shorter, hairs of hind basitarsus and front and middle tibiae dark, despite yellow cuticular coloration; halter yellowish.

Abdomen: Tergites (Fig. 26) with yellowish areas not especially translucent, first tergite mostly yellow, tergites 2–5 with yellow narrowly reaching lateral margin at bases, tergite 6 with medial yellow spot on anterior half, tergite 7 with vague lateral yellow spots; sternites entirely yellow; cerci with first segment longer than the short-ovoid second segment, first segment yellow, second slightly infused with brownish coloration, hairs pale on first segment, mostly dark on second.

Length: 6.3-8.3 mm.

Distribution. Known only from the state of Chiapas, Mexico.

Type material. I have re-examined the male holotype and 6 female paratypes (all CNC) originally cited by Woodley (1995: 137). The specimens have slightly differing elevations cited on their labels ranging from 9400 feet (2865 m) to 9600 feet (2927 m) from the locality: **MEXICO:** Chiapas, Mt. Tzontehuitz, 16°50'N, 92°35'W. The elevations cited indicate a locality near the summit of the peak

Additional material. One male (USNM), MEXICO: Chiapas, El Triunfo (49 km S of Jaltenango, 15°39.4'N, 92°48.5'W), 1300–2000 meters, 13–15.v.1985, W.N. Mathis.

Etymology. The species epithet, *tzontehuitza* (Woodley 1995: 136), is a noun in apposition referring to the type locality.

Remarks. The male specimen from El Triunfo has the hind femur a little more extensively darkened apically, and the knob of the halter is brownish. The male genitalia are identical to the holotype male so these slight differences are attributable to infraspecific variation.

Acknowledgments

I am grateful to Jeffrey Cumming (CNC) for the loan of type material of *P. tzontehuitza*. I also wish to thank Lucrecia Rodriguez of the Systematic Entomology Laboratory (SEL) for taking the photographic images. Taina Litwak (SEL) prepared the line drawings of the male terminalia, for which I am grateful. USDA is an equal opportunity provider and employer.

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RESEARCH ARTICLE



Genetic variation corroborates subspecific delimitation in the Namib fog-basking beetle, Onymacris unguicularis (Haag) (Tenebrionidae, Coleoptera)

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Academic editor: P. Bouchard | Received 11 September 2013 | Accepted 13 November 2013 | Published 20 November 2013

Citation: Lamb T, Pollard R, Bond JE (2013) Genetic variation corroborates subspecific delimitation in the Namib fog-basking beetle, *Onymacris unguicularis* (Haag) (Tenebrionidae, Coleoptera). ZooKeys 353: 47–60. doi: 10.3897/ zookeys.353.6228

Abstract

The fog-basking beetle, *Onymacris unguicularis* (Haag, 1875), is currently listed as a polytypic form comprising two subspecies. A flightless substrate specialist, the beetle is endemic to vegetationless dunes in the Namib, where southern populations constitute the nominate subspecies, *O. u. unguicularis*, and populations some 300 km to the north compose *O. u. schulzeae* Penrith, 1984. Their taxonomic descriptions are based on minor differences in pronotal and prosternal shape, and the phylogenetic validity of these subspecies has yet to be ascertained. Here we reassess the polytypic status of *O. unguicularis* by (1) examining diagnostic phenotypic characters in conjunction with a geometric morphometric analysis, and (2) conducting phylogenetic analysis of mitochondrial DNA sequences. Our results confirm pronotal and prosternal differences, which are complemented by geometric morphometric resolution of the subspecies. Phylogenetic analysis recovered two reciprocally monophyletic lineages that exhibit perfect phylogeographic congruence with phenotypic variation. Our genetic data identify southern and northern populations as distinct lineages, corroborate morphometric data regarding subspecific delimitation, and therefore support the recognition of *O. u. unguicularis* and *O. u. schulzeae* as valid taxa under the general lineage concept.

Keywords

Subspecies, integrative taxonomy, Namib Desert, Onymacris, Tenebrionidae

Introduction

Darkling beetles (family Tenebrionidae) figure prominently in the arthropod fauna of Africa's Namib Desert, where they compose ~80% of all coleopterans (Louw 1983). Many exhibit unique adaptations to the Namib's substrate, thermal, and moisture conditions (Endrödy-Younga 1978; Seely et al. 2005), the most remarkable of which involves water-gathering behavior practiced by the fog-basking beetle, *Onymacris unguicularis* (Hamilton and Seely 1976). As its common name implies, *O. unguicularis* 'basks' in the advective fogs that characterize this coastal desert and provide an important water source for Namib biota in general (Henschel and Seely 2008). Fog basking typically occurs before dawn, at which time these otherwise diurnal beetles ascend the dunes (at temperatures 20–30°C below optimal activity conditions), tilt headwards into incoming fog, and drink condensate that forms on their dorsum (Hamilton and Seely 1976; Seely et al. 1983). Although fog basking has been observed in a second species from the northern Namib (*Onymacris bicolor* (Haag, 1875)), investigations have centered largely on *O. unguicularis* (Seely et al. 1983, Nøgaard and Dacke 2010; Nøgaard et al. 2012), making it one of the more widely recognized beetles worldwide.

Onymacris unguicularis has also been the subject of taxonomic investigation; Penrith (1984) examined morphological variation throughout the species' range, which is apportioned south to north in a patchy network along the Namib's coastal segment (Fig. 1). Ecologically, these flightless beetles exhibit further restriction, being habitually if not exclusively confined to vegetationless dunes within the desert's major sand seas. Penrith (1984) identified phenotypic distinctions between northern vs. southern populations, which are separated by ~300 km of duneless plains. Based on their morphological differentiation and apparent absence of gene flow, she proposed northern and southern populations be recognized as subspecies (Figs 2-3). Thus, Penrith (1984) designated southern populations as the nominate subspecies and named the northern populations Onymacris unguicularis schulzeae-honoring Lieselotte Prozesky-Schulze, who first reported differences between northern/southern populations on the basis of larval characteristics (Schulze 1964). Penrith's (1984) view of subspecies reflects the classic use of this taxonomic category in recognizing "geographic forms which cannot rank as full species" by noting that her morphological diagnosis could not "on the present evidence, separate the northern population more than subspecifically from southern populations."

Efforts in subspecies delimitation mirror those of species delimitation conceptually if not methodologically and, similarly, engage controversy (Mayr and Ashlock 1991; Mortiz 1994; Burbrink et al. 2000; Zink 2004; Cronin 2006; Phillimore and Owens 2006; Jorgensen et al. 2013). Despite contention over subspecific rank, its taxonomic utility, and evolutionary validity, the category nonetheless remains the sole infraspecific unit recognized by the International Code of Zoological Nomenclature (ICZN 1999). Moreover, certain animal groups (e.g., birds, butterflies, beetles) still contain significant numbers of traditionally-recognized subspecies. Braby et al. (2012) recently provided a critical update of the subspecies concept, justifying viability and recommending that it correspond closely in theory and practice to the general lineage species



Figure 1. Map illustrating the range and disjunct distribution of *Onymacris unguicularis* in the Namib Desert. Subspecies distributions are approximated by oval overlays; localities for genetic sampling, listed from south to north, are: **I** Luderitz **2** Gobabeb **3** Walvis Bay, and **4**, **5** Torra Bay.

concept (de Queiroz 1998, 2007). They proposed its application be restricted to extant groups of populations "representing partially isolated lineages of a species that are allopatric, phenotypically distinct, have at least one fixed diagnosable character state, and that these character differences are correlated with evolutionary independence according to population genetic structure."

Subspecific taxa are now routinely reassessed using molecular phylogenetic analysis as a key component of integrative or coalescent approaches to recover evolutionarily independent lineages. These investigations generally yield one of two outcomes. Patterns of genetic variation may exhibit discordance with traditionally defined subspecies, either phenotypically or geographically, if not both (Burbrink et al. 2000, Zink



Figures 2-3. Dorsal habitus of Onymacris unguicularis unguicularis (2) and O. u. schulzeae (3).

2004, Joyce et al. 2009, Spinks et al. 2013). In effect, the subspecies fail to be recovered as historically independent lineages—their morphological distinctions being reinterpreted as local adaptation, clinal variation, etc.—and are dismissed as valid taxonomic entities. Alternatively, genetic differentiation corroborates the phenotypic variation defining subspecies, in which case researchers may justify trinomial retention (Braby et al. 2012), elevation to full species (Glor and Laport 2012), or some combination thereof (Fuchs et al. 2011).

In this study we examined morphological and genetic variation in *O. unguicularis*, adopting Braby et al.'s (2012) criteria to evaluate the validity of its polytypic status. Our inquiry involved a reassessment of Penrith's (1984) diagnostic morphological characters in conjunction with (1) morphometric analysis of additional phenotypic variation and (2) phylogenetic analysis of mitochondrial DNA sequences.

Materials and methods

Morphological analysis

Penrith's (1984) morphological evidence for subspecific recognition involved shape differences of the pronotum and prosternal process (Figs 4–7). The pronotum is more strongly transverse in *O. u. schulzeae*, and its prosternal process is generally broader, featuring a blunt apex that is largely hidden in lateral aspect (Fig. 8). Conversely, the prosternal process in *O. u. unguicularis* is evident in lateral aspect, its apex often appearing as tooth-like projection (Fig. 9).



Figures 4–7. Pronotum (**4–5**) and prosternum (**6–7**) of *Onymacris u. unguicularis* (**4**, **6**) and *O. u. schulzeae* (**5**, **7**). Measurements for ratio calculations are marked on **5** and **7**.

To reassess Penrith's (1984) diagnostic morphological characters and explore additional aspects of phenotypic variation, we examined a series of pinned specimens from the Ditsong National Museum of Natural History (formerly Transvaal Museum), Pretoria, South Africa. Material included 93 *O. u. unguicularis* representing 11 populations and 30 *O. u. schulzeae* (all paratypes) representing four populations (Appendix). Two and five additional specimens of *O. u. unguicularis* and *O. u. schulzeae*, respectively (representing a portion of our genetic sample), were also examined. Specimens were photographed to provide dorsal and ventral images of each beetle. Images were made using a Visionary Digital Imaging System (Visionary Digital[™], Richmond, VA).

Penrith (1984) quantified pronotal shape differences between subspecies as a ratio of pronotal length divided by pronotal width (PL/PW). We repeated these measurements as part of our morphological analysis, and also quantified differences in the prosternal process as a length/width ratio (Figs 5 and 7), using the software program Image J. Additionally, Penrith (1984) noted possible differences in elytral shape, which appears to be "less elongate, broader, and more abruptly tapered posteriorly" in *O. u. schulzeae*. We therefore used a geometric morphometric analysis to assess putative differences in dorsal (elytral) shape. Mindful that sexual dimorphism may contribute to elytral shape variation, we acknowledge its potential to confound signal attributable to subspecific variation. However, only one species of *Onymacris*, *O. plana* (Péringuey,1888), exhibits pronounced sexual dimorphism in elytral shape; in all others the female's elytra are only "slightly broader than those of the male," with "much overlap"



Figures 8–9. Lateral aspects of the prosternal process depicting a blunt apex (**8**) in *Onymacris unguicularis schulzeae* and toothed apex (**9**) in *O. u. unguicularis*.



Figure 10. Landmarks for the geometric morphometric analysis of dorsal (elytral) shape.

(Penrith 1975). In *O. unguicularis*, frequency distributions of maximal elytral width, expressed as a percentage of elytral length, exhibit complete overlap between the sexes (Penrith 1975), and elytral shape in northern populations has been dismissed as being "scarcely dimorphic" (Penrith 1984). We should note that sexual dimorphism is evident in *O. unguicularis*: males possess longer legs and, uniquely within *Onymacris*, bear setose brushes on the anterior femora (Penrith 1975). Given the species' limited elytral dimorphism and our relatively small sample of *O. u. schulzeae* (n = 35), we elected to combine the sexes in our morphometric analysis.

We identified eleven dorsal landmarks—eight type 1 and three type 3 (Brookstein 1991; Fig. 10)—and used the programs tps-Util and tps-DIG2 (Rohlf 2012) to assemble dorsal (elytral) image files for analysis and score landmarks, respectively. Landmarks were aligned and scaled to size using the generalized least squares Procrustes superim-

position method (Rohlf and Slice 1990), which removes information not relevant to shape (location, scale, and rotational effects). Relative warps were calculated with *a* set to zero, thus weighting all principal warps equally. Superimposition, calculation of relative warps, and calculation of centroid size were preformed using the program MorphoJ (Klingenberg 2011).

Molecular phylogenetic analysis

Sixteen beetles were captured, preserved (100% ethanol), and processed for DNA analysis; the specimens included 12 *O. u. unguicularis*, representing three geographic localities, and four *O. u. schulzeae*, representing two relatively close localities (Fig. 1; Appendix). *Onymacris laeviceps* Gebien, 1938 and *O. plana*, identified as sister taxa to *O. unguicularis* in a generic-level phylogeny (Lamb and Bond 2013), served as outgroups. The mitochondrial genes cytochrome oxidase I (*cox1*) and cytochrome oxidase II (*cox2*) were amplified using the primers and PCR conditions listed in Table 1.

Amplification products were cleaned using exoSAP-IT (USB Corp.) and sequenced on an Applied Biosystems 3130 capillary sequencer. Sequences were edited and assembled in Sequencher 4.9 (GeneCodes, Ann Arbor, MI) and aligned using ClustalX ver. 2.0 (Larkin et al. 2007), after which sequences were translated to ensure a correct reading frame.

We used Bayesian inference (BI) and maximum likelihood (ML) methods to analyze the concatenated gene (*cox1-cox2*) dataset. We used Kakusan 4 (Tanabe 2007) to select nucleotide substitution models for BI, partitioning protein-coding genes by codon position and assessing each gene/codon partition using the Bayesian Information Criteria (BIC4 criterion). BI analysis was conducted in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and involved two concurrent runs of four simultaneous Markov Chain Monte Carlo chains for 20,000,000 generations, with trees sampled every 1,000 generations. Topologies in the first 25% of the posterior distribution were discarded as burn-in. Likelihood values for all post-analysis trees and parameters were evaluated for convergence and burn-in using the "sump" command in MrBayes and the computer program Tracer ver. 1.5 (Rambaut and Drummond; http://evolve.zoo.ox.ac.uk/software.html?id=tracer). Trees remaining after burn-in were used to calculate posterior probabilities using the "sumt" command. The ML analysis, executed in RAxML ver. 7.2.8 (Stamatakis 2006), comprised 1,000 random sequence addition replicates (RAS)

Gene	Primer	Annealing	Cycles	Reference
cox1	TY-J-1460	5000	35	Simon et
	TL2-N-3014	30°C		
	C1-J-2183	sequencing only		
cox2	TL2-J-3037	5000	25	al.(1994)
	TK-N-3785	30°C	55	

Table 1. PCR primers and amplification conditions.

using the commands "-# 1000" and "-m GTRGAMMA." Bootstrap support values were calculated using the same search parameters with 1,000 replicates, and bootstrap results were applied to the best tree recovered in the RAS search.

Results and discussion

Morphometrics

Ratios generated for both the protonal and prosternal datasets differed significantly between subspecies (p < 0.0001), with minimal overlap for each character (Table 2). In the geometric morphometric analysis, the first two principal components based on the non-uniform components of dorsal (elytral) shape account for 78.54% of the variation between subspecies. An ordination plot of PC1 and PC2 revealed that the two subspecies are relatively well separated along the PC1 axis (Fig.11). Dorsal shape separation probably reflects proportionally longer elytra in O. u. unguicularis, which become apparent in side-by-side comparisons with O. u. schulzeae (Figs 2–3). In light of these findings, we measured elytral length and width (at the midpoint of elytral length) for all specimens to determine whether a simple ratio (EL/EW) might reflect the subspecific separation observed in our geometric morphometric analysis. We also noted the position of greatest elytral width for each specimen, scored as midpoint, anterior to midpoint, or posterior to midpoint. Despite broad overlap, elytral ratios differed significantly (p < 0.0001) between subspecies (Table 2); elytral width is widest anterior to midpoint in both subspecies but is positioned closer to the pronotal suture in O. u. schulzeae. Of the three ratios, we consider that for the pronotum to be the strongest diagnostic metric.

Molecular phylogenetics

Concatenated sequence data for the *cox1* (1574 bp) and *cox2* (680 bp) genes yielded eleven haplotypes among the 16 beetles surveyed: eight haplotypes for *O. u. unguicula-ris* and three for *O. u. schulzeae* (Genbank accession numbers KF835703-KF835721). No haplotypes were shared between subspecies. Mean haplotype divergence (calculated from uncorrected pair-wise distance values) was limited across geographic localities for both *O. u. unguicularis* (*cox1* = 0.046%; *cox2* = 0.027%) and *O. u. schulzeae* (*cox1* = 0.008%; *cox2* = 0.014%) but differed substantially between the two subspecies (*cox1* = 3.87%; *cox2* = 3.01%). The BI (harmonic mean $-\ln = 4690.15$) and ML ($-\ln = 4172.41$) analyses generated topologically identical trees in which subspecies were shown to be reciprocally monophyletic (Fig. 12). Moreover, subspecific monophyly was strongly supported (Bayesian posterior probabilities = 1.0; ML bootstraps = 100%), in contrast to the marginal to moderate support observed for haplotype relationships of geographic localities within subspecies (Fig. 12).

Character	Subspecies	N	Mean	Range
	unguicularis	95	1.66 ± 0.08	1.47-1.83
pronotum	schulzeae	35	1.97 ± 0.13	1.73-2.35
	unguicularis	94	2.22 ± 0.17	1.86-2.71
prosternum	schulzeae	33	2.01 ± 0.14	1.65-2.34
1	unguicularis	95	1.44 ± 0.08	1.25-1.61
eiytra	schulzeae	34	1.35 ± 0.07	1.24–1.47

Table 2. Pronotal, prosternal, and elytral ratio means and ranges.



Figure 11. Scatterplot of the principal component scores derived from geometric morphometric analysis of dorsal shape.

Conclusions

We employed Braby et al.'s (2012) integrative approach to evaluate the polytypic status of *O. unguicularis* and found support for each criterion in their template for subspecies delimitation. *Onymacris unguicularis* is an ultrapsammophile confined to major dune fields within the northern (Cunene, Skeleton Coast) and southern (Namib) sand seas. Separated by 300 km of unsuitable substrate, these populations are unquestionably allopatric, satisfying Braby et al.'s first criterion. Regarding criterion two, phenotypic distinctiveness, we confirmed qualitative differences in pronotal and prosternal shape and verified putative distinctions in elytral shape. Patterns in larval variation—the ninth abdominal tergum being shorter and broader in northern populations (Schulze



Figure 12. Bayesian consensus tree showing relationships within *Onymacris unguicularis*. Bold branches subtend nodes with Bayesian posterior probabilities of 1.0; numbers below the branches are ML bootstrap values.

1964)—complement differences in adult morphology and augment the case for phenotypic distinctiveness. Support for Braby et al.'s third criterion, character difference correlations with genetic variation, involves a phylogeographic profile that is perfectly congruent with the north-south partition in phenotypic variation. More importantly, the reciprocal monophyly observed between northern and southern haplotypes (with associated levels of genetic divergence) identifies respective paths of evolutionary independence. These data demonstrate that northern and southern populations of *O. unguicularis* are phylogenetically distinct under the general lineage concept. Thus, we endorse Penrith's (1984) taxonomic interpretation in recognizing *O. u. unguicularis* and *O. u. schulzeae* as valid taxa.

Acknowledgements

We thank Ruth Müller of the Ditsong National Museum of Natural History, Pretoria, South Africa, for arranging specimen loan and shipment in a timely manner. Tom Fink graciously provided time and expertise with digital imagine capture and measurements. This project was supported in part by an East Carolina University Undergraduate Research Award and George T. Barthalmus Undergraduate Research Grant to Rachel Pollard. Specimens used for DNA sequencing were collected under a permit provided by Namibia's Ministry of Environment and Tourism.

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Appendix

Localities for genetic and morphometric samples.

Subspecies	Dataset	Ν	Locality	Voucher numbers*
unguicularis	morphometric	10	Anigab	
		9	Blauberg	
		10	Bogenfels	
		1	Chaneis	
		12	Gobabeb	
		8	Grillental	
		9	Hottentot Bay	
		5	Luderitz	
		10	Spencer Bay	
		10	Swakopmund	
		11	Walvis Bay	
	genetic	4	Dune 7, near Walvis Bay; -22.9691, 14.5946	TL022 TL023 TL024 TL025
		5	Gobabeb; -23.5691, 15.0424	TL026 TL027 TL028 TL029 TL030
		3	20 km E Luderitz; -26.7110, 15.2828	TL031 TL032 TL033
schulzeae –	morphometric	3	near Foz du Cunene, Angola	
		4	Lacrau, 13 km N Fos du Cunene, Angola	
		11	Kaokoveld coast, between Koichab and Unjab rivers	
		12	Unjab River, 8 km from mouth	
	genetic	5	near Torra Bay; -20.3345, 13.2929	
		3	near Torra Bay; -20.3345, 13.2929	TL034 TL035 TL036
		1	near Torra Bay; -20.2738; 13.2655	TL037

*as coded in Genbank

RESEARCH ARTICLE



Two new species and one subspecies of *Craniophora* Snellen, 1867 (Lepidoptera, Noctuidae, Acronictinae) from China

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Academic editor: D. Lafontaine | Received 18 July 2013 | Accepted 22 October 2013 | Published 20 November 2013

http://zoobank.org/A4AFB65E-7E05-4705-B292-9F742F098F6E

Citation: Kiss Á, Gyulai P (2013) Two new species and one subspecies of *Craniophora* Snellen, 1867 (Lepidoptera, Noctuidae, Acronictinae) from China. ZooKeys 353: 61–70. doi: 10.3897/zooKeys.353.5990

Abstract

Three *Craniophora* taxa from China, *C. fujianensis* Kiss and Gyulai, **sp. n.**, *C. fujianensis hainanensis* Kiss and Gyulai, **ssp. n.** and *C. sichuanensis* Kiss, Gyulai and Saldaitis, **sp. n.**, are newly described. Adult habitus and male genitalia are illustrated and compared with those of *C. harmandi* (Poujade) and *C. praeclara* (Graeser). Females of the new taxa are unknown.

Keywords

New taxa, Noctuidae, Acronictinae, Craniophora, China, Fujian, Hainan, Sichuan

Introduction

Craniophora Snellen, 1867 is an Old World genus of the Acronictinae restricted to the Palaearctic, Oriental, Australian and Ethiopian regions (Fibiger et al. 2009). Most of the 20 described species occur in eastern Asia (Poole 1989; Han and Kononenko 2010). Only a few taxonomic studies of the genus have been undertaken (e.g., Hampson 1909; Kozhanchikov 1950; Chen 1999; Han and Kononenko 2010, Kononenko

2010), and most of the publications mentioning *Craniophora* are faunistic works or check lists (e.g., Draudt 1937, 1950; Inoue et al. 1982; Holloway 1989; Kononenko et al. 1998; Yoshimoto 1994; Kononenko 2005). A diagnosis of the genus is given by Fibiger et al. (2009) and Han and Kononenko (2010).

In the subfamily Acronictinae there are two main branches according to the external and genital features. The first branch consists of the genera *Acronicta* Ochsenheimer, *Gerbathodes* Warren, *Moma* Hübner, *Oxicesta* Hübner, *Simyra* Ochsenheimer, *Subleuconycta* Kozhanchikov with diverse external features, but with similar genitalia with a heavily-sclerotised clasping apparatus and a simple structure of the vesica. The second group consists of the genus *Craniophora* with very similar external features, weakly sclerotised valvae and more complex vesica than in the first group.

The majority of the taxa of *Craniophora* and *Acronicta* are separable without checking their genital structures. In smaller species groups, however, the separation of species using external features requires thorough study due to the similarity in forewing pattern and shared features of the typical noctuid maculation. Forewing traits shared by many *Craniophora* and *Acronicta* include the variably strong basal dash, and the black streak of the tornus situated in the submedial fold, often extending from the medial area or the postmedial line to the terminal line. Moreover, in the three main species-groups of *Craniophora*, such as the *pontica-*, *harmandi-* and the *fasciata-*groups (although these have not previously been proposed formally, the common external and genital features suggest it), identification of some species requires detailed study of the genitalia.

Additionally, the species of *Cranionycta* de Lattin, which is a distinct lineage, probably with intermediate position between *Acronicta* and *Craniophora* (distributed in the Russian Far East, China, Nepal) externally can also be very similar to *Craniophora*, although they are usually smaller with narrower wings than most of the *Craniophora* species. The main differences are found in the specific features of the genitalia of both sexes (see de Lattin 1949; Inoue and Sugi 1958; Kononenko et al. 1998); in the males of *Craniophora*, the most conspicuous features are the much broader, but shorter, less sclerotised valvae, slighter corona, more diverticulated or twisted and more or less larger vesica; in the females the corpus bursae is more developed, without signa.

Method

Specimens were collected in China using ultraviolet light traps. Genital slides were prepared following standard techniques (abdominal integument cut lengthwise after KOH maceration male and female genitalia were dissected and mounted in euparal or in Canada balsam on glass slides). Additional material for comparison was borrowed from the Hungarian Natural History Museum, Budapest (HNHM). Additionally, numerous genitalia dissections were examined, both material on loan and own material.

The genital slides were digitalized with an Olympus SZX12 zoom stereo microscope with an Olympus DP 70 digital microscope camera in the Hungarian Natural History Museum, Budapest. After the digitalization, the pictures were converted to greyscale mode

and the unnecessary grey background was deleted by photo editing software (Gimp). The habitus pictures were taken with Nikon D200 with Micro-Nikkor 200mm F/4 lens and Nikon D90 with Nikkor 200mm F/4 lens, after deleted the background by software.

The authors of all of the newly described taxa are the authors of this paper; however, *Craniophora sichuanensis* sp. n. is described in co-authorship with Aidas Saldaitis.

Systematic part

Genus Craniophora Snellen, 1867

Craniophora Snellen, 1867, De Vlinders van Nederland, Macrolepidoptera systematisch beschreven: 262.

Type species. Noctua ligustri [Denis et Schiffermüller], 1775, Ankündung eines systematischen Werkes von den Schmetterlingen der Wienergegend: 70.

Craniophora fujianensis Kiss & Gyulai, sp. n. http://zoobank.org/E032E47F-3F27-44A3-8AEA-78C7683AC46B http://species-id.net/wiki/Craniophora_fujianensis Figs 1, 7, 8

Type material. Holotype: Male (Fig. 1), China, Fujian, Dai Mao Shan, 20 km NW of Longyan, 25°32'N, 116°51'E, 1300 m, 21–30.Nov.2004, leg V. Siniaev and team; slide No.: 3207 Gyulai (coll. P. Gyulai, to be deposited in Hungarian Natural History Museum (HNHM), Budapest). **Paratypes:** None. We exclude specimens of *C. fujianensis* from Hainan, China, as these represent a separate subspecies, described below.

Diagnosis and description. Wingspan 37 mm. *Craniophora fujianensis* resembles *C. praeclara* (Graeser, 1890) (Fig. 6) and especially *C. harmandi* (Poujade, 1898) (Fig. 5) externally. Reliable separation of the three taxa does not require genitalic study, since *C. fujianensis* exhibits unique external characteristics. The shared features of the two related taxa are the more or less similar forewing pattern and noctuid maculation, the presence of the strong or weaker black streaks in the basal area, in the termen and the tornus (the latter streak in the submedial fold, regularly from the medial area or the postmedial line towards the terminal line; the oblique, wavy antemedial line, the double, crenulate postmedial line and the less wavy whitish-grey subterminal line. *Craniophora fujianensis* can be distinguished from *C. praeclara* and *C. harmandi* by its more uniform vestiture of thorax, light brownish-grey (and not chequered white) forewing fringe and the less evenly broad, somewhat shorter blackish streak extending through the submedial fold from the medial area outwards to the lowest part of the terminal line. In comparison with *C. harmandi*, the new species has a more unicolorous, lighter brownish-grey forewing ground colour; lighter, more obsolescent, narrower dark suf-



Figures 1–6. Adults. I *Craniophora fujianensis* sp. n., holotype, male, China, Fujian, coll. P. Gyulai 2 *C. fujianensis hainanensis* ssp. n., holotype, male, China, Hainan, coll. P. Gyulai 3 *C. fujianensis hainanensis* ssp. n., paratype, male, China, Hainan, coll. G. Ronkay 4 *C. sichuanensis* sp. n., holotype, male, China, Sichuan, coll. P. Gyulai 5 *C. harmandi*, male, Nepal, coll. HNHM 6 *C. praeclara*, male, North Korea, coll. HNHM.

fusion in the medial area, conspicuous clear white colouration of the small quadrangular basal spot, which is not confluent with the whitish spot of the costal field; rather ashy grey (and not white), less conspicuous comma-like tiny spot beside the claviform stigma; the stigmata are smaller, the orbicular spot is not evenly white encircled. *C. fujianensis* is distinguished from *C. praeclara* by its smaller average size, more unicolorous, lighter brownish-grey forewing ground colour, without mossy green shades; lighter, more obsolescent, narrower dark suffusion in the medial area; clear white small quadrangular basal spot; less crenulate postmedial line, much smaller, blackish-filled stigmata and especially by the almost white hindwing of the male. **Male genitalia** (Figs 7, 8): a close relationship with *C. harmandi* (Figs 13, 14) is evident; however, the differences are very conspicuous. *C. fujianensis* can be easily distinguished from both of the allied taxa by its much larger, longer uncus, larger juxta and vinculum, large bundle of long hairs on the tegumen, strikingly elongate, curved valvae with straighter dorsal and almost evenly curved (with one angle medially) ventral costa and broader corona with much longer setae. The aedeagus is larger, the vesica ventrally curved; the two medial spines are straight, almost evenly thin and parallel, the third, weaker medial spine weakly sclerotised and hardly visible; whereas the two large spines are oppositely positioned in *C. harmandi*, and the third, medial, cornutus is weaker than the others but stronger than in *C. fujianensis*; *C. praeclara* has no cornuti in the vesica (Figs 15, 16). Additionally, *C. fujianensis* has a tiny semiglobular, sclerotised medial diverticulum, finely serrate on its surface, from which a longitudinal, wavy-ribbed, sclerotised area is situated towards the terminal section of the vesica.

Female. Unknown.

Etymology. The species name refers to Fujian Province, China, where the species was discovered.

Distribution. The species is known from Fujian and Hainan Provinces, China, with the nominate subspecies known only from the type locality in Fujian; subspecies *hainanensis* occurs in Hainan. *Craniophora fujianensis* is the allopatric sister taxa of *C. harmandi*, which occurs from the western Himalaya to Taiwan, in the region with monsoonic influence.

Craniophora fujianensis hainanensis Kiss & Gyulai, ssp. n. http://zoobank.org/3A6BE9D1-5170-4B00-925A-5A8E250C5909 http://species-id.net/wiki/Craniophora_fujianensis_hainanensis Figs 2, 3, 9, 10

Type material. Holotype: Male (Fig. 2), China, prov. Hainan, Wuzhi Shan, 1333 m, 03–10.Jan.2008, leg local collector; slide No.: 3502 Gyulai (coll. P. Gyulai, to be deposited in HNHM, Budapest). **Paratype:** Male (Fig. 3), same data as holotype; slide No.: 3209 Gyulai (coll. G. Ronkay, Budapest, Hungary).

Diagnosis and description. *C. fujianensis hainanensis* is endemic to the island of Hainan. It can be separated at first sight from similar *Craniophora* by its whitish-ochreous forewing ground colour, indistinct wing pattern, with a more doubleangled inner edge of the medial fascia, the whitish fringe and the conspicuous clear white hindwing. Wingspan 35–38 mm. **Male genitalia** (Figs 9, 10): In the male genitalia, ssp. *hainanensis* has a somewhat shorter uncus, with the ventral costa evenly rounded, and a distally more dilated valvae compared to the nominate subspecies. More specimens are needed to evaluate if these differences represent individual or subspecific variation. The two thin medial spines of the vesica are not parallel, but V-shaped, arising from the same sclerotised plate; the tiny semiglobular, medial diverticulum is hardly visible because the surface is not sclerotised or spinulose; the longitudinal, wavy-ribbed, sclerotised area towards the end of the vesica is bifurcate anteriorly then confluent.



Figures 7–12. Male genitalia. 7, 8 *Craniophora fujianensis* sp. n., holotype, male genitalia, China, Fujian, slide No.: 3207 Gyulai, coll. P. Gyulai 7 valvae 8 aedeagus. 9, 10 *C. fujianensis hainanensis* ssp. n., holotype, male, China, Hainan, slide No.: 3502 Gyulai, coll. P. Gyulai 9 valvae 10 aedeagus 11, 12 *C. sichuanensis* sp. n., holotype, male genitalia, China, Sichuan, slide No.: 2883 Gyulai, coll P. Gyulai 11 valvae 12 aedeagus.

Female. Unknown.

Etymology. The name refers to the island of Hainan where this taxon occurs.

Distribution. The subspecies is known only from the type-locality, China, Hainan Island.

Craniophora sichuanensis Kiss, Gyulai & Saldaitis, sp. n. http://zoobank.org/E24039A7-3A32-448D-BEE0-C56FED2881A4 http://species-id.net/wiki/Craniophora_sichuanensis Figs 4, 11, 12

Type material. Holotype: Male (Fig. 4), China, W. Sichuan, road Yaan/Kangding, Erlang Shan Mt., 2200 m, 02.Aug.2011, 29°87.340"N, 102°30.970"E, leg. Floriani and Saldaitis; slide No.: 2883 Gyulai (coll. P. Gyulai, to be deposited in HNHM, Budapest). **Paratypes:** None.

Diagnosis and description. Wingspan 32 mm. Externally most similar to C. harmandi and to a lesser degree to C. fujianensis. The shared features with the two related taxa are the more or less similar forewing pattern and noctuid maculation and the less sinuous whitish-grey subterminal line. It can be distinguished from C. fujianensis by its smaller size, with a wingspan of 32 mm compared to 35-38 mm in the two subspecies of C. fujianensis and 33-40 mm in C. harmandi; the slight black circle in the centre of the thoracic tuft; the white, curved, fine, comma-like basal mark (which is not quadrangular as in the two related taxa); the conspicuous, clear white inner stripe of the medial area along the broad black medial fascia; the more recognisable white outline of the orbicular and reniform stigmata; the longer basal black streak, the diluted blackish streak extending in the submedial fold from the middle of the medial line outward to the lowest part of the terminal line (tornal area) and the more uniform, light brownish-grey hindwing with a faint dark-brown discal spot, sinuous medial line and darker suffused terminal area. Additionally, in comparison with C. harmandi, C. sichuanensis has darker and narrower dark suffusion in the medial area, and lacks the large whitish area extending outward from the reniform stigma toward the apex and in the postmedial line. C. sichuanensis is more distinct from C. fujianensis, especially in the conspicuous clear white inner third of medial area. Male genitalia (Figs 11, 12): Uncus almost evenly slender and apically hooked, valvae spatulate, lacking corona, vesica almost even in width with two equally long, weak, slender spines and one shorter, broader, stout cornutus and the broad, sclerotised distal area covered by numerous almost straight parallel ribs. These genitalia features, as well as the overall smaller male genitalia, shorter, more asymmetrical, medially broadened valvae, and V-shaped vinculum, separate the new species from the two close relatives.

Female. Unknown.

Etymology. The species name refers to the type locality in the Province of Sichuan, China.

Distribution. The new species is known only from the Erlang Shan at the eastern edge of the Tibetan plateau in China's Sichuan province. The single male was collected at ultraviolet light. The new species appeared with a very local distribution, as it was discovered in only one valley in mountainous region. The new species was collected in virgin mixed forest habitat dominated by various broad-leaved trees such as oaks (*Quercus dentata* Thunb., *Q. glauca* Thunb.), poplars (*Populus cathayana* Rheder, *P. simonii* Car-



Figures 13–16. Male genitalia. 13, 14 *C. harmandi*, male genitalia, Nepal, slide No.: KA138m, coll. HNHM 13 valvae 14 aedeagus. 15, 16 *C. praeclara*, male genitalia, North Korea, slide No.: KA054m, coll. HNHM 15 valvae 16 aedeagus.

rière), elm (*Ulmus parvifolia* Jacq.), rhododendrons (*Rhododendron brachycarpum* D. Don ex G. Don, *R. dauricum* L.), and bamboos (*Phyllostachys* ssp., *Borinda* ssp., *Fargesia* spp.). Adults are on the wing with many other late summer Noctuidae species, such as *Pareuplexia chalybeate* (Moore, 1867), *Blepharosis bryocharis* Boursin, 1964, *B. lamida* (Draudt, 1950) and *Amphipyra amentet* Babics, Benedek & Saldaitis, 2013.

Acknowledgements

The authors would like to thank Aidas Saldaitis (Vilnius, Lithuania) and Alessandro Floriani (Milano, Italy) for providing the HT of *Craniophora sichuanensis* to the Hungarian Natural History Museum, Budapest; Zsolt Bálint (Budapest, Hungary) for allowing us the use of a microscope to digitalize the slides, and for loaning material from the HNHM, Budapest; Zoltán Varga (Debrecen, Hungary) and László Ronkay

(Budapest, Hungary) for their suggestions and checking the manuscript; Edvárd Mizsei (Debrecen, Hungary) for providing technical help with the photographs of adults.

This paper was supported by the OTKA (K-84071) and TÁMOP 4.2.4. A/1-11-1-2012-0001 "National Excellence Program – Elaborating and operating an inland student and researcher personal support system." The project was subsidized by the European Union and co-financed by the European Social Fund.

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RESEARCH ARTICLE



Two new species of Abernessia Arlé (Pompilidae, Ctenocerinae)

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Academic editor: Michael Engel | Received 9 September 2013 | Accepted 16 October 2013 | Published 20 November 2013

http://zoobank.org/148EE18B-BDEB-456C-A4A6-6C6A265E8E2E

Citation: Waichert C, Pitts JP (2013) Two new species of *Abernessia* Arlé (Pompilidae, Ctenocerinae). ZooKeys 353: 71–79. doi: 10.3897/zookeys.353.6223

Abstract

Two new species are added to the rare pompilid genus *Abernessia* Arlé. *Abernessia capixaba* **sp. n.** and *A. giga* **sp. n.** are described and illustrated. This is the first record of the genus from the states of Espírito Santo and Minas Gerais, Brazil. The genus now contains four species. A brief discussion of generic characters, illustrations, and a key to the known species of *Abernessia* are provided.

Keywords

Ctenocerinae, Neotropical, new record, spider wasp, taxonomy, key

Introduction

The spider wasps (Pompilidae) form a cosmopolitan family comprised of approximately 5,000 species and more than 120 genera (Pitts et al. 2006), with the greatest species richness in the tropical areas (Wasbauer 1995). Four subfamilies are currently recognized in Pompilidae: Ceropalinae, Ctenocerinae, Pepsinae, and Pompilinae (Pitts et al. 2006).

Ctenocerinae has 18 described genera and is found in South America, Africa, and Australia (Arnold 1932; Evans 1972; Fernández 2000; Waichert and Pitts 2011). Rodriguez (personal communication) has found the first fossil of Ctenocerinae from the Florissant Fossil Beds, Colorado, USA. This fossil indicates a wider distribution of extinct lineages of Ctenocerinae in the New World. Currently, the subfamily is diverse in the Afrotropical region, and rare in the Neotropical region (excluding *Epipompilus* Kohl). Neotropical Ctenocerinae are identified by 1) lacking subapical spine-like setae in grooves or pits on the meso and metafemur; 2) the fore wing with the Cu1 vein simple at the base; 3) the pronotum with dorsal and lateral faces; 4) the clypeus and face flattened and polished with a bilobed clypeus; 5) the face with a deep and broad antennal scrobe; 6) the scale-like setae clothing the integument; 7) and the head with a prolonged vertex (Waichert and Pitts 2011).

The Neotropical genus *Abernessia* was described by Arlé (1947) to include a single female from Rio de Janeiro, Brazil. The genus remained monotypic until Waichert and Pitts (2011) described the first male of Ctenocerinae from the Neotropics. This male was placed in *Abernessia* due to similarities in morphology, especially the flat clypeus that is undifferentiated from the face and the large antennal scrobes. Herein, we describe two species of *Abernessia* – one species based on the female sex and another based on the male sex, which is the second Neotropical record for males in the subfamily. Finally, a key for the genus and illustrations are provided.

Material and methods

Abbreviations used in the descriptions are the same as those used by Wasbauer and Kimsey (1985). They are defined as follows: FD, the facial distance; LA3, the length of third antennal segment; MID, the middle interocular distance; OOL, the ocellocular length; POL, the postocellar length; TFD, the transfacial distance; UID, the upper interocular distance; and WA3, the width of third antennal segment. Measurements of clypeus are as follow: WC, width of clypeus, measured from the widest points; and LC, highest length of clypeus. Additional to the standard measurements, we determined the height of eye, measured in frontal view (HE).

The males described here were collected as part of the project "N.E.S.H. – Nucleus of Excellence in Systematics of Hymenoptera: broadening agricultural and environmental frontiers of Espírito Santo", grant FAPES/CNPq #52263010/2011, coordinated by Dr. Celso O. Azevedo.

Material examined is deposited in Coleção Entomológica da Universidade Federal do Espírito Santo (UFES), Vitória, Brazil and Zoological Museum University of Copenhagen (ZMUC), Copenhagen, Denmark, as indicated.
Systematics

Subfamily Ctenocerinae Dahlbom Genus *Abernessia* Arlé, 1947

Abernessia capixaba sp. n. http://zoobank.org/D39506D7-B032-4242-9223-BC9BCA273E5E http://species-id.net/wiki/Abernessia capixaba

Holotype. \eth (Figs 1–3), pinned, with genitalia in a separate vial, labeled "BRA-ZIL: E[spírito] S[anto], Laranja da Terra, Joatuba, Fazenda Betzel, 19°50'25"S, 40°49'40"W, Malaise Bosque 9, 280–430 m, 05–12.x.2012, M.T. Tavares & eq. col. (UFES #135382)".

Paratypes. 2♂: BRAZIL: E[spírito] S[anto], Laranja da Terra, Joatuba, Fazenda Betzel, 19°50'25"S, 40°49'40"W, 280–430 m, 05-12.x.2012, M.T. Tavares & eq. col., Malaise Bosque 3 (1♂) (UFES #134333), Malaise Bosque 12 (1♂) (UFES #134542).

Diagnosis. This species can be recognized by the following unique combination of characters: the integument is black with scale-like setae reflecting greenish metallic (Fig. 1); the face has small whitish spots on inner margin of the eyes (Fig. 2); and the wing is darkened without pale maculations (Fig. 1).

Description. Body length 2.00 cm; fore wing 1.82 cm; maximum wing width 0. 57 cm. **Coloration.** Integument black with pale yellow maculation on inner margin of eyes; body covered with pubescence with bluish-green metallic reflections (Fig. 1); clypeus, antennae, labial and maxillary palpi black; wings black with weak purple reflections; veins dark castaneous; legs with greenish-purple-blue reflections.

Head. Head wide; TFD 1.2 × FD; MID 0.7 × FD; punctuation conspicuous, small, shallow. Ocelli in obtuse angle; lateral ocelli closer to each other than to compound eyes; POL 0.8 × OOL. Mandible narrow, base about 2.0 × wider than apex, with two sharp apical teeth; 1/3 of base covered by thin copper pubescence. Clypeus undifferentiated from frons, flat, bilobed, apical median margin invaginated (Fig. 2); clypeal lobes rounded (Fig. 2); LC 0.4 × WC. Labrum partially exposed. Maxillary beard not present. Flagellum elongate; length of second flagellomere 2.5 × width; ratio of the scape, pedicel, and flagellomeres 1-2 11:4:14:15; WA3 0.5 × LA3; LA3 0.4 × UID; scape with erect setae on internal margin. Torulus circular, antennal scrobe large.

Mesosoma. Pronotum not elongated (Fig. 1), width $3.3 \times$ length; posterior margin arched, anterior margin slightly invaginated medially; propodeal disc with thinshallow median sulcus. Notauli shallow, present on 1/5 of anterior margin. Postnotum striated. Propodeum with punctures small, almost inconspicuous under setae; propodeal disc covered with short-apressed pubescence, setae equally abundant on propodeal disc; propodeal disc slightly elevated medially, edges of disc rounded. Wing elongate; maximum width $0.3 \times$ length; third submarginal cell about as long as second submarginal cell; second recurrent vein straight, meeting third submarginal cell half distance



Figures 1–3. Male holotype of *Abernessia capixaba* sp. n. **I** Lateral habitus **2** Head, frontal view **3** Apex of metasoma in lateral view.

from base to apex of cell (Fig. 1). Fore tibia with short, sharpened spines, posterior edge angulated. Front tarsal claw bifid, mid and fore claw dentate. Tarsi spinose.

Metasoma. Metasoma covered by short, scale-like setae. Sternum 7 covered by thick, long setae, marginal setae longer than remaining setae, apex of setae sinuous and dilated (Fig. 3).

Genitalia. (Figs 4–6) Parapenial lobe bifid; lobe wide, short, almost shieldlike; outer apex lanceolate, higher than inner apex; inner apex rounded, broad. Dorsal lobe of digitus slightly longer than edeagus, apex wide, rounded, with small extension ventrally; ventral lobe of digitus, short, length $0.3 \times$ paramere length, spatulate, base with long, thin setae (Figs 4, 5). Aedeagus short, total length $0.6 \times$ length of paramere + gonobase, split, lateral margins rolling inwards, apex rounded (Figs 4, 5). Paramere as long as aedeagus, constricted on base, wide apically; apex lanceolate, covered with long setae, inner face flat, outer rounded; setae long, longer marginally, apex dilated. Subgenital plate elongate, wide; apex narrower, rounded; apical margin of apex polished, glabrous; abundant setae, long, thin along entire length (Fig. 6).

Variation. No significant morphological variation was observed.

Etymology. The specific epithet refers to the type locality. *Capixaba* refers to a person born in Espírito Santo State, Brazil.

Remarks. Males of *A. capixaba* are distinguished from those of *A. prima* Waichert & Pitts (2011) by the lack of pale maculation on the metasoma and the fully fuscous



Figures 4–6. Male genitalia, paratype of *Abernessia capixaba* sp. n. 4 Dorsal view 5 Ventral view 6 Genital plate.

fore wing (Fig. 1). In *A. prima* the fore wing is partially yellow and maculations are present on the face, metasoma, and fore wing. Finally, the setae on the subapical metasomal sternite are longer on the outer margin in *A. capixaba*.

Abernessia giga sp. n.

http://zoobank.org/2DB96132-8EE5-498D-936A-DFF88AC8C599 http://species-id.net/wiki/Abernessia_giga

Holotype. ♀ (Figs 7–9), labeled "[BRAZIL]: Minas Gerais, Reinhardt. Mus: Drenis (ZMUC)".

Diagnosis. This species can be recognized by the following unique combination of characters: the integument is black, with scale-like setae reflecting metallic bluish-green (Fig. 7); the antennal scape is red apically (Fig. 8); the clypeus is slightly folded ventrally on the apical-lateral margin, and the wing is dark with a large pale brown band (Fig. 9).

Description. Body length 2.81 cm; fore wing 2.00 cm; maximum wing width 0.63 cm. **Coloration.** Head black; mesosoma black, legs brown with purple reflections (Fig. 7); metasoma brown, tergites distally reddish, hypopygium reddish; body pubescence with bluish-green metallic reflections; wings dark brown with pale brown spots, hind wing with pale brown band (Fig. 9); wing venation brown, pale brown on pale spots; stigma brown (Fig. 9).

Head. Head as long as wide; TFD $1.0 \times$ FD; MID $0.7 \times$ FD; punctuation conspicuous, small, shallow. Pubescence abundant, short, thin, apressed, with metallic reflections from above ocelli to vertex. Eye short, HE $0.6 \times$ FD; vertex long, distance from posterior ocellus to vertex $0.2 \times$ FD. Ocelli in an obtuse angle; lateral ocelli closer to each other than to compound eyes; POL $0.3 \times$ OOL. Mandible wide, elbowed, with two sharpened apical teeth; distal margin with a row of setae. Clypeus undifferentiated from frons, flat, bilobed, apical median margin invaginated; clypeal lobes rounded, sides slightly turned downwards (Fig. 8); LC $0.4 \times$ WC. Labrum partially exposed, setose (Fig. 8). Maxillary beard not present. Flagellum elongate; length of second flagellomere $3.1 \times$ width; ratio of



Figures 7–9. Female holotype of *Abernessia giga* sp. n. 7 Lateral habitus 8 Face, frontal view 9 Fore and hind wings.

the scape, pedicel, and flagellomeres 1-2 15:3:16:14; WA3 0.3 × LA3; LA3 0.4 × UID; scape curved, internal margin flat. Torulus circular, antennal scrobe large.

Mesosoma. Pronotum not elongate (Fig. 7), width $1.6 \times$ length; posterior margin arched, anterior margin slightly invaginated medially; propodeal disc with thin-shallow median sulcus, lateral margins rounded. Notauli shallow, complete. Postnotum striated. Propodeum with punctures small, almost inconspicuous under setae; propodeal disc covered with short-apressed pubescence, setae equally abundant; propodeal disc slightly elevated medially, edges rounded. Wing long; maximum width $0.3 \times$ length; third submarginal cell about as long as second submarginal cell; second recurrent vein straight, meeting third submarginal cell about half the distance from base to apex of cell; 2r-m straight (Fig. 9). Fore tibia with short, sharpened spines, posterior edge angulated. Front tarsal claw bifid, mid dentate (hind tarsi broken in holotype). Tarsi spinose.

Metasoma. Metasoma long (Fig. 7), total length $1.4 \times$ mesosoma + head lengths, wide; covered by short, scale-like setae. Apical tergite setose.

Etymology. The specific epithet was taken from Greek, *giga*, meaning "giant" in English. It refers to the large size of the specimen.

Remarks. This species can be distinguished from *A. irmgardae* Arlé by having the wing brown, with venation both pale and dark brown. In *A. irmgardae* the wing is yellow and the venation is only dark brown. Additionally, the eyes are convergent on vertex in *A. giga* and the vertex expanded unlike in *A. irmgardae*.

Key to the species of Abernessia

Females

1	Wings yellow, slightly darkened at the base; fore wing with pale brown vein;
	head with vertex weakly prolonged posteriorly; eyes not convergent on vertex
	Abernessia irmgardae Arlé
_	Wings darkened, with spots in pale brown (Fig. 9) near the base; fore wing
	with venation both pale and dark brown; hind wing with a pale brown band;
	head with vertex strongly prolonged posteriorly; eyes convergent on vertex
	(Fig. 8)
	801

Males

Discussion

Abernessia is a species-poor genus totaling four described species. All four species of *Abernessia* appear to be restricted to Brazil. Two species have precise collecting data (*A. prima* and *A. capixaba*) indicating that they were collected in ecotones of the Brazilian cerrado and Atlantic forest. Additionally, this is the first record of *Abernessia* in the states of Espírito Santo and Minas Gerais and the first time that more than one specimen was collected in the same locality providing the opportunity to account for morphological variation for the group. Although males and females differ in several features, all four species of *Abernessia* are diagnosed by the following characteristics: the clypeus is flat and undifferentiated from the face; the antennal scrobe is large (Figs 2, 8); the labrum is partially exposed with setae present apically; the metasoma is longer

than the mesosoma; and the pronotal disc is flat dorsally with a median sulcus on the anterior margin. Additionally, males have yellowish pale spots on the inner side of the eyes (Fig. 2); the subapical sternite and paremere have long setae that are swollen on the apex (Fig. 3); and the genitalia has a short base, which gives a false impression of long parameres (Figs 4, 5).

We were unable to associate the sexes of the newly described species. Unfortunately, the type of *A. irmgardae* could not be located for morphological studies. Based on the wing and body coloration, *A. capixaba* could be the male of *A. irmgardae*, and *A. prima* of *A. giga*. However, *A. capixaba* differs from *A. irmgardae* by lacking reddish color on the median flagellomeres and on the apex of tarsi, and by having the wings black, whereas in *A. irmgardae* the wings are yellow but dark brown basally. Additionally, the second submarginal cell is slightly different from the type by having the vein inclined downwards, while in the female type it is bent upwards. *Abernessia giga* resembles *A. prima* by having pale spots on the wing; *A. prima*, however, has a different pattern of wing coloration and presents whitish spots on metasoma. In other genera of Pompilidae (e.g. *Priocnemella* Banks, *Phanochilus* Banks), wing venation and coloration usually matches between sexes of a single species, even when sexual dimorphism is present. Although, wing venation in *Abernessia* seems only slightly variable, differences between specimens are obvious. Because the dimorphism sexual is not understood in the genus yet, we believe it unwise to associate these sexes prematurely.

Acknowledgments

We are grateful to Dr. Celso Azevedo and N.E.S.H program for supporting the trip to study the UFES collection through the grant FAPES/CNPq #52263010/2011; to the ZMUC collection for loaning specimens; to Dr. Felipe Vivallo for sharing information about the type of *A. irmgardae*; to Felipe B. Braga for kindly taking pictures of *A. capixaba*; and to Dr. Lynn Kimsey and an anonymous reviewer for improving this manuscript. This work was supported by the National Science Foundation award DEB-0743763 to JPP and CDvD, and by the Utah Agricultural Experiment Station, Utah State UAES #8599.

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CATALOGUE



A catalog of bird specimens associated with Prince Maximilian of Wied-Neuwied and potential type material in the natural history collection in Wiesbaden

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Academic editor: G. Servat | Received 26 October 2012 | Accepted 12 November 2013 | Published 20 November 2013

Citation: Hoffmann D, Geller-Grimm F (2013) A catalog of bird specimens associated with Prince Maximilian of Wied-Neuwied and potential type material in the natural history collection in Wiesbaden. ZooKeys 353: 81–93. doi: 10.3897/ zookeys.353.4198

Abstract

Bird specimens collected by 19th century explorer and ornithologist Prince Maximilian of Wied-Neuwied form one of the foundation collections of the American Museum of Natural History in New York. However, parts of his collection remained in Germany and came to the Museum Wiesbaden. Since Wied described numerous new species without designating types, some of these specimens might be type material. Here we present a catalog of the 30 Wiesbaden specimens associated with him and discuss their potential type status. We conclude that 17 individuals in 11 species are potential type specimens that should be considered in future taxonomic work.

Keywords

Aves, types, museum specimens, Brazil, Mata Atlântica

Introduction

The natural history collection at the Hessian state museum in Wiesbaden (MWNH) owns several specimens that either originate from the collection of Prince Maximilian of Wied-Neuwied or were described by him as new species. In the early 19th cen-

tury Wied was among the first explorers to travel to Brazil and the especially diverse ecoregion Mata Atlântica, where he collected large numbers of ethnographic objects, plants and animals. In 1870 part of the Wied collection comprising 4,000 birds, 600 mammals and 2,000 fish and reptiles was purchased by the American Museum of Natural History in New York (AMNH) and constitutes the cornerstone of its scientific collection (Myers 2000).

Besides mammals, reptiles, and amphibians, Wied described 160 species and subspecies of birds. The names of more than 50 of these taxa are still valid today (Encyclopedia of Life). Potential type specimens of the majority (ca. 120) could be recognized at the AMNH. At least some of the specimens presented here came to the collection in Wiesbaden in Wied's lifetime. The AMNH type catalogs published to date (Allen 1889, 1891; Greenway 1973, 1978, 1987; LeCroy and Sloss 2000; LeCroy 2003, 2005, 2012) do not offer any information on material in Wiesbaden. However, we must assume that more material exists outside the AMNH. To begin with, the corresponding types of 40 taxa are not in the AMNH collection, and furthermore, Wied himself recorded only limited information on the material he studied and, as was common practice at that time, did not designate types. A systematic research for more material is still outstanding, and since at that time it was usual to barter, trade and give away undocumented specimens, some surprising discoveries may still be expected. Berger (1995) reports later divestitures of material by the AMNH to other institutions, like the Smithsonian in Washington, D.C. De Avila-Pires (1965) and Engländer (1995) mention other collections; for instance besides New York, mammal types have also been deposited in Leiden and Paris.

Like at the AMNH, in Wiesbaden as well many of the originally mounted specimens were later dismounted and added to the study skin collection. Most of the series of the main Wied collection at the AMNH still bear their original labels, but not every individual specimen has such a label by Wied (see Carter 1942). It appears that in Wiesbaden no original labels are preserved. Although this is unfortunate and complicates interpretation, it does not disqualify the material as potential types, since bartered series often remained unlabeled and at the museums labels were replaced in the course of time (which of course today would be an unpardonable sin).

Wied's work on birds is significant not only because of the huge number of species and forms described for the first time, but it also gives information on distribution and biology of numerous animals. Even today records on the biology of many organisms are completely lacking. As Berger (1995) notes, Wied's diaries remain unstudied to date, even though they most certainly contain further biological details on many animals. Unfortunately, most of his travel journals and handwritten catalogs are privately owned and not accessible. Even so, it is important to make at least his museum specimens known to a broader scientific public, since several of Wied's taxa are still in taxonomic transition today.

Catalog

In our catalog we follow the systematic classification of the *Handbook of the Birds of the World* (del Hoyo et al. 1992–2011). Additionally, English and German names are given. The data of the original labels are recorded in full and unaltered, if necessary supplemented by details from the inventory catalog and the digital database of the MWNH.

Different types of printed labels are indicated as follows:

- [*] Label "Naturhist. Museum Wiesbaden." or "Naturh. Museum Wiesbaden", after 1917;
- [**] Display label, after [*];
- [***] Label " Neues Museum Wiesbaden Naturwissenschaftliche Sammlung", after 1937.

A. Bird specimens from Prince Maximilian of Wied-Neuwied in the MWNH collection, definitely not type material (Plate 1)

1. Sterna paradisaea Pontoppidan, 1763 (Charadriiformes – Sternidae)

Arctic Tern – Küstenseeschwalbe

Inv. nr. 2209: 1 ad., breeding plumage, mounted specimen

Labels: a) [*] 2209. Cat. Birds Br. Mus. XXV, 62. *Sterna macrura* Naum. Südamerika Frühjahr 1846 G.: Prinz Max v. Wied; b) [**] 2209 Rchw. 1, 116. *Sterna macrura* Naum. Küsten-Seeschwalbe (Sommerkleid) Süd-Amerika G.: Prinz Max v. Wied.

2. Anser albifrons albifrons (Scopoli, 1769) (Anseriformes – Anatidae)

Greater White-fronted Goose – Bläßgans

Inv. nr. 2504: 1 fem. juv., mounted specimen

Labels: a) Anser albifrons, Bechst. Bläßgans ♀ N.Europa; b) [*] 2504. Cat. Birds Brit. Mus. 27 pag. 92. Anser albifrons (Scop.) ♀ N.Europa Novbr. 1847 G.: Prinz Max v. Wied; c) [**] 2504. Anser albifrons (Scop.) Weißstirngans, Bläßgans ♀ juv. Europa XI/1847 G.: Prinz Max v. Wied

3. Meleagris gallopavo osceola Scott, 1890 (Galliformes – Meleagrididae)

Florida Wild Turkey – Florida-Truthuhn

Inv. nr. 1853: 1 fem. ad., mounted specimen

Labels: a) *Meleagris Gallopavo* Nord-Amerika; b) [*] 1853 Cat. B. Br. Mus. XXII, 389/90 *Meleagris americana* Bartr. subsp. *osceola* Scott ♀ Nord-Amerika 1835. G.: Prinz Max v. Wied; c) [**] 1853 Rchw. 1, 304. *Meleagris americana* Bartr. subsp. *osceola* Scott Wildes Truthuhn Nord-Amerika S.G.: S. H. Prinz M. v. Wied



Plate 1. Bird specimens from Prince Maximilian of Wied-Neuwied in the MWNH collection that are definitely not type material. Clockwise from upper left: *Sterna paradisaea, Anser albifrons, albifrons, Meleagris gallopavo osceola, Aulacorhynchus prasinus atrogularis, Colaptes auratus auratus*-group, *Melanerpes erythrocephalus, Sporophila caerulescens.*

4. *Aulacorhynchus prasinus atrogularis* (Sturm, 1841) (Piciformes – Ramphastidae) Emerald Toucanet – Laucharassari

Inv. nr. 848: 1 ad., mounted specimen

Labels: a) *Pteroglossus atrogularis* Gould. Amerika Von Prinz Max erkauft(?) [purchased from Prince Max]; b) [*] 848. Br. C. B. XIX p. 160 *Aulacorhamphus atrogularis* Sturm S.Amerika S. Prinz Max v. Wied; c) [**] 848 Rchw. 2, 37. *Aulacorhynchus atrogularis* (Sturm.) Schwarzkehliger Arassari Südamerika.; on pedestal: 1835 ans Museum [to the museum in 1835]

5. Colaptes auratus auratus-group (Linnaeus, 1758) (Piciformes – Picidae)

Yellow-shafted Flicker - Goldspecht

Inv. nr. 1002: 1 fem. ad., study skin

Labels: a) *Picus auratus* Nord-Amerika; b) [*] 1002 Cat. Birds Brit. Mus. 18.p.12. *Colaptes auratus* (L.) \bigcirc Nord-Amerika; catalog: Prinz Max von Wied, 1835

6. Melanerpes erythrocephalus (Linnaeus, 1758) (Piciformes – Picidae)

Red-headed Woodpecker – Rotkopfspecht

Inv. nr. 1027: 1 ad., study skin

Label: [***] Kat.Nr. 1027 Br.C.B. XVIII 145 *Melanerpes erythrocephalu* (L) Rotkopfspecht Nord-Amerika; database: erworben 1835 von Prinz Max v. Wied [purchased 1835 from Prince Max of Wied]

7. Sporophila caerulescens (Vieillot, 1823) (Passeriformes – Thraupidae)

Double-collared Seedeater – Schmuckpfäffchen

Inv. nr. 5238: 1 male ad., study skin

Label: [***] Kat.Nr. 5238. *Sporophila ornata* (Licht.) \mathcal{E} Brasilien leg. Prinz Max v. Wied; reverse: *Sporophila* (-) *caerulescens* (Vieill.) Schmuckpfäffchen

B. Bird specimens from Prince Maximilian of Wied-Neuwied with potential type status (Plates 2 and 3)

1. Touit melanonota (Wied, 1820) (Psittaciformes – Psittacidae)

Brown-backed Parrotlet - Braunrückenpapagei

Inv. nr. 748: 1 ad., mounted specimen

Syn.: Psittacus melanonotus Wied, 1820: 275; Urochroma melanota (Stephens, 1826)
Labels: a) Psittacus melanotus Brasilien; b) [*] 748 Br.C.B. XX. p. 352. Urochroma wiedi Allen Brasilien.; c) [**] 748 Rchw. 1, 484. Urochroma melanota (Lcht.) Wied Schwarzrückiger Zwergpapagei Brasilien.; catalog: Prinz Max v. Wied, 1835

Remarks: According to the original description, Wied saw several animals and noted that this new species was displayed at the Berlin museum under the name *Psittacus melanonotus*. Berger (1995: 291 with pictures from the AMNH, inv. nr. 6302) states that there



Plate 2. Bird specimens from Prince Maximilian of Wied-Neuwied with potential type status. Clockwise from upper left: *Touit melanonota, Glaucidium minutissimum, Spizaetus tyrannus* (mounted), *S. tyrannus* (study skin), *Conopias trivirgatus, Lipaugus vociferans* (mounted), *L. vociferans* (study skin), *Todirostrum poliocephalum*.

is one type specimen in New York. According to M. LeCroy (AMNH, pers. comm.), this type was listed by Allen (1889: 264-265) but, when included in the genus *Psittacus*, Wied's name was preoccupied by *Psittacus melanotus* Shaw, 1804. Allen provided a replacement name, *Urochroma wiedi*. Wied's form is now placed in the genus *Touit* (del Hoyo et al. 1997, Vol. IV: 456) and his species name, *melanonotus*, is being used again. *Urochroma wiedi* Allen 1889 is a synonym sharing the same type/types. Greenway (1978: 86) was apparently in error in listing AMNH 6302 as a holotype, as Allen (1889: 265) noted that there was at least one additional syntype and perhaps others. Presently there is nothing to be said against the assumption that the specimen at the MWNH is also a syntype.

2. Glaucidium minutissimum (Wied, 1830) (Strigiformes - Strigidae)

Least Pygmy-owl - Kleinstzwergkauz

Inv. nr. 202: 1 ad., study skin

Syn.: Strix minutissima Wied, 1830: 242

Label: [*] 202 G. (Glaucidium) minutissimum (Wied) Zwergkauz Brasilien

Remarks: According to the original description, Wied examined males and females, but does not give any information about the quantities or the whereabouts. Allen (1889: 266) and Greenway (1978: 126) report two syntypes of *Strix minutissima* at the AMNH with the inventory numbers 6345 (male) and 6345bis (female). The MWNH specimen might belong to the original type series, although there is no proof.

3. Spizaetus tyrannus (Wied, 1820) (Falconiformes – Accipitridae)

Black Hawk-eagle – Tyrannenadler

I. Inv. nr. 2989: 1 male ad., mounted specimen

II. Inv. nr. 2990: 1 juv., study skin

Syn.: Falco tyrannus Wied, 1820: 360

Labels: I. a) No. 109 Falco Tyrannus Pr. Max Temm. Pl. col. 73. Seite 61 Cuv. Seite 384. Iris gelb.; b) [*] 2989. Brit. Cat. I. 264 Spizaetus tyrannus Wied. I Surinam; c) [**] 2989 Rchw. 1, 386. Spizaetus tyrannus (Wied.) Tyrann-Adler I Surinam.; II. [***] Kat.Nr. 2990 R. I. 386 Spizaetus tyrannus (Wied) Tyrann-Adler Surinam

Remarks: According to the original description, Wied had at least one male at his hands. The AMNH has a specimen (inv. nr. 6381) that Greenway (1973: 270) called a lectotype. Of the specimens at the MWNH, the more recent labels give "Surinam" as the country of origin, but the older label contains a reference to "Pr. Max". Wied may have had specimens from Surinam in his collection that he did not collect himself (M. LeCroy, pers. comm.). Thus it appears quite possible that these two specimens are type material.

4. Conopias trivirgatus (Wied, 1831) (Passeriformes – Tyrannidae)

Three-striped Flycatcher - Olivbrust-Maskentyrann

Inv. nr. 5313: 1 ad., study skin

Syn.: Muscicapa trivirgata Wied, 1831: 871

Labels: a) *Muscicapa trivirgata* unserer(?) Beiträge; b) *Muscicapa trivirgata* M. v. Wied, Brasilien; c) [*] 5313. Cat. Birds Brit. Mus. C. (Conopias) trivirgata (Wied) Subsp. Dreistreifentyrann Brasilien



Plate 3. Bird specimens from Prince Maximilian of Wied-Neuwied with potential type status, continued. Clockwise from upper left: *Conopophaga lineata, Myrmeciza ruficauda, Cyanocorax cyanopogon, Tangara velia cyanomelas, Coryphospingus pileatus, Schistochlamys ruficapillus capistratus, Thraupis palmarum palmarum* (mounted), *T. p. palmarum* (study skin), *Oryzoborus maximiliani.*

Remarks: According to the original description, Wied examined one female from Bahia. Greenway (1987: 34) listed a female holotype at the AMNH under the number 4926. Allen (1889: 234) noted that "Femina" was not written in Wied's catalog, but AMNH 4926 is sexed as a female. Since Wied himself only mentions one female, the specimen at the AMNH probably is the holotype.

5. Lipaugus vociferans (Wied, 1820) (Passeriformes – Cotingidae)

Screaming Piha – Tiefland-Graupiha

I. Inv. nr. 150: 1 ad., mounted specimen

II. Inv. nr. 3989: 1 fem. ad., study skin

Syn.: Muscicapa vociferans Wied, 1820: 242

Labels: I. a) [*] 150 Cat. Birds Brit. Mus. 14 p.352. *Lathria cinerea* (Vieill.) Surinam; b) [**] 150 Rchw. 2, 191. *Lipaugus cinereus* (Vieill.) Surinam.; II. [*] 3989. R. II. 191. *Lipaugus cinereus* (Vieill.) Brasilien G.: Geschw. Brambeer.; reverse: Cotingidae

Remarks: In the original description Wied does not state the quantity of examined specimens. According to him, this new species was displayed at the Berlin museum under the name *Muscicapa ampelina*. Greenway (1987: 42) listed two syntypes in the AMNH collection, one female with the number 5099 and one male with 5098. Allen (1889: 239) misquotes the numbers as 5198 and 5199. Due to the reference "G: Brambeer" the study skin (inv. nr. 3989) at the MWNH is probably not a type specimen. The mounted specimen (inv. nr. 150) might be type material, since Wied may have used specimens that he did not collect himself (see above).

6. Todirostrum poliocephalum (Wied, 1831) (Passeriformes – Tyrannidae)

Grey-headed Tody-flycatcher - Gelbzügel-Todityrann

I. Inv. nr. 120 a: 1 ad., study skin

II. Inv. nr. 120 b: 1 ad., study skin

III. Inv. nr. 120 c: 1 ad., study skin

Syn.: Todus poliocephalus Wied, 1831: 964

Labels: I. a) *Todus poliocephalus*, Pr. Max Grauköpfiger Plattschnabel Brasilien; b) [*] 120 3/a Cat. Birds Brit. Mus. 14 p. 71. *Todirostrum poliocephalum* (Wied) Brasilien.; II. a) *Todus poliocephalus* Max v. Wied. Grauköpfiger Plattschnabel Brasilien; b) [*] 120 3/b Cat. Birds Brit. Mus. 14. 71 *Todirostrum poliocephalum* (Wied.) Brasilien; III. a) *Todus policephalus*, Max v. [abgeschnitten] Grauköpfiger Plattschnabel Brasilien; b) [*] 120 3/c Cat. Birds Brit. Mus. 14 p. 71 *Todirostrum poliocephalum* (Wied) Brasilien.; reverse of b) (all): Tyrannidae

Remarks: In the original description Wied examined males and females, but did not note the quantities and whereabouts. Allen (1889: 228) listed a male (nr. 6790) and a female syntype (nr. 6791) in the AMNH collection. The original Wied label pasted to the reverse of the AMNH label on the male indicates both sexes and originally served for both specimens. The three MWNH specimens might well stem from Wied and must hence be regarded as potential type material. 7. Conopophaga lineata (Wied, 1831) (Passeriformes – Conopophagidae) Rufous Gnateater – Rotkehl-Mückenfresser I. Inv. nr. 106 a: 1 ad., study skin II. Inv. nr. 106 b: 1 ad., study skin III. Inv. nr. 107: 1 ad., study skin Syn.: Myiagrus lineatus Wied, 1831: 1046 Labels: I. [*] 106a. Cat. Birds Brit. Mus. 15. p. 333 Conopophaga lineata (Wied)

Brasilien; II. [*] 106b. Cat. Birds Brit. Mus. 15 p. 333. *Conopophaga lineata* (Wied.) Brasilien; III. [*] 107 Cat. Birds Brit. Mus. 15 p. 333. *Conopophaga lineata* (Wied.) Brasilien

Remarks: In the original description Wied mentions only one individual. The AMNH has one female holotype (Nr. 6777) listed by Allen (1889: 256; see also LeCroy and Sloss 2000: 65). Since Wied referred to only one individual, and as we have no proof that the MWNH specimens originate from him, they are probably not type material.

8. Myrmeciza ruficauda (Wied, 1831) (Passeriformes – Thamnophilidae)

Scalloped Antbird – Nördlicher Schuppenameisenvogel

Inv. nr. 121: 1 ad., mounted specimen

Syn.: Myiothera ruficauda Wied, 1831: 1060

Labels: a) *Formicivora loricata* ♀ Swains. Bahia; b) *Formicivora* ♂. Bahia. 3. Gust. Schneider, Basel.; c) [*] 121. Cat. Birds Brit. Mus. 15 p. 281. *Myrmeciza ruficauda* Wied Bahia S.: G. Schneider, Basel.; d) [**] 121 Rchw. 2, 231. *Myrmeciza ruficauda* Wied. Bahia.

Remarks: In the original description Wied examined males and females, but does not specify the quantities and whereabouts. There are four syntypes at the AMNH: males nr. 5388 and 6829, juvenile male nr. 5386 and female nr. 5385 (Allen 1889: 254; LeCroy and Sloss 2000: 56-57). Because of its origin the specimen at the MWNH can be ruled out as type material.

9. Cyanocorax cyanopogon (Wied, 1821) (Passeriformes – Corvidae)

White-naped Jay – Weißnacken-Blaurabe

Inv. nr. 609: 1 ad., mounted specimen

Syn.: Corvus cyanopogon Wied, 1821: 137

Labels: a) *Corvus cyanopogon* Brasilien; later altered: *Cyanocorax Corvus cyanopogon* (Max Neuwied) III, 123.; b) [*] 609 Br. C. B. III p. 123 *Cyanocorax cyanopogon* (Neuwied) Brasilien.

Remarks: In the original description Wied had several individuals at hand. Allen (1889: 227) lists two syntypes (juvenile female nr. 6773 and male nr. 6774) at the AMNH. It cannot be excluded that the mounted specimen at the MWNH is also type material.

10. Tangara velia cyanomelas Wied, 1830 (Passeriformes – Thraupidae)

Silvery-breasted Tanager - Rotbauchtangare; group of 3 individuals

I. Inv. nr. 3792: 1 male ad., mounted specimen

II. Inv. nr. 3793: 1 fem. ad., mounted specimen

III. Inv. nr. 3794: 1 fem. ad., mounted specimen

Syn.: Tangara cyanomelas Wied, 1830: 453

Labels: a) *Tanagrella velia* $\Diamond \heartsuit$? Gmel Bahia; b) [*] 3792/94 Brit.Cat.11 p. 88. *Tanagrella cyanomelaena* (Wied.) $\Diamond \heartsuit \heartsuit$ Bahia; c) [**] 3792/94 Rchw. 2, 436. *Calospiza cyanomlaena* (Wied.) 1 \Diamond , 2 \heartsuit Bahia, Brasilien.

Remarks: In the original description Wied mentions several males, while the female was unknown to him. According to Allen (1889: 218) and LeCroy (2012) there are no types at the AMNH. If no other material turns up, the specimens at the MWNH must be classified as potential types. First of all, they need to be sexed, as Wied had no females at hand. The sexes assigned on the labels were possibly inferred from the individuals' positions (male on top, females below) by a curator. On first glance the three specimens are indistinguishable and must be compared to a series.

11. Coryphospingus pileatus (Wied, 1821) (Passeriformes – Thraupidae)

Pileated Finch - Graurückenkronfink

Inv. nr. 5102: 1 male ad., study skin

Syn.: Fringilla pileata Wied, 1821: 160

Label: [*] 5102. Cat. Birds Brit. Mus. *Tanagra cristatella* Spix. A Brasilien; reverse: Tanagridae = Thraupidae *Coryphospingus pileatus* (Wied) Subsp. Graurücken-Kronfink

Remarks: In the original description Wied described the male without giving details on quantity or whereabouts of the specimens. According to Allen (1889: 225) and LeCroy (2012) there are three male syntypes at the AMNH (nr. 4618, 4619, and 4621). Comparison of the skins at the AMNH and the MWNH should help to decide whether the specimen in Wiesbaden, with identical or similar taxidermy, belongs to the type series.

12. Schistochlamys ruficapillus capistratus (Wied, 1821) (Passeriformes – Thraupidae)

Cinnamon Tanager – Gimpeltangare

Inv. nr. 5095: 1 ad., study skin

Syn.: Tanagra capistrata Wied, 1821: 179

Label: [*] 5095. Brit.Cat.11 p. 301. *Schistochlamys capistratus* (Max) Brasilien.; reverse: Tanagridae = Thraupidae

Remarks: In the original description, and also in 1831 (p. 500), Wied examined males and females, but did not note specifics on quantities and whereabouts. According to Allen (1889: 222) and LeCroy (2012) the AMNH has only one male syntype (nr. 6861). Since at least the female is still missing, and also the number of individuals remains unclear, the specimen in Wiesbaden may well be classified as potential type material. In this species the sexes look alike, so it will be difficult to determine the specimen's sex.

13. Thraupis palmarum palmarum (Wied, 1821) (Passeriformes – Thraupidae)

Palm Tanager – Palmentangare

I. Inv. nr. 43: 1 ad., mounted specimen

II. Inv. nr. 5096: 1 ad., study skin

Syn.: Tanagra palmarum Wied, 1821: 76

Labels: I. a) *Tanagra palmarum* Mexiko; b) [*] 43 Brit.Cat. 11 p. 159. *Tanagra palma-rum* Max. Brasilien. Mexiko auf alt/Schauetik.; c) [**] 43 Rchw. 2, 435. *Tanagra palmarum* Max. Brasilien.; II. [*] 5096. Brit.Cat.11 p.159. *Tanagra palmarum* Max. Brasilien.

Remarks: In the original description Wied refers to both sexes, but does not specify the quantity and whereabouts of the examined specimens. Allen (1889: 219) notes a male syntype at the AMNH (nr. 6765), which LeCroy (2012) confirms. Both specimens at the MWNH are unsexed. The oldest label of nr. 43 gives Mexico as origin, while the more recent ones point out a mistake. The study skin is a formerly mounted specimen and does not have an old label. Whether or not these specimens are type material cannot be determined.

14. Oryzoborus maximiliani Cabanis, 1851 (Passeriformes – Thraupidae)

Great-billed Seed-finch - Dickschnabel-Reisknacker

Inv. nr. 5235: 1 fem. ad., study skin

Syn.: Oryzoborus crassirostris Wied, 1830: 564, preocc. Oryzoborus crassirostris (Gmelin, 1789)

Labels: a) *Fringilla crassirostris*, Max v. Wied *Pyrrhula crassirost*. \bigcirc Brasilien; b) [*] 5235. Brit.Cat.12 p. 78 *Oryzoborus maximiliani* Cab. \bigcirc Brasilien.

Remarks: Unfortunately we could not obtain the original description. Cabanis (1851: 151) replaced the homonym. According to Allen (1889: 222) and LeCroy (2012) there is no specimen at the AMNH. If no other specimen turns up, the study skin at the MWNH must be regarded as type material.

Conclusion

In conclusion, it can be assumed that 17 individuals in 11 species of birds in the Wiesbaden collection are potential type specimens that should be considered in future taxonomic work. Close examination, comparison with other material, or even genetic tests will be necessary to make a final decision on the specimens' type status.

Acknowledgements

We gratefully acknowledge Mary LeCroy (AMNH, New York) for information about the AMNH types, valuable references and extensive comments on an earlier draft of the manuscript. We are also indebted to Sylvain Hodvina (MWNH, Wiesbaden) for help with deciphering of old labels. Morton Isler (Smithsonian Institution, Washington) and Roger Lederer (Ornithology.com) kindly provided expertise on tanager morphology, and Malte Seehausen (MWNH, Wiesbaden) gave additional taxonomic advice. Two anonymous referees commented on an earlier version of the manuscript.

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