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Brazilian obligatory subterranean fauna and threats to the hypogean environment

Jonas Eduardo Gallão[1], Maria Elina Bichuette[1]

1 *Laboratório de Estudos Subterrâneos, Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos, São Carlos, São Paulo, Brasil*

Corresponding author: *Jonas Eduardo Gallão* (jonasgallao@gmail.com)

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Abstract

The subterranean environment harbors species that are not capable of establishing populations in the epigean environment, i.e., the obligatory subterranean species. These organisms live in a unique selective regime in permanent darkness and usually low food availability, high air humidity in terrestrial habitats, and low temperature range allied to other unique conditions related to lithologies and past climatic influences. The pressure to increase Brazil's economic growth relies on agricultural/pastoral industries and exporting of raw materials such as iron, limestone, ethanol, soybean, cotton, and meat, as well as huge reservoir constructions to generate electricity. Mining (even on a small scale), agricultural expansion, and hydroelectric projects are extremely harmful to subterranean biodiversity, via the modification and even destruction of hypogean habitats. The Brazilian subterranean species were analyzed with respect to their distributions, presence on the IUCN Red List, and current and potential threats to hypogean habitats. A map and three lists are presented, one with the described obligatory subterranean species, one with undescribed taxa, and one with the current and potential threats to the hypogean environment. To date, 150 obligatory subterranean species have been recorded in Brazil, plus at least 156 undescribed troglomorphic taxa, totaling 306 Brazilian troglobites/obligatory cave fauna. We also analyzed the current and potential cave threats and the conservation actions that are underway to attempt to compensate for loss of these habitats. In according to the Brazilian legislation (Decree 6640) only caves of maximum relevance are fully protected. One strategy to protect the subterranean fauna of Brazil is the inclusion of these species in the IUCN Red List (one of attributes that determines maximum relevance for caves); however, one of the IUCN assumptions is that the taxa must be formally described. It is clear that the description and proposed protection of Brazilian subterranean biodiversity depends on more systematics studies.

Keywords

caves, Neotropical region, IUCN Red list, troglobites

Introduction

The most obvious intrinsic feature of subterranean environments is the absence of light, which results in energy restriction (Poulson and White 1969, Poulson and Lavoie 2000). Furthermore, subterranean environments tend to be environmentally stable in terms of low temperature, high relative humidity, and complete darkness (Moore and Sullivan 1997). Consequently, few organisms are capable of effectively colonizing these environments (Barr 1968).

Obligatory subterranean species have evolved in isolation under particular selective conditions, such as complete darkness, low food quantity (with exceptions), and high and constant air humidity for terrestrial species. Obligatory subterranean species have accumulated specializations that are not present in their epigean relatives, which have culminated in exclusively subterranean populations that are no longer capable of colonizing the epigean realm (Trajano 2012).

The importance and fragility of hypogean environments was acknowledged when subterranean species were placed on the IUCN Red List by the environmental government agency in 2004 (IBAMA) and 2014 (ICMBio) (Machado et al. 2008, ICMBio 444 2014 and ICMBio 445 2014). The inclusion of obligatory subterranean species in the IUCN Red List elevates caves to the maximum relevance level (out of four levels of relevance - maximum, high, median, and low), meaning that the cave habitat must be protected (Decree 6640 from November 7, 2008 (Brasil 2008), Normative Instruction [NI] number 2 from August 20, 2009; Normative Instruction [NI] number 2 from August 30, 2017). The biological attributes present in the Normative Instructions that elevates caves to maximum relevance are species included in official Red Lists; presence of endemic or relict troglobites; presence of rare troglobites; and occurrence of unique ecological interactions.

The hypogean environment is fragile and, thus, highly vulnerable to environmental changes; it typically presents high endemism and small population sizes with low restoration capacity, which implies that obligatory subterranean fauna is sensitive to habitat changes, such as chemical pollution, eutrophication, deforestation close to the outcrops and drainages, uncontrolled tourism, mining, dams, etc. (Poulson 1964, Culver and Pipan 2009).

Extinction rates and disturbances caused by human activities are significant (Pimm et al. 1995), thus the knowledge of biodiversity becomes a fundamental tool to recognize threats to biodiversity. Financial resources for documenting biodiversity must be prioritized, as they are essential to establishing and developing best conservation policies (Brooks et al. 2006).

Knowledge of the geographical distribution of obligatory subterranean fauna in Brazil is fragmented compared to Europe and Asia, where a higher level of knowledge has been achieved (Botosaneanu 1986, Juberthie and Decu 2001, Deharveng et al. 2009, Stoch et al. 2009, Brancelj et al. 2013). The first list of obligatory subterranean fauna of Brazil was published in the 1980s and comprised five areas (Dessen et al. 1980). Since then, these lists have been constantly reviewed (Trajano 1987, Trajano

and Gnaspini-Netto 1991, Gnaspini and Trajano 1994, Pinto-da-Rocha 1995, Trajano and Bichuette 2010a). Herein we update and elaborate on the list of Brazilian obligatory subterranean fauna, mapping in detail the areas/regions with this fauna and its main threats.

Materials and methods

To construct the list, species descriptions, literature data, and sampling conducted by our group were utilized. The undescribed taxa were confirmed by specialists and are deposited in Brazilian collections (Museu Nacional do Rio do Janeiro/Universidade Federal do Rio de Janeiro, Instituto Butantan, Museu de Zoologia da Universidade de São Paulo). The information contained in two existing faunistic lists is expanded upon: one with the formally described obligatory subterranean fauna and the other containing the troglomorphic taxa (possible obligatory subterranean fauna detailed to as accurate taxonomic level as possible).

The purpose of the inclusion of undescribed troglomorphic taxa was to propose potential areas for conservation (since they are not included in the IUCN Red List). To avoid overestimation of taxa, we did not use data from environmental impact assessment reports.

The geomorphologic units used follow Karmann and Sanchez (1979). Groups: main uninterrupted limestone rocks (Una-Irecê, Corumbá, Bambuí, Açungui, Rio Pardo, Araras, Brusque, Apodi); supergroups: main interrupted limestone rocks (Canudos); sandstone: main sandstone rocks (Altamira-Itaituba, Chapada Diamantina); formation: main iron ore rocks (Carajás, Quadrilátero Ferrífero). Since the Bambuí group is huge, we divided it into regions, based on municipalities (Presidente Olegário, Mambaí, São Domingos, São Desidério, Itacarambi, Jaíba, Montes Claros, Cordisburgo, Unaí, Distrito Federal) or based on continuous outcrops (Serra da Canastra, Serra do Ramalho). Other minor geomorphological units used are Serra do Mar and Serra da Mantiqueira (quartiztic), Vargem Alta (marble), and Itirapina (sandstone).

The threats listed herein are those that directly disturb the hypogean environment and its fauna, such as small and large hydroelectrical projects, mining projects, deforestation, uncontrolled tourism, chemical pollution, and lowering of the water table due to extraction of water; and indirect threats such as roads, land conflicts and gas extraction. The main threats were listed for municipalities and for some Brazilian geomorphologic units.

The map was created on QuantumGis Essen 2.14 with shapefiles of South America and Brazil. Besides these, we used the shapefile of Brazilian karst areas, available at the CECAV/ICMBIO website. Circle size is proportional to the number of species occurring in each area and was plotted using Adobe Illustrator CS6.

To evaluate the addition of Brazilian subterranean species in the IUCN Red lists, we compared the number of species presented in the 2004 IUCN Red List (Machado et al. 2008) and the 2014 IUCN Red List (ICMBio 444 2014 and ICMBio 445

2014). We distinguished between the species not rated in the IUCN Red List as "not reported" and "not included". "Not reported" refers to species that were not revised and "not included" are species that were revised and do not fit into any threat category: vulnerable (VU), endangered (EN), and critically endangered (CR). The term IUCN Red List used herein correspond to the Brazilian List of Threatened Fauna.

Results

Presently, Brazil has 150 described obligatory subterranean species, distributed over 12 states and located in different lithologies and geomorphologic groups (Figure 1, Table 1). The majority of these species occur in limestone rocks (123 species), mainly owing to the vast size of limestone geomorphologic units and the higher sampling effort in this lithology. Even with the high number of impact reports (mainly mining) regarding iron ore lithologies, and the increase in studies and inventories over the last ten years after publication of Decree 6640, there has been few described species (twelve species). In the other lithologies, sandstone contains less described species than does iron ore (ten species); for quartzitic and marble lithologies, we recorded only two obligatory subterranean species, one for each. Besides, there are two hyporheic fishes, one from Pará State and another from Rondônia State.

At least 156 troglomorphic/stygomorphic taxa are undescribed (Figure 1, Table 2), representing possible obligatory subterranean populations; these collections, deposited in different museums, await further taxonomic studies. Most of these specimens have not been identified to even a generic taxonomic level. In total, we listed approximately 306 obligatory and potentially obligatory subterranean species for Brazil (Tables 1 and 2). The Brazilian states with the highest number of species are Bahia (Serra do Ramalho karst area and São Desidério region, part of the Bambuí group, the Una-Irecê and Rio Pardo groups, the Canudos supergroup and the sandstone Chapada Diamantina; at least 90 obligatory subterranean species) and São Paulo (including part of the Açungui group, with at least 66 obligatory subterranean species) (Figure 1). Considering the geomorphological units used here, the Bambui group is the richest with 100 obligatory subterranean species followed by the Açungui group with 73 obligatory subterranean species.

In total, eight threats are identified in the Bahia State (Table 3) and the majority of the caves in this State are outside conservation units (natural areas liable for protection by law owing to special features), the exception being in the Andaraí and Lençois regions, where the sandstone caves are recorded inside a conservation unit. For São Paulo State, the amount of threats are fewer (five, Table 3), but there is a concentration of them in areas that contain a high number of subterranean species, e.g., the Alto do Ribeira region – deforestation, land conflicts, pollution of subterranean drainage, small hydroelectric power-stations buildings (SHPS) and uncontrolled tourism.

The most common threat to the hypogean environment (Figure 1, Table 3) was miscellaneous impacts, with historical threats (e.g., deforestation related to agriculture/pastures and mining). For example, from the 29 impacted regions, deforestation for

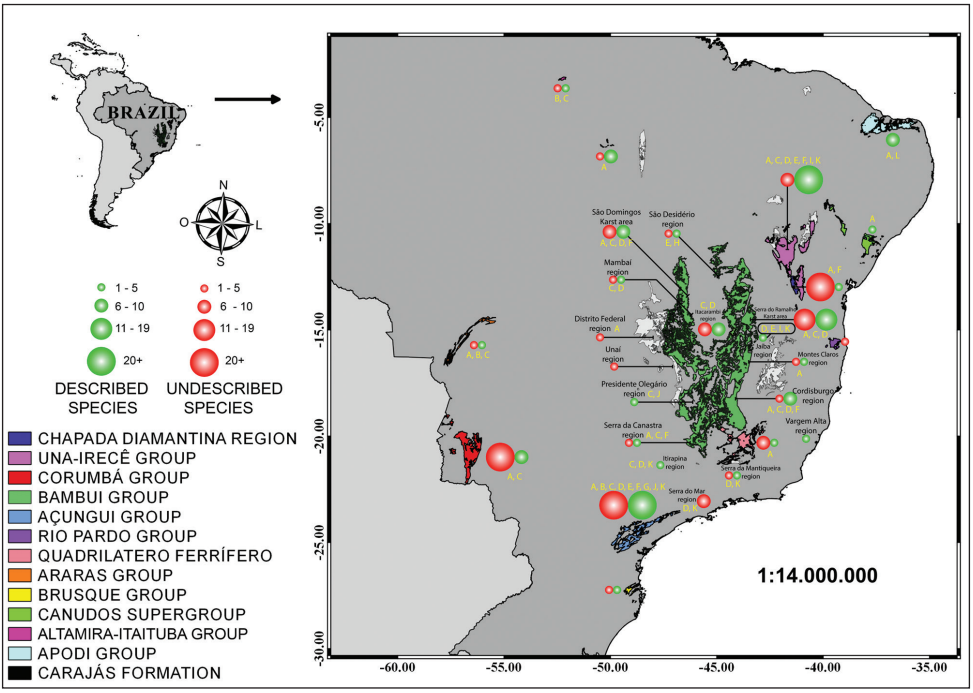

Figure 1. Map of Brazil with main rock groups, karst areas, and formations with obligatory cave-dwelling species. Threats are indicated by letters as follows: **A** Minig **B** Reservoir construction **C** Deforestation for pastures **D** Deforestation for agriculture **E** Pollution of subterranean drainages **F** Tourism **G** Land conflict **H** Road construction, **I** Lowering of water table **J** Small hydroelectric power station buildings, **K** Pesticides **L** Natural gas and oil exploration. For Bambuí group, we grouped as follows (see Table 1 for distinction): Mambaí region - Mambaí and Posse municipalities; Distrito Federal region - Distrito Federal region plus Formosa and Padre Bernardo municipalities; Presidente Olegário region - Presidente Olegário and Vazante municipalities; Serra da Canastra region - São Roque de Minas, Arcos and Pains municipalities; Cordisburgo region - Cordisburgo, Matozinhos, Sete Lagoas, Morro do Pilar, Monjolos and Lagoa Santa municipalities; Montes Claros region - Montes Claros, Coração de Jesus and Luislândia municipalities.

agricultural and/or pastures occurred in 17 (58.6 %); mining in 15 (51.7 %), uncontrolled tourism in six, as is also the case for pollution (20.7 % each); hydroelectric projects are present in five (17.2 %). Roads, land conflicts, gas extraction, and lowering of the water table are more widespread and are present in five regions (17.2 %). Caves included in conservation units are not fully protected - for example, the Açungui group in southeastern Brazil (where there are three State Parks) is under five different threats (Figure 1, Table 3). Specifically, considering the Carajás region in North Brazil, we observed that only mining had an impact that would deplete the entire subterranean environment and lead to the total destruction of landscapes and caves (by mining), with the possible pollution of soil and drainage ways.

Considering the described subterranean species up to the end of 2003, only 33 were included in the Brazilian Red List of 2004 and another 30 species were "not re-

Table 1. Obligatory subterranean fauna described in Brazil (149 species) and IUCN Red List threatened species categories. VU – vulnerable; EN – endangered; CR – critically endangered; LC – least concern; DD – data deficient. SNR – still not rated, see text for explanations. States: BA – Bahia, GO – Goiás, MG – Minas Gerais, MS – Mato Grosso do Sul, MT – Mato Grosso, PA – Pará, PR – Paraná, RO – Rondônia, RN – Rio Grande do Norte, SP – São Paulo.

Higher taxon	Species	Lithology / Geomorphological Unit / Karstic area or Region (State)	Category 2004	Category 2014
Phylum Platyhelminthes				
Class Turbellaria				
Order Tricladida				
Dimarcusidae	*Hausera hauseri* Leal-Zanchet & Souza, 2014	Limestone / Apodi group / Felipe Guerra region (RN)	–	SNR
Dugesiidae	*Girardia multidiverticulata* Souza, Morais, Cordeiro & Leal-Zancheti, 2015	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	–	SNR
	Girardia desiderensis Souza & Leal-Zancheti, 2016	Limestone / Bambuí group / São Desidério region (BA)	–	SNR
Phylum Porifera				
Class Demospongiae				
Order Haplosclerida				
Spongillidae	*Racekiela cavernicola* Volkmer-Ribeiro, Bichuette & Machado, 2010	Limestone / Una-Irecê group / Morro do Chapéu region (BA)	–	CR
Phylum Arthropoda				
Class Malacostraca				
Order Amphipoda				
Hyalellidae	*Hyalella caeca* Pereira, 1989	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	SNR
	Hyalella spelaea Bueno & Cardoso, 2011	Sandstone / Itirapina region (SP)	–	SNR
	Hyalella veredae Cardoso & Bueno, 2014	Limestone / Bambuí group / Vazante formation / Presidente Olegário region (MG)	–	SNR
	Hyalella formosa Cardoso &Araujo, 2014	Limestone / Açungui group / Alto do Ribeira karst area (PR)	–	SNR
	Hyalella epikarstica Rodrigues, Bueno & Ferreira, 2014	Limestone / Açungui group / Alto do Ribeira karst area (SP)	–	SNR
Artesiidae	*Megagidiella azul* Koenemann & Holsinger, 1999	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	"Not reported"	SNR
	Spelaeogammarus bahiensis Brum, 1975	Limestone / Una-Irecê group / Curaça region (BA)	"Not reported"	SNR
	Spelaeogammarus santanensis Koenemann & Holsinger, 2000	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	"Not reported"	SNR
	Spelaeogammarus spinilacertus Koenemann & Holsinger, 2000	Limestone / Una-Irecê group / Iraquara region (BA)	"Not reported"	SNR
	Spelaeogammarus trajanoae Koenemann & Holsinger, 2000	Limestone / Una-Irecê group / Campo Formoso region (BA)	"Not reported"	SNR
	Spelaeogammarus titan Senna, Andrade, Castelo-Branco & Ferreira, 2014	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	–	SNR
	Spelaeogammarus sanctus Bastos-Pereira & Ferreira, 2015	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	–	SNR
	Spelaeogammarus uai Bastos-Pereira & Ferreira, 2017	Limestone / Bambuí group / Itacarambi region (MG)	–	–
Mesogammaridae	*Potiberaba porakuara* Fiser, Zagmajter & Ferreira, 2013	Limestone / Apodi group / Felipe Guerra region (RN)	–	SNR
Seborgiidae	*Seborgia potiguar* Fiser, Zagmajter & Ferreira, 2013	Limestone / Apodi group / Governador Dix-Sept Rosado region (RN)	–	SNR
Order Decapoda				
Aeglidae	*Aegla cavernicola* Turkay, 1972	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	CR

Higher taxon	Species	Lithology / Geomorphological Unit / Karstic area or Region (State)	Category 2004	Category 2014
Aeglidae	*Aegla leptochela* Bond-Buckup & Buckup ,1994	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	CR
	Aegla microphthalma Bond-Buckup & Buckup, 1994	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	CR
Order Isopoda				
Calabozoidae	*Pongycarcinia xiphidiourus* Messana, Baratti & Benvenuti, 2002	Limestone / Una-Irecê group / Campo Formoso region (BA)	"Not reported"	SNR
Brasileirinidae	*Brasileirinho cavaticus* Prevorcnik, Ferreira & Sket, 2011	Limestone / Canudos supergroup / Paripiranga region (BA)	–	SNR
Philosciidae	*Benthana iporangensis* Lima & Serejo, 1993	Limestone / Açungui group / Alto do Ribeira karst area (SP)	"Not reported"	SNR
	Leonardossia hassalli Campos-Filho, Araujo & Taiti, 2014	Sandstone / Altamira-Itaituba group / Altamira region (PA)	–	SNR
Pudeoniscidae	*Iansaoniscus iraquara* Campos-Filho, Araujo & Taiti, 2017	Limestone / Una-Irecê group / Iraquara region (BA)	–	–
	Iansaoniscus georginae Campos-Filho, Araujo & Taiti, 2017	Limestone / Canudos supergroup / Paripiranga region (BA)	–	–
Scleropactidae	*Amazoniscus eleonorae* Souza, Ferreira & Araujo, 2006	Sandstone / Altamira-Itaituba group / Altamira region (PA)	–	SNR
	Amazoniscus leistikowi Campos-Filho, Araujo & Taiti, 2014	Sandstone / Altamira-Itaituba group / Altamira region (PA)	–	SNR
	Circoniscus buckupi Campos-Filho & Araujo, 2011	Iron ore / Carajás formation / Parauapebas region (PA)	–	SNR
	Circoniscus carajasensis Campos-Filho & Araujo, 2011	Iron ore / Carajás formation / Canãa dos Carajás region (PA)	–	SNR
Styloniscidae	*Spelunconiscus castroi* Campos-Filho, Araujo & Taiti, 2014	Limestone / Bambuí group / Matozinhos region (MG)	–	SNR
	Xangoniscus aganju Campos-Filho, Araujo & Taiti, 2014	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	–	SNR
	Xangoniscus odara Campos-Filho & Taiti, 2016	Limestone / Bambuí group / Itacarambi region (MG)	–	SNR
	Iuiuniscus iuiuensis Souza, Ferreira & Senna, 2015	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	–	SNR
	Xangoniscus itacarambiensis Bastos-Pereira, Souza & Ferreira, 2017	Limestone / Bambuí group / Itacarambi region (MG)	–	–
Order Spelaeogriphacea				
Spelaeogriphidae	*Potiicoara brasiliensis* Pires, 1987	Limestone / Corumbá and Araras groups / Serra da Bodoquena karst area (MS) and Rosário Oeste region (MT)	"Not reported"	SNR
Class Chelicerata				
Order Amblypygi				
Charinidae	*Charinus troglobius* Baptista & Giupponi, 2002	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	CR	CR
	Charinus eleonorae Baptista & Giupponi, 2003	Limestone / Bambuí group / Itacarambi region (MG)	"Not reported"	CR
	Charinus caatingae Vasconcelos & Ferreira, 2016	Limestone / Una-Irecê group / Várzea Nova region (BA)	–	–
	Charinus taboa Vasconcelos, Giupponi & Ferreira, 2016	Limestone / Bambuí group / Sete Lagoas region (MG)	–	SNR
	Charinus ferreus Giupponi & Miranda, 2016	Iron ore / Carajás formation / Serra de Carajás (PA)	–	SNR
	Charinus spelaeus Vasconcelos & Ferreira, 2017	Limeston / Bambuí group / Presidente Juscelino region (MG)	–	–
Order Araneae				
Theraphosidae	*Tmesiphantes hypogeous* Bertani, Bichuette & Pedroso, 2013	Sandstone / Chapada Diamantina region (BA)	–	CR
Dipluridae	*Harmonicon cerberus* Pedroso & Baptista, 2014	Iron ore / Carajás formation / Parauapebas region (PA)	–	CR
Ctenidae	*Isoctenus corymbus* Polotow, Brescovit & Pellegatti-Franco, 2005	Limestone / Bambuí group / São Domingos karst area (GO)	–	CR
Ochyroceratidae	*Speocera eleonorae* Baptista, 2003	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	"Not reported"	EN

Higher taxon	Species	Lithology / Geomorphological Unit / Karstic area or Region (State)	Category 2004	Category 2014
Ochyroceratidae	*Ochyrocera ibitipoca* Baptista, Gonzalez & Tourinho, 2008	Quartzitic / Serra da Mantiqueira / Lima Duarte (MG)	–	EN
Pholcidae	*Metagonia diamantina* Machado, Ferreira & Brescovit, 2011	Limestone / Una-Irecê group / Itaetê region (BA)	–	CR
	Metagonia potiguar Ferreira, Souza, Machado & Brescovit, 2011	Limestone / Apodi group / Felipe Guerra region (RN)	–	CR
Prodidomidae	*Lygromma ybyguara* Rheims & Brescovit, 2004	Limestone / Bambuí group / Cordisburgo region (MG)	–	CR
Symphytognathidae	*Anapistula guyri* Rheims & Brescovit, 2003	Limestone / Bambuí group / São Domingos karst area (GO)	VU	LC
Order Opiliones				
Gerdesiidae	*Gonycranaus pluto* Bragagnolo, Hara & Pinto-da-Rocha, 2015	Limestone / Bambuí group / Morro do Pilar region (MG)	–	–
Gonyleptidae	*Pachylospeleus strinatii* Šilhavý, 1974	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	EN
	Iandumoema uai Pinto-da-Rocha, 1996	Limestone / Bambuí group / Itacarambi region (MG)	CR	CR
	Iandumoema smeagol Pinto-da-Rocha, Fonseca-Ferreira & Bichuette, 2015	Limestone / Bambuí group / Monjolos region (MG)	–	–
	Iandumoema setimapocu Hara & Pinto-da-Rocha, 2008	Limestone / Bambuí group / Coração de Jesus region (MG)	–	EN
	Giupponia chagasi Pérez & Kury, 2002	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	CR	CR
	Discocyrtus pedrosoi Kury, 2008	Sandstone / Chapada Diamantina region (BA)	–	LC
	Eusarcus elinae Kury, 2008	Limestone / Una-Irecê group / Iraquara region (BA)	–	EN
	Spinopilar moria Kury & Pérez-González, 2008	Limestone / Bambuí group / Cordisburgo region (MG)	–	CR
Escadabiidae	*Spaeleoleptes spaeleus* Soares, 1966	Limestone / Bambuí group / Cordisburgo region (MG)	EN	EN
Kimulidae	*Relictopiolus galadriel* Pérez-González, Monte & Bichuette, 2017	Limestone / Bambuí group / Itacarambi region (MG)	–	–
Order Palpigradi				
Eukoeneniidae	*Eukoenenia maquinensis* Souza & Ferreira, 2010	Limestone / Bambuí group / Cordisburgo region (MG)	–	CR
	Eukoenenia spelunca Souza & Ferreira, 2011	Marble / Vargem Alta region (ES)	–	CR
	Eukoenenia virgemdalapa Souza & Ferreira, 2012	Limestone / Bambuí group / Vazante formation / Vazante region (MG)	–	EN
	Eukoenenia sagarana Souza & Ferreira, 2012	Limestone / Bambuí group / Cordisburgo region (MG)	–	CR
	Eukoenenia jequitinhonha Souza & Ferreira, 2016	Granitic / Caraí region (MG)	–	–
	Eukoenenia cavatica Souza & Ferreira, 2016	Limestone / Bambuí group / Arcos region	–	–
Order Pseudoscorpiones				
Bochicidae	*Spelaeobochica allodentatus* Mahnert, 2001	Limestone / Una-Irecê group / Palmeiras region (BA)	"Not reported"	CR
	Spelaeobochica muchmorei Andrade & Mahnert, 2003	Limestone / Açungui group / Alto do Ribeira karst area (SP)	"Not reported"	EN
	Spelaeobochica iuiu Ratton, Mahnert & Ferreira, 2012	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	–	CR
Chthoniidae	*Maxchernes iporangae* Mahnert & Andrade, 1998	Limestone / Açungui group / Alto do Ribeira karst area (SP)	EN	CR
	Pseudochthonius strinatii Beier, 1969	Limestone / Açungui and Bambuí groups / Alto do Ribeira karst area (SP-PR) and Sete Lagoas region (MG)	VU	DD
	Pseudochthonius biseriatus Mahnert, 2001	Limestone / Bambuí group / Itacarambi region (MG)	"Not reported"	CR
Ideoroncidae	*Ideroncus cavicola* Mahnert 2001	Limestone / Açungui group / Alto do Ribeira karst area / Iporanga (SP) and Rio Branco do Sul regions (PR)	"Not reported"	VU

Higher taxon	Species	Lithology / Geomorphological Unit / Karstic area or Region (State)	Category 2004	Category 2014
Order Scorpiones				
Buthidae	*Troglorhopalurus translucidus* Lourenço, Baptista & Giupponi, 2004	Sandstone / Chapada Diamantina region (BA)	–	EN
	Troglorhopalurus lacrau (Lourenço & Pinto-da-Rocha, 1997)	Limestone / Una-Irecê group / Itaetê region (BA)	"Not reported"	EN
Class Chilopoda				
Order Scolopendromorpha				
Cryptopidae	*Cryptops* (*Trygonocryptops*) *iporangensis* Ázara & Ferreira, 2013	Limestone / Açungui group / Alto do Ribeira karst area (SP)	–	EN
	Cryptops (*Cryptops*) *spelaeoraptor* Ázara & Ferreira, 2014	Limestone / Una-Irecê group / Campo Formoso region (BA)	–	VU
	Scolopocryptops troglocaudatus Chagas-Jr. & Bichuette, 2015	Sandstone / Chapada Diamantina region (BA)	–	SNR
Scolopocryptopidae	*Newportia* (*Newportia*) *spelaea* Ázara & Ferreira, 2014	Limestone / Una-Irecê group / Campo Formoso region (BA)	–	–
	Newportia (*Newportia*) *potiguar* Ázara & Ferreira, 2014	Limestone / Apodi group / Apodi and Felipe Guerra regions (RN)	–	–
Class Diplopoda				
Order Glomeridesmida				
Glomeridesmidae	*Glomerides musspelaeus* Iniesta & Wesewer, 2012	Iron ore / Carajás formation / Curionópolis region (PA)	–	CR
Order Spirostreptida				
Pseudonannolenidae	*Pseudonannolene spelaea* Iniesta & Ferreira, 2013	Iron ore / Carajás formation / Parauapebas region (PA)	–	CR
	Pseudonannolene ambuatinga Iniesta & Ferreira, 2013	Limestone / Bambuí group / Pains region (MG)	–	EN
	Pseudonannolene lundi Iniesta & Ferreira, 2015	Limestone / Bambuí group / Luislândia region (MG)	–	SNR
Order Polydesmida				
Chelodesmidae	*Leodesmus yporangae* (Schubart, 1946)	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	CR
Cryptodesmidae	*Peridontodesmella alba* Schubart, 1957	Limestone / Açungui group / Alto do Ribeira karst area / Iporanga (SP) and Adrianópolis regions (PR)	VU	EN
Fuhmannodesmidae	*Phaneromerium cavernicolum* Golovatch & Wytwer, 2004	Limestone / Bambuí group / Serra do Ramalho karstarea (BA)	"Not reported"	–
Pyrgodesmidae	*Yporangiella stygius* Schubart ,1946	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	VU
Class Entognatha				
Order Diplura				
Campodeidae	*Oncinocampa trajanoae* Condé, 1997	Limestone / Açungui group / Alto do Ribeira karst area (SP)	"Not reported"	SNR
Order Collembola				
Arrhopalitidae	*Arrhopalites amorimi* Palacios-Vargas & Zeppelini, 1995	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	CR
	Arrhopalites gnaspinii Palacios-Vargas & Zeppelini, 1995	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	CR
	Arrhopalites lawrencei Palacios-Vargas & Zeppelini, 1995	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	CR
	Arrhopalites alambariensis Zeppelini, 2006	Limestone / Açungui group / Alto do Ribeira karst area (SP)	–	CR
	Arrhopalites botuveraensis Zeppelini, 2006	Limestone / Brusque group / Botuverá region (SC)	–	CR
	Arrhopalites heteroculatus Zeppelini, 2006	Limestone / Açungui group / Alto do Ribeira karst area (SP)	–	CR
	Arrhopalites paranaensis Zeppelini, 2006	Limestone / Açungui group / Alto do Ribeira karst area (PR)	–	CR
Hypogastruridae	*Acherontides eleonorae* Palacios-Vargas & Gnaspini-Neto, 1992	Limestone / Açungui group / Alto do Ribeira karst area / Iporanga (SP) and Rio Branco do Sul regions (PR)	"Not reported"	EN

Higher taxon	Species	Lithology / Geomorphological Unit / Karstic area or Region (State)	Category 2004	Category 2014
Paronellidae	*Troglobius brasiliensis* Palacios-Vargas & Zeppelini, 1995	Sandstone / Altamira-Itaituba region / Medicilândia region (PA); Limestone / Açunguigroup / Alto do Ribeira karst area (SP)	"Not reported"	CR
	Troglobius ferroicus Zeppelini, Silva & Palácios-Vargas, 2014	Iron ore / Quadrilátero Ferrífero formation / Itabirito region (MG)	–	CR
	Trogolaphys aaelleni Yossi, 1988	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	VU
	Trogolaphysa hauseri Yossi, 1989	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	VU
Sminthuridae	*Pararrhopalites wallacei* (Palacios-Vargas & Zeppelini, 1995)	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	CR
	Pararrhopalites papaveroi (Zeppelini & Palacios-Vargas, 1999)	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	VU	EN
Class Insecta				
Order Zygentoma				
Nicoletiidae	*Cubacubana spelaea* Galán, 2001	Limestone / Una-Irecê group / Campo Formoso region (BA)	"Not reported"	SNR
Order Blattaria				
Blattellidae	*Litoblatta camargoi* Gutierrez, 2005	Limestone / Una-Irecê group / Iraquara region (BA)	–	SNR
Order Coleoptera				
Carabidae	*Schizogenius ocellatus* Whitehead, 1972	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	EN
	Coarazuphium tessai (Godoy & Vanin, 1990)	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	VU	CR
	Coarazuphium bezerra Gnaspini, Vanin & Godoy, 1998	Limestone / Bambuí group / São Domingos karst area (GO)	VU	VU
	Coarazuphium cessaima Gnaspini, Vanin & Godoy, 1998	Limestone / Una-Irecê group / Itaetê region (BA)	VU	CR
	Coarazuphium pains Alvares & Ferreira, 2002	Limestone / Bambuí group / Pains region (MG)	VU	EN
	Coarazuphium formoso Pellegrini & Ferreira, 2011	Limestone / Una-Irecê group / Campo Formoso region (BA)	–	VU
	Coarazuphium tapiaguassu Pellegrini & Ferreira, 2011	Iron ore / Carajás formation / Curionópolis region (PA)	–	CR
	Coarazuphium caatinga Pellegrini & Ferreira, 2014	Limestone / Una-Irecê group / Campo Formoso region (BA)	–	EN
	Coarazuphium ricardoi Bená & Vanin, 2014	Limestone / Açungui group / Alto do Ribeira karst area (PR)	–	CR
	Coarazuphium spinifemur Pellegrini & Ferreira, 2017	Iron ore / Carajás formation / Curionópolis region (PA)	–	–
	Coarazuphium amazonicus Pellegrini & Ferreira, 2017	Iron ore / Carajás formation / Flona de Carajás (PA)	–	–
Dytiscidae	*Copelatus cessaima* Caetano, Bená & Vanin, 2013	Iron ore / Carajás formation / Parauapebas region (PA)	–	CR
Staphylinidae	*Metopiellus painensis* Asenjo, Ferreira & Zampaulo, 2017	Limestone / Bambuí group / Pains region (MG)	–	–
Order Hemiptera				
Cixiidae	*Ferricixius davidi* Hoch & Ferreira, 2012	Iron ore / Quadrilátero Ferrífero formation / Itabirito region (MG)	–	SNR
Kinnaridae	*Kinnapotiguara troglobia* (Hoch & Ferreira, 2013)	Limestone / Apodi group / Felipe Guerra and Governador Dix-Sept Rosado regions (RN)	–	SNR
	Iuiuia caeca Hoch & Ferreira, 2016	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	–	SNR
Order Orthoptera				
Phalangopsidae	*Endecous apterus* Bolfarini & Souza-Dias, 2013	Limestone / Una-Irecê group / Ituaçu region (BA)	–	SNR
	Endecous peruassuensis Bolfarini, 2015	Limestone / Bambuí group / Itacarambi region (MG)	–	–

Higher taxon	Species	Lithology / Geomorphological Unit / Karstic area or Region (State)	Category 2004	Category 2014
Phylum Mollusca				
Class Gastropoda				
Order Mesogastropoda				
Hydrobiidae	*Potamolithus troglobius* Simone & Moracchiolli, 1999	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	CR
Order Caenogastropoda				
Pomatiopsidae	*Spiripockia punctata* Simone, 2012	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	–	EN
Phylum Chordata				
Class Osteichtyes				
Order Characiformes				
Characidae	*Stygichthys typhlops* Brittan & Böhlke, 1965	Limestone / Bambuí group / Jaíba region (MG)	VU	EN
Order Gymnotiformes				
Sternopygidae	*Eigenmannia vicentespelaea* Triques, 1996	Limestone / Bambuí group / São Domingos karst area (GO)	VU	VU
Order Siluriformes				
Callychthyidae	*Aspidoras mephisto* Tencatt & Bichuette, 2017	Limestone / Bambuí group / Posse region (GO)	"Not reported"	EN
Heptapteridae	*Pimelodella kronei* (Ribeiro, 1907)	Limestone / Açungui group / Alto do Ribeira karst area (SP)	VU	EN
	Pimelodella spelaea Trajano, Reis & Bichuette, 2004	Limestone / Bambuí group / São Domingos karst area (GO)	–	EN
	Rhamdia enfurnada Bichuette & Trajano, 2005	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	–	LC
	Rhamdiopsis krugi Bockmann & Castro, 2010	Limestone / Una-Irecê group / Itaetê region (BA)	–	VU
Loricariidae	*Ancistrus cryptophthalmus* Reis, 1987	Limestone / Bambuí group / São Domingos karst area (GO)	"Not reported"	EN
	Ancistrus formoso Sabino & Trajano, 1997	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	VU	VU
Trichomycteridae	*Trichomycterus itacarambiensis* Trajano & de Pinna, 1996	Limestone / Bambuí group / Itacarambi region (MG)	VU	CR
	Trichomycterus dali Rizzato, Costa-Jr, Trajano & Bichuette, 2011	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	–	VU
	Trichomycterus rubbioli Bichuette & Rizzato, 2012	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	–	VU
	Ituglanis mambai Bichuette & Trajano, 2008	Limestone / Bambuí group / Posse region (GO)	–	EN
	Ituglanis bambui Bichuette & Trajano, 2004	Limestone / Bambuí group / São Domingos karst area (GO)	–	CR
	Ituglanis passensis Fernandez & Bichuette, 2002	Limestone / Bambuí group / São Domingos karst area (GO)	"Not reported"	VU
	Ituglanis epikarsticus Bichuette & Trajano, 2004	Limestone / Bambuí group / São Domingos karst area (GO)	–	VU
	Ituglanis ramiroi Bichuette & Trajano, 2004	Limestone / Bambuí group / São Domingos karst area (GO)	–	VU
	Ituglanis boticário Rizzato & Bichuette, 2014	Limestone / Bambuí group / Mambaí region (GO)	–	SNR
	Glaphyropoma spinosum Bichuette, de Pinna & Trajano, 2008	Sandstone / Chapada Diamantina region (BA)	–	VU
Incertae sedis	*Phreatobius cisternarum* Goeldi, 1905	Hyporheic / Ilha de Marajó (PA)	–	"Not reported"
	Phreatobius dracunculus Shibatta, Muriel-Cunha & de Pinna, 2007	Hyporheic / Rio Pardo basin (RO)	–	"Not reported"

Table 2. Obligatory subterranean undescribed. References: A – Dessen et al. 1980; B – Chaimowicz 1984; C – Trajano 1987; D – Trajano and Gnaspini-Netto 1991; E – Trajano and Moreira 1991; F – Gnaspini and Trajano 1994; G – Trajano and Sanchez 1994; H – Pinto-da-Rocha 1995; I – Bichuette 1998; J – Lourenço et al. 2004; K – Deharveng 2005; L – Trajano and Bichuette 2010a; M – Cordeiro et al. 2014; TS – this study. spp – widespread taxa possibly meaning several species. States: BA-Bahia, GO-Goiás, MG-Minas Gerais, MS-Mato Grosso do Sul, MT-Mato Grosso, PA-Pará, PR-Paraná, RJ-Rio de Janeiro, RN-Rio Grande do Norte, SC-Santa Catarina, SP-São Paulo.

Taxon	Lithology / Geomorphological Unit / karst area or region	References
Phylum Annelida		
Class Clitellata		
Subclass Oligochaeta	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	M
Phylum Platyhelminthes		
Order Tricladida		
Dugesiidae indet. 1	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	TS
Dugesiidae indet. 2	Limestone / Açungui group / Alto do Ribeira karst area (SP)	L
Phylum Onychophora		
Order Euonychophora		
Peripatidae indet.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	M
Phylum Arthropoda		
Order Amphipoda		
Bogidiellidae		
Megagidiella sp.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	L
Hyalellidae		
Hyalella aff. *pernix*	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, G, H
Hyalella sp.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	A, D, G, H
Order Isopoda		
Indet. 1	Limestone / Bambuí group / Montes Claros region (MG)	A, B, H
Indet. 2	Limestone / Araras group / Nobres region (MT)	L
So. Oniscidea	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	M
Armadillidae		
Venezillo sp. 1	Magnesita / Padre Bernardo region (GO)	F, H
Venezillo sp. 2	Limestone / Bambuí group / Distrito Federal region (GO)	L
Bathytropidae		
Neotroponiscus sp.	Iron ore / Quadrilátero Ferrífero formation / Brumadinho region (MG)	Cardoso & Araujo pers. comm.
Philosciidae indet. 1	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, H
Philosciidae indet. 2	Sandstone / Chapada Diamantina region (BA)	TS
Benthana sp.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, G, H
Platyarthridae		
Trichorhina spp.	Limestone / Bambuí group / several regions (BA, MG, SP, PR); Iron ore / Quadrilátero Ferrífero (MG)	H, L
Scleropactidae indet.	Sandstone / Altamira-Itaituba group / Altamira region (PA)	E, F, G, H, L
Styloniscidae indet. 1	Limestone / Bambuí group / Itacarambi region (MG)	TS
Styloniscidae indet. 2	Limestone / Bambuí group / Itacarambi region and Serra do Ramalho karst area (MG and BA)	L, TS
Styloniscidae indet. 3	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	TS
Styloniscidae indet. 4	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	TS
Styloniscidae indet. 5	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	F, G, H
Styloniscidae indet. 6	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, H
Styloniscidae indet. 7	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, H
Styloniscidae indet. 8	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	B, G, H

Taxon	Lithology / Geomorphological Unit / karst area or region	References
Styloniscidae indet. 9	Sandstone / Chapada Diamantina region (BA)	TS
Pectenoniscus sp. 1	Limestone / Brusque group / Botuverá region (SC)	L
Pectenoniscus sp. 2	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	TS
Pectenoniscus sp. 3	Limestone / Bambuí group / Lagoa Santa region (MG)	L
Pectenoniscus sp. 4	Limestone / Açungui group / Alto do Ribeira karst area (SP)	H, L
Order Decapoda		
Palaeomonidae		
Macrobrachium indet.	Sandstone / Altamira-Itaituba group / Prainha region (PA)	E, G, H
Subclass Acari indet. 1	Sandstone / Chapada Diamantina region (BA)	TS
Subclass Acari indet. 2	Sandstone / Chapada Diamantina region (BA)	TS
Order Amblypygi		
Charinidae		
Charinus sp.	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	L, TS
Order Araneae		
Symphytognathidae		
Anapistula sp.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, G, H
Hahniidae indet.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	E, F, H, L
Amaurobiidae indet.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	L
Ctenidae indet.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	M
Isoctenus sp.	Sandstone / Chapada Diamantina region (BA)	J, L
Enoploctenus sp.	Sandstone / Chapada Diamantina region (BA)	TS
Gnaphosidae indet.	Quartzitic / Serra da Mantiqueira / Ibitipoca region (MG)	L
Nesticidae		
Nesticus sp. 1	Limestone / Una-Irecê group / Chapada Diamantina region (BA)	L
Nesticus sp. 2	Limestone / Bambuí group / Lagoa Santa region (MG)	L
Ochyroceratidae indet. 1	Sandstone / Chapada Diamantina region (BA)	TS
Ochyroceratidae indet. 2	Sandstone / Chapada Diamantina region (BA)	J, L
Ochyroceratidae indet. 3	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	L, TS
Ochyrocera sp. 1	Limestone / Bambuí group / São Domingos karst area (GO)	L
Ochyrocera sp. 2	Granitic / Serra do Mar / Rio de Janeiro region (RJ)	L
Prodidomidae indet.	Sandstone / Chapada Diamantina region (BA)	TS
cf. Prodidomidae indet.	Limestone / Bambuí group / Serra da Canastra region (MG)	TS
Order Opiliones		
Gonyleptidae indet. 1	Limestone / Brusque group / Botuverá region (SC)	L
Gonyleptidae indet. 2	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	TS
Gonyleptidae indet. 3	Limestone / Açungui group / Alto do Ribeira karst area (SP)	L
Gonyleptidae indet. 4	Sandstone / Chapada Diamantina region (BA)	J
Eusarcus sp. 1	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	L, M
Eusarcus sp. 2	Limestone / Bambuí group / São Domingos karst area (GO)	L
Eusarcus sp. 3	Sandstone / Chapada Diamantina region (BA)	TS
Eusarcus sp. 4	Limestone / Bambuí group / Serra da Canastra region (MG)	TS
Escadabiidae indet	Limestone / Bambuí group / Itacarambi region (MG)	TS
Spaeleoleptes sp.	Limestone / Una-Irecê group / Chapada Diamantina region (BA)	L
Order Palpigradi spp.	Limestone / Açunguiand Bambuí groups / Alto do Ribeira karst area and Mambai region (SP and GO)	L
Eukoenenia sp.	Sandstone / Chapada Diamantina region (BA)	TS
Eukoenenia sp.	Limestone / Araras group / Nobres region (MT)	TS
Order Pseudoscorpiones		
Chernetidae indet.	Sandstone / Chapada Diamantina region (BA)	TS

Taxon	Lithology / Geomorphological Unit / karst area or region	References
Chthoniidae indet. 1	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, H
Chthoniidae indet. 2	Sandstone / Chapada Diamantina region (BA)	TS
Chthoniidae indet. 3	Iron ore / Quadrilátero Ferrífero (MG)	L
Class Diplopoda indet. 1	Limestone / Una-Irecê group / Chapada Diamantina region (BA)	F, H
Diplopoda indet. 2	Limestone / Bambuí group / Unaí region (MG)	D, H
Order Polydesmida indet. 1	Limestone / Bambuí group / Formosa region (GO)	F, G, H
Polydesmida indet. 2	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, H
Polydesmida indet. 3	Iron ore / Quadrilátero Ferrífero (MG)	L
Polydesmida indet. 4	Limestone / Bambuí group / Itacarambi region (MG)	K, TS
Chelodesmidae indet.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, H
Alecodesmus sp.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, H
Cryptodesmidae indet.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	D, F, G, H
Cryptodesmus indet.	Limestone / Açungui group / Adrianópolis region (PR)	H
Cryptodesmus sp. 1	Limestone / Açungui group / Alto do Ribeira karst area (SP)	L
Cryptodesmus sp. 2	Limestone / Açungui group / Alto do Ribeira karst area (SP)	L
cf. Cryptodesmidae indet.	Limestone / Una-Irecê group / Chapada Diamantina region (BA)	F, H
Oniscodesmidae indet. 1	Limestone / Una-Irecê group / Chapada Diamantina region (BA)	F, H
Oniscodesmidae indet. 2	Granitic / Serra do Mar / Ribeirão Pires region (SP)	F, H
Oniscodesmidae indet. 3	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, H
Crypturodesmus sp. 1	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	L, M
Crypturodesmus sp. 2	Limestone / Açungui group / Alto do Ribeira karst area (SP)	L
Crypturodesmus sp. 3	Limestone / Brusque group / Botuverá region (SC)	L
Katandodesmus spp.	Limestone / Açungui group / several regions (PR and SP)	F, G, H
Katandodesmus sp.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	F, G, H, M
Paradoxosomatidae indet.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	M
Pyrgodesmidae indet.	Limestone / Una-Irecê group / Chapada Diamantina region (BA)	TS
Order Spirostreptida		
Pseudonannolenidae indet.	Sandstone / Chapada Diamantina region (BA)	TS
Class Chilopoda		
Order Geophilomorpha		
Geophilidae indet.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	L
Order Scolopendromorpha		
Cryptopidae		
Cryptops sp.	Iron ore / Carajás Formation / Carajás region (PA)	L
Scolopendridae indet.	Sandstone / Chapada Diamantina region (BA)	TS
Order Lithobiomorpha indet.	Iron ore / Quadrilátero Ferrífero (MG)	L
Class Pauropoda indet.	Sandstone / Altamira-Itaituba group / Altamira region (PA)	TS
Class Symphyla indet.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	L
Scutigerellidae indet.	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	TS
cf. *Hanseniella* sp.	Limestone / Rio Pardo group (BA)	L
Class Entognatha		
Order Collembola indet.	Limestone / Bambuí group / Itacarambi region (MG)	K, TS
Arrhopalitidae indet.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	F, G, H
Arrhopalites sp.	Iron ore / Quadrilátero Ferrífero (MG)	L
Hypogastruridae		
Acherontides spp.	Limestone / Brusque and Rio Pardo groups (SC and BA)	L
Onychiuridae indet.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, H
Isotomidae spp.	Granitic, Limestone and Iron ore / Serra do Mar, Bambuí group and Quadrilátero Ferrífero / several regions (SP and MG)	D, F, G, H, L

Taxon	Lithology / Geomorphological Unit / karst area or region	References
Entomobryidae spp.	Limestone and Sandstone / Açungui, Bambuí, Corumbá groups and Chapada Diamantina region (BA, GO, MS, PR and SP)	F, G, H, L, M
Heteromurus sp.	Sandstone / Chapada Diamantina region (BA)	TS
Verhoefiella sp.	Sandstone / Chapada Diamantina region (BA)	TS
Cyphoderidae spp.	Granitic and Limestone / Serra do Mar, Bambuí and Corumbá groups / several regions (BA, GO, MS and SP)	F, G, H, M
Cyphoderus sp.	Limestone / Bambuí group / Montes Claros region (MG)	F, H
Paronellidae spp.	Limestone / Açungui, Una-Irecê and Corumbá groups / Alto do Ribeira karst area, Chapada Diamantina region and Serra da Bodoquena karst area (SP, BA and MS)	D, F, H, G
Trogolaphysa sp.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	M
Troglopedetes sp. 1	Sandstone / Chapada Diamantina region (BA)	TS
Troglopedetes sp. 2	Limestone / Brusque group / Botuverá region (SC)	L
Troglobius sp. 1	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, H
Troglobius sp. 2	Sandstone / Altamira-Itaituba / Prainha region (PA)	E, F, H
Class Insecta		
Order Blattaria		
Blattellidae indet.	Sandstone / Chapada Diamantina region (BA)	TS
Order Coleoptera		
Carabidae indet.	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	TS
Oxydrepanus sp.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, G, H, L
Dytiscidae indet.	Sandstone / Chapada Diamantina region (BA)	TS
Staphylinidae		
Pselaphinae indet. 1	Limestone / Açungui group / Alto do Ribeira karst area (SP)	C, F, H
Pselaphinae indet. 2	Limestone / Bambuí group / São Domingos karst area (GO)	TS
Pselaphinae indet. 3	Sandstone / Chapada Diamantina region (BA)	TS
Arthimius sp.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, G, H, L
Syrbatus sp. 1	Limestone / Bambuí group / Pains region (MG)	F, H, L
Syrbatus sp. 2	Granitic / Serra do Mar / Rio de Janeiro region (RJ)	F, H, L
Tr. Brachyglutini indet.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, H
cf. *Strombopsis* sp.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	F, G, H, L
Tenebrionidae indet.	Granitic / Serra do Mar / Rio de Janeiro region (RJ)	F, H, L
Order Hemiptera		
Dipsocoridae indet.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	M
Enicocephalidae indet.	Iron ore / Quadrilátero Ferrífero (MG)	L
Ortheziidae indet.	Iron ore / Quadrilátero Ferrífero (MG)	L
Hydrometridae indet.	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	TS
Order Hymenoptera		
Formicidae		
Formicinae indet.	Limestone / Bambuí group / São Desidério karst area (BA)	TS
Ponerinae indet.	Limestone / Bambuí group / São Domingos karst area (GO)	F, G, H, L
Order Orthoptera		
Phalangopsidae indet.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	TS
Phylum Mollusca		
Order Caenogastropoda		
Pomatiopsidae		
cf. *Spiropockia* sp.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	M
Order Mesogastropoda		
Potamolithus sp. 1	Limestone / Açungui group / Alto do Ribeira karst area (SP)	I
Potamolithus sp. 2	Limestone / Açungui group / Alto do Ribeira karst area (SP)	I
Potamolithus sp. 3	Limestone / Açungui group / Alto do Ribeira karst area (SP)	I
Potamolithus sp. 4	Limestone / Açungui group / Alto do Ribeira karst area (SP)	I

Taxon	Lithology / Geomorphological Unit / karst area or region	References
Potamolithus sp. 5	Limestone / Açungui group / Alto do Ribeira karst area (SP)	D, F, H
Potamolithus sp. 6	Limestone / Açungui group / Alto do Ribeira karst area (SP)	I, M.E. Bichuette pers. obs.
cf. *Potamolithus* sp.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	L
Order Pulmonata		
Endodontidae indet.	Limestone / Açungui group / Alto do Ribeira karst area (SP)	L
Systrophiidae		
Happia sp.	Sandstone / Chapada Diamantina region (BA)	TS
Phylum Chordata		
Order Siluriformes		
Loricariidae		
Ancistrus sp.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	M
Trichomycteridae		
Trichomycteridae indet.	Limestone / Bambuí group / Pains region (MG)	TS
Trichomycterus sp. 1	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	TS
Trichomycterus sp. 2	Limestone / Bambuí group / Serra do Ramalho karst area (BA)	TS
Copionodon sp.	Sandstone / Chapada Diamantina region (BA)	TS
Heptapteridae		
Heptapteridae indet.	Limestone / Bambuí group / Posse region (GO)	TS
Rhamdia sp.	Limestone / Corumbá group / Serra da Bodoquena karst area (MS)	M
Rhamdiopsis sp. 1	Limestone / Bambuí group / Cordisburgo region (MG)	E. Trajano pers. comm.
Rhamdiopsis sp. 2	Limestone / Una-Irecê group / Chapada Diamantina region (BA)	E. Trajano pers. comm.

Table 3. Threats recorded for different Brazilian regions with subterranean taxa. Highlighted in bold, intense degradation activities nowadays; highlighted in italics, potential threats in the near future. SHPS – small hydroelectric power-station buildings.

State / Region	Municipality	Lithology / Geomorphological Unit	Threats
Pará / North Brazil	Altamira region	Sandstone / Altamira-Itaituba group	**Reservoir construction** (Belo Monte) / **Deforestation for pastures**
–	Parauapebas, Curionópolis and Canaã dos Carajas region	Iron ore / Carajás Formation	**Mining**
Mato Grosso do Sul / Central Brazil	Bonito and Jardim regions	Limestone / Corumbá group	**Deforestation for pastures** / Mining projects
Mato Grosso / Central Brazil	Nobres region	Limestone / Araras group	*Hydroelectric project* /*Mining* / *Deforestation for agriculture*
Rio Grande do Norte / Northeastern Brazil	Felipe Guerra and Governador Dix-Spet Rosado regions	Limestone / Apodi group	Mining / Natural gas and oil exploration
Bahia / Northeastern Brazil	Morro do Chapéu region	Limestone / Una-Irecê group	Pollution of subterranean drainages / Deforestation for agriculture / *Mining projects*
–	Iraquara region	Limestone / Una-Irecê group	**Lowering of the water table** / **Uncontrolled tourism**

State / Region	Municipality	Lithology / Geomorphological Unit	Threats
–	Carinhanha, Coribe, Santana and Santa Maria da Vitória regions	Limestone / Bambuí group - Serra do Ramalho karst area	Deforestation for charcoal production and agriculture / *Mining projects*
–	São Desidério region	Limestone / Bambuí group	*Road construction* (collapses of rock) / *Pollution of subterranean drainage*
–	Itaetê region	Limestone / Una-Irecê group	**Uncontrolled tourism / Deforestation for pastures and agriculture**
–	Andaraí and Lençóis regions	Sandstone / Chapada Diamantina	Illegal garimpo / Uncontrolled tourism
	Paripiranga region	Limestone / Canudos supergroup	*Mining projects*
Goiás / Central Brazil	São Domingos region	Limestone / Bambuí group - São Domingos karst area	Uncontrolled tourism / Ilegal mining / Deforestation for pastures and charcoal production
–	Posse and Mambaí regions	Limestone / Bambuí group	Deforestation for pastures, agriculture and charcoal production
	Distrito Federal region	Limestone / Bambuí group	*Mining projects*
Tocantins / Central Brazil	Aurora do Tocantins	Limestone / Bambuí group	Deforestation for pastures and agriculture / *Mining projects*
Minas Gerais / Southeastern Brazil	São Roque de Minas	Limestone / Bambuí group - Serra da Canastra region	Uncontrolled tourism / Deforestation for pastures
–	Jaíba region	Limestone / Bambuí group	**Lowering of the water table /** Pollution of subterranean drainage
–	Presidente Olegário region	Limestone / Bambuí group	**SHPS** / Deforestation for pastures
–	Caeté, Moeda and Brumadinho regions	Iron ore / Quadrilátero Ferrífero	**Mining**
–	Itacarambi and Januária regions	Limestone / Bambuí group	Deforestation for pastures and charcoal production.
–	Cordisburgo region	Limestone / Bambuí group	Uncontrolled tourism (Maquiné cave) / Deforestation for pastures and agriculture
–	Sete Lagoas region	Limestone / Bambuí group	**Mining**
–	Pains region	Limestone / Bambuí group	**Mining**
	Serra da Mantiqueira region	Quartizitic	Deforestation for agriculture / Pollution by pesticides
São Paulo / Southeastern Brazil	Iporanga, Apiaí and Eldorado regions	Limestone / Açungui group - Alto do Ribeira karst area	Uncontrolled tourism / Land conflicts / Pollution of subterranean drainage due to illegal mining and tomatoes plantation / SHPS
–	Itirapina region	Sandstone	Deforestation for pastures and agriculture / Pollution of subterranean darinages
–	Serra do Mar region	Quartizitic	Deforestation for agriculture / Pollution by pesticides
Paraná / South Brazil	Adrianópolis and Rio Branco do Sul regions	Limestone / Açungui group - Alto do Ribeira karst area	SHPS / Deforestation for pastures and agriculture

ported". This corresponds to 53 % of the known described subterranean species being included in the IUCN Red List at that time. From 2004 to 2014 we observe augmentation of the Red List, from 33 to 83 species, as well as an increase in the number of described obligatory subterranean species. The majority of these are in the Endangered (EN) or Critically Endangered (CR) categories, compared with the previous Red List, corroborating the fragility of this fauna. Besides there are many species that have not been evaluated (Table 1).

Discussion

Considering the small number of Brazilian subterranean species recorded to date (150 species plus 156 troglomorphic taxa), we highlight the extreme difficulty in effectively protecting these species. Taxonomic impediment (Linnean shortfall - most of the species have not been described and catalogued (Brown and Lomolino 1998)) is reflected in our results, including specimens of known taxa that have been stored for over 20 years that still are undescribed (e.g., Pseudoscorpiones and Diplopoda). Thus, there is an urgent need for training new taxonomists, since they can accelerate the descriptions, conduct revisionary works, and then include obligatory subterranean species in the IUCN Red List.

As observed in other studies, São Paulo and Bahia States have the highest numbers of obligatory subterranean species, since the São Paulo cave fauna is the best studied in Brazil (Dessen et al. 1980, Trajano 1987, Trajano and Gnaspini-Netto 1991). Regarding the Bahia State, the extended limestone area associated with the current semi-arid climate conditions and the history of past climates has allowed many possibilities for faunistic isolations (Trajano 1995, Trajano et al. 2016). Indeed, it is in this state that we recorded the highest number of obligatory subterranean species occurring also in other kinds of previously neglected lithologies, such as sandstone (Gallão and Bichuette 2015).

Publication of Decree 6640 and the corresponding Normative Instructions (2009, 2017), which classifies caves in terms of relevance degrees, resulted in suppression of Brazilian cave listings. The NIs recommend that subterranean studies for environmental impact assessment reports (for commercial use of the cave/subterranean habitat, such as mining) include two cave sampling campaigns, one in the dry season and one in the rainy season. Highlighting conceptual problems of the NIs, Deharveng et al. (2009) show that even after 110 samplings in European karstic areas, obligatory subterranean species were found. Subterranean fauna inventories may be so inadequate that many species become extinct, before they are discovered and identified (Schneider and Culver 2004, Zagmajster et al. 2014). Thus, adequate sampling methods in different habitats are extremely relevant (Brancelj 2002, Bichuette et al. 2015). Poor subterranean studies represent another problem considering cave conservation. Trajano and Bichuette (2010b) and Trajano et al. (2012) stressed that inadequate sampling designs for evaluation of taxonomic and ecological characteristics leads to biased conclusions, and consequently compromises the conservation of these habitats.

According to Primack and Rodrigues (2001), some species are especially vulnerable to extinction and occur in the following categories: *limited occurrence area*; *one or few known populations*; *small populations*; declining populations; *low population density*; need huge habitats; large species; *species that are not effective dispersers*; seasonal migrants; *low genetic variability*; *species that require special niches*; *species that occur in stable environments*; permanent or temporary aggregations species; and hunting or consumed species. Among these fourteen categories, obligatory subterranean fauna fit at least eight of them (highlighted in italics), revealing the fragility and vulnerability of this fauna.

Although the extent and intensity of deforestation have been relatively high in our study area, reservoir construction for hydroelectric power stations and mining projects are worse threats because these can cause total destruction or irreversible impacts (total removal or flooding) of subterranean habitats, which could lead to fauna extinction as a result of physical destruction of the habitat (Culver 1986). According to Groombridge (1992), habitat loss is the most harmful threat to vertebrates as well as invertebrates, reinforcing the harm caused by the above activities, which can decimate cave fauna.

Recognition of the importance and fragility of subterranean environments by government agencies is becoming apparent with inclusion of obligatory subterranean fauna in threatened species lists. Gallão and Bichuette (2012) stressed the importance of the IUCN Red List for the protection of obligatory subterranean fauna in Brazil. When there is such inclusion, the cave is categorized as 'maximum totally avoiding cave destruction/suppression', thus, the IUCN Red List becomes one of the most important tools for protecting caves in Brazil. The IUCN Red List is also an important tool for obligatory subterranean species conservation, since it is one element (among others, see Trajano and Bichuette (2010b) for a review) that includes hypogean habitats as having maximum relevance according to the new Brazilian speleological laws (Decree 6640; see Trajano 2010, 2013, Trajano and Bichuette 2010b). Another relevant and critical point is that, with the inclusion of subterranean species in the IUCN Red List, the whole habitat is being protected. Despite caves with several subterranean species being existing conservation priorities, inclusion of a single subterranean species should be enough to protect the entire cave. However, it is important that we try to protect the entire system, i.e., the cave itself, the surroundings, and the hydrographic basin and/or landscape (Gallão and Bichuette 2012).

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RESEARCH ARTICLE

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Species of the fungivorous genus *Psalidothrips* Priesner from China, with five new species (Thysanoptera, Phlaeothripidae)

Chao Zhao[1], Hongrui Zhang[2], Xiaoli Tong[1]

1 *Department of Entomology, College of Agriculture, South China Agricultural University, Guangzhou 510642, China* **2** *Plant Protection College, Yunnan Agricultural University, Kunming 650201, China*

Corresponding author: *Xiaoli Tong* (xtong@scau.edu.cn)

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Abstract

An identification key and review is provided of fifteen species of the fungivorous genus *Psalidothrips* Priesner from China, with five new species, *P. angustus* **sp. n.**, *P. comosus* **sp. n.**, *P. fabarius* **sp. n.**, and *P. latizonus* **sp. n.**, and *P. nigroterminatus* **sp. n.** In addition, *Psalidothrips consimilis* Okajima, previously known only from Ryukyu Islands, Japan, is newly recorded in China.

Keywords

fungus-feeding, leaf-litter thrips, new species, Phlaeothripinae

Introduction

Psalidothrips Priesner is one of the most common phlaeothripine genera in tropical and subtropical regions. The members of the genus are fungus-feeders, and are particularly associated with leaf litter, their body and wing form probably being an adaptation to such habitats (Mound 1970; Okajima 1983). The genus appears to be derived from

Hoplothrips and belongs to the *Phlaeothrips*-lineage (Mound and Marullo 1996; Okajima 2006; Dang et al. 2014). Okajima (1983) reviewed the genus worldwide and provided an identification key to 17 known species. Subsequently, eleven further species were added to the genus: from New Zealand (three species), Japan (four species), and China (four species) (respectively Mound and Walker 1986; Okajima 1992; Zhang and Tong 1997; Wang et al. 2007). Okajima and Urushihara (1992) transferred *Trichothrips lewisi* Bagnall, to *Psalidothrips* and treated *P. alaris* Haga as a synonym of *P. lewisi* (Bagnall). Later, *P. lepidus* zur Strassen was considered a synonym of *P. conciliatus* Hood, and *Hennigithrips ananthakrishnani* Johansen was transferred to *Psalidothrips* by Mound and Marullo (1996). Up to the present, 28 *Psalidothrips* species are known worldwide, 15 of these being from Asia (ThripsWiki 2017). The purpose of this paper is to review the *Psalidothrips* species now recognized from China, to provide an updated identification key to these 15 species, including five new species and one newly recorded species from China, together with male pore plate illustrations of thirteen species.

Materials and methods

All thrips specimens in this study were extracted by using Tullgren funnels from leaf litter unless otherwise noted, and the specimens then were sorted and preserved in 90% alcohol. Examined specimens were mounted in Canada balsam using the method outlined by Zhang et al. (2006). Slide-mounted specimens were examined and photographed under the microscope with a digital camera attached. The following abbreviations are used for the pronotal setae:

am anteromarginal
aa anteroangular
ml midlateral
epim epimeral
pa posteroangular

Slide-mounted specimens of *P. lewisi* (male and female), *P. longiceps*, and *P. simplus* (male and female) have also been examined; these were provided by Professor Okajima of Tokyo University of Agriculture (**TUA**, Japan). All type specimens are preserved in the Insect Collection, South China Agricultural University (**SCAU**) unless otherwise noted.

Taxonomy

Key to *Psalidothrips* species from China

1 Antennal segment III with 2 sense cones..... **2**
– Antennal segment III with 3 sense cones..... **6**

2 Abdominal tergites III to VII each with one pair of simple wing-retaining setae; antennal segment IV with 2–4 sense cones; abdominal tergite II concolourous with the other tergites; pelta trapezoidal or broadly hat-shaped; postocellar setae much shorter than hind ocellus; male pore plate transversely long oval (Fig. 78)..... ***simplus***

– Abdominal tergites III to VII each with two pairs of wing-retaining setae; antennal segment IV with 2 sense cones only..... **3**

3 Head largely yellow..... **4**

– Head uniformly brown..... **5**

4 Antennal segments I–III yellow, IV–VIII dark brown; abdominal tergite II concolourous with the other tergites; male pore plate (Fig. 59) arched with slightly straight anterior margin..... ***nigroterminatus* sp. n.**

– Antennal segments I–II pale brown, III–VIII gradually from yellow to pale brown towards apex; abdominal tergite II darker than other tergites; male pore plate broad and arched, reaching to lateral margins (Fig. 70)..... ***latizonus* sp. n.**

5 Antennal segments I–II and VI–VIII brown, other segments yellow; postocellar setae slightly longer than hind ocellus; postocular setae slightly longer than eyes and pointed at apex; antennal segments IV–VI not globular; male pore plate narrow and slightly arched (Fig. 73)..... ***chebalingicus***

– Antennal segments I and basal half of II light brown, segment III yellow, IV–VIII yellowish brown, gradually darkened distally; postocellar setae twice as long as hind ocellus; postocular setae approximately 1.5 times longer than eyes, blunt or weakly expanded at apex; antennal segments IV–VI globular; male pore plate arched with a projection medially (Fig. 69)..... ***fabarius* sp. n.**

6 Antennal segment IV with 3 sense cones..... **7**

– Antennal segment IV with 4 sense cones..... **11**

7 Fore tarsal tooth present in both sexes..... **8**

– Fore tarsal tooth absent in female..... **9**

8 Postocular setae blunt apically; antennal segment VIII longer than segment VII; pelta irregularly trapezoidal without campaniform sensilla; male pore plate slightly arched (Fig. 77)..... ***longidens***

– Postocular setae expanded apically; antennal segment VIII as long as segment VII; pelta hat-shaped with a pair of campaniform sensilla; male unknown..... ***armatus***

9 Postocular setae longer than eyes..... **10**

– Postocular setae shorter than eyes with apices acute; antennal segments brown except III–IV yellow, segment VIII longer than VII; male pore plate arched and reaching to near the margins (Fig. 72)..... ***bicoloratus***

10 Postocular setae and pronotal epim with apices expanded; sense cones on antennal segment IV about two thirds as long as the segment; abdominal tergite II concolourous with other tergites; male unknown..... ***amens***

– Postocular setae and pronotal epim with apices pointed; sense cones on antennal segment IV about half as long as the segment; abdominal tergite II darker than other tergites; male pore plate shuttle-shaped (Fig.75)..... ***elagatus***

11 Fore tarsal tooth present in female **12**
– Fore tarsal tooth absent in female..... **13**
12 Antennae brown except basal third of III yellowish brown, surface without sculpture; postocular setae expanded at apex; abdominal tergites II to VII each with two pairs of wing-retaining setae; male pore plate slightly arched and incomplete, not reaching lateral margins (Fig. 67)..... ***angustus* sp. n.**
– Antennae almost uniformly yellow, segments III–VII with lines of sculpture; postocular setae pointed at apex; abdominal tergites II to VII each with one pair of wing-retaining setae; male pore plate on abdominal sternite VIII narrow and arched, reaching to near lateral margins (Fig. 68) ..... ***comosus* sp. n.**
13 Postocellar setae much longer than diameter of hind ocellus; antennal segment VIII as long as segment VII; male pore plate banded and complete, reaching lateral margins (Fig. 76)..... ***lewisi***
– Postocellar setae usually as long as or shorter than diameter of hind ocellus..... **14**
14 Antennal segment VIII slightly longer than segment VII, segments III and IV somewhat globular; male pore plate arched and incomplete (Fig. 74) .... ***consimilis***
– Antennal segment VIII as long as or shorter than segment VII, segments III and IV slender, not globular; male pore plate arched and complete (Fig. 71) .... ***ascitus***

Psalidothrips amens Priesner

Psalidothrips amens Priesner, 1932: 62.

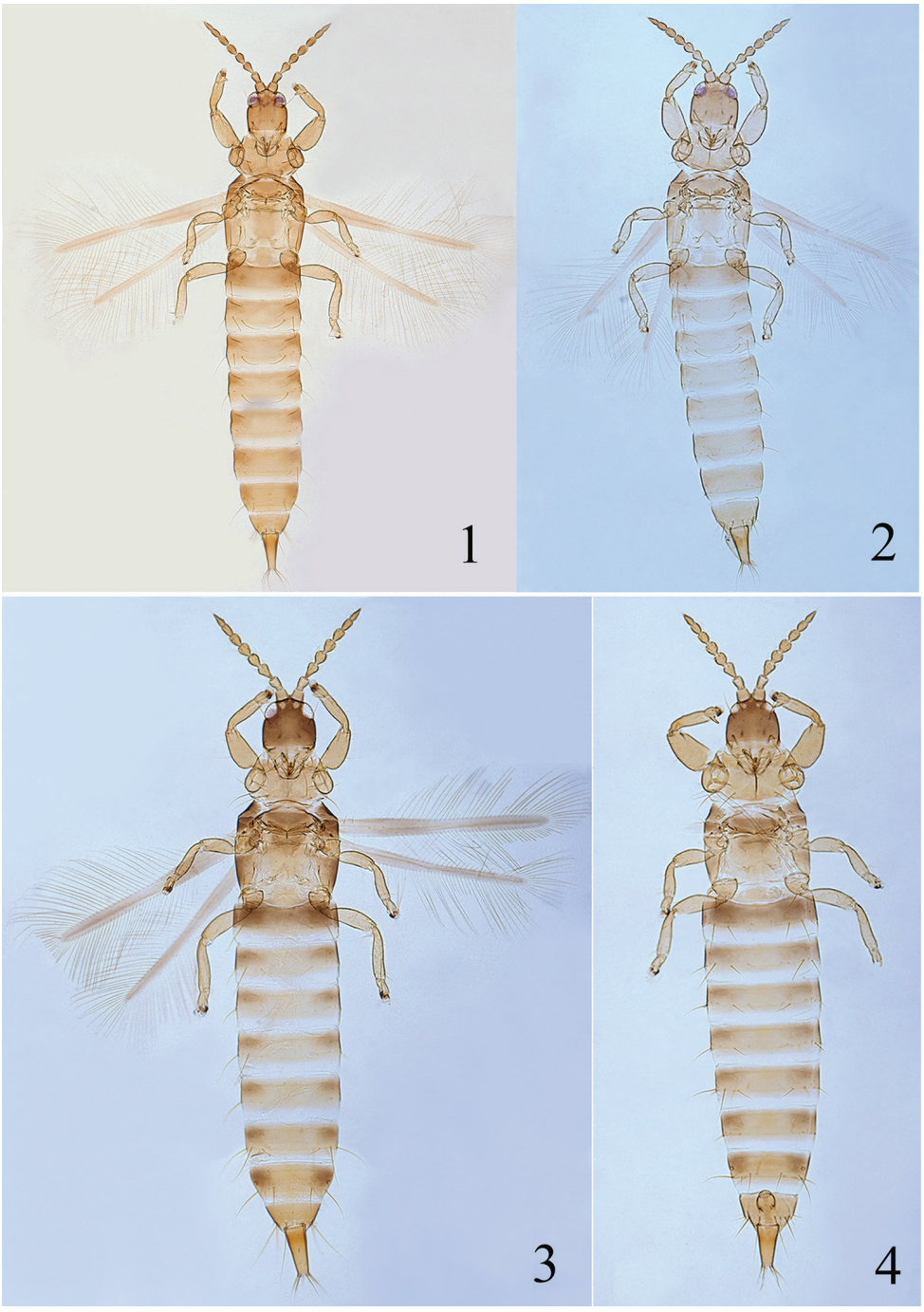
Comments. This is the type species of the genus, and was based on a single female from Java, Indonesia. It belongs to the group that have antennal segments III–IV each with three sense cones. Two females listed here are identified as *P. amens* based on the original description and the key in Mound (1970). Wang et al. (2007) mentioned that a single male of *P. amens* was recorded from Hainan, China. We examined that slide-mounted specimen, labelled as the male of *P. amens* by Wang et al. (2007), and consider that it represents the male of *P. latizonus* sp. n., described below. In China, *P. amens* is found, so far, only from Hainan.

Distribution. China (Hainan); Indonesia (Java).

Psalidothrips angustus sp. n.

http://zoobank.org/14F2A513-EDFD-4FD2-ADE8-75A00A92B4EC
Figs 1–2, 29–36, 67

Material examined. Holotype female: **CHINA**, **Guangdong**: Guangzhou, Arboretum of South China Agricultural University (23°09'22"N, 113°21'15"E), 10.x.2014 (Chao Zhao).



Figures 1–4. New *Psalidothrips* species. *Psalidothrips angustus* sp. n.: **1** female **2** male; *Psalidothrips comosus* sp. n. **3** female **4** male.

Figures 5–8. New *Psalidothrips* species. *Psalidothrips fabarius* sp. n.: **5** female **6** male; *Psalidothrips latizonus* sp. n. **7** female **8** male.

Paratypes. Four females and 1 male, collected with holotype; 42 females and 14 males, same locality as holotype, 29.xii.2013 (Jingna Li), 5 females and 1 male, 14.vii.2014 (Chao Zhao). **Guangdong**: Guangzhou City, Longdong (23°14'N, 113°24'E), 1 female, 1.xii.2006 (Jun Wang); Panyu, Dafushan Forest Park (22°57'33"N, 113°18'0"E),

2 females, 10.x.2014 (Chao Zhao). **Hainan**: Ledong County, Jianfengling National Nature Reserve (18°44'N, 108°51'E), 1 female, 31.x.1986 (Xiaoli Tong); Qiongzhong County, Limushan National Forest Park (19°12'40"N, 113°12'39"E, alt. 1200 m), 3 females and 1 male, 24.x.2017 (Chao Zhao).

Description. Female macropterous (Fig. 1). Head and antennae brown (but basal third of segment III paler), pronotum pale brown; mesonotum, abdominal segment II and sides of tergites III–VIII brown; the rest of body yellow or yellowish brown; fore wings greyish brown but paler medially.

Head almost as long as broad; dorsal surface smooth, faintly sculptured posteriorly; cheeks almost straight and constricted behind eyes. Eyes approximately one-third of head length; postocular setae much longer than eyes, expanded at apex (Fig. 29); postocellar setae fine and acute, longer than diameter of hind ocellus. Antennae 8-segmented (Fig. 36), somewhat moniliform, surface without sculpture; segment III vasiform and IV–V globular, segment VIII longer than segment VII; segments III–IV with three and four sense cones respectively, sense cones usually long and thick, those on segment IV usually longer than half of the segment. Maxillary stylets reaching approximately half way to postocular setae and placed far apart, often V-shaped.

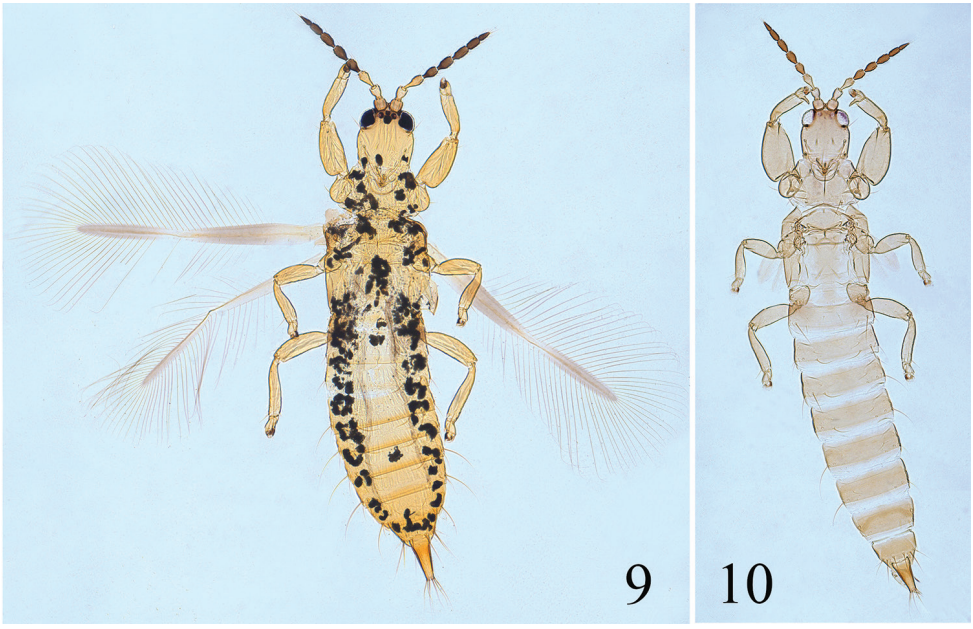
Pronotum broad (Fig. 30), dorsal surface smooth with a weak median longitudinal line; pronotal am and aa setae minute; ml, epim, and pa setae well developed, ml with expanded apex, epim and pa bluntly acute. Mesonotum sculptured on anterior third, lateral setae minute. Metanotum largely smooth with faint sculpture laterally. Mesopresternum boat-shaped, often eroded medially (Fig. 31). Fore tarsal tooth present (Fig. 32). Fore wing wide at base and constricted medially, sub-basal setae S1 minute, S2 slightly longer than S3.

Pelta hat-shaped with flat anterior margin, faintly reticulate medially, a pair of campaniform sensilla present (Fig. 34). Abdominal tergites II–VII with two pairs of weakly sigmoid wing-retaining setae; tergite IX setae S1 subequal to tube in length and shorter than S2; S2 slightly longer than tube, both pointed at apex (Fig. 35).

Measurements (holotype female in microns). Distended body length 1890. Head length 195, width 175; eye length 65; postocular setae length 80; diameter of posterior ocellus 22; postocellar setae length 32. Antennal length 360, segments I–VIII length (width) as follows: 36 (42); 40 (30); 55 (35); 48 (37); 43 (33); 42 (30); 38 (22); 46 (35). Pronotum median length 145, median width 270; length of major setae: ml 70, pa 85, epim 75. Fore wing length 800, subbasal setae S2–S3 length: 22, 15. Abdominal tergite IX S1 setae length 145, S2 setae length 165. Tube length 150, basal width 75, apical width 32; anals 140.

Male macropterous (Fig. 2). Similar in colour and structure to female, but body smaller; fore tarsal tooth present (Fig. 33); pore plate on abdominal sternite VIII disconnected and slightly arched (Fig. 67); abdominal tergite IX setae S1 as long as tube and longer than S2.

Measurements (paratype male in microns). Distended body length 1450. Head length 170, width 160; eye length 55; postocular setae length 65; diameter of posterior ocellus 15; postocellar setae length 25. Antennal length 310, segments I–VIII length (width) as follows: 31 (40); 32 (27); 46 (28); 38 (29); 38 (28); 37 (25); 32 (22); 40



Figures 9–10. *Psalidothrips nigroterminatus* sp. n.: **9** female **10** male.

(18). Pronotum median length 135, median width 260; length of major setae: ml 60, pa 75, epim 57. Fore wing length 520, subbasal setae S2–S3 length: 13, 21. Abdominal tergite IX S1 setae length 100, S2 setae length 75. Tube length 120, basal width 80, apical width 20, anals 105.

Etymology. The specific epithet, *angustus*, is from the Latin adjective, meaning narrow and refers to the narrow pore plate.

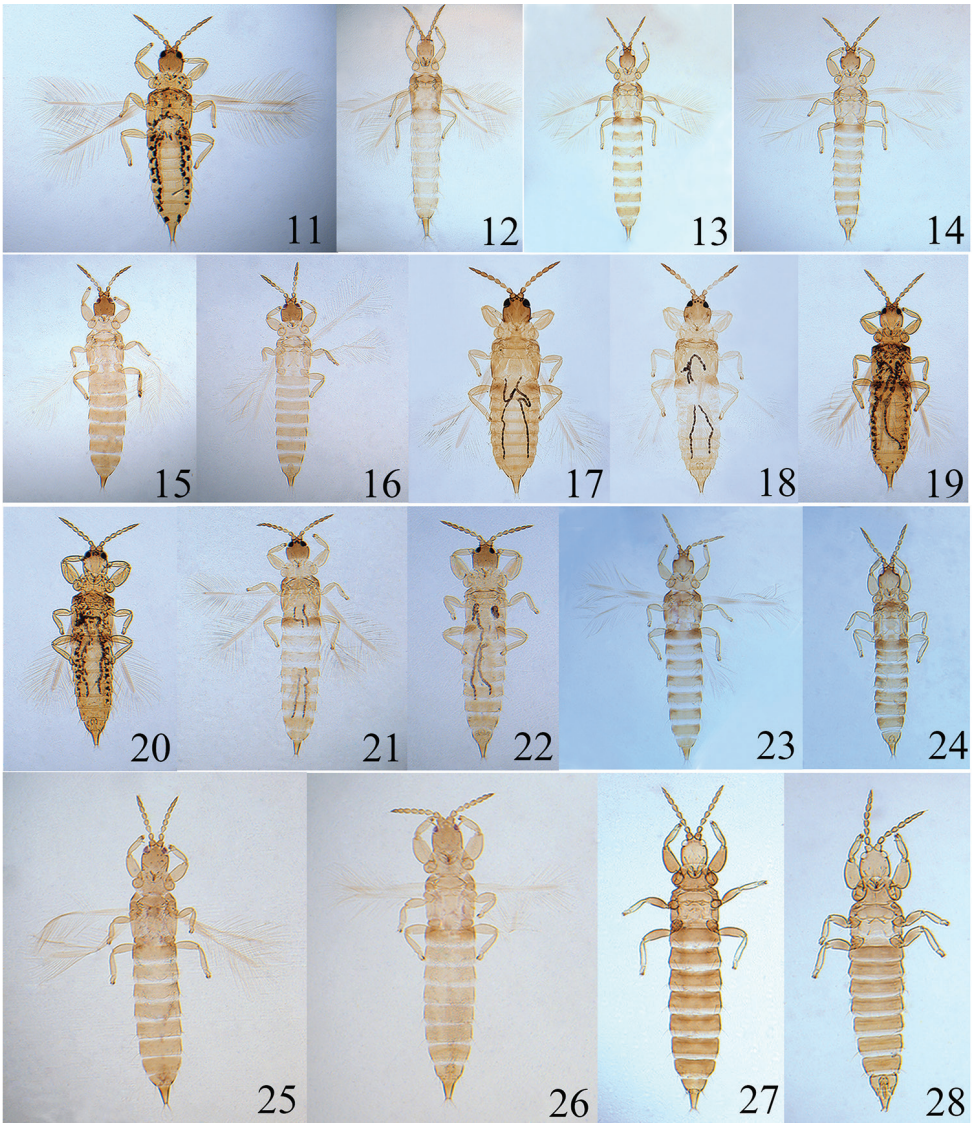
Distribution. China (Guangdong, Hainan).

Comments. This new species appears to be closely related to *P. comosus* sp. n., by sharing moniliform antennae and antennal segments III–IV with three and four sense cones, and the fore tarsal tooth present in female. However, it differs from the latter by the following characteristics: (1) the surface of antennae is without sculpture (apical half of antennal segments III–VII with lines of sculpture in *comosus*); (2) postocular setae with expanded apex (whereas *comosus* with pointed postocular setea); (3) abdominal tergites II to VII each with two pairs of wing-retaining setae (only one pair of wing-retaining setae on these segments in *comosus*); (4) male's pore plate on abdominal sternite VIII narrow and slightly arched, occasionally disconnected (whereas pore plate with wider band which reaches lateral margins in *comosus*).

Psalidothrips armatus Okajima

Fig. 12

Psalidothrips armatus Okajima, 1983: 6.



Figures 11–28. *Psalidothrips* species. *P. amens* **11** female; *P. armatus* **12** female; *P. ascitus* **13** female **14** male; *P. bicoloratus* **15** female **16** male; *P. chebalingicus* **17** female **18** male; *P. consimilis* **19** female **20** male; *P. elagatus* **21** female **22** male; *P. lewisi* **23** female **24** male; *P. longidens* **25** female **26** male; *P simplus* **27** female **28** male.

Comments. This species (Fig. 12) belongs to the group in which the fore tarsus is armed with a tooth in both sexes. Wang et al. (2007) recorded a male of this species in Hainan, China. In this study, we examined the male specimen labelled as *P. armatus* by Zhang and Tong (1997) and considered that this single male specimen seems to be an unknown species which is similar to the male of *P. angustus* sp. n. or *P. longidens.* In China, this species so far is only recorded in Hainan.

Distribution. China (Hainan); Thailand.

***Psalidothrips ascitus* (Ananthakrishnan)**
Figs 13–14, 71

Callothrips ascitus Ananthakrishnan, 1969: 176.
Psalidothrips ascitus (Ananthakrishnan): Okajima, 1983: 6.

Comments. This species is found widely across the tropical and subtropical areas of China, also in other parts of the world. It is most closely related to *P. lewisi* (Bagnall). Their males are difficult to distinguish from each other as their pore plates are very similar in shape (Figs 14, 71, 76), but the females can be distinguished from those of *P. lewisi* by the length of the postocellar setae that are usually as long as the diameter of an ocellus or shorter, and the colour of the antennae and head that are almost uniformly brown (Fig. 13) (Okajima 2006). However, these two species were always collected together at the same sampling sites from leaf litter. Overall, in structure these two species are very similar to each other and the possibility exists that they may represent a single, widespread species.

Distribution. China (Hubei, Guizhou, Hunan, Jiangxi, Yunnan, Guangdong, Hainan, Taiwan); Japan; Malaysia; India.

***Psalidothrips bicoloratus* Wang, Tong & Zhang**
Figs 15–16, 72

Psalidothrips bicoloratus Wang, Tong & Zhang, 2007: 28.

Comments. This species is very similar to *P. amens* in general appearance. However, it is distinguished from the latter by the following characters: head and antennal segments I–II, V–VIII brown, rest of body yellow (Fig. 15–16); postocular setae shorter than eyes, cheeks straight and weakly constricted behind eyes.

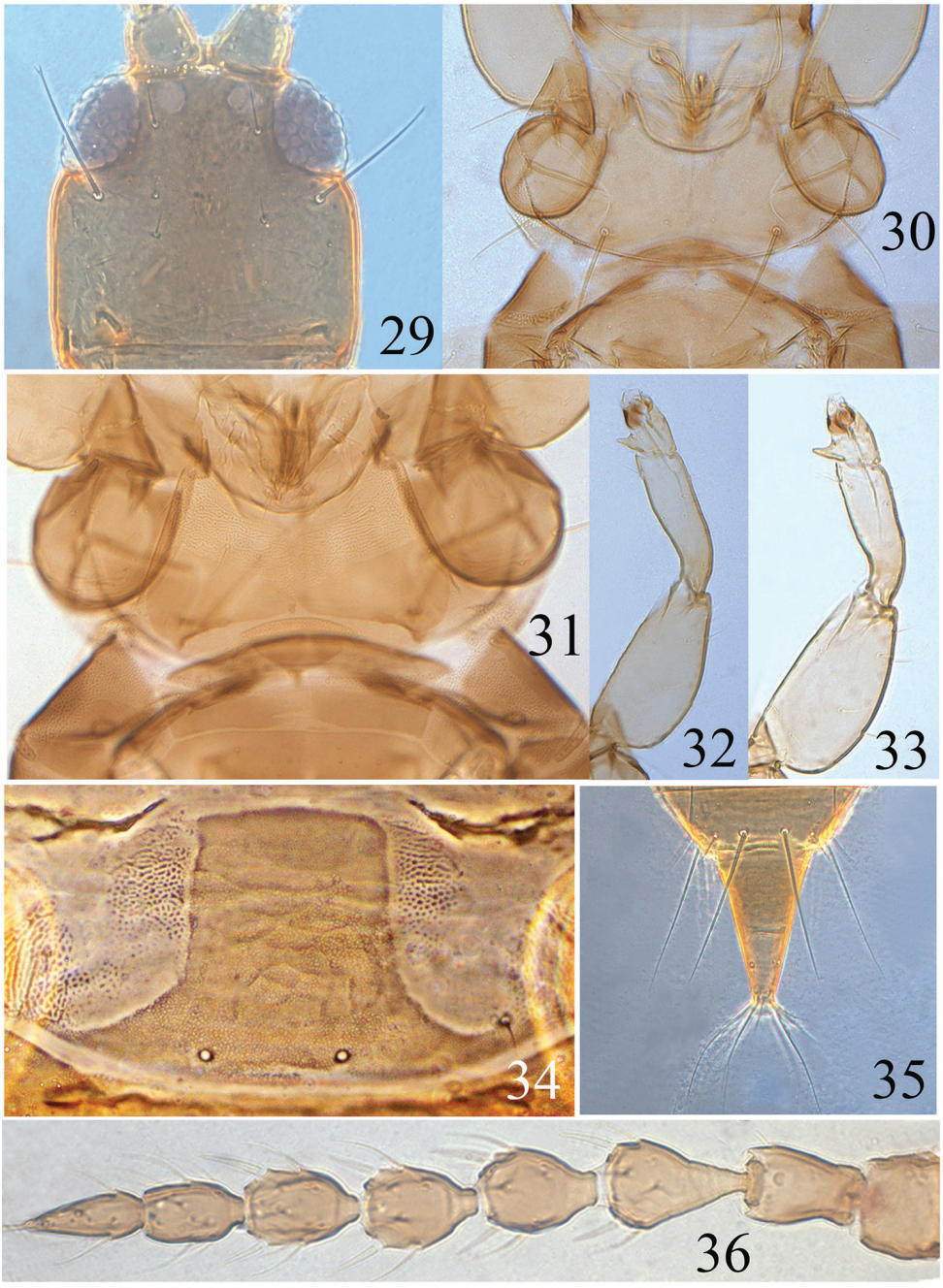
Distribution. China (Guangdong).

***Psalidothrips chebalingicus* Zhang & Tong**
Figs 17–18, 62–66, 73

Psalidothrips chebalingicus Zhang & Tong, 1997: 87.

Diagnosis. This species was originally described in Chinese with the male as holotype (Zhang & Tong 1997). Unfortunately, in original paper the illustrations were distorted by compression in the process of printing and the female was described very briefly, which meant that the female of *P. chebalingicus* could be confused with other similar species. The diagnosis is emended as follows:

Female macropterous (Fig. 17). Body yellow except head, antennal segments I–II and VI–VIII (Fig. 66), margins of pterothorax brown; abdominal tergite II brown with median portion yellow; antennal segments III–V yellowish brown but gradually dark-



Figures 29–36. *Psalidothrips angustus* sp. n. **29** head **30** pronotum **31** ventral view of prothorax **32** fore leg of female **33** fore leg of male **34** pelta **35** tube **36** antenna.

ened distally. Head (Fig. 62) slightly wider than long, dorsal surface smooth; cheeks weakly swollen and constricted just behind eyes; postocellar setae slightly longer than hind ocellus; postocular setae slightly longer than eyes and pointed at apex. Antennae

8-segmented (Fig. 66), surface without sculpture; segments III–IV each with two sense cones; segment VIII slightly longer than segment VII. Maxillary stylets reaching approximately half distance to postocular setae and far apart, V-shaped. Pronotum dorsal surface smooth, am and aa minute, ml apex blunt, epim and pa pointed at apex (Fig. 63). Fore tarsal tooth absent. Pelta hat-shaped, weakly sculptured on anterior half, with a pair of campaniform sensilla posteriorly (Fig. 64); abdominal tergites II–VII with two pairs of weakly sigmoid wing-retaining setae; abdominal tergite IX setae S1 subequal to tube in length (Fig. 65), setae S2 longer than tube, both S1 and S2 pointed at apex.

Male macropterous (Fig. 18). Similar in colour and structure to females, but body smaller; fore tarsal tooth present; pore plate on abdominal sternite VIII narrow and slightly arched medially (Fig. 73); abdominal tergite IX setae S1 slightly shorter than tube but much longer than S2.

Comments. This species is somewhat similar to *P. ascites* in colour and structure. However, it can be distinguished from the latter by the following main features: antennal segments III–IV each with two sense cones; pore plate on abdominal sternite VIII located medially, not reaching lateral margin.

Distribution. China (Hunan, Guangdong).

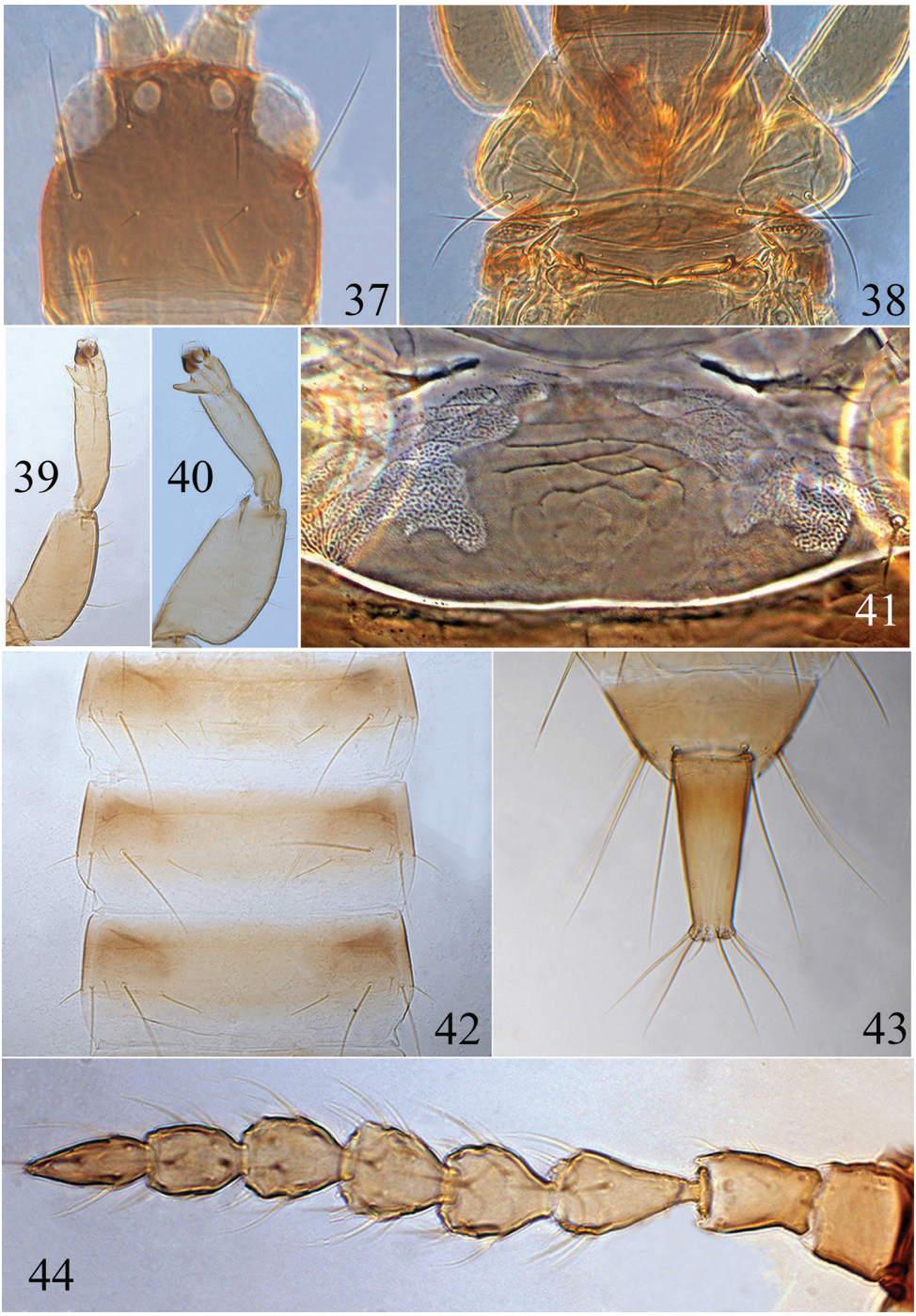
***Psalidothrips comosus* sp. n.**
http://zoobank.org/5ADD2A6A-82F9-4603-BEE8-4E794C91B985
Figs 3–4, 37–44, 68

Material examined. Holotype female (macropterous): **CHINA. Guangdong**: Shenzhen City, Honghu Park (22°33'N, 114°07'E), collected from leaf litter of *Araucaria heterophylla* (Araucariaceae), 2.xi.2017 (Chao Zhao).

Paratypes. 13 macropterous females, 4 apterous females, and 6 apterous males, collected with holotype. Nineteen apterous females, 5 macropterous females, and 8 apterous males, collected at the same locality as holotype, 30.x.2014 (Chao Zhao).

Description. Female macropterous (Fig. 3). Body yellow except for head, anterior and lateral margins of pterothorax, abdominal segment II dark brown; abdominal tergites III–VIII yellow with light brown shadings laterally; tube yellow with light brown basally. Wings shaded with greyish brown.

Head (Fig. 37) wider than long, faintly sculptured on posterior margin; cheeks almost straight or slightly widened towards base, but constricted behind eyes; eyes approximately 1/3 of head length; postocellar setae long and acute, approximately 1.5–2.0 times longer than the diameter of hind ocellus; postocular setae long and acute, approximately 2.0 times longer than eyes. Antennae 8-segmented (Fig. 44) and slightly moniliform, apical half of antennal segments III–VII with lines of sculpture; segment III vasiform, IV–V globular, segment VIII distinctly longer than segment VII; segments III–IV with 3 and 4 sense cones respectively. Maxillary stylets reaching approx. half way to postocular setae and wide apart.



Figures 37–44. *Psalidothrips comosus* sp. n. **37** head **38** pronotum **39** fore leg of female **40** fore leg of male **41** pelta **42** abdominal tergites III–V **43** tube **44** antenna.

Pronotum (Fig. 38) broad, surface smooth with a weak median longitudinal line; three pairs of major setae (ml, epim, pa) well developed, elongate and acute, aa setae fine and long, slightly shorter or subequal to interocular setae in length. Mesopresternum eroded with small irregular sclerites laterally. Fore wing sub-basal setae S1 shortest, S2 longer than S3. Fore tarsal tooth present (Fig. 39).

Pelta irregularly hat-shaped (Fig. 41), sculptured on anterior half, campaniform sensilla absent in holotype. Abdominal tergites II to VII each with one pair of straight wing-retaining setae (Fig. 42); tergite IX setae S1 and S2 long and acute, setae S1 as long as or slightly longer than S2, setae S2 longer than tube; tube slightly longer than head.

Female apterous. Similar to macropterous female in structure, but eyes smaller, approximately 1/4 of head length; postocellar setae approximately 2.5–3.5 times longer than diameter of hind ocellus; postocular setae elongate and acute, approximately 2.5 times longer than eyes.

Measurements (holotype female in microns). Distended body length 1680. Head length 155, width 178; eye length 60; postocular setae length 120; diameter of posterior ocellus 20; postocellar setae length 50. Antennal length 360, segments I–VIII length (width) as follows: 35 (39); 45 (30); 57 (33); 40 (35); 37 (39); 40 (35); 35 (27); 55 (20). Pronotum median length 135, median width 260; length of major setae: aa 50, ml 90, pa 130, epim 95. Abdominal tergite IX S1 setae length 212, S2 setae length 200. Tube length 165, basal width 80, apical width 40; anals 100.

Male apterous (Fig. 4). Colour and chaetotaxy similar to apterous females, but femora thickened and fore tarsal tooth well developed (Fig. 40); pore plate on abdominal sternite VIII narrow and arched, nearly reaching lateral margins (Fig. 68); tergite IX setae S1 much longer than S2; setae S2 shorter than tube (Fig. 43).

Measurements (paratype male in microns). Distended body length 1620. Head length 165, width 170; eye length 40; postocular setae length 100; diameter of posterior ocellus 10; postocellar setae length 25. Antennal length 340, segments I–VIII length (width) as follows: 37 (38); 40 (28); 55 (28); 38 (32); 36 (33); 38 (30); 35 (25); 47 (17). Pronotum median length 125, median width 255; length of major setae: aa 35, ml 87, pa 120, epim 80. Abdominal tergite IX S1 setae length 135, S2 setae length 85. Tube length 125, basal width 70, apical width 33; anals 100.

Etymology. Specific epithet from Latin *comosus* which means long haired, and refers to the new species having relatively long body setae.

Distribution. China (Guangdong).

Comments. This new species is similar to *P. taylori* Mound & Walker from Australia and New Zealand in sharing the elongate postocular setae, distinct pronotal aa setae, and only one pair of wing-retaining setae on abdominal tergites (Mound and Walker 1986). However, it can be readily distinguished from the latter by the antennal segments III–VII with lines of sculpture, segments III–IV with three and four sense cones respectively, and a fore tarsal tooth present in both sexes. Campaniform sensilla are absent from the pelta of the holotype, but are present on the pelta of paratypes from the same population. This new species is also similar to the new species, *P. angustus*, as discussed above.

***Psalidothrips consimilis* Okajima**
Figs 19–20, 74

Psalidothrips consimilis Okajima, 1992: 541.

Material examined. CHINA. Guangdong: Foshan City, Suoluo Nature Reserve (22°29'N,111°30'E), 2 females and 1 male, 27.iii.2005 (Jun Wang), 2 females and 1 male, 3.vii.2014 (Chao Zhao).

Distribution. China (Guangdong); Japan (Ryukyu Islands).

Comments. Described originally from Ryukyu Islands, Japan (Okajima 1992), this thrips is here newly recorded from China. In the description (Okajima 1992), the postocellar setae are minute, usually shorter than the diameter of the hind ocellus, but in the specimens listed here these setae are variable in length: some of them are much longer than the hind ocellus. The female (Fig. 19) is very similar to that of *P. ascites* (Okajima 1992, 2006). However, the males (Fig. 20) can be easily distinguished from *P. ascitus* by the narrow and incomplete pore plate on abdominal sternite VIII (Fig. 74).

***Psalidothrips elagatus* Wang, Tong & Zhang**
Figs 21–22, 75

Psalidothrips elagatus Wang, Tong & Zhang, 2007: 26.

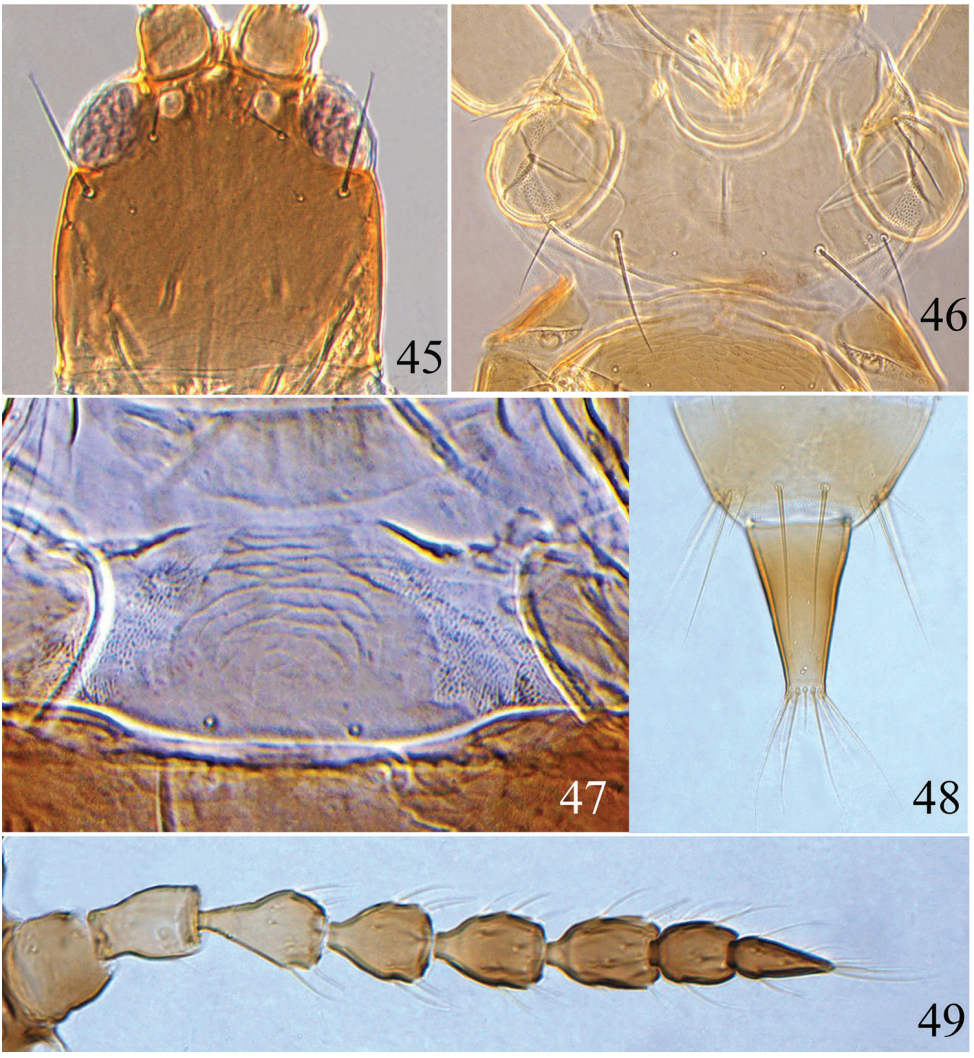
Comments. Wang and Tong (2007) stated that this species has two sense cones on antennal segment III, but re-examination of the type material has found this segment to have three sense cones, although the ventral one is small and short. Therefore, *P. elagatus* belongs to the group that have antennal segments III and IV each with three sense cones. This species (Figs 21–22) is similar to *P. bicoloratus*, but can be distinguished by the key above.

Distribution. China (Guangdong).

***Psalidothrips fabarius* sp. n.**
http://zoobank.org/6CB4F657-6301-4695-9E8D-95D17CB66609
Figs 5–6, 45–49, 69

Materials examined. Holotype female: **CHINA. Guangdong**: Guangzhou City, Arboretum of South China Agricultural University (23°09'N, 113°21'E), 29.xii.2013 (Jingna Li).

Paratypes. Ten females and 3 males, collected with holotype; same locality as holotype: 2 females, 31.iii.2011 (Tao Song), 1 female and 1 male, 26.viii.2013 (Jingna Li), 1 female and 1 male, 29.xii.2013 (Jingna Li), 3 females and 1 male, 26.vii.2014 (Chao Zhao), 6 females and 3 males, 14.vii.2014 (Chao Zhao); Guangzhou City, Huolushan Forest Park (23°10'47"N, 113°22'44"E), 2 females and 2 males, 1.vi.2014 (Jingna Li).



Figures 45–49. *Psalidothrips fabarius* sp. n. **45** head **46** pronotum **47** pelta **48** tube **49** antenna.

Description. Female macropterous (Fig. 5). Body yellow except head, mesothorax, abdominal tergite II brown, the rest of body yellow. Antennal segments I and basal half of II light brown, segment III yellow, IV–VIII yellowish brown gradually darkened distally. Wings shaded with greyish brown but paler medially.

Head (Fig. 45) almost as long as wide, dorsal surface smooth, faintly sculptured at base; cheeks weakly swollen, slightly constricted just behind eyes. Eyes one-third as long as head; postocular setae approximately 1.5 times longer than eyes and weakly expanded at apex; postocellar setae approximately twice as long as hind ocellus or longer. Antennae 8-segmented, somewhat moniliform, surface without sculpture (Fig. 49); segment VIII longer than segment VII; segments III and IV each with two sense

cones. Maxillary stylets reaching about one-third way to postocular setae and wide apart, often V-shaped.

Pronotum (Fig. 46) broad, surface smooth with a weak median longitudinal line; pronotal am and aa minute, the other three pairs of major setae well developed, pa longest, ml slightly longer than epim, epim and pa pointed, but ml expanded at apex. Fore tarsal tooth absent. Sub-basal wing seta S1 minute, S2 longer than S3, both pointed at apex.

Pelta nearly bell-shaped with short lateral lobes (Fig. 47), anterior half distinctly sculptured, a pair of campaniform sensilla present. Abdominal tergites II–VII with two pairs of simply curved wing-retaining setae; S1 and S2 on tergite IX subequal in length (Fig. 48), slightly shorter than tube, all pointed at apex; basal width of tube approximately 3.0 times wider than apical width; anal setae much shorter than tube.

Measurements (holotype female in microns). Body length 1680. Head length 180, head maximum width 175; eye length 55, postocular setae length 80; diameter of posterior ocellus 16, postocellar setae length 32. Antennal length 360, segments I–VIII length (width) as follows: 42(42); 42 (33); 56 (35); 46 (35); 50 (32); 46 (31); 33 (27); 42 (19). Pronotum median length 145, median width 240; length of major setae: ml 65, pa 80, epim 60. Fore wing length 720, subbasal setae S1–S3 length: 4, 35, 23. Abdominal tergite IX S1 setae length 100, S2 setae length 95. Tube length 120, tube basal width 70, apical width 23; anals 100.

Male apterous (Fig. 6). Similar to female in structure and colour, but smaller and fore tarsus armed with a tooth; setae S2 much shorter than S1 on abdominal tergite IX; abdominal sternite VIII pore plate arch-shaped with a projection medially (Fig. 69).

Measurements (paratype male in microns). Distended body length 1370. Head length 150, width 140; eye length 40; postocular setae length 68; diameter of posterior ocellus 12; postocellar setae length 28. Antennal length 295, segments I–VIII length (width) as follows: 32 (42); 35 (30); 46 (30); 35 (32); 35 (30); 38 (28); 32 (26); 42 (19). Pronotum median length 130, median width 210; length of major setae: ml 44, pa 64, epim 44. Abdominal tergite IX S1 setae length 85, S2 setae length 60. Tube length 100, basal width 55, apical width 25; anals 60.

Distribution. China (Guangdong).

Etymology. The specific epithet, *fabarius*, is from the Latin word meaning bead-like, referring to the moniliform antennal segments.

Comments. This new species appears to be most similar in appearance to *P. ochraceus* Okajima from Ryukyu Islands, Japan, particularly in having two sense cones on antennal segments III and IV, and the elongate postocellar setae, but it can be readily distinguished from the latter by the following characteristics: (1) postocular setae expanded at apex (pointed in *ochraceus*); (2) pronotal ml setae expanded at apex (ml pointed in *ochraceus*); (3) pelta distinctly sculptured on anterior half (whereas pelta indistinctly sculptured in *ochraceus*); (4) abdominal tergite IX setae S1 and S2 subequal in length, but shorter than tube (whereas in *ochraceus*, S1 slightly shorter than tube, S2 longer than tube); (5) three sub-basal wing setae present on fore wing (only one minute sub-basal wing seta present in *ochraceus*).

***Psalidothrips latizonus* sp. n.**
http://zoobank.org/25FD6569-3677-4870-A161-00EFA248D28E
Figs 7–8, 50–54, 70

Material examined. Holotype: CHINA. Hainan: 1 female, Ledong County, Jianfengling National Nature Reserve (18°44'N, 108°51'E), 30.x.1986 (Xiaoli Tong).

Paratypes. Two females 2 males, same data as holotype. **Guangdong**: 1 male, Haifeng County, Mt. Lianhuashan (23°03'N, 115°15'E), 14.ix.2005 (Jun Wang).

Female macropterous (Fig. 7). Head largely yellow or yellowish brown with dark brown margins; mesonotum yellowish brown with dark brown margin, abdominal tergite II brown, darker than other tergites, abdominal segments yellow shaded with pale brown laterally, the rest of body yellow. Antennal segments I–II pale brown, III–VIII shading gradually from yellow to pale brown towards apex. Wings shaded with greyish brown but paler medially.

Head (Fig. 50) almost as long as broad, dorsal surface smooth with a few lines of sculpture posteriorly; cheeks slightly swollen and constricted just behind eyes. Eyes approximately one-third of head length; postocellar setae approximately 2.5 times longer than hind ocellus; postocular setae bluntly acute, as long as or slightly longer than eyes . Antennae 8-segmented (Fig. 54), surface without sculpture; segments III and IV each with two sense cones, segment VIII longer than segment VII. Maxillary stylets short and wide apart, often V-shaped.

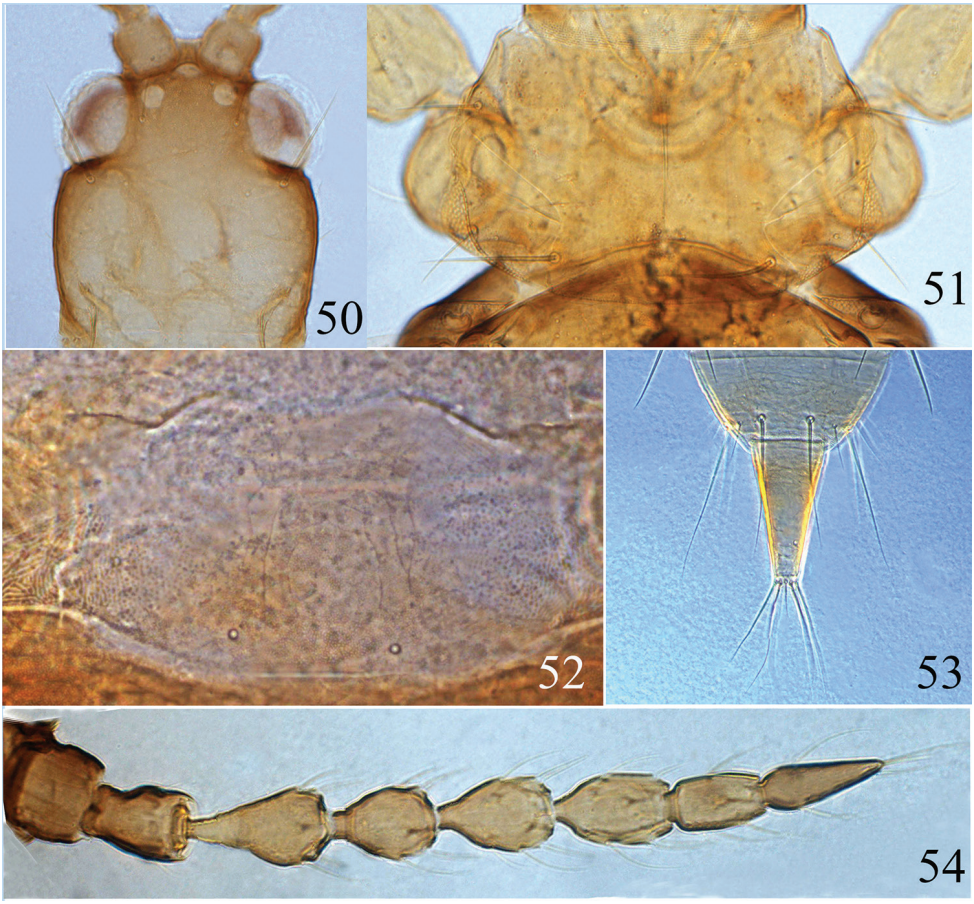
Pronotum about 0.8 times as long as head, almost smooth (Fig. 51); ml and epim subequal in length, pa longest, all bluntly acute. Fore tarsal tooth absent. Fore wing sub-basal wing seta S1 minute, S2 longer than S3, both pointed at apex.

Pelta nearly hat-shaped (Fig. 52), faintly sculptured, with a pair of campaniform sensilla. Abdominal tergites II to VII each with two pairs of sigmoid wing-retaining setae; tergite IX setae S1 shorter than S2 which slightly longer than tube (Fig. 53); basal width of tube 3–4 times wider than apical width.

Measurements (holotype female in microns). Distended body length 2050. Head length 205, width 203; eye length 70; postocular setae length 90; diameter of posterior ocellus 16; postocellar setae length 32. Antennal total length 425, segments I–VIII length (width): 42 (39); 47 (28); 63 (30); 57 (30); 59 (28); 59 (25); 43 (23); 55 (17). Pronotum median length 160, median width 260; length of major setae: ml length 45, pa length 65, epim length 50. Fore wing length 800, subbasal setae S1–S3 length: 3, 15, 13. Abdominal tergite IX setae S1 length 130, S2 length 165. Tube length 145, tube basal width 80, apical width 27, anals 100.

Male macropterous (Fig. 8). Similar in colour and structure to female except for fore tarsal tooth present and setae S1 slightly longer than S2 on abdominal tergite IX; pore plate on abdominal sternite VIII broadly arched (Fig. 70).

Measurements (paratype male in microns). Distended body length 1230. Head length 190, width 175; eye length 55, postocular setae length 75; diameter of posterior ocellus 15; postocellar setae length 38. Antennal total length 370, segments I–VIII length (width): 35 (40); 36 (28); 52 (25); 45 (29); 44 (25); 48 (28); 34 (24); 45 (20). Pronotum median length 140, median width 240; length of major setae: ml length 45,



Figures 50–54. *Psalidothrips latizonus* sp. n. **50** head **51** pronotum **52** pelta **53** tube **54** antenna.

pa length 65, epim length 50. Abdominal tergite IX setae S1 length 105, S2 length 85. Tube length 115, basal width 67, apical width 22, anals 85.

Distribution. China (Guangdong, Hainan).

Etymology. The specific epithet, *latizonus*, is from the Latin adjective meaning broad band, in reference to the broad male pore plate.

Comments. The new species is closely similar to *P. chebalingicus* in general appearance, but differs from it as follows: head largely yellowish brown but darkened laterally (head uniformly brown in *chebalingicus*); antennal segments I–II pale brown, III–VIII shading gradually from yellowish brown to pale brown towards apex (antennae yellow except segments I–II and VI–VIII brown in *chebalingicus*); postocellar setae about 2.5 times as long as hind ocellus (postocellar setae slightly longer than hind ocellus in *chebalingicus*); antennal segment VIII longer than segment VII (antennal segment VIII as long as segment VII in *chebalingicus*). Moreover, the males have the broad pore plate reaching the lateral margin of sternite VIII, whereas the pore plate of *P. chebalingicus* is narrow and slightly arched, not reaching the lateral margin.

***Psalidothrips lewisi* (Bagnall)**
Figs 23–24, 76

Trichothrips lewisi Bagnall, 1914: 30.
Psalidothrips alaris Haga, 1973: 76. Synonymised by Okajima and Urushihara 1992: 167.
Psalidothrips lewisi (Bagnall): Okajima and Urushihara 1992: 167.

Comments. This species has a wide geographical range from Shandong province to Hainan province in China. The Chinese specimens listed here have been compared with the Japanese specimens (provided by S. Okajima) and, despite antennal segments III–VIII of the Japanese specimens being almost uniformly yellow and much paler than the Chinese specimens (Figs 23–24), they are considered to represent *P. lewisi*. Moreover, the macropterous form in both sexes is more common than the apterous form among the Chinese specimens.

Distribution. China (Shandong, Guizhou, Hunan, Jiangxi, Yunnan, Guangdong, Hainan); Japan.

***Psalidothrips longidens* Wang, Tong & Zhang**
Figs 25–26, 77

Psalidothrips longidens Wang, Tong & Zhang, 2007: 30.

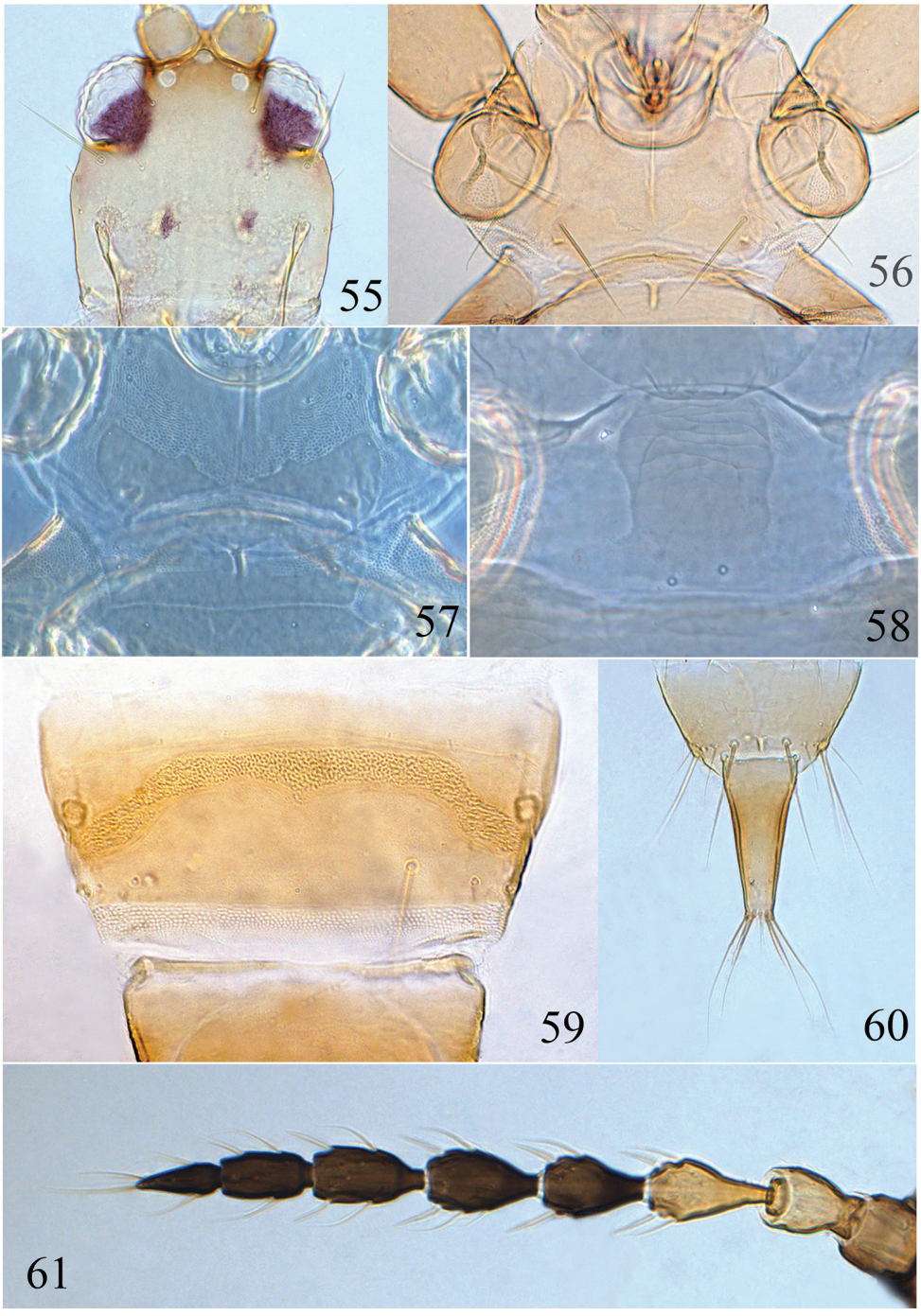
Comments. This species belongs to the group in which the fore tarsal tooth is present in both sexes (Figs 25–26). The following characters can distinguish it from congeneric species: antennal segments III and IV each with three sense cones; fore tarsus of females armed with a long and strong tooth, pelta irregularly rectangular without campaniform sensilla, and male with a transverse and weakly curved pore plate (Fig. 77) which does not reach the lateral margins.

Distribution. China (Guangdong).

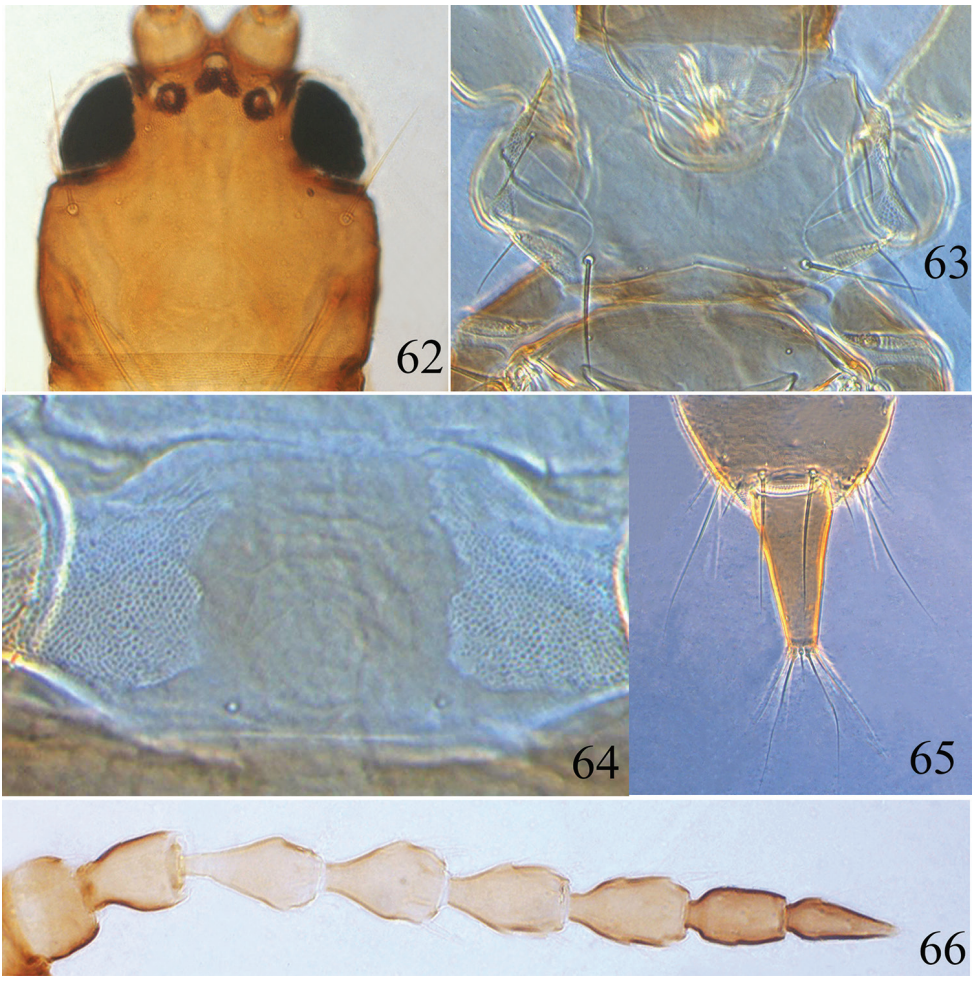
***Psalidothrips nigroterminatus* sp. n.**
http://zoobank.org/2703C57E-AD67-4E79-A282-EC10A3E10123
Figs 9–10, 55–61

Material examined. Holotype: CHINA. Hainan: 1 female, Qiongzhong County, Limushan National Forest Park (19°12'40"N, 113°12'39"E, alt. 1200m), 24.x.2017 (Chao Zhao).

Paratypes. Four females and 1 male, collected with holotype. **Yunnan:** One female and 1 male, Mengla County (21°56'N, 101°15'E), from leaf litter of bamboo,



Figures 55–61. *Psalidothrips nigroterminatus* sp. n. **55** head **56** pronotum **57** ventral view of prothorax **58** pelta **59** male pore plate **60** tube **61** antenna.

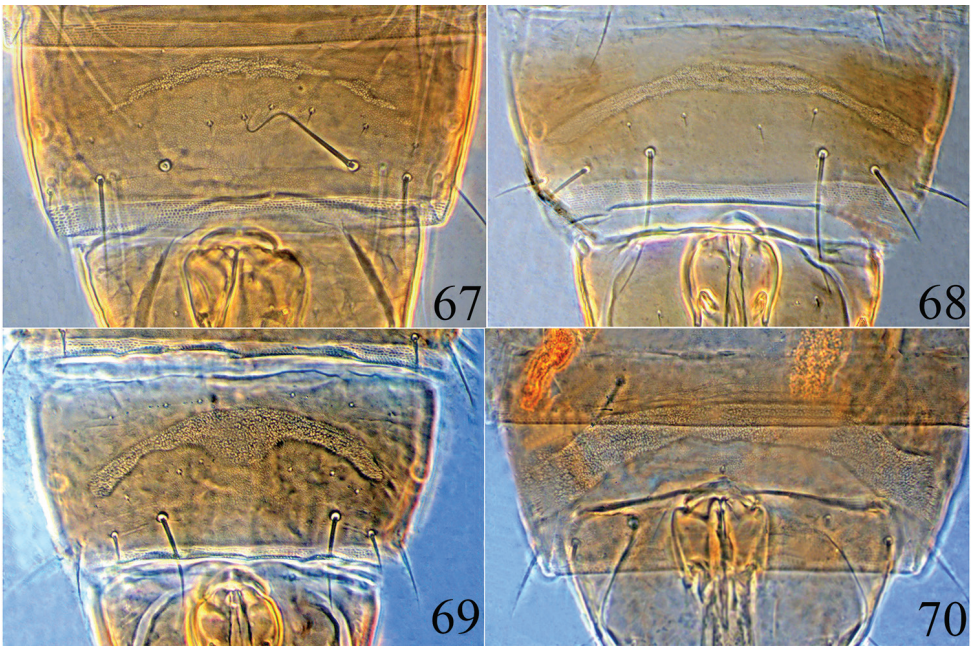


Figures 62–66. *Psalidothrips chebalingicus* Zhang & Tong **62** head **63** pronotum **64** pelta **65** tube **66** antenna.

3.x.2010 (Nie Jing & Sun Jun); 13 females and 10 males (preserved in the Insect Collection, Yunnan Agricultural University, YAU), Mengla County (21°56'N, 101°15'E), collected from leaf litter of bamboo, 3.x.2010 (Nie Jing & Sun Jun).

Female macropterous (Fig. 9). *Body* largely yellow, head yellow tinged with light brown anteriorly, abdominal segments shading gradually from yellow to yellowish brown towards tube; antennal segments I–III yellow, IV–VIII dark brown. Wings shaded greyish brown but paler medially.

Head (Fig. 55) wider than long, faintly sculptured on posterior margin; cheeks slightly swollen and constricted behind eyes; eyes approximately 2/5 of head length; postocellar setae long and acute, approximately 2.5 times longer than diameter of hind ocellus; postocular setae blunt or weakly expanded at apex, as long as or slightly longer than eyes. Antennae 8-segmented (Fig. 61), surface without sculpture; antennal seg-



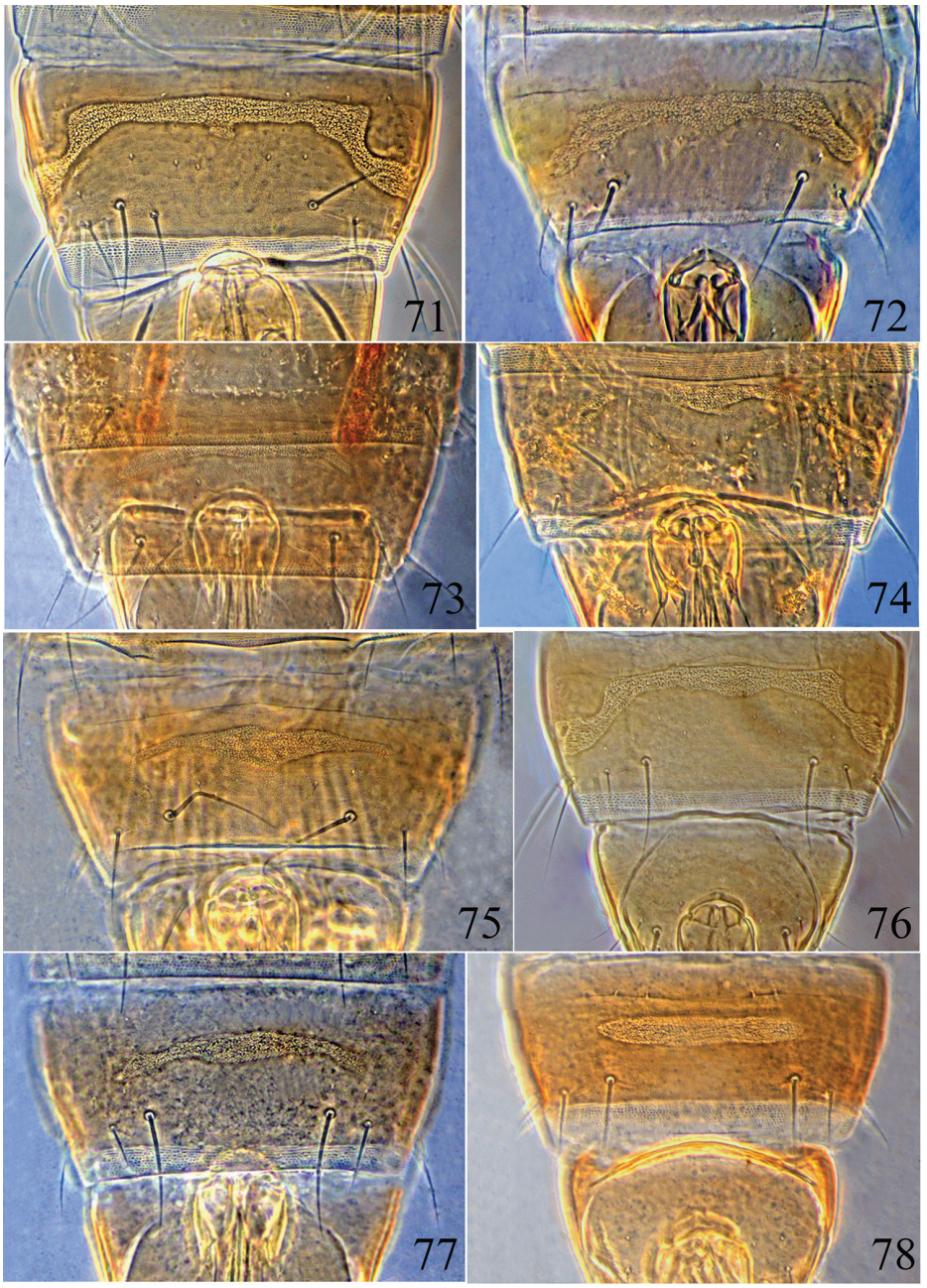
Figures 67–70. Male pore plate of *Psalidothrips* species: **67** *P. angustus* sp. n. **68** *P. comosus* sp. n. **69** *P. fabarius* sp. n. **70** *P. latizonus* sp. n.

ments III and IV each with two sense cones, segment VIII shorter than segment VII. Maxillary stylets reaching about half way to postocular setae and wide apart.

Pronotum broad (Fig. 56), surface smooth with a median longitudinal line; three pairs of major setae well developed, pa longest and acute at apex; ml subequal to epim in length, both slightly expanded apically. Fore tarsal tooth absent. Sub-basal wing seta S1 minute, S2 longer than S3, both pointed at apex. Mesonotum weakly sculptured on anterior third; metanotum smooth with longitudinal sculpture laterally. Mesopresternum complete and boat-shaped (Fig. 57).

Pelta (Fig. 58) hat-shaped with a pair of campaniform sensilla posteriorly, surface sculptured on anterior half. Abdominal tergites II to VII each with two pairs of wing-retaining setae; tergite IX setae S1 and S2 pointed (Fig. 60), S1 shorter than S2 which are subequal to tube in length; basal width of tube approximately 2.5 times wider than apical width.

Measurements (holotype female in microns). Distended body length 1810. Head length 170, width 170; eye length 67; postocular setae length 67; diameter of posterior ocellus 12; postocellar setae length 30. Antennal total length 365, segments I–VIII length (width): 36 (40); 44 (32); 56 (28); 44 (30); 49 (33); 51 (25); 44 (21); 41 (16). Pronotum median length 130, median width 250; length of major setae: ml length 42, pa length 75, epim length 42. Fore wing length 710, subbasal setae S1–S3 length: 3, 15, 3. Abdominal tergite IX setae S1 length 110, S2 length 130. Tube length 130, tube basal width 62, apical width 25, anals 125.



Figures 71–78. Male pore plate of *Psalidothrips* species: **71** *P. ascitus* **72** *P. bicoloratus* **73** *P. chebalingicus* **74** *P. consimilis* **75** *P. elagatus* **76** *P. lewisi* **77** *P. longidens* **78** *P. simplus*

Male micropterous (Fig. 10). Similar in colour and structure to female except for fore tarsal tooth present; pore plate on abdominal sternite VIII arched, slightly straight anteriorly and reaching lateral margins (Fig. 59).

Measurements (paratype male in microns). Distended body length 1640. Head length 160, width 160; eye length 60, postocular setae length 60; diameter of posterior ocellus 12; postocellar setae length 32. Antennal total length 335, segments I–VIII length (width): 33 (35); 39 (26); 52 (24); 43 (24); 44 (24); 45 (23); 41 (19); 39 (14). Pronotum median length 140, median width 230; length of major setae: ml length 48, pa length 70, epim length 43. Abdominal tergite IX setae S1 length 100, S2 length 85. Tube length 105, basal width 56, apical width 22, anals 100.

Distribution. China (Yunnan, Hainan).

Etymology. The species name is an arbitrary combination of two Latin adjectives, *niger* meaning black and *terminatus* meaning terminal, in reference to the antennae with dark brown distal segments.

Comments. The new species belongs to the group in which antennal segments III and IV both have two sense cones. It can be distinguished from the other members of the group by the following combination of features: (1) body largely yellow but antennal segments IV–VIII dark brown; (2) mesopresternum complete and boat-shaped; (3) pronotal posteroangular setae acute at apex and much longer than other major pronotal setae; (4) abdominal tergite II concolourous with the other tergites, and (5) male pore plate arched but slightly straight anteriorly.

***Psalidothrips simplus* Haga**
Figs 27–28, 78

Psalidothrips simplus Haga, 1973: 77.

Comments. This species (Figs 27–28) is easily separated from congeneric species by the following combination of characters: body largely yellowish brown, abdominal tergite II almost concolourous with other tergites; abdominal tergites III to VII each with one pair of simple wing-retaining setae; pelta broad hat-shaped or trapezoidal and male with a transversely long oval pore plate (Fig. 78). Okajima (2006) pointed out that antennal segment III always has two sense cones, but those on segment IV are variable in number from two to four, which is the same in the Chinese specimens.

Distribution. China (Hubei, Guizhou, Hunan, Jiangxi, Yunnan, Guangdong, Hainan); Japan.

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RESEARCH ARTICLE

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Deep intraspecific DNA barcode splits and hybridisation in the *Udea alpinalis* group (Insecta, Lepidoptera, Crambidae) – an integrative revision

Richard Mally[1], Peter Huemer[2], Matthias Nuss[3]

1 *University Museum of Bergen, Natural History Collections, Realfagbygget, Allégaten 41, 5007 Bergen, Norway* **2** *Tiroler Landesmuseen Betriebsges.m.b.H., Natural History Department, Collections and Research Center, Krajnc-Str. 1, 6060 Hall, Austria* **3** *Senckenberg Museum of Zoology, Königsbrücker Landstraße 159, 01109 Dresden, Germany*

Corresponding author: *Richard Mally* (richard.mally@uib.no)

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Abstract

The analysis of mitochondrial COI data for the European-Centroasian montane *Udea alpinalis* species group finds deep intraspecific splits. Specimens of *U. austriacalis* and *U. rhododendronalis* separate into several biogeographical groups. These allopatric groups are not recovered in the analyses of the two nuclear markers wingless and Elongation factor 1-alpha, except for *U. austriacalis* from the Pyrenees and the French Massif Central. The latter populations are also morphologically distinct and conspecific with *Scopula donzelalis* Guenée, 1854, which is removed from synonymy and reinstated as *Udea donzelalis* (Guenée, 1854) **stat. rev.** Furthermore, *Udea altaica* (Zerny, 1914), **stat. n.** from the Mongolian central Altai mountains, *U. juldusalis* (Zerny, 1914), **stat. n.** from the Tian Shan mountains of Kazakhstan, Kyrgyzstan and NW China, and *U. plumbalis* (Zerny, 1914), **stat. n.** from the Sayan Mountains of Northern Mongolia are raised to species level, and lectotypes are designated. Evidence of introgression of *U. alpinalis* into *U. uliginosalis* at three localities in the Central Alps is presented. A screening for *Wolbachia* using the markers wsp, gatB and ftsZ was negative for the *U. alpinalis* species group, but *Wolbachia* was found in single specimens of *U. fulvalis* and *U. olivalis* (both in the *U. numeralis* species group). We do not find evidence for the conjecture of several authors of additional subspecies in *U. rhododendronalis*, and synonymise *U. rhododendronalis luquetalis* Leraut, 1996, **syn. n.** and *U. r. ventosalis* Leraut, 1996, **syn. n.** with the nominal *U. rhododendronalis* (Duponchel, 1834).

Keywords
Alps, Central Asia, montane, nuclear genes, introgression, *Wolbachia*

Introduction

With currently 217 recognised species (Nuss et al. 2003–2018), *Udea* Guenée (in Duponchel), 1845 is the most diverse genus of Spilomelinae within Crambidae. Munroe (1966) revised the North American species of *Udea*. Mally and Nuss (2011) proposed a phylogenetic framework for the majority of the 39 currently recognised European species and described four monophyletic species groups: the *U. ferrugalis*, *U. itysalis*, *U. numeralis* and *U. alpinalis* species groups. Whereas the first three species groups are also represented on other continents, the *U. alpinalis* group occurs, to the present knowledge, only in the mountain systems from Europe to Central Asia. Currently, this group contains nine species: *Udea alpinalis* (Denis & Schiffermüller, 1775), *U. austriacalis* (Herrich-Schäffer, 1851), *U. bourgognealis* Leraut, 1996, *U. carniolica* Huemer & Tarmann, 1989, *U. cretacea* (Filipjev, 1925), *U. murinalis* (Fischer von Röslerstamm, 1842), *U. nebulalis* (Hübner, 1796), *U. rhododendronalis* (Duponchel, 1834), *U. uliginosalis* (Stephens, 1834). Furthermore, *U. uralica* Slamka, 2013 exhibits the group-specific apomorphies (see below) and is here added to the *U. alpinalis* group.

The *U. alpinalis* species group is characterised by a homogenous wing colouration with an inconspicuous maculation. Species of this group exhibit sexual dimorphism, with females having shorter, more acute forewings and the dorsal side of the hindwings being usually darker than in males. Furthermore, the *U. alpinalis* group is distinguished from other *Udea* species groups by the presence of a sclerotised protrusion of variable shape on the posterior phallus apodeme. The species inhabit montane regions, and the larvae exhibit a range of feeding habits from monophagy to polyphagy on a variety of herbaceous plants (Lhomme 1935; Huemer and Tarmann 1989; Slamka 2013).

Several authors suspect that the actual species diversity in the *U. alpinalis* group in Europe is higher than formal descriptions in the literature indicate, specifically in relation to *U. austriacalis* and *U. rhododendronalis*. This suspicion is based on small differences in genitalia structure and wing maculation (Zerny 1914, Filipjev 1925, Leraut 1996, Slamka 2013). For *U. alpinalis*, Galvagni (1933), Weber (1945), and Panigaj and Kulfan (2012) found considerable variability in the forewing maculation. In preliminary COI Barcode cluster analyses, several specimens that have been identified as *U. uliginosalis* based on wing maculation clustered with *U. alpinalis*, raising questions about the correct species identification.

In this study, these taxonomic suspicions are addressed through the analysis of morphological as well as mitochondrial and nuclear genetic data. We present, investigate, and, where possible, explain this unsettled question of intraspecific diversity of *U. rhododendronalis*, *U. austriacalis* and the *U. uliginosalis*-*U. alpinalis* species pair.

Materials and methods

The study is based on adult specimens of *Udea alpinalis*, *U. austriacalis*, *U. cretacea, U. rhododendronalis,* and *U. uliginosalis*, collected at different localities in Europe and Central Asia. The genetic dataset was complemented with sequences of *U. bourgognealis*, *U. carniolica*, *U. murinalis,* and *U. nebulalis*. *Udea ruckdescheli* Mally, Segerer & Nuss, 2016 of the *U. numeralis* species group (sensu Mally and Nuss 2011) served as outgroup. The morphologically investigated material is summarised in the 'material examined' sections of the respective species in the taxonomic results, the genetic data are summarised in Table 2.

Molecular data from three different genes were used for the dataset: the 5' half of the mitochondrial Cytochrome c oxidase subunit 1 (COI) gene (the "DNA Barcode"), 657 base pairs (bp) in length, the 5' part of the nuclear Elongation factor 1-alpha (EF1a) gene (780 bp), and the nuclear Wingless gene (372 bp). In addition, a screening for molecular traces of *Wolbachia* infections was done by amplifying the bacterial markers *Wolbachia* surface protein (wsp), aspartyl/glutamyl-tRNA(Gln) amidotransferase subunit B (gatB) and Filamenting temperature-sensitive mutant Z (ftsZ).

COI Barcode sequences and specimen data for the *Udea* species of interest were obtained from ongoing Barcoding projects of PH and MN on the Barcoding of Life Database (BOLD, www.boldsystems.com), Version 4. The DNA lab protocols at the Canadian Centre for DNA Barcoding (CCDB) are available at http://www.ibolproject.org/resources.php. Barcodes with less than 500 bp were excluded, and public records retrieved from NCBI GenBank were included. In addition, DNA Barcode sequences were obtained for several specimens through PCR and sequencing in the DNA labs of the Senckenberg Natural History Collections Dresden, Germany (SNSD) and the Institute of Biology at the University of Bergen, Norway (UiB).

For DNA lab protocols at SNSD see Mally and Nuss (2011). The DNA lab protocols at UiB are as follows: The abdomen was detached from the dried specimen and DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's protocol. Gene sequences were amplified in 25 µl reactions from 2 µl DNA extract using 400 nM of each primer, 800 µM dNTP mix, 2.5 µl Taq buffer (incl. $MgCl_2$), 0.75u TaKaRa Ex Taq DNA Polymerase and distilled water added up to 25 µl in total per reaction. COI primers were HybLCO (forward) and HybNancy (reverse) (Folmer et al. 1994, Wahlberg and Wheat 2008), EF1a primers were HybOscar-6143 (forward) and Bosie-6144 (reverse) (Wahlberg and Wheat 2008, Haines and Rubinoff 2012), and Wingless primers were HybLepWg1 (forward) and HybLepWg2 (reverse) (Wahlberg and Wheat 2008). Those primers contained the universal primer tail pair T7/T3 ('Hyb' in the primer names; Wahlberg and Wheat 2008), which were used for sequencing. The wsp gene was amplified using the primers WspecF and WspecR (Werren and Windsor 2000). In cases of lacking amplification success the internal primers INTF1 and INTR2 (Sakamoto et al. 2006) were used. The genes gatB and ftsZ were amplified using the primers of Baldo et al. (2006) in combination with the universal forward (T7 promoter) and reverse (T3) tails (Wahlberg and Wheat 2008).

The PCR programme for COI was: initial phase at 95 °C for 5 min, 38 cycles with 95 °C for 30 s, 50 °C for 30 s and 72 °C for 60 s, final phase at 72 °C for 10min and cooling at 8 °C. For EF1a and Wingless a touchdown PCR was performed: 24 cycles at 95 °C for 30 s, 55 °C with -0.4 °C/cycle for 30 s and 72 °C for 60 s +2s/cycle, then 12 cycles at 95 °C for 30 s, 45 °C for 30 s and 72 °C for 120 s +3 s/cycle, final phase at 72 °C for 10min and cooling at 8 °C. The PCR protocol of Sakamoto et al. (2006) was used for For wsp, and the protocol of Baldo et al. (2006) for gatB and ftsZ.

PCR results were examined via gel electrophoresis on a 1 % agarose gel and GelRed as dye agent. Successful PCR samples were cleaned with ExoSAP and subsequently amplified in Sanger-sequencing PCR reactions for both primers using the BigDye kit and this setup: 0.5–3.0 µl of PCR sample (depending on the sample's band thickness on the agarose gel), 160 nM primer, 1 µl buffer, 0.5 µl BigDye, and adding up distilled water to 10 µl in total per reaction. Sequencing was conducted at the sequencing facility of UiB, Dept. of Molecular Biology. PCR and sequencing PCR were performed on a Bio-Rad 1000 thermal cycler; ExoSAP clean-up was done on a MJ Research PTC-200 thermal cycler.

The three gene datasets (COI, Wingless, EF1a) were aligned with PhyDE 0.9971 (Müller et al. 2008) and analysed individually with raxmlGUI v. 1.5b2 (Stamatakis 2006; Silvestro and Michalak 2012), using a Maximum Likelihood (ML) search under the GTRGAMMA model (Rodriguez et al. 1990) and with a thorough bootstrap of 1,000 Bootstrap replicates. Phylograms were edited in TreeGraph version 2.13.0-748 beta (Stöver and Müller 2010). The corresponding alleles and supergroups of successful *Wolbachia* sequences were sought in the BIGSdb database (Jolley and Maiden 2010).

Dissection of genitalia was performed according to Robinson (1976), with modifications. In order to preserve the tympanal organ, the abdomen was cut open longitudinally along one pleural membrane, detached from the genitalia, cleaned, and embedded under a separate cover slip next to the cover slip with the genitalia. Morphological structures were investigated using a Leica M125 stereomicroscope. Photographic documentation of imagines was done with a Canon EOS 60D in combination with a Canon EF 100mm 1:2,8 Macrolens and Canon EOS Utility Version 2.10.2.0 on a Windows PC. A Leica CTR6000 microscope in combination with a Leica DFC420 camera and Leica Application Suite programme (Version 3.8.0) on a Windows PC was used for documentation of the genitalia. Images were edited in GIMP 2.8.6. The distribution maps were generated with DIVA-GIS version 7.5.0.0 (Hijmans et al. 2001) and SRTM 90 m digital elevation data (Jarvis et al. 2008).

Abbreviations

The abbreviations of the insect collections follow Evenhuis (2017).

EF1a	Elongation Factor 1-alpha
ftsZ	Filamenting temperature-sensitive mutant Z
gatB	aspartyl/glutamyl-tRNA(Gln) amidotransferase, subunit B

GTR	General time reversible substitution model (see Rodriguez et al. 1990)
ML	Maximum Likelihood
MLST	Multilocus sequence typing
MTD	Senckenberg Natural History Collections, Museum of Zoology Dresden
NHMUK	Natural History Museum London, UK
NHMW	Natural History Museum Vienna, Austria
SMNK	State Museum of Natural History Karlsruhe, Germany
TLMF	Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria
wsp	*Wolbachia* surface protein
ZMBN	Zoological Museum, University of Bergen, Norway
ZMHB	Zoological Museum, Humboldt University Berlin, Germany
ZMUC	Zoological Museum, University Copenhagen, Denmark
ZSM	Zoological State Collections Munich, Germany

Results

Molecular results

In total, genetic data were analysed for 80 specimens (see Table 2) of the *U. alpinalis* group, specifically for *U. alpinalis*, *U. austriacalis*, *U. rhododendronalis* and *U. uliginosalis*. COI data were available for 77 specimens, EF1a for 31 specimens, and wingless for 29 specimens. The low coverage of nuclear genetic data is due to the age of most specimens, with the nuclear genome being too fragmented to be sequenced with the classical Sanger approach.

Analysis of COI resulted in a gene tree (Fig. 1) with several deep intraspecific, geographically coherent clades for *U. austriacalis* and *U. rhododendronalis*. *Udea austriacalis* splits into three groups: (aus1) Pyrenees and the French Massif Central (green clade in Fig. 1), (aus2) the French and Italian Maritime Alps (red clade), and (aus3) the Central and Eastern Alps as well as the Balkan Mountains (black clade). A fourth clade within *U. austriacalis* is represented by a single specimen of *U. cretacea*, indicating that *U. austriacalis* is non-monophyletic. *Udea rhododendronalis* splits into three COI clades: (rho1) Pyrenees (orange clade in Fig. 1); (rho2) Alps (black clade); (rho3) Southern Balkan Mountains (blue clade). Seven specimens of *U. uliginosalis* (marked in pink in Fig. 1) group with the *U. alpinalis* clade instead of with the other *U. uliginosalis* specimens. These seven specimens originate from three different localities in the Central Alps (see Tab. 2); the specimens collected at Hahntennjoch (Tyrol, Austria) group together, while the specimen from Styria (Austria) groups with the specimen from Belluno (Italy). All seven mismatched *U. uliginosalis* specimens are males. The two specimens identified as *U. juldusalis* are sister to the clade *U nebulalis* + *U. murinalis*.

In the ML analysis of EF1a under the GTRGAMMA model, values for alpha were often above 10, and the EF1a dataset was analysed with the GTR model in-

stead. In the resulting EF1a gene tree (Fig. 2), two *U. austriacalis* clades are found: one clade containing all three successfully sequenced specimens from group aus1, originating from the Pyrenees and the Massif Central (green clade), and one clade containing specimens from groups aus2 and aus3 (*U. austriacalis* samples marked in red and black). In comparison to the COI results, *U. rhododendronalis* does not group into distinct clades, and specimens from groups rho1 and rho2 form a single clade instead; no specimen from the group rho3 could be sequenced successfully. All specimens of *U. uliginosalis*, including those that group with *U. alpinalis* in the COI gene tree, form a monophyletic clade that is sister to the monophyletic *U. alpinalis* clade.

In the wingless gene tree (Fig. 3), specimens of the *U. austriacalis* clade aus1 form a monophylum on a long branch that is nested in the clade containing specimens from group aus3 and the single successfully amplified specimen from group aus2. *Udea rhododendronalis* specimens of all three COI Barcode groups (rho1–3) form a single monophyletic clade. *Udea alpinalis* and *U. uliginosalis* are not distinguished in two clades but form a common clade instead, including the *U. uliginosalis* specimens with the COI Barcode mismatch.

The three bacterial markers wsp, gatB, and ftsZ were used in order to screen for *Wolbachia* in a number of *Udea* specimens. Sequencing was successful in only two of the twelve tested specimens: a specimen of *U. fulvalis* from Crete collected 5.44 years before DNA extraction, and a specimen of *U. olivalis* from Armenia collected 4.26 years before DNA extraction. For the other ten tested specimens, PCR amplification produced a band in some cases, but sequencing failed.

For the two successful samples, wsp sequences produced no match in BIGSdb (Jolley and Maiden 2010). For the specimen of *U. fulvalis* (voucher ZMBN Lep125), the gatB sequence had the closest match with gatB allele 196, found in MLST profile 306; the ftsZ sequence had the closest match with ftsZ allele 36, present in 12 MLST profiles (41, 42, 109, 145, 146, 150, 151, 156, 157, 235, 305, 374). Both gatB and ftsZ place the *Wolbachia* strain from the *U. fulvalis* specimen into supergroup B. For the specimen of *U. olivalis* (voucher ZMBN Lep156), gatB had the closest match with gatB allele 7, present in seven MLST profiles (19, 112, 118, 261, 266, 268, 272); ftsZ resulted in an exact match with ftsZ allele 3, present in 31 MLST profiles (10, 13, 14, 17, 19, 24, 25, 54, 80, 83, 89, 91, 92, 107, 112, 118, 122, 123, 127, 132, 133, 165, 199, 234, 330, 404, 432, 434, 449, 451, 454). Both gatB and ftsZ place the *Wolbachia* strain from the *U. olivalis* specimen into supergroup A. The gatB and ftsZ sequences obtained from *U. fulvalis* and *U. olivalis* can be obtained from RM.

Taxonomy

Based on morphological, genetic, and biogeographical data, the following changes in the taxonomy of the species of the *U. alpinalis* species group are proposed, and redescriptions are provided.

***Udea austriacalis* (Herrich-Schäffer, 1851), stat. rev.**
Figs 1–4, 7–10, 23–28, 45

Botys austriacalis Herrich-Schäffer, 1851: 288 [1851 – binominal], pl. 20 fig. 142 [1849 - uninominal].
= *Botys nitidalis* Heinemann, 1865: 83.
= *Botys sororialis* Heyden, 1860: 93.

Type locality. Austria, Carinthia, Hohe Tauern, Grossglockner.

Material examined. Central Alps and Balkan clade (BOLD BIN AAD2364; aus3 in Fig. 1): AUSTRIA. 1♂ "Austria merid., Kärnten | Dellach im Drautal | Zollnertörl | 13°04'24"E, 46°35'58"N | 1830 m, 1.7.2009 | leg. Huemer | TLMF 2009-138", [pale green label] "BC TLMF Lep 00837", [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 419", Mally prep. no. 1041 (TLMF); 1♂ same data but without DNA voucher label and with [pale green label] "BC TLMF Lep 00838", Mally prep. no. 1109 (TLMF); 1♂ "[handwritten] Vent | Oetztal | [handwritten] 19.VII1942 | E. Möbius", "Coll. STARKE / Bautzen | Ankauf 1953 | Übernahme 1969", Mally prep. no. 9 (MTD); 1♂ "Teriolis | Ötztal [handwritten] 2000 m | Vent [handwritten] 31/7 1926" (NHMW); **SWITZERLAND.** 1♂ "Lukmanierpass | W. Heinitz | 1910"; "zu prüfen! | Pionea nebulalis | Pyrausta austriacalis | sororialis", "20069", "Coll. W. Heinitz | Ankauf 1950", Mally prep. no. 417 (MTD); 1♂ "Fusio, | [handwritten] 20. July, 1917, | (K.J. & N.C.R.)", [handwritten] Pyrausta | austriacalis | ♂ H.-S.", "Rothschild | Bequest | B.M.1939-1.", Mally prep. no. 1092 (NHMUK); 1♀ "[handwritten] Pontresi- | na. 61 10/4", "♀", " 21513", "Coll. W. Heinitz | Ankauf 1950", Mally prep. no. 10 (MTD); 1♀ [handwritten] "Pontresina | Switz | 12.VII.1965 | [printed] S.N.A.JACOBS.", "Brit. Mus. | 197 [handwritten]2 305", Mally prep. no. 1091 (NHMUK); **ITALY.** 1♂ "Italien, Prov. Südtirol | Kastelruth, Saltner Hütte SE | 1870 m, 17.6.2007 | leg. Huemer | TLMF 2008-009", "Udea austriacalis | det. P. Huemer 2008", [orange label] "DNA voucher | Lepidoptera | M. Nuss 2007 | [transverse] no. 238", Mally prep. no. 69 (TLMF); 1♂ "Italy, Südtirol, Sellagruppe | Sela de Culac, 2018m, | Wirtschaftswiese, Tagfang | 05.08.2008, leg. Nuss et al.", [pale green label] "BC MTD 00758", [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 421", Mally prep. no. 1043 (MTD); 1♂ "Italien, Prov. Belluno | Passo di Valparola E - Passo | Falzarego | 12°0'25,1"E, 46°31'20,6"N | 2150 m, 20.-21.7.2009 | leg. Huemer | TLMF 2009-138", [pale green label] "BC TLMF Lep 00570", [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 422", Mally prep. no. 1044 (TLMF); 1♂ [handwritten] "Prad. Alp. | b.Trafoi | Juli 871." (NHMW); 1♂ [handwritten] "Rognhofr | Stilfser | Joch | 1871." (NHMW); 1♂ "Sella-Joch, | Rbl. VII" (NHMW); 1♀ "Sellajoch | W. Heinitz | 1912", "♂", [handwritten] "25736", "Coll. W. Heinitz | Ankauf 1950", Mally prep. no. 50 (MTD); 1♀ "Italy, Südtirol, Sellagruppe | Sela de Culac, 2018m, | Wirtschaftswiese, Tagfang | 05.08.2008, leg. Nuss et al.", [orange label] "DNA voucher | Lepidoptera | M. Nuss 2007 | [transverse] no. 292", Mally prep. no. 114 (MTD); **ALBANIA and**

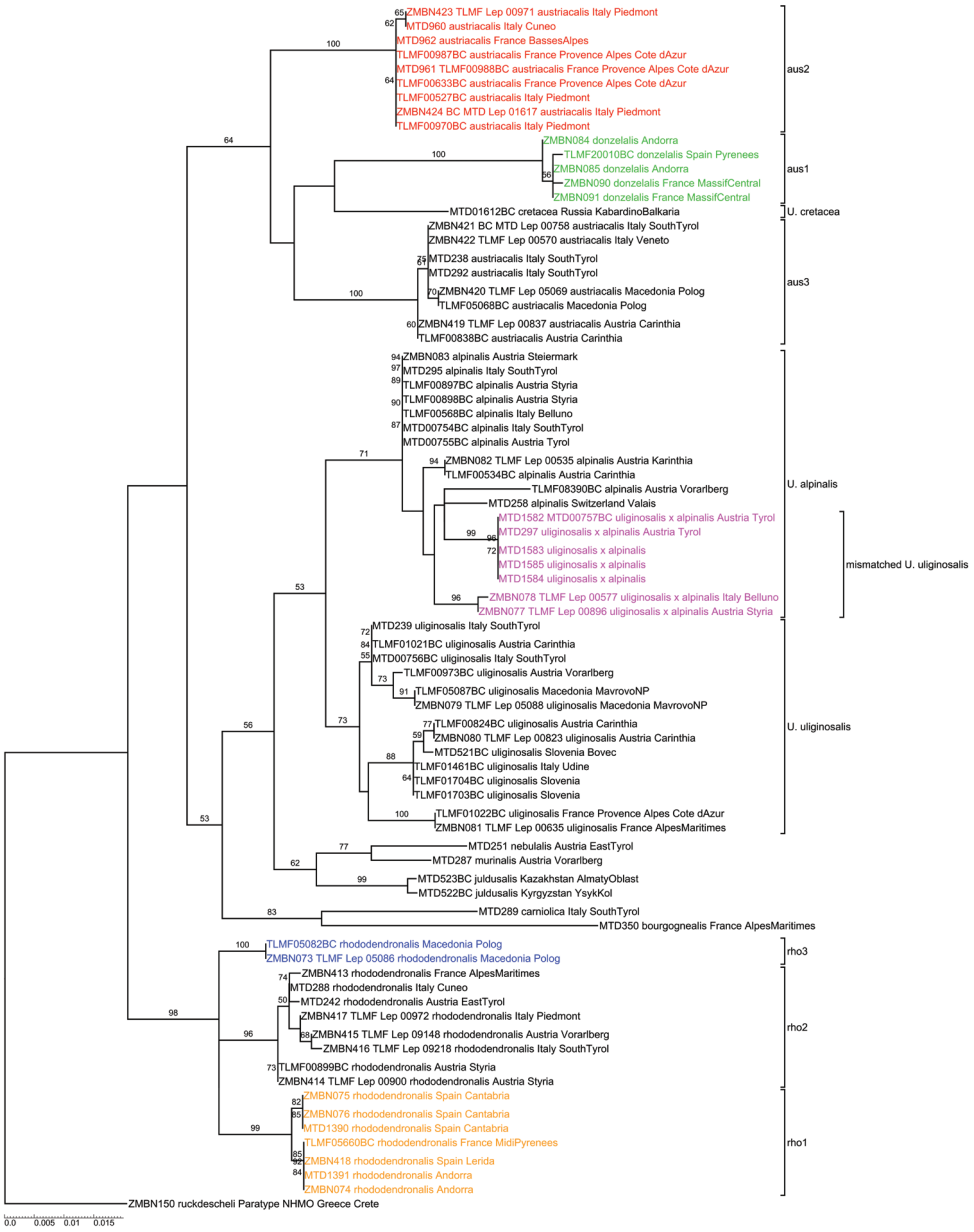
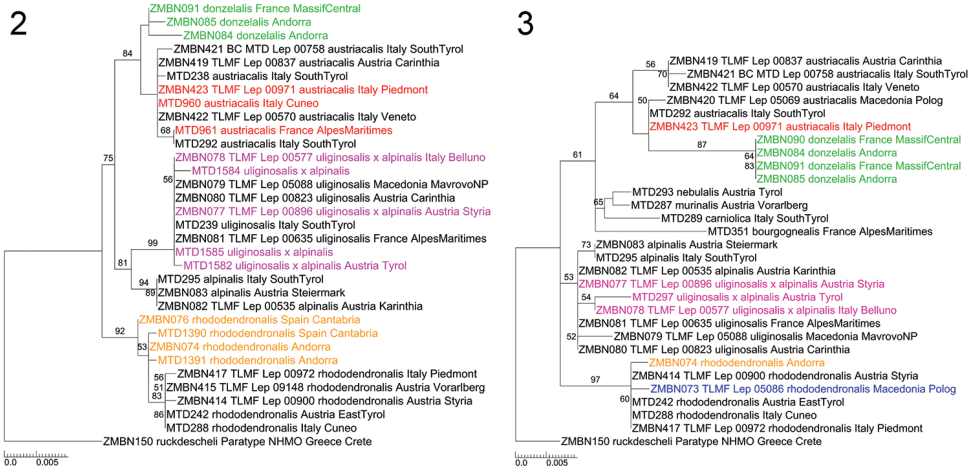


Figure 1. Maximum Likelihood analysis of COI Barcode data of the *Udea alpinalis* species group. Numbers on branches represent bootstrap values of ≥ 50 % inferred from 1,000 replicates, scale bar represents substitutions per site.

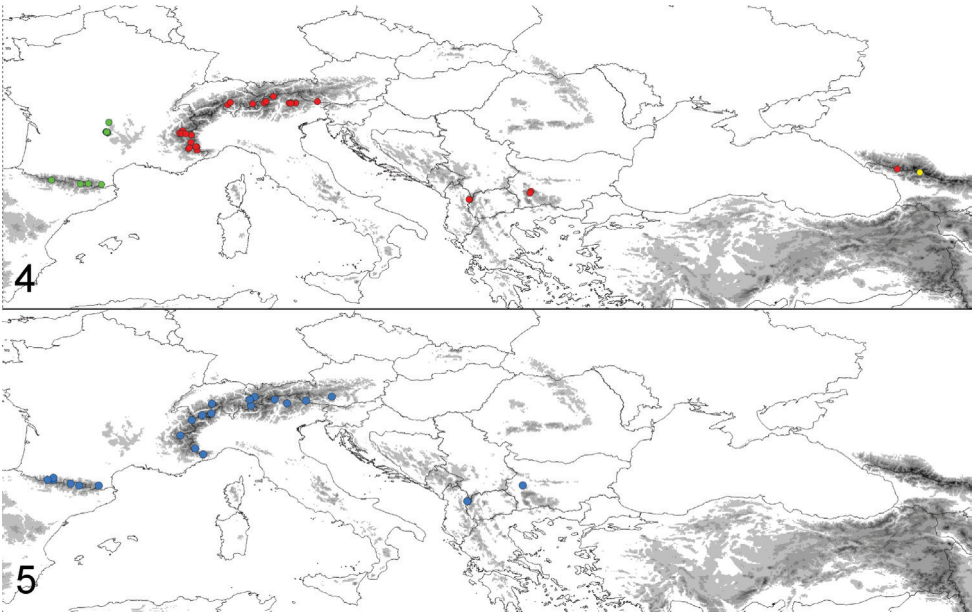
MACEDONIA. 1♂ "Macedonia, NP Mavrovo | Korab, Korabska jezero, | Kobilino pole, 2080–2180 m | 20°34'55"E, 41°46'42"N | 28.7.–1.8.2011 | leg. Huemer & Tarmann", [pale green label] "BC TLMF Lep 05069", [salmon-pink label] "DNA



Figures 2–3. Maximum Likelihood analysis of EF1a (**2**) and wingless (**3**) data of the *Udea alpinalis* species group. Numbers on branches represent bootstrap values of ≥ 50% inferred from 1,000 replicates. Note that the taxon set is not identical for the two analyses, scale bars represent substitutions per site.

voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 420", Mally prep. no. 1042 (TLMF); 1♂ 1♀ "Alban.Exp.1918 | Korab,23–31.VII", Mally prep. no. 1096 (♀) (NHMW); **BULGARIA.** 2♂ "Ende | Juli", "Bulgarien | Rebel '02. | [handwritten] Rila 1300 [underlined] m" (NHMW); 1♂ [handwritten] "Rila | c.1800 m | 25.VII 02" (NHMW); **RUSSIA.** 1♂ "Kaukasus | [handwritten] Dombai | Tsihutsihur- | Tal leg. Alberti; [Unterseite] 2300 m | 5.7.1968", [green label, handwritten] "1 | Amsel", [handwritten] austriacalis", Mally prep. no. 205 (ZSM);

Maritime Alps Barcode clade (BOLD BIN AAD2363; aus2 in Fig. 1): FRANCE. 1♂ "Frankreich, Alpes-Maritimes | PN Mercantour | N Col de la Cayolle | Col de la Boucharde N | 6°44'36"E, 44°17'00"N | 1930–1950m, 26.7.2009 | leg. Huemer", [pale green label] "BC TLMF Lep 00988", [orange label] "DNA voucher | Lepidoptera | Mally 2011 | [transverse] no. LEP961", Mally prep. no. 449 (TLMF); 1♂ [handwritten] "NÉVACHE | HAUTES-ALPES | July 29-Aug.16, 1924 | Wm Fassnidge.", "Brit. Mus. | 197[handwritten]2 305", [folded label] "2059.austriacalis [handwritten] 123 | Herrich-Schaffer." (NHMUK); 1♂ "Valloire, Savoie, | 11. July 1910. | (W. R. & K. J.)", "Rothschild | Bequest | B.M.1939-1.", Mally prep. no. 1105 (NHMUK); 1♂ "La Grave, Hautes Alpes, | 1500–1800 m. | 21. July 1908. | (W. R. & K. J.)", "Rothschild | Bequest | 1939-1." (NHMUK); 1♂ "Le Lautaret, | Hautes Alpes, | 2000–2300 m., | 4. August 1908. | (W. R. & K. J.)", "Rothschild | Bequest | 1939-1." (NHMUK); 1♂ "Frankreich, Alpes-Maritimes | PN Mercantour | N Col de la Cayolle | Col de la Boucharde N | 6°44'36"E, 44°17'00"N | 1930–1950 m, 26.7.2009 | leg. Huemer", [turquoise label] "BC TLMF Lep 00633" (TLMF); 1♂ "Frankreich, Alpes-Maritimes | PN Mercantour | N Col de la Cayolle | Col de la Boucharde N | 6°44'36"E, 44°17'00"N | 1930–1950 m, 26.7.2009 | leg. Huemer", [turquoise label] "BC TLMF Lep 00987" (TLMF); 1♂ "MAURIN.B-ALPES | [handwritten] 25



Figures 4–5. Distribution of investigated specimens of the *Udea austriacalis* species complex (**4**) and *U. rhododendronalis* (**5**) in Europe **4** *U. austriacalis* (red), *U. donzelalis* (green), *U. cretacea* (yellow) **5** *U. rhododendronalis* (blue); altitudes ≥ 1,000 m are marked in increasingly darker grey shades every 500 m.

8 1932 | W.FASSNIDGE.", "Brit. Mus. | 197[handwritten]2 305", Mally prep. no. 1106 (NHMUK); 1♀ same data but "1 8 1932", Mally prep. no. 1101 (NHMUK); 1♀ "Frankreich, Dep. Basses Alpes | SW Castel de Restfond | Ste. De Caire Brun N | 2420m, 25.–26.7.1990 | leg. Huemer & Tarmann", [orange label] "DNA voucher | Lepidoptera | Mally 2011 | [transverse] no. 962", Mally prep. no. 450 (TLMF); **ITALY.** 1♂ "Italien, Prov. Cuneo | Alpi Cozie, Demonte NW | 44°23'04"N 7°6'23"E | 2.8.2010 | leg. Huemer | TLMF 2011-010", [orange label] "DNA voucher | Lepidoptera | Mally 2011 | [transverse] no. 960", Mally prep. no. 448 (TLMF); 1♂ "Italy, Prov. Piemonte, | Colle di Lombarda, | 2300 m, 25.vii.2006, | Peder Skou leg.", [yellow label] "Coll. ZMUC | Copenhagen, DK", [pale green label] "BC MTD 01617", [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 424", Mally prep. no. 1046 (ZMUC); 1♂ "Italien, Prov. Cuneo | Alpi Cozie, Demonte NW | Gias Valcavera | 7°8,2'E, 44°22,6'N | 2050 m, 23.7.2009 | leg. Huemer | TLMF 2009-138", [turquoise label] "BC TLMF Lep 00971", [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 423", Mally prep. no. 1045 (TLMF); 1♀ "Piemont,Colle di | Sestrières,18–2100m | 23.–31.VII.'37,Zerny", Mally prep. no. 1097 (NHMW).

Diagnosis. Outer side of labial palps' 2nd and 3rd palpomeres pronouncedly darker than the rest of the labial palps; in *U. donzelalis*, labial palps' 2nd and 3rd palpomeres on outside barely darker than rest of labial palps. Maculation of forewing usually less pronounced than in *U. donzelalis*, the apical brown streak often narrower; hindwing

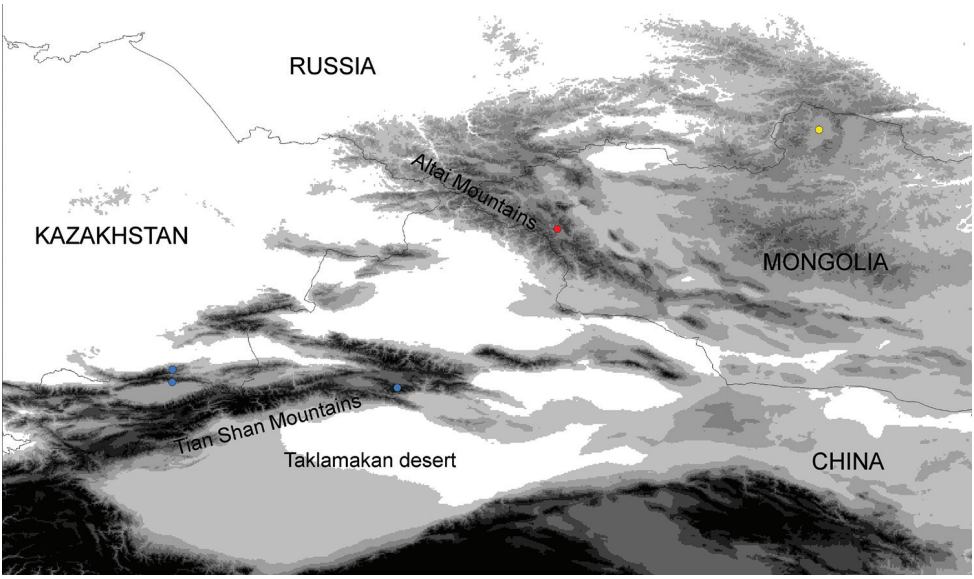


Figure 6. Distribution of investigated specimens of *Udea juldusalis* (blue), *U. altaica* (red) and *U. plumbalis* (yellow) in Central Asia; altitudes ³ 1,000 m are marked in increasingly darker grey shades every 500 m, altitudes ³ 4,000 m are in black. Note that the eastern locality of *U. juldusalis* and the localities of *U. altaica* and *U. plumbalis* are only approximations of the type localities.

in females dorsally evenly dark brown, whereas in *U. donzelalis* the inner area is greyish cream-white, contrasted by a darker outer band. In the male genitalia, the fibula of *U. austriacalis* is generally a bit narrower and more evenly broad from the base to the subapex; in *U. donzelalis* the fibula is somewhat broader and elongate triangular. In the female genitalia, the signum of *U. austriacalis* is 3.4–4.4 times as long as broad (n = 8), whereas in *U. donzelalis* the signum is on average narrower, being 4.1–5.1 times as long as broad (n = 6) (Tab. 1). *Udea austriacalis* can furthermore be distinguished by the COI Barcode from all other sequenced *Udea* species; the two allopatric DNA barcode clades of *U. austriacalis*, the first confined to the Maritime Alps and the second from other parts of the Alps, the Balkan Mountains and the Caucasus, do not differ from each other morphologically and in the nuclear genes wingless and EF1-alpha, so that they are considered conspecific. The two COI Barcode clades are the nearest neighbours to each other, and they differ by a minimum of 2.38 % p-distance of nucleotide divergence from each other; the Maritime Alps clade also has *U. cretacea* as nearest neighbour with 2.38% p-distance of minimum nucleotide divergence (Tab. 3).

Redescription. *Head*. Frons and vertex covered with beige scales; frons evenly convex, covered with beige to light brown scales; labial palps projecting forward, third segment pointed, palps covered with beige scales, outer sides of labial palps' second and third segment light to dark brown; maxillary palps approximately one quarter as long as labial palps, with beige apical scale tuft; compound eyes hemispherical; proboscis well developed, its base covered in pale beige and dark brown scales; antennae filiform,

Table 1. Ratios of length versus breadth of the main signum of female genitalia in selected species of the *Udea alpinalis* species group.

Ratio length to breadth of main signum	*Udea austriacalis* (n = 8)	*U. donzelalis* (n = 7)	*U. altaica* (n = 4)
minimum	3.44	4.06	3.09
maximum	4.375	5.08	3.44
Average	**3.85**	**4.56**	**3.25**
standard deviation	0.36	0.41	0.15

posterior side covered in pale beige scales, anterior side in males densely covered with cilia shorter than the basal antennal radius, shorter still in females, antennal length approx. 50–60 % of forewing length in males, approx. 70 % in females; ocellus posterior to antenna base.

Thorax. Cream-white, with collar and anterior part of tegulae scales more caramel-coloured; legs cream-white except for dark brown inner side of fore- and midlegs as well as distal half of hindlegs; tibial spurs on fore-/mid-/hindleg 0/2/4, as in other species of the genus; midleg outer spur ca. 2/3 length of inner spur; hindleg outer spurs ca. 2/3 length of inner spurs.

Wings. Forewing length 11–13 mm in males, 8–10 mm in females. Males and females with one frenulum bristle. Females with more acute apex due to the straight costa (distally curved in males). Forewing ground colour glossy cream-white to beige, with maculation more or less prominent: basal two thirds of costa with light brown streak, distal discoidal stigma a diffuse brownish dot, postmedial line brownish, arching around distal discoidal stigma, then turning basad until below distal discoidal stigma, sharply arching back outwards (the typical "*Udea* loop"), then following course of arch in postmedial line's anterior part and meeting with dorsum at about two thirds of dorsum length; apical streak more or less pronounced, narrow; ends of veins on dorsum in some specimens with minute dark brown dots, but often missing; fringe light cream-white. Hindwing in males cream-white to brownish, postmedial line brown, more or less clear, terminal band brown, often only at apex; hindwing in females evenly dark brown, some specimens with a slightly darker terminal band. Underside of forewing uniformly brown with a slightly darker distal discoidal stigma and, in some specimens, with a slightly darker postmedial line; underside of hindwing greyish white to grey, with costal area somewhat darker, a diffuse brownish grey postmedial line might be visible in males, in females a more or less pronounced terminal band is present.

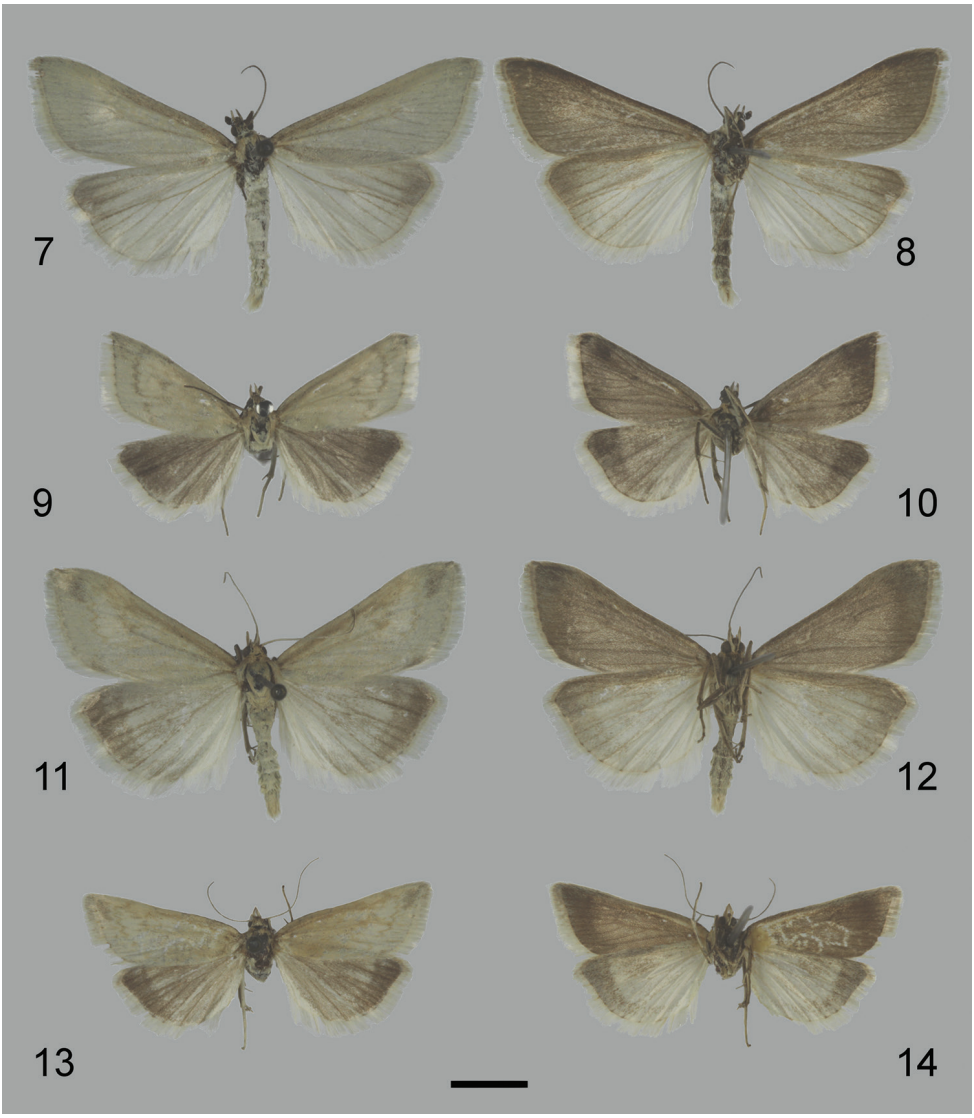
Abdomen. Dorsal side of abdomen covered with cream-white glossy scales, ventral side with dark brown scales, interspersed by cream-white scales; male 8th segment with long beige posterior scales. Tympanum with broad short lobulus; 2nd sternite with broad U-shaped sclerotisation, more so in females, inner part less sclerotised; 3rd sternite anterior edge with broad sclerotised lobe on each side of the centre, tapering anterolaterad into thin apex; 4th sternite anterior edge with oval hole in sternite sclerotisation on each side of the centre; centre of anterior edge of sterites V-VII with

broad, short rectangular protrusion; male 8th sternite with U-shaped sclerotisation along borders, posterior ends broadened; male 8th tergite with broad central longitudinal sclerotisation, somewhat broadening anteriorly, leading laterally into pointed triangular process.

Male genitalia. (Figs 23–28) Uncus broadly attached to tegumen, the attachment site laterally constricted; apical part of uncus constricted into slim, strap-like neck leading into flattened oval uncus head, dorsally covered densely with stiff, deeply bifid setae. Tegumen broad, rectangular. Broad, weakly sclerotised gnathos band with a central conical dorsad protrusion. Transtilla arms forming equilateral triangles, dorsal surface sparsely set with thin long simple setae. Vinculum broad, well sclerotised, sides elongate drop-shaped to oval, ventral part mediodorsally forming broad bell-shaped protrusion towards juxta, ventrally forming U-shaped saccus with prominent ventromedial keel. Juxta nearly rhomboidal to almost circular, apex sharply bifid with medial incision about one quarter as long as juxta. Valva long, slender, slightly tapering towards apex; costa slightly concave, broader at base, surface sparsely set with thin, long, simple setae; sacculus broad, roughly oval, dorsodistal edge close to fibula base, ventrodistal part concavely curving towards ventral valva edge; ventral valva edge straight apart from a slightly concave recess in the area of the fibula tip; valva apex rounded towards distal end of costa; slender, evenly broad, strongly sclerotised fibula directed towards sacculus apex, apical half narrowed to pointed, ventrad curved claw, ventral side of claw flat; fibula emerging from oval sclerotised lobe near base of costa, sparsely set with thin, long simple setae. Phallus tubular, slightly curved, evenly sclerotised; posterior phallus apodeme bent sinistrad, dorsally and ventrally with elongate unsclerotised strip, right posterior phallus apodeme forming sclerotised spatulate lobe with medially protruding ridge containing three (rarely two) teeth of variable shape apically.

Female genitalia. (Fig. 45) Corpus bursae membranous, oval; elongate lens-shaped longitudinal signum extending through corpus bursae, 3.4- to 4.4-times as long as broad (n = 8). Ductus bursae membranous apart from short sclerotised section in posteriormost part and oval, longitudinally folded auxiliary signum in anterior section where it transitions into corpus bursae; length of auxiliary signum 29–60% of main signum length (n=8); ductus bursae anteriorly broad, tapering posteriad to half its diameter; posterior section and colliculum less strongly sclerotised, with thickened mesocuticle, colliculum forming short 'S'; ductus seminalis membranous, emerging at colliculum. Antrum broad, tubular to conical, strongly sclerotised, without thickened mesocuticle; length of sclerotisation equal to antrum diameter (longer in dissected female from Korab), with variably pronounced unsclerotised longitudinal strip in antrum's dorsal sclerotisation. Postvaginal area membranous, sparsely covered with evenly spread tiny spicules. Segment 8 a broad, strongly sclerotised, ventrally open band, with anterior side broadly recessed where apophyses anteriores attach; apophyses anteriores slender, with broadened triangular to rhomboidal muscle attachment area at ca. 1/3 of their length. Apophyses posteriores slender, ca. 2/3 as long as apophyses anteriores. Papillae anales simple, with long setae on outer margin and shorter ones elsewhere.

Immature stages. Not studied and, to our knowledge, not described in the literature.



Figures 7–14. Adult specimens of *Udea* species. **7–10** *U. austriacalis* **7–8** male, dorsal (**7**) and ventral (**8**) **9–10** female, dorsal (**9**) and ventral (**10**), abdomen removed **11–14** *U. donzelalis* **11–12** male, dorsal (**11**) and ventral (**12**) **13–14** female, dorsal (**13**) and ventral (**14**), abdomen removed. Scale bars: 500 µm.

Distribution. Alps, Southern Balkan and Caucasus, where it might occur sympatrically with *U. cretacea* (Fig. 4).

Food plants. Lhomme (1935) reports *U. austriacalis* from *Plantago major* L. (Plantaginaceae), and the synonymous *"Pyrausta" sororialis* Heyden, 1860 as polyphagous.

DNA data. See Table 2. In BOLD, *U. austriacalis* is represented by the BINs AAD2363 and AAD2364. The seven DNA barcoded specimens forming the clade from the Central and East Alps and Macedonia differ between 0% and 0.95% in p-distance,

Table 2. Origin and gene sequence data of the genetically investigated *Udea* material.

species	Origin	BOLD sample no.	DNA extraction no.	COI accession no.	EF1a accession no.	wingless accession no.
U. austriacalis	Austria, Carinthia	TLMF Lep 00837	ZMBN Lep419	HQ968213	MG523969	MG523989
	Macedonia, Mavrovo Nat. Park	TLMF Lep 05069	ZMBN Lep420	KX042511	–	MG523990
	Italy, South Tyrol	BC MTD 00758	ZMBN Lep421	JF852277	MG523970	MG523991
	Italy, Belluno	TLMF Lep 00570	ZMBN Lep422	HM381412	MG523971	MG523992
	Italy, Cuneo	TLMF Lep 00971	ZMBN Lep423	HM381536	MG523972	MG523993
	Italy, Piedmont	BC MTD 01617	ZMBN Lep424	MG191924	–	–
	Italy, South Tyrol	–	MTD Lep238	JF497036	MG523942	–
	Italy, South Tyrol	–	MTDLep292	–	MH078064	JF497077
	Italy, Cuneo	–	MTD Lep960	MG523926	MG523946	–
	France, Alpes-Maritimes	TLMF Lep 00988	MTD Lep961	HQ968447	MG523947	–
	France, Basses-Alpes	–	MTD Lep962	MG523927	–	–
	Italy, Cuneo	TLMF Lep 00527	–	HM381372	–	–
	France, Alpes-Maritimes	TLMF Lep 00633	–	HM381456	–	–
	Austria, Carinthia	TLMF Lep 00838	–	HQ968214	–	–
	Italy, Cuneo	TLMF Lep 00970	–	HM381535	–	–
	France, Provence-Cote d'Azur	TLMF Lep 00987	–	HM381552	–	–
	Macedonia, Mavrovo Nat. Park	TLMF Lep 05068	–	KX042766	–	–
U. donzelalis	Andorra	–	ZMBN Lep084	MG523936	MG523962	MG523983
	Andorra	–	ZMBN Lep085	MG523937	MG523963	MG523984
	France, Cantal	–	ZMBN Lep090	MG523938	–	MG523985
	France, Cantal	–	ZMBN Lep091	MG523939	MG523964	MG523986
	Spain, Huesca	TLMF Lep 20010	–	MG191926	–	–
U. cretacea	Russia, Kabardino-Balkaria	BC MTD Lep 01612	–	MG191928	–	–
U. rhododendronalis	Macedonia, Mavrovo Nat. Park	TLMF Lep 05086	ZMBN Lep073	KX042769	–	MG523974
	Andorra	–	ZMBN Lep074	MG523933	MG523953	MG523975
	Spain, Cantabria	–	ZMBN Lep075	MG523934	–	–
	Spain, Cantabria	–	ZMBN Lep076	MG523935	MG523954	–
	France, Alpes-Maritimes	–	ZMBN Lep413	MG523940	–	–
	Austria, Styria	TLMF Lep 00900	ZMBN Lep414	HQ968270	MG523966	MG523987
	Austria, Vorarlberg	TLMF Lep 09148	ZMBN Lep415	KP253729	MG523967	–

species	Origin	BOLD sample no.	DNA extraction no.	COI accession no.	EF1a accession no.	wingless accession no.
U. rhododendronalis	Italy, South Tyrol	TLMF Lep 09218	ZMBN Lep416	MG191932	–	–
	Italy, Cuneo	TLMF Lep 00972	ZMBN Lep417	HM381537	MG523968	MG523988
	Spain, Lerida	–	ZMBN Lep418	MG523941	–	–
	Austria, East Tyrol	BC MTD Lep 00768	MTD Lep242	JF852287	MG523944	MG523973
	Italy, Cuneo	–	MTD Lep288	MG523923	MG551293	JF497100
	Spain, Cantabria	–	MTD Lep1390	MG523928	MG523948	–
	Andorra	–	MTD Lep1391	MG523929	MG523949	–
	Austria, Styria	TLMF Lep 00899	–	HM381472	–	–
	Macedonia, Mavrovo Nat. Park	TLMF Lep 05082	–	KX042320	–	–
	France, Midi-Pyrenees	TLMF Lep 05660	–	MG191933	–	–
U. alpinalis	Austria, Carinthia	TLMF Lep 00535	ZMBN Lep082	HM381379	MG523960	MG523981
	Austria, Styria	TLMF Lep 00897	ZMBN Lep083	HM381470	MG523961	MG523982
	Switzerland, Valais	–	MTD Lep258	JF497035	–	–
	Italy, South Tyrol	–	MTD Lep295	MG523924	MG523945	JF497076
	Italy, South Tyrol	BC MTD Lep 00754	–	JF852273	–	–
	Austria, Tyrol	BC MTD Lep 00755	–	JF852274	–	–
	Austria, Carinthia	TLMF Lep 00534	–	HM381378	–	–
	Italy, Belluno	TLMF Lep 00568	–	HM381410	–	–
	Austria, Styria	TLMF Lep 00897	–	HM381470	–	–
	Austria, Styria	TLMF Lep 00898	–	HM381471	–	–
	Austria, Vorarlberg	TLMF Lep 08390	–	KP253445	–	–
U. uliginosalis	Macedonia, Mavrovo Nat. Park	TLMF Lep 05088	ZMBN Lep079	KX042480	MG523957	MG523978
	Austria, Carinthia	TLMF Lep 00823	ZMBN Lep080	HQ968199	MG523958	MG523979
	France, Alpes-Maritimes	TLMF Lep 00635	ZMBN Lep081	HM426003	MG523959	MG523980
	Italy, South Tyrol	–	MTD Lep239	JF497067	MG523943	–
	Slovenia, Bovec	BC MTD Lep 521	–	HQ960224	–	–
	Italy, South Tyrol	BC MTD Lep 00756	–	JF852275	–	–
	Austria, Carinthia	TLMF Lep 00824	–	HQ968200	–	–
	Austria, Vorarlberg	TLMF Lep 00973	–	HM381538	–	–

species	Origin	BOLD sample no.	DNA extraction no.	COI accession no.	EF1a accession no.	wingless accession no.
U. uliginosalis	Austria, Carinthia	TLMF Lep 01021	–	HM381585	–	–
	France, Provence-Cote d'Azur	TLMF Lep 01022	–	HM381586	–	–
	Italy, Udine	TLMF Lep 01461	–	HQ968677	–	–
	Slovenia	TLMF Lep 01703	–	HQ968907	–	–
	Slovenia	TLMF Lep 01704	–	HQ968908	–	–
	Macedonia, Mavrovo Nat. Park	TLMF Lep 05087	–	KX042181	–	–
U. uliginosalis* x *alpinalis	Austria, Tyrol	–	MTD Lep297	MG523925	–	JF497102
	Austria, Styria	TLMF Lep 00896	ZMBN Lep077	HQ968269	MG523955	MG523976
	Italy, Belluno	TLMF Lep 00577	ZMBN Lep078	HM381419	MG523956	MG523977
	Austria, Tyrol	BC MTD Lep 00757	MTD Lep1582	JF852276	MG523950	–
	Austria, Tyrol	–	MTD Lep1583	MG523930	–	–
	Austria, Tyrol	–	MTD Lep1584	MG523931	MG523951	–
	Austria, Tyrol	–	MTD Lep1585	MG523932	MG523952	–
U. juldusalis	Kyrgyzstan, Ysyk-Kol	BC MTD Lep 522	–	HQ960225	–	–
	Kazakhstan, Almaty Region	BC MTD Lep 523	–	HQ960226	–	–
U. murinalis	Austria, Vorarlberg	–	MTD Lep287	JF497057	–	JF497094
U. nebulalis	Austria, East Tyrol	–	MTD Lep251	JF497057	–	–
	Austria, East Tyrol	–	MTD Lep293	–	–	JF497095
U. bourgognealis	France, Alpes-Maritimes	–	MTD Lep350	JF497038	–	–
	France, Alpes-Maritimes	–	MTD Lep351	–	–	JF497078
U. carniolica	Italy, South Tyrol	–	MTD Lep289	JF497039	–	JF497079
U. ruckdeschelli **(outgroup)**	Greece, Crete	–	ZMBN Lep150	LT595885	MG523965	LT595888

whereas the nine specimens from the Maritime Alps are identical in their DNA barcodes. The two clades are closest to each other in COI p-distances, with a minimum of 2.38%; furthermore, the Maritime Alps clade has *C. cretacea* as closest nearest COI neighbour (see Tab. 3). The next-closest neighbours are *U. rhododendronalis*, *U. uliginosalis* and *U. ruckdeschelli* with a minimum interspecific COI p-distance of 4.29%. *Udea donzelalis* (see below) differs by 5.24–5.71% COI p-distance from *U. austriacalis*.

Remarks. The type material was not stated in the original description of Herrich-Schäffer (1847–1855 ["1849"]), however, on pl. 20 fig. 142 a male imago is illustrated. Type material is also not stated in Herrich-Schäffer (1843–1856 ["1856"]).

Table 3. COI Barcode p-distances of the systematically investigated *Udea* populations.

DNA Barcode group	n	range of intraspec. p-distance [%]	average intraspec. p-distance [%]	nearest neighbour(s) (NN)	range of interspec. p-distance to NN [%]	average interpec. p-distance to NN [%]
U. austriacalis (C- & E-Alps, Macedonia)	7	0–0.95	0.45	*U. austriacalis* (Maritime Alps)	2.38–3.33	2.86
U. austriacalis (Maritime Alps)	9	0	0	*U. austriacalis* (C- & E-Alps, Macedonia) / *U. cretacea*	2.38–3.33 / 2.38	2.86 / 2.38
U. donzelalis	5	0	0	*U. cretacea*	4.29	4.29
U. cretacea	1	n/a	n/a	*U. austriacalis* (Maritime Alps)	2.38	2.38
U. rhododendronalis (Pyrenees)	7	0–0.48	0.27	*U. rhododendronalis* (Alps)	2.38–3.33	3.07
U. rhododendronalis (Alps)	8	0–0.48	0.12	*U. rhododendronalis* (Macedonia)	0.95–1.43	1.01
U. rhododendronalis (Macedonia)	2	0	0	*U. rhododendronalis* (Alps)	0.95–1.43	1.01
U. juldusalis	2	0	0	*U. uliginosalis*	2.86–4.29	3.30
U. uliginosalis	14	0–2.86	1.03	*U. alpinalis*	1.43–4.29	2.64
U. alpinalis	11	0–2.86	0.99	*U. uliginosalis* / *U. alpinalis* x *uliginosalis* hybrids	1.43–4.29 / 0.95–3.33	2.64 / 2.76
U. alpinalis x uliginosalis hybrids	7	0–2.38	1.11	*U. alpinalis*	0.95–3.33	2.71

***Udea donzelalis* (Guenée, 1854), stat. rev.**
Figs 1–4, 11–14, 29–33, 46

Scopula donzelalis Guenée, 1854: 392, pl. 6 fig. 12.

Type locality. France, Auvergne-Rhône-Alpes region, Département Puy-de-Dôme, Arrondissement Clermont-Ferrand, Mont-Dore.

Material examined. Type specimens. Lectotype ♀ "Puy de Dôme | Mont Dore | Guenée", [orange label] "Cotype", [circular label with yellow margin] "Co- | type", "Paravicini Coll. | B. M. 1937-383.", NHMUK loan label NHMUK010589059, [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2017 | [transverse] no. 473", Mally prep. no. 1123 ♀ (NHMUK); **3 Paralectotypes:** 1♂ with labels as Lectotype, NHMUK loan label NHMUK010589061, [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2017 | [transverse] no. 474", Mally prep. no. 1124 ♂ (NHMUK); 1♂ with labels as Lectotype plus [brown label] "Donzelalis | Gn. Mont Dore", NHMUK loan label NHMUK010589062, [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2017 | [transverse] no. 475", Mally prep. no. 1125 ♂ (NHMUK); 1♀ [abdomen missing] "Puy de Dôme | Mont Dore | Guenée", [orange label] "Cotype", [circular label with yellow margin] "Co- | type" (NHMUK).

Additional material (aus1 i Fig. 1): FRANCE. 1♂ "Plomb du Cantal | 13-7-2007 | D. Tourlan", [yellow label] "DNA voucher | Lepidoptera | ZMBN 2015 | [transverse] no. 089", Mally prep. no. 1022 (coll. D. Tourlan); 1♂ [handwritten] "Le Lioran | Puy Griou | CANTAL | 10.07.1988 | D. TOURLAN" (coll. D. Tourlan); 1♂ same data but with date "25.6.1989" (coll. D. Tourlan); 1♂ [handwritten] "Le Lioran | Bataillouze | CANTAL | 10.07.1991 | D. TOURLAN" (coll. D. Tourlan); 1♂ "S.-Frankr.,Pyr.or. | Mt. Canigou | 12–16.VI.'24.Zerny" (NHMW); 1♂ "Le Lioran | CANTAL | [handwritten] 25.6.2011 | D. Tourlan", [yellow label] "DNA voucher | Lepidoptera | ZMBN 2015 | [transverse] no. 091", Mally prep. no. 1024 (coll. D. Tourlan); 2♀ same collection data but with date "28.7.1997" and "25.7.2001", Mally prep. no. 1103 & 1104 (coll. D. Tourlan); 1♀ "Pas de Peyrol | CANTAL | [handwritten] 31.7.2010 | D. Tourlan", [yellow label] "DNA voucher | Lepidoptera | ZMBN 2015 | [transverse] no. 090", Mally prep. no. 1023 (coll. D. Tourlan); 1♀ [handwritten] "Gavarnie | H.Pyr. France | 15. VII. 1958 | [printed] S.N.A.JACOBS.", "Brit. Mus. | 197[handwritten]2-305"", Mally prep. no. 1047 (NHMUK); 1♀ [handwritten] "Col de Puy Morens | Pyr.Or. France | 26. VII. 1958 | [printed] S.N.A.JACOBS.", "Brit. Mus. | 197[handwritten]2-305", Mally prep. no. 1048 (NHMUK); 1♂ 1♀ "Pyrénées Orientales | Mt. Canigou | Bellier", Mally prep. no. 1102 (♀) (NHMUK); **ANDORRA.** 3♂ "ANDORRA | Port de Cabús, 2290 m | 1°25'13´E [sic], 42°32'45"N | 16.7.2012 | leg. Huemer | TLMF 2012-011", [yellow label] "DNA voucher | Lepidoptera | ZMBN 2014 | [transverse] no. 084" and "085", Mally prep. no. 866 and 867 (TLMF); 5♂ "ANDORRA | Port de Cabús, 2290 m | 1°25'13"E, 42°32'45"N | 16.7.2012 | leg. Huemer | TLMF 2012-011"; **SPAIN.** 1♂ "Spain, Huesca | Balneario de Panticosa | 42.75, -0.217, 1650 m | 14.07.2012, leg. P. Huemer", "BC TLMF Lep 20010" (TLMF).

Diagnosis. Labial palps in males approx. 20% longer than in *U. austriacalis* and *U. cretacea*; outer side of labial palps' 2nd and 3rd palpomeres barely darker than rest of labial palps. Maculation of forewing usually more pronounced than in *U. austriacalis*, the apical brown streak often broader; hindwing in both sexes with greyish cream-white inner area contrasted by a prominent dark brown postmedial line and dark brown terminal band, especially in specimens where the postmedial line and the outer band are fused into a broad band; in contrast, the hindwings' upper side is evenly dark brown in females of *U. austriacalis*. In the male genitalia, the fibula of *U. donzelalis* is generally broader and elongate triangular, tapering from the broad base towards the apex; in *U. austriacalis* the fibula is narrower and evenly broad from the base to the subapex. In the female genitalia, the signum of *U. donzelalis* is 4.1–5.1 times as long as broad (n = 6), whereas in *U. austriacalis* the signum is 3.4–4.4 times as long as broad (n = 8) (Tab. 1). *Udea donzelalis* can furthermore be distinguished by the DNA Barcode from all other sequenced *Udea* species; the nearest neighbour is *U. cretacea* with 4.29% minimum p-distance.

Redescription. *Head*. As for *U. austriacalis*, apart from: frons and vertex covered with cream-white to beige scales; labial palps covered with light brown scales, outer sides of labial palps' second and third segment sometimes slightly darker with dirty

light brown scales; maxillary palps approximately one third as long as labial palps, with beige to light brown apical scale tuft; antennal length approx. 60 % of forewing length in males, approx. 70 % in females.

Thorax. As for *U. austriacalis*, apart from: legs cream-white except for dark brown inner side of fore- and midlegs; hindleg proximal outer spur ca. 2/3 length of inner spur, distal spurs almost equal in length, outer slightly shorter.

Wings. Forewing length 12–13 mm in males, 8–10 mm in females. Males and females with one frenulum bristle. Female forewing with more acute apex due to the straight costa (distally curved in males). Forewing ground colour glossy cream-white, with maculation more or less prominent: a light brown streak parallel to the basal two thirds of the costa, distal discoidal stigma a diffuse brownish area, postmedial line brownish, arching around distal discoidal stigma, then turning basad until below distal discoidal stigma, sharply arching back outwards (the "*Udea* loop" typical for most species in the genus), then following the course of the arch in the postmedial line's anterior part and meeting with the dorsum at about two thirds of the dorsum length; apical streak more or less pronounced, usually relatively broad; ends of veins on dorsum with minute dark dots; fringe light cream-white. Hindwing in both sexes cream-white to grey-brown, postmedial line and terminal band dark brown, can be fused into one broad band. Underside of forewing uniformly brown, sometimes with grey-grown strip along cell; underside of hindwing greyish white with a brown tinge, postmedial line brownish grey, rather diffuse; colour of external area as internal area, or brownish grey as postmedial line, with which it can form a broad band.

Abdomen. As for *U. austriacalis*.

Male genitalia. (Figs 29–33) As for *U. austriacalis*, apart from: juxta nearly rhombical to broad drop-shaped, apex sharply bifid with medial incision about one fifth of juxta length; ventral valva edge straight to slightly convex; elongate triangular, apically tapering, strongly sclerotised fibula directed towards distal sacculus apex, apical half narrowed to pointed, ventrad curved claw, ventral side of claw flat; right posterior phallus apodeme forming sclerotised spatulate lobe with medially protruding ridge containing three teeth of varying shape at posterior end.

Female genitalia. (Fig. 46) As for *U. austriacalis*, apart from: signum 4.1- to 5.1-times as long as broad (n = 6); length of auxillary signum 42–62 % of main signum length (n=6); length of antrum sclerotisation 1–1.5 times the antrum diameter.

Immature stages. Unknown.

Distribution. Massif Central (France), Pyrenees (France, Andorra, Spain) (Fig. 4).

Food plants. Unknown.

DNA data. See Table 2. On BOLD, *U. donzelalis* is represented by BIN ADB6837. The five DNA barcoded specimens are identical in their DNA barcodes. The nearest neighbour is *U. cretacea* with 4.29 % minimum p-distance in the COI Barcode (see Tab. 3). The next-closest neighbour is *U. rhododendronalis* with a minimum interspecific COI p-distance of 4.76 %.

Remarks. Lederer (1863) synonymised *donzelalis* with *U. austriacalis*, and following authors (La Harpe 1864, Marion 1973, Leraut 1996) came to the same con-

clusion. However, Leraut (1996) mentioned that the specimens from the Pyrenees, conspecific with the revised *U. donzelalis,* differ from other specimens of *U. austriacalis* in their clearer maculation on the forewings in both sexes. Based on the investigation of the four syntypes of *U. donzelalis*, a lectotype and three paralectotypes are designated (see material examined).

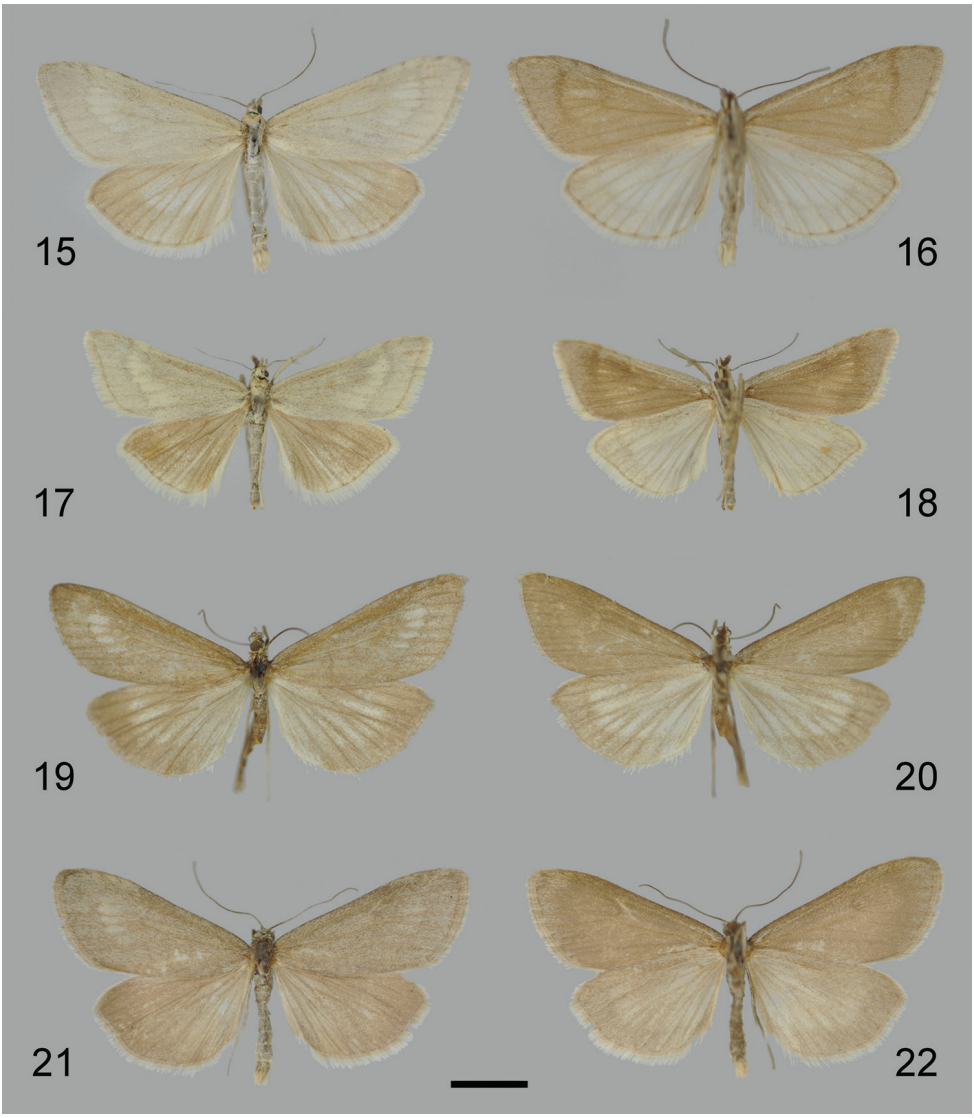
***Udea altaica* (Zerny, 1914), stat. n.**
Figs 6, 15–18, 36–37, 47

Pyrausta austriacalis v. altaica Zerny, 1914: 334–335.

Type locality. Mongolia, central Altai mountains.

Material examined. Type specimens. Lectotype ♀ "Altai centr. | mont.", "Stgr. | [handwritten] 1914", "667.", [handwritten] "P. austriacalis | v. altaica | Zerny ♀ [in red] Type", Mally prep. no. 1084 (NMW); **Paralectotype** ♂ (abdomen lost) "Altai centr. | mont.", "Stgr. | [handwritten] 1914", "666.", [handwritten] "P. austriacalis | v. altaica | Zerny ♂ [in red] Type" (NMW). – **Additional material. MONGOLIA.** 3♂ 2♀ "Altai", one of the ♂ also with [handwritten] "Alticolalis | BH i L", Mally prep. no. 1099 (♀), 1100 (♀), 1117–1119 (♂) (ZMHB); 1♂ 1♀ [handwritten] "Pyrausta | Alticolalis | Altai BH", Mally prep. no. 1090 (♂) & 1098 (♀) (ZMHB).

Diagnosis. Proximal outer spur of hindleg minute (as in *U. alpinalis*, *U. juldusalis* and *U. plumbalis*), whereas in *U. austriacalis*, *U. cretacea*, *U. donzelalis* and *U. uliginosalis* it is ca. half to two thirds the length of the proximal inner spur. The wing maculation of *U. altaica* can be confused with that of *U. austriacalis*, *U. cretacea*, *U. donzelalis*, *U. juldusalis*, *U. plumbalis*, *U. uliginosalis* and untypically maculated specimens of *U. alpinalis* (see Fig. 4 in Panigaj and Kulfan 2012), but it can be distinguished from all those species by the more or less distinct proximal brown section of the postmedial line on the ventral side of the forewing in both sexes (Figs 16, 18); in males, the proximal subterminal area of the ventral forewing side is as light brown as the central area (Fig. 16), whereas in the other species it is darker than the central area; on the hindwing ventral side, the subterminal area is only faintly darker than the central wing area in both sexes (Figs 16, 18), whereas the other species have a darker subterminal area, at least in the apex. In male genitalia only distinguishable from *U. cretacea* and *U. uliginosalis* by the small dentate ridge-like process on the posterior phallus apodeme, whereas in *U. cretacea* the sclerotisation at posterior phallus apodeme is a slim, elongate, apically dentate process emerging from the posteriormost end (Fig. 35), in *U. uliginosalis* a large hooked spine. In the female genitalia, the main signum is 3.1- to 3.4-times as long as broad, whereas in *U. austriacalis* the main signum is 3.4- to 4.4-times longer and in *U. donzelalis* 4.1- to 5.1-times longer than its maximum width (Tab. 1). The antrum is conical, widening posteriorly and is about twice as long as broad (Fig. 47), whereas in *U. austriacalis* and *U. donzelalis* the sclerotised antrum is predominantly tubular and 1- to 1.5-times as long as broad (Figs 45–46).



Figures 15–22. Adult specimens of *Udea* species. **15–18** *U. altaica* **15–16** male, dorsal (**15**) and ventral (**16**) **17–18** Lectotype (NHMW) female, dorsal (**17**) and ventral (**18**) **19–20** *U. juldusalis* Lectotype (NHMW) male, dorsal (**19**) and ventral (**20**) **21–22** *U. plumbalis* Holotype (NHMW) male, dorsal (**21**) and ventral (**22**). Scale bar: 500 μm.

Redescription. *Head*. As for *U. austriacalis*, part from: frons and vertex with cream-white scales; distal half of labial palps brown on the outside, basal half and inner sides cream white; maxillary palps cream-white with some brown scales mixed in; antennal length approx. 60 % of forewing length in males, approx. 70 % in females.

Thorax. As for *U. austriacalis*, apart from: legs cream-white on inner and outer sides; proximal pair of metatarsal spurs with outer spur minute and inner spur long,

distal pair ca. half the length of the proximal inner spur, distal inner spur a bit longer than distal outer spur.

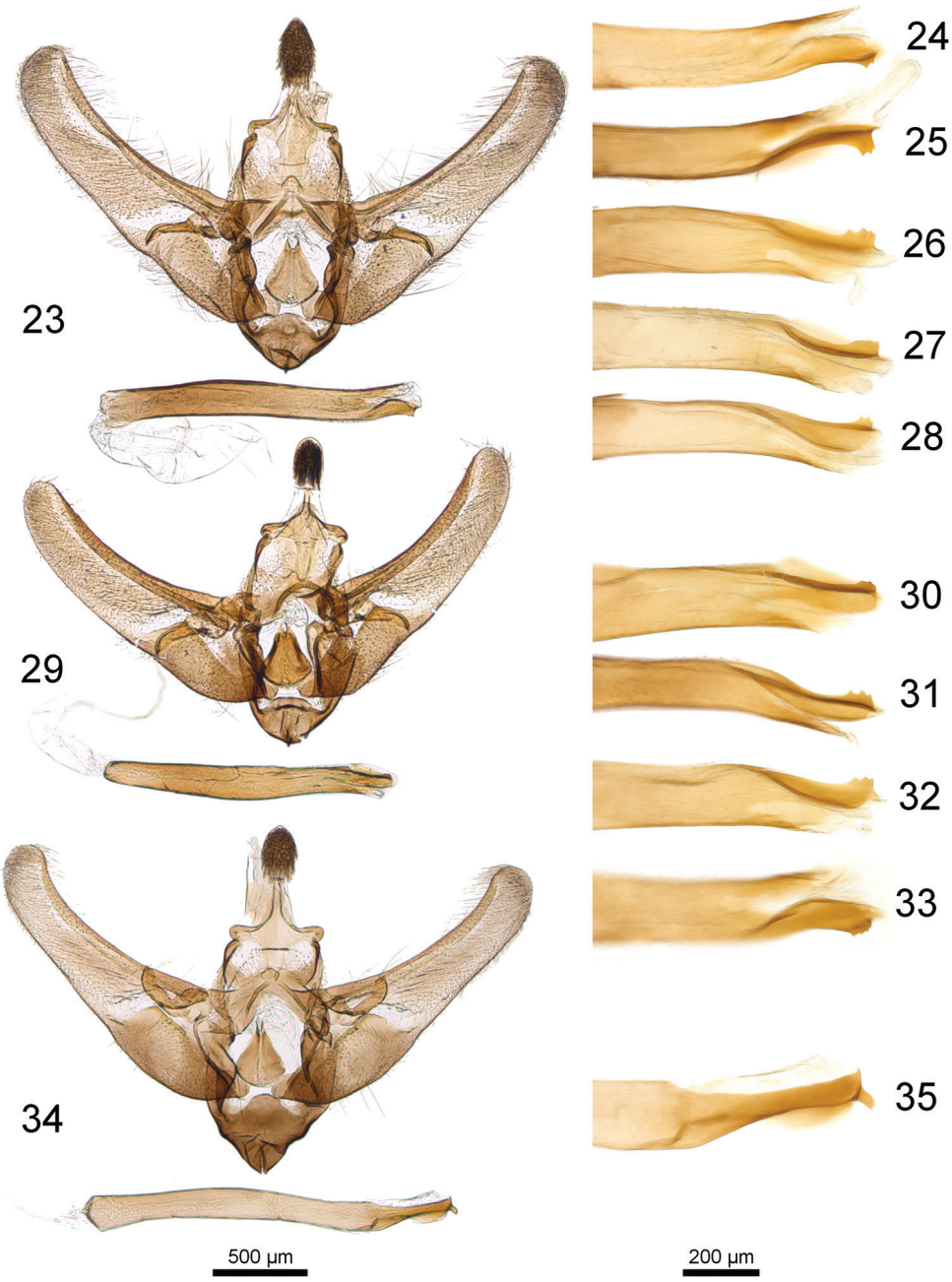
Wings. Forewing length 14 mm in males, 11 mm in females. Males and females with one frenulum bristle. Female forewing with more acute apex due to the straight costa (distally curved in males), and with outer wing margin (termen) of hindwing cut straight. Forewing upper side cream white with brown scales interspersed, giving it a dirty appearance; brownish subcostal line along the basal two third of the forewing; cell margin facing the forewing centre demarcated by a thin brown line, less prominent in females; outer medial area with a transverse cream white band lacking the interspersed brown scales; outer margin of cream white band delimited by diffuse grey postmedial line which leaves the costa in a right angle, bends inward at vein M1 and parallels the termen until the line reaches the dorsum; postmedial area homogenous greyish white, apex with more or less darker streak; termen with a slim brown margin and long, cream-white fringes. Hindwing upper side in males pale yellowish brown with diffuse light brown apex, in females light brown with a faint, slightly brighter medial band. Forewing underside in females vivid brown, with a darker, diffuse outer cellular spot and a darker postmedial area, demarcated by the postmedial line, maculation paler in males; slim whitish subcostal line along the basal two third of the forewing; fringes cream-white. Hindwing underside cream white with the subcostal and terminal areas tinted slightly brownish, the postmedial line more or less prominent.

Abdomen. Pale grey dorsally, slightly darker grey ventrally; distal segment margins greyish white, scales on terminal segment pale yellowish. Tympanum without broad short lobulus.

Male genitalia. (Figs 36–37) As for *U. austriacalis*, apart from: juxta nearly rhombical to broad drop-shaped, with small indention on each side dorsal of its greatest width, apex sharply bifid with narrow V-shaped medial incision ca. 1/4 of juxta length; ventral valva edge convex, with a slight bulge in the area to which the fibula is pointing; valva apex evenly rounded. An elongate triangular, apically tapering, strongly sclerotised fibula directed towards the distal sacculus, apical half narrowed to pointed, ventrad curved claw, ventral side of claw with flat 'blade'; fibula emerging from an oval sclerotised lobe near base of costa which is very sparsely studded with thin long simple setae; posterior phallus apodeme dorsally and ventrally with elongate unsclerotised strip, right posterior phallus apodeme forming a sclerotised spatulate lobe with a medially protruding ridge containing at the posterior end two equal-sized teeth and a third tiny posterior-most tooth.

Female genitalia. (Fig. 47) As for *U. austriacalis*, apart from: signum 3.1- to 3.4-times as long as broad (n = 4); auxillary signum 52–67 % length of main signum (n=4); antrum conical, widening posteriorly, sclerotised section about twice as long as its diameter, with or without a narrowly V-shaped unsclerotised longitudinal indentation in the antrum's dorsodistal sclerotisation; apophyses posteriores slender, approx. 60–80 % the length of the apophyses anteriores.

Immature stages. Unknown.



Figures 23–35. Male genitalia of the *Udea austriacalis* species complex. **23–28** *U. austriacalis* **23** male genitalia (Mally prep. 1092) **24–28** posterior phallus apodeme **24** Mally prep. 1042 **25** Mally prep. 1043 **26** Mally prep. 1044 **27** Mally prep. 1045 **28** Mally prep. 1046 **29–33** *U. donzelalis* **29** male genitalia (Mally prep. 1024) **30–33** posterior phallus apodeme **30** Mally prep. 1024 **31** Mally prep. 866 **32** Mally prep. 867 **33** Mally prep. 1022 **34–35** *U. cretacea* (Mally prep. 523) **34** male genitalia **35** posterior phallus apodeme; 500 µm scale bar refers to male genitalia, 200 µm scale bar to posterior phallus apodemes.

Distribution. The species is known from the central Altai mountains in NW Mongolia, the Küngöy Ala-Too (Kungey Alatau) Range in Kazakhstan and Kyrgyzstan, and the Yulduz mountains in NW China (see Fig. 6); the Küngöy Ala-Too and Yulduz mountain ranges are part of the Tian Shan mountains.

Food plants. Unknown.

DNA data. Unavailable.

***Udea juldusalis* (Zerny, 1914), stat. n.**
Figs 1, 6, 19–20, 38–41

Pyrausta austriacalis v. *juldusalis* Zerny, 1914: 335.

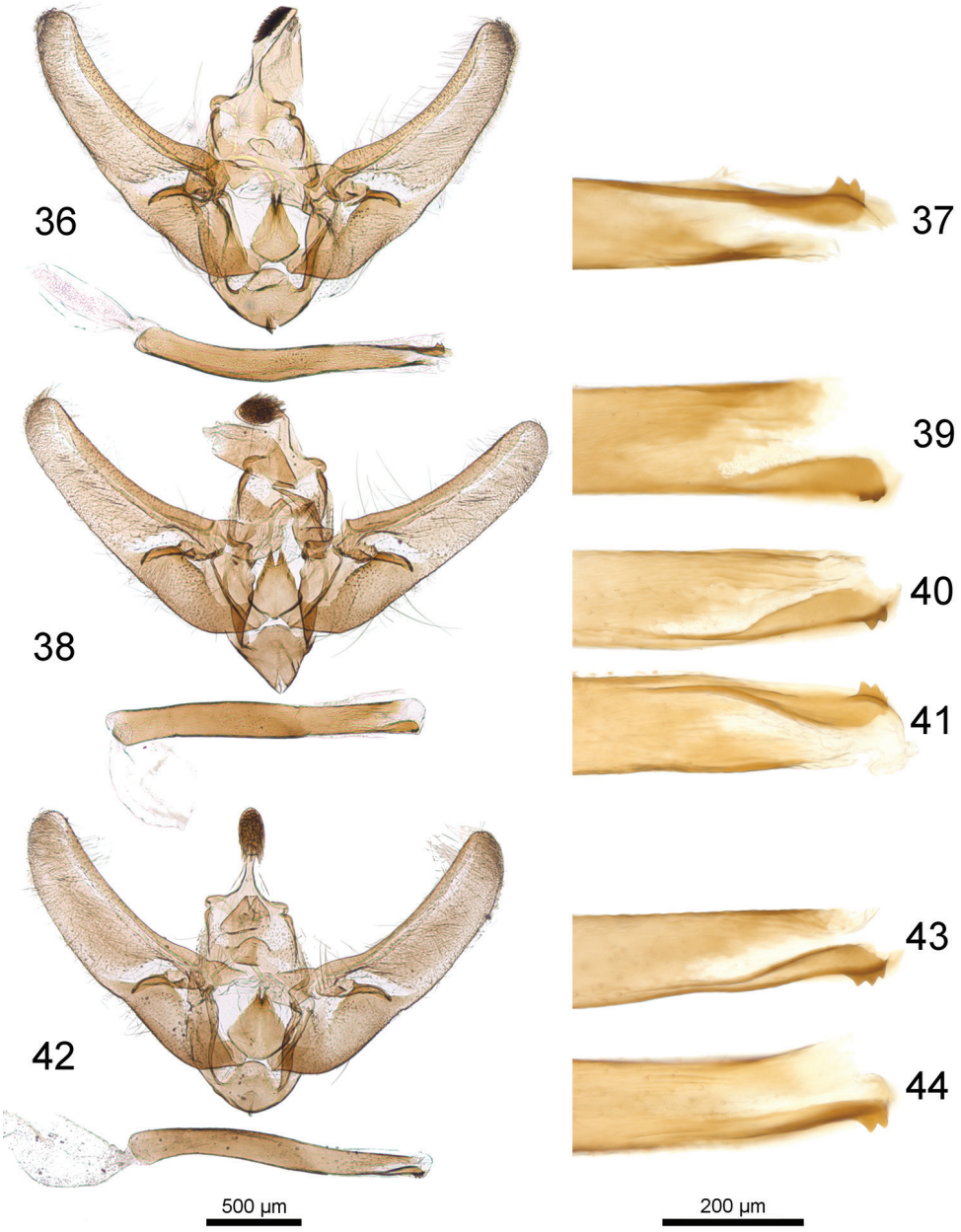
Type locality. China, Xinjiang, Tian Shan, Yulduz mountains.

Material examined. Type specimens. Lectotype ♂ "Asia centr. | Thian-Schan | Juldus Geb. | Coll.Wagner", [handwritten] "P. austriacalis | v. juldusalis | Zerny ♂ [in red] Type", Mally prep. no. 1082 (NMW); **Paralectotype** ♂ [handwritten] "Thian-Schan | Juldus Geb | Coll. Wagner", Mally prep. no. 1081 (NMW).

Additional material. CHINA. 1♂ [handwritten] "Pyrausta | Plumbealis | v. Juldusalis | Juldus BH.", Mally prep. no. 1089 (ZMHB); **KYRGYZSTAN.** 1♂ "Kyrgyzstan, Ysyk-Kol, Chong Oruktu, 42.796 77.86, 1900 m, 22-Jun-1998, L. Kuehne", [light green label] "DNA Barcode | BC MTD 00522", Mally prep. no. 1126 (MTD); **KAZAKHSTAN.** 1♂ "Kazakhstan, Almaty Oblysy, Zailijskij Alatau, Turgen valley, 43.217 77.867, 2660 m, 19-Jun-2000, M. Nuss" [light green label] "DNA Barcode | BC MTD 00523", Nuss prep. no. 1127 (MTD).

Diagnosis. *Udea juldusalis* has a wing maculation similar to that of *U. altaica*, *U. plumbalis*, *U. uliginosalis* and untypically maculated specimens of *U. alpinalis* (see Fig. 4 in Panigaj and Kulfan 2012). It can be distinguished from *U. uliginosalis* by the minute proximal outer spur of the hindleg, which is well developed in *U. uliginosalis* and about half to two thirds the length of its proximal inner spur; furthermore, *U. uliginosalis* has a large hooked spine on the posterior phallus apodeme, whereas the posterior phallus apodeme carries a small dentate ridge-like process in *U. juldusalis*. *Udea altaica* specimens have lighter forewings dorsally and ventrally, and the proximal section of the postmedial line is a diffuse, though well visible brown arch in both sexes. *Udea plumbalis* has darker, broader and more rounded fore- and hindwings (at least in the male). *Udea alpinalis* is distinguished by the dark subterminal band on the hindwings' dorsal and ventral side that contrasts with the white inner hindwing area. The COI sequences (DNA Barcode) of *U. juldusalis* are unique and not shared with any other DNA-barcoded organism, and the nearest neighbour is *U. uliginosalis* with a minimum of 2.86 % p-distance.

Redescription. *Head*. Frons and vertex covered with cream-white scales; frons evenly convex, covered with beige to light brown scales; labial palps projecting forward, third segment pointed, palps covered with beige scales, outer sides of labial palps' sec-



Figures 36–44. Male genitalia of *Udea* species. **36–37** *U. altaica* (Mally prep. 1090) **36** male genitalia **37** posterior phallus apodeme **38–41** *U. juldusalis* **38** male genitalia, Paralectotype (Mally prep. 1081) **39–41** posterior phallus apodeme **39** Paralectotype (Mally prep. 1081) **40** Lectotype (Mally prep. 1082) **41** (Mally prep. 1089) **42–44** *U. plumbalis* **42** male genitalia, Holotype (Mally prep. 1083) **43–44** posterior phallus apodeme **43** Holotype (Mally prep. 1083) **44** (Mally prep. 1094); 500 µm scale bar refers to male genitalia, 200 µm scale bar to posterior phallus apodemes.

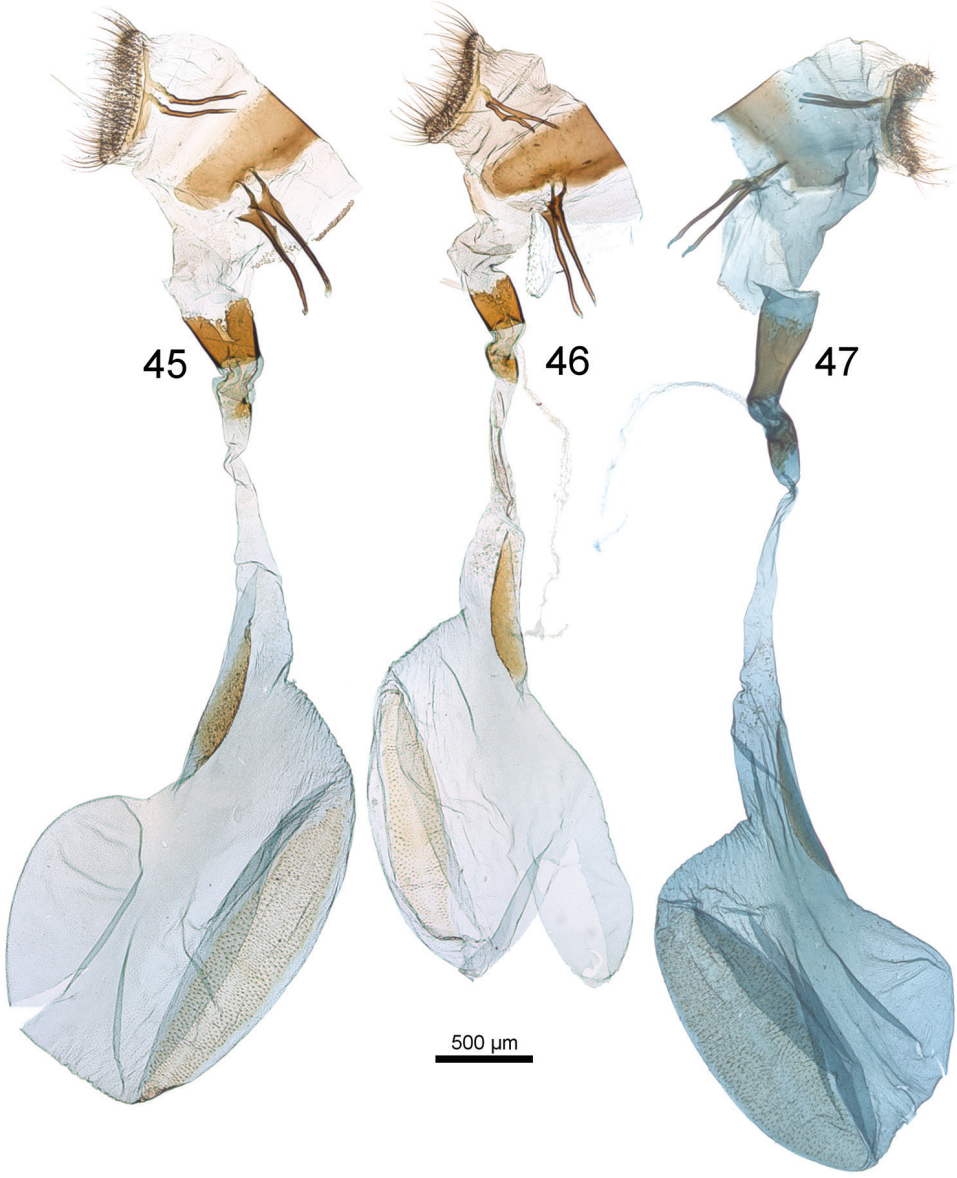
ond and third segment brown; maxillary palps brown on outside, cream-white on the inside apart from subapical area with brown scaling; compound eyes hemispherical; proboscis well developed, its base covered in brown and greyish scales; antennae filiform, dorsal side covered in beige scales, anterior side in males densely covered with cilia shorter than the basal antennal radius, shorter still in females, antennal length approx. 50% of forewing length in males, females unknown; ocellus posterior to antenna base.

Thorax. As for *U. austriacalis*, apart from: greyish brown ground colour; hindlegs and outer side of fore- and midlegs cream-white, inner side of fore- and midleg brown; proximal pair of metatarsal spurs with outer spur minute and inner spur long, distal pair about half the length of the proximal inner spur, distal inner spur a bit longer than distal outer spur.

Wings. Forewing length 13–14 mm in males, females unknown. Males with single frenulum bristle. Forewing dorsal side pale brownish to brownish yellow white, somewhat darker between cell and costa; veins delimiting cell pale brown; outer medial area behind cell cream white and clear, intersected by the brownish coloured veins R5, M1–3 and Cu1, outer margin of cream white area sharply defined by postmedial line; postmedial line slightly darker than brown ground colour, indistinct at anterior and posterior wing margins, smoothly curving around whitish area of medial wing; outer wing margin with two thin brownish lines separated by a thin yellowish cream-white line; distal part of fringe pale whitish. Hindwing dorsal side with dirty white to brownish ground colour, intersected by the brown-tinted veins; a broad brown subterminal band along the outer margin, blurrily demarcated from the somewhat lighter inner area; hindwing outer margin with a thin yellowish line between subterminal band and brownish basal half of fringe; distal half of fringe whitish. Forewing ventral side homogenous brown, subcostal area and veins delimiting the cell somewhat darker; more or less prominent white line along costal side of cell; outer wing margin and fringe as on dorsal side. Hindwing ventral side with dirty white basal and central area, intersected by the brown-tinted veins; brown subterminal band more prominent and clearly marked-off from the inner area; outer wing margin and fringe as on dorsal side.

Abdomen. As for *U. austriacalis*, apart from: abdomen pale grey dorsally, dark grey ventrally; distal segment margins on dorsal side cream white, scales on terminal segment pale yellow.

Male genitalia. (Figs 38–41) As for *U. austriacalis*, apart from: vinculum ventrally forming a roundly V-shaped saccus with a prominent ventromedial keel; juxta nearly rhombical to broad drop-shaped, apex sharply two-pointed with a narrow V-shaped medial incision ca. 1/4 of the juxta length; valva long, relatively broad, slightly tapering towards apex; costa concave, evenly tapering towards apex, surface sparsely studded with thin long simple chaetae; sacculus broad, roughly rectangular, dorsodistal edge close to fibula base, ventrodistal part concavely curving towards ventral valva edge; ventral valva edge straight from mid-sacculus to valva subapex, with a very slight bulge in the area where the fibula is pointing to; valva apex evenly rounded; elongate, apically tapering, strongly sclerotised fibula directed towards the distal sacculus, fibula base



Figures 45–47. Female genitalia of *Udea* species. **45** *U. austriacalis* (Mally prep. 1047) **46** *U. donzelalis* (Mally prep. 1023) **47** *U. altaica* (Mally prep. 1084).

somewhat constricted, apical half narrowed to a pointed, ventrad curved claw; right posterior phallus apodeme forming a sclerotised spatulate lobe with a central raised ridge bearing two small, laterally protruding teeth at its posterior end, sometimes with one or two additional, much smaller teeth posterior to the larger ones.

Female genitalia. Unknown.

Immature stages. Unknown.

Distribution. The species is known from the Küngöy Ala-Too (Kungey Alatau) Range in Kazakhstan and Kyrgyzstan, and from the Yulduz mountains in NW China; both mountain ranges are part of the Tian Shan mountain system (Fig. 6).

Food plants. Unknown.

DNA data. See Table 2. On BOLD, *U. juldusalis* is represented by the BIN AAO4297. The two available DNA Barcodes are identical with each other. The nearest COI Barcode neighbour is *U. uliginosalis* with 2.86–4.29 % p-distance (see Tab. 3); the next-closest neighbour is *U. alpinalis* with 3.33–4.29 % p-distance.

***Udea plumbalis* (Zerny, 1914), stat. n.**
Figs 6, 21–22, 42–44

Pyrausta austriacalis v. *plumbalis* Zerny, 1914: 335.

Type locality. Mongolia, Khövsgöl Province, eastern Sayan mountains, Darhad basin, Arsain Gol river.

Material examined. Type specimen. Holotype ♂ "Arasagun-gol | Sajan", "Stgr. | [handwritten] 1914", [handwritten] "Pyrausta | plumbealis B.H.iL.", [handwritten] "P. austriacalis | v. plumbalis | Zerny ♂ [in red] Type", Mally prep. no. 1083 (NMW). – **Additional material. MONGOLIA.** 1♂ "Arasagun-gol | Sajan", [handwritten] "Pyrausta | Plumbealis | [crossed out "Juldus"] BH.", Mally prep. no. 1094 (ZMHB).

Diagnosis. *Udea plumbalis* can be confused with *U. juldusalis*, *U. uliginosalis*, *U. uralica* and untypically maculated specimens of *U. alpinalis* (see Fig. 4 in Panigaj and Kulfan 2012). It differs from *U. uliginosalis* in the minute proximal outer spur of the hindleg, which is well developed in *U. uliginosalis*; furthermore, *U. uliginosalis* has a large hooked spine on the posterior phallus apodeme, while *U. plumbalis* has a small dentate ridge-like process. In *U. alpinalis*, the forewing apex is more acute and the hindwings have a white inner area (with a more or less broad brown area along the dorsum, sometimes occupying the majority of the inner wing area) contrasted with a clear dark-brown terminal band along the termen, whereas in *U. plumbalis* the hingwings' dorsal side is evenly brown (Fig. 19), and on the ventral side the inner wing area is only faintly lighter than the subterminal band (Fig. 20). *Udea juldusalis* has lighter, narrower, and more acute fore- and hindwings in the male, and the hindwings' inner area is lighter. *Udea plumbalis* is distinguished from *U. uralica* by the rounded apex and dark fringe in the forewing of males, and by the absence of a prominent bulge in the centre of the ventral valva edge (compare Slamka 2013, pl. 27 fig. 129); it is not clear whether this bulge on the ventral valva edge or its absence is a reliable character to distinguish the two species; the length of the hindlegs' proximal outer spur in comparison to the proximal inner spur is not known for *U. uralica*.

Redescription. *Head*. As for *U. austriacalis*, apart from: frons and vertex with greyish brown scales; proboscis base covered in brown and greyish scales; labial palps directed forward, dirty- to cream white, distal half of the outside greyish brown; maxil-

lary palps brown on outside, cream white on the inside; dorsal side of antennae with line of metallic brown scaling; antennal length approx. 60 % of forewing length in males, females unknown.

Thorax. As for *U. austriacalis*, apart from: greyish brown thorax; front legs and inner side of mid- and hindlegs cream-white, side of mid- and hindlegs pale brownish with many cream-white scales intermixed; hindleg proximal outer spur minute, inner spur the longest one on the hindleg, distal outer spur ca. 80% the length of the inner spur.

Wings. Forewing length 12–13 mm in males, females unknown. Males with one frenulum bristle. Forewing dorsal side dirty pale brown, distal of the postmedial line brown-grey to dirty greyish. Maculation absent apart from a more or less prominent oval whitish area basal of the postmedial line on the M veins. Area between costa and cell and veins encircling the cell somewhat darker brown. Slim band along outer wing margin consisting of three thin brown lines alternated with two thin pale yellowish brown lines; distal fringe pale white. Hindwing pale brown, apical area somewhat darker; outer wing margin with thin bands as in forewing, outermost band somewhat fainter. Ventral side of forewing uniformly brown, costa blended with darker brown and greyish scales and with a thin whitish line running in parallel on the length of the cell; distal end of cell with a faint darker brown arc; subterminal area slightly darker; dirty greyish brown area on wing apex, running along outer wing margin, narrowing towards posterior end of termen; outer wing margin and fringe as on dorsal side. Ventral side of hindwing dirty brownish grey with a more or less prominent broad pale brown subterminal band; outer wing margin and fringe as on dorsal side.

Abdomen. As for *U. austriacalis*, apart from: abdomen brownish grey, distal segment margins on dorsal side and scales on terminal segment pale yellow.

Male genitalia. (Figs 42–44) As for *U. austriacalis*, apart from: juxta rhombical to broad drop-shaped, apex sharply bifid with a narrow V-shaped medial incision 20–25% of the juxta length; Valva long, relatively broad to slim, slightly tapering towards apex; costa concave, central costa somewhat narrowed, costa surface sparsely studded with thin long simple setae; sacculus broad, roughly rectangular, dorsodistal edge close to fibula base, ventrodistal part concavely curving towards ventral valva edge; ventral valva edge straight to convex, with or without a slight bulge in the area where the fibula is pointing to; fibula elongate, apically tapering, strongly sclerotised, directed towards the distal sacculus, dorsal fibula edge inflated to a narrow tube, apical half narrowed to a pointed, ventrad curved claw, ventral side of claw with flat surface; right posterior phallus apodeme forming a sclerotised spatulate lobe with a central raised ridge bearing two to three small, laterally protruding teeth at its posterior end.

Female genitalia. Unknown.

Immature stages. Unknown.

Distribution. So far only known from the eastern Sayan mountains in the Khövsgöl Province in N Mongolia (Fig. 6).

Food plants. Unknown.

DNA data. No data available.

***Udea rhododendronalis* (Duponchel, 1834)**
Figs 1–3, 5

Botys rhododendronalis Duponchel, 1834: 363–364, pl. 235 fig. 5.
= *Udea rhododendronalis luquetalis* P. Leraut, 1996: 216–217, syn. n.
= *Udea rhododendronalis ventosalis* P. Leraut, 1996: 215–216, syn. n.

Material examined. Alpine DNA Barcode clade (BOLD BIN AAH7703; rho2 in Fig. 1): FRANCE. 1♂ "France, Alpes | Maritimes, 2000m | 6 km NW Tende | Mont Chajol | 5.vii.2008 | O. Karsholt"; "ZMUC | 00400001"; [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 413", Mally prep. no. 1035 (ZMUC); 1♂ "Htes. Alpes | Lauteret | Fletcher Coll. | [handwritten] 31.VII. 1932" (BMNH); **SWITZERLAND.** 1♂ "Heuthal | Graubuenden | [handwritten] 4.VIII.1902", "Coll. E. Möbius | Ankauf 1946", Mally prep. no. 47 (MTD); 1♂ [handwritten] "Pontresina | Switz. | 12.VII.1965 | [printed] S.N.A.JACOBS.", "Brit. Mus. | 197[handrwritten]2 305" (BMNH); 1♂ "Valais | Arolla | 6500 ft. | [handwritten] 5.Aug 1925 | Fletcher coll.", [handwritten] "Pyrausta | rhododendronalis, Dup | (V.1594)" (BMNH); 1♂ 1♀ "Switzerland. | [underlined]Scheidegg- | Wengen. | 8. vii. 1948 | S.N.A.Jacobs"; "Brit. Mus. | 197[handwritten]2-305", Mally prep. no. 1030 ♀ & 1031 ♂ (BMNH); 1♂ 1♀ [handwritten] "SaasFee | Switz. | 26.VI.1967 | [printed] S.N.A.JACOBS", "Brit. Mus. | 197[handwritten]2 305" (BMNH); 1♂ 1♀ "Graubünden | Davos, Dorfthäli | Pfr. Hauri", "Paravicini Coll. | B.M. 1937-383." (BMNH); **AUSTRIA.** 1♂ [red-bordered label, handwritten] "Tirol | Ötztal | 22.8.26 | Starke | Bautzen | [transverse] 21ov", "Coll. STARKE / Bautzen | Ankauf 1953 | Übernahme 1969", Mally prep. no. 5 (MTD); 1♂ "Austria, Osttirol | Obertilliach | Golzentipp, 2070–2317m | 01.07.2007 | leg. A. Stübner", [orange label] "DNA voucher | Lepidoptera | M. Nuss. 2007 | [transverse] no. 242", Mally prep. no. 56 (MTD); 2♂ "Austria merid., Steiermark | Turracher Höhe NW | 1750–1850 m | 13°52'05"E, 46°55'41"N | 4.7.2009 | leg. Huemer", [pale green label] " BC TLMF Lep 00899" and "00900", one ♂ with [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 414", Mally prep. no. 1036 (TLMF); 1♂ "Austria, Vorarlberg | Partenen, 2,5 km W | Silvrettastausee, 1980 m | 10°03'43"E, 46°55'21"N | 3.7.2010, leg. Huemer | TLMF 2010-020", [turquoise label] "BC TLMF Lep 09148", [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 415", Mally prep. no. 1037 (TLMF); 1♀ [red-bordered label, handwritten] "Tirol | Ötztal | 22.8.26 | Starke | Bautzen | [transverse] 22ov", "Coll. STARKE / Bautzen | Ankauf 1953 | Übernahme 1969", Mally prep. no. 6 (MTD); **ITALY.** 1♂ [handwritten] "Südtirol, Grödner Tal | Plan dla Gran Costa | nördl.St.Ulrich 2150 m | 9.7.1990 leg Sutter"; [handwritten] "[transverse] 253 | ♂ Udea | det.R.Sutter | Dup | rhododendronalis"; [light green label] "DNA Barcode | BC MTD 00792", [orange label] "DNA voucher | Lepidoptera | ZMBN 2014 | [transverse] no. 116", Mally prep. no. 1025 (SMNK); 1♂ "Italien, Prov. Cuneo | Alpi Cozie, Demonte NW | Gias Valcavera | 7°8,2'E, 44°22,6'N | 2050 m, 23.7.2009 | leg. Huemer | TLMF 2009-138"; [turquoise label] "BC TLMF

Lep 00972", [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 417", Mally prep. no. 1039 (TLMF); 1♀ "ITALIA, Südtirol, | Pfelders, Pfelderer Alm, | 11°03'28" E, 46°46'50" N | 1850 m, 25.-26.6.2010 | leg. Huemer"; [turquoise label] "BC TLMF Lep 09218", [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 416", Mally prep. no. 1038 (TLMF); 1♂ [handwritten] "Courmayeur | Aosta Italy | 13.VI.1964. | [printed] S.N.A.JACOBS.", "Brit. Mus. | 197[handwritten]2 305" (BMNH).

Pyrenean and Cantabrian DNA Barcode clade (BOLD BIN ABZ6001; rho1 in Fig. 1): SPAIN. 1♂ "ESPANA, Prov. Cantabria | PN Picos de Europa | Fuente De, El Cable Bergst. | 4°48,53´W, 43°09,55´N | 1870 m, 11.7.2012 | leg. Huemer | TLMF 2012-011", [light red label] "DNA voucher | Lepidoptera | R. Mally 2012 | [transverse] no. 1390", Mally prep. no. 560 (TLMF); 1♂ same data, without orange DNA voucher label, Mally prep. no. 569 (TLMF); 1♂ same data, but [yellow label] "DNA voucher | Lepidoptera | ZMBN 2014 | [transverse] no. 075", Mally prep. no. 870 (TLMF); 1♂ same data, but [yellow label] "DNA voucher | Lepidoptera | ZMBN 2014 | [transverse] no. 076", Mally prep. no. 871 (TLMF); 2♂ "Spain, prov. Lerida | 42°39'13" N, 00°60 E | East of Port de la | Bonaigua, 1925 m | 31.vii.2007 | leg. B.Skule & P.Skou", [salmon-pink label] "DNA voucher | Lepidoptera | ZMBN 2016 | [transverse] no. 418", Mally prep. no. 1040 (ZMUC); **ANDORRA.** 1♂ "ANDORRA | Port de Cabús, 2290 m | 1°25'13´[sic!]E, 42°32'45"N | 16.7.2012 | leg. Huemer | TLMF 2012-011"; [light red label] "DNA voucher | Lepidoptera | R. Mally 2012 | [transverse] no. 1391", Mally prep. no. 561 (TLMF); 1♂ same data, but [yellow label] "DNA voucher | Lepidoptera | ZMBN 2014 | [transverse] no. 074", Mally prep. no. 869 (TLMF); **FRANCE.** 1♂ [handwritten] "Franz. Zentralpyrenäen | Res.Nat. Neouvielle | Lac d'Aumar | 2200m; 15.VIII.1991 | [printed] leg.M. Sommerer", [pale turquoise label] BC TLMF Lep 05660", Mally prep. no. 1032 (TLMF); 1♂ [handwritten] "NEL Jacques | Lac de Gaube | 65.Cauterets. PN | 28.VII.2002", "gen ♂ | 14454" (TLMF); 1♂ 1♀ [handwritten] "20.7.94" (♂) and [handwritten] "1.7.47" (♀), "Pyrénées Orientales | Vernet-les-Bains | R. Oberthür", "Paravicini Coll. | B.M. 1937-383.", Mally prep. no. 1107 (♂), 1108 (♀) (BMNH); 1♀ [handwritten] "NEL Jacques | Pic Midi Bigorre | 2350m.65 | 07.VIII.2002", [yellow label] "DNA voucher | Lepidoptera | ZMBN 2015 | [transverse] no. 088", Mally prep. no. 873 (TLMF); 1♀ [handwritten] "NEL Jacques | Pic Midi Bigorre | 2350m.65 | 07.VIII.2002", [handwritten] "gen ♀ | 14437" (TLMF).

Balkan DNA Barcode clade (rho3 in Fig. 1): MACEDONIA. 1♂ "Macedonia, NP Mavrovo | Korab, summit ridge | ca. 2700–2750 m | 20°32'48"E, 41°47'20"N | 28.7.–1.8.2011 | leg. Huemer & Tarmann", [pale green label] "BC TLMF Lep 05086", [yellow label] "DNA voucher | Lepidoptera | ZMBN 2014 | [transverse] no. 073", Mally prep. no. 868 (TLMF); 1♀ "Macedonia, NP Mavrovo | Korab, eastern ridge | ca. 2325–2400 m | 20°34'46"E, 41°47'08"N | 28.7.–1.8.2011 | leg. Huemer & Tarmann"; [pale green label] "BC TLMF Lep 05082", Mally prep. no. 848 (TLMF); **BULGARIA.** 4♂ 3♀ "Bulg. Vitoscha | 2290 m,23.7.83 | leg.J Ganev"; "Brit. Mus. | 198[handwritten]5·189" or "Brit. Mus. | 198[handwritten]5·282", Mally prep. no. 1026 ♀, 1027 ♂, 1110 ♂ (BMNH).

DNA data. See Table 2. On BOLD, *U. rhododendronalis* is represented by the BINs AAH7703 and ABZ6001.

Remarks. We conclude from the data that none of the genitalia characters stated by Leraut (1996) for his subspecies are diagnostic: The shape of the valva apex and the size and extent of the protruding tooth ridge on the posterior phallus apodeme is variable in *U. rhododendronalis* (and in other species of the *U. alpinalis* species group); the number of cornuti ranges from four to six and is not fixed for any of the proposed subspecies. In the female genitalia, the width of the ductus bursae is variable and can be significantly influenced by the uptake of spermatophore(s) during copulation. One of the dissected females contained two spermatophores in its genital tract, indicating the possibility of multiple mating. Due to the absence of distinct morphological characters among the three clades, the two subspecies *U. r. luquetalis* and *U. r. ventosalis*, both described by Leraut (1996), are synonymised with *U. rhododendronalis*.

The *U. alpinalis* species group now consists of the following taxa (in alphabetical order):

Udea alpinalis (Denis & Schiffermüller, 1775)
= *Phalaena grisealis* Fabricius, 1794
= *Pyrausta alpinalis* ab. *prolongata* Weber, 1945
= *Pyrausta alpinalis valerialis* Galvagni, 1933
= *valerianalis* Speidel, 1996 (misspell.)
Udea altaica (Zerny, 1914), **stat. n.**
Udea austriacalis (Herrich-Schäffer, 1851)
= *Botys nitidalis* Heinemann, 1865
= *Botys sororialis* Heyden, 1860
Udea bourgogealis Leraut, 1996
Udea carniolica Huemer & Tarmann, 1989
Udea cretacea (Filipjev, 1925)
Udea donzelalis (Guenée, 1854), **stat. rev.**
Udea juldusalis (Zerny, 1914), **stat. n.**
Udea murinalis (Fischer von Röslerstamm, 1842)
Udea nebulalis (Hübner, 1796)
= *Botys pratalis* Zeller, 1841
= *Pyralis squalidalis* Hübner, 1809
Udea plumbalis (Zerny, 1914), **stat. n.**
Udea rhododendronalis (Duponchel, 1834)
= *Udea rhododendronalis luquetalis* P. Leraut, 1996, **syn. n.**
= *Udea rhododendronalis ventosalis* P. Leraut, 1996, **syn. n.**
Udea uliginosalis (Stephens, 1834)
= *Pyrausta monticolalis* La Harpe, 1855
= *uliginosalis* (Stephens, 1829), **nom. nud.**
Udea uralica Slamka, 2013

Discussion

Deep intraspecific splits are observed in the results of a phylogenetic analysis of COI sequence data of the *U. alpinalis* species group. However, further investigation of these clades based on nuclear genetic and morphological data does not indicate species-specific differences, with the exception of the *U. austriacalis* clade from the French Massif Central and the Pyrenees. We conclude that the latter clade represents a species distinct from *U. austriacalis*, as it differs in morphology and in all three investigated genetic markers from *U. austriacalis*. This distinct species is identified as *U. donzelalis*, after comparison with the respective type material, and consequently revoked from synonymy with *U. austriacalis*.

In the COI phylogram (Fig. 1), the *U. rhododendronalis* clade rho1 corresponds with the geographic distribution of Leraut's (1996) subspecies *U. rhododendronalis luquetalis*, but no morphological character could be found to separate it from the nominate *U. rhododendronalis* from the Alps. Similarly, the Balkan specimens of *U. rhododendronalis*, forming clade rho3 in the COI phylogram, are morphologically not distinct from the specimens of the Alps and Pyrenees, and thus not supporting Slamka's (2013) assumption of as potential Balkan subspecies. Specimens from the Maritime Alps, from where Leraut's (1996) subspecies *U. r. ventosalis* is described, do not form a distinct COI clade, like the *U. r. luquetalis* specimens, but group with the *U. rhododendronalis* specimens from the Central Alps instead. Furthermore, the COI groups are not reflected in the phylogenetic results of the two nuclear markers (Figs 2–3). In conclusion, no conclusive evidence for either additional species or subspecies in *U. rhododendronalis* is found.

Morphological investigation of the type material of Zerny's (1914) three *U. austriacalis* subspecies reveals that they are good species, and they are consequently raised to species level. For *U. juldusalis*, this decision is further supported by COI Barcode sequences which are not shared with other *Udea* species.

Similar cases of deep intraspecific, although not necessarily allopatric, divergences in COI data of Lepidoptera have been observed in other groups, e.g. by Charlat et al. (2009), Dasmahapatra et al. (2010), Muñoz et al. (2011), Mutanen et al. (2012), Nieukerken et al. (2012) and Ritter et al. (2013). In *U. rhododendronalis* and *U. austriacalis*, geographically well-separated COI groups are found. The observed mitochondrial genetic divergences could therefore be explained with allopatry, and the absence of (congruent) clades in the nuclear data could be due to slower substitution rates and incomplete lineage sorting. However, the specimens of *U. donzelalis* form distinct, congruent clades among all three investigated genetic markers. No such congruent pattern is found for the COI subclades of *U. austriacalis* and *U. rhododendronalis*, respectively, and the species status is therefore rejected for the named subclades of *U. rhododendronalis* based on the available data.

The uniformity of male genitalia in the *U. alpinalis* species group makes species discrimination based on this character complex difficult to impossible, as is also the case for example in the *U. fimbriatralis* complex (Mally et al. 2016). The female genitalia, however, are suitable for species distinction: the sclerotised parts of antrum, colliculum and signa as well as the ratio of length to breadth of the main signum are useful for species identification. Munroe (1966) points out the usefulness of the hindleg's

proximal outer spur for distinguishing species of the *U. itysalis* group, a character that is also useful for distinguishing species of the *U. alpinalis* group.

Introgression between *U. alpinalis* and *U. uliginosalis*

Some specimens morphologically identified as *U. uliginosalis* have been found grouping with specimens of *U. alpinalis* in the COI Barcode ML analysis (Fig. 1). All those 'mismatched' specimens are males, and their genitalia present the diagnostic characters of *U. uliginosalis*. Analyses of two nuclear markers result in different topologies: The ML analysis of wingless data (Fig. 2) does not provide a differentiation between *U. alpinalis* and *U. uliginosalis*. In the ML analysis of EF1-alpha data (Fig. 3), all 'mismatched' specimens are monophyletic with all other specimens of *U. uliginosalis* and are sister to *U. alpinalis*. The contradicting placements of those 'mismatched' *U. uliginosalis* specimens in the mitochondrial and nuclear ML analyses leads us to the assumption that introgression must have occurred between *U. uliginosalis* and *U. alpinalis*. This could be explained by the following scenario: A female of *U. alpinalis* successfully mates with a male of the sympatrically occurring *U. uliginosalis*. The F1 generation has the mitochondrial genotype of *U. alpinalis*, and the nuclear genotype is heterozygotic. In concordance with Haldane's rule (Haldane 1922), the sex that is heterogametic for sex factors – in Lepidoptera this is the female – is rare or sterile or absent. The specimens of *U. uliginosalis* collected at the localities of assumed hybridisation are exclusively male, without a single female. (However, females are less frequently collected than males - they may be poor flyers due to their shorter wings.) Despite this, assuming that a few fertile hybrid females exist in the F1 generation and these mate with males of *U. uliginosalis*, the F2 (backcrossing) generation retains the mitochondrial genotype of *U. alpinalis*, since the mitochondrial genome is maternally inherited; the nuclear genotype of the offspring consists to three quarters of that of *U. uliginosalis*. Additional backcrossings further increase the proportion of the *U. uliginosalis* nuclear genome, while the mitochondrial genotype remains that of *U. alpinalis*.

The deep intraspecific splits led us to test for *Wolbachia* infection which might play a role in the *U. alpinalis* group. The intracellular bacterium *Wolbachia* is known to have an impact on the reproduction of a wide range of arthropods, including Lepidoptera (e.g. Werren et al. 2008). The observed deep intraspecific COI Barcode splits in species of the *U. alpinalis* group could be explained by *Wolbachia*-mediated cytoplasmic incompatibility between different populations, or by geographic isolation, or a mix of both processes. In the present screening, no evidence of a *Wolbachia* infection was found in specimens of the *U. alpinalis* species group. However, several instances of false negative results exist that could obscure the presence of *Wolbachia* in these species: The mean *Wolbachia* infection prevalence is 21 % among Crambidae (Ahmed et al. 2015), so that *Wolbachia* can remain undetected if the sampling of a population is not comprehensive enough. The amplification with wsp primers resulted in sequences from *U. fulvalis* and *U. olivalis* that could not be matched with *Wolbachia* or any other sequences in GenBank or BIGSdb, indicating suboptimal primer sequences which can lead to failure of PCR amplification or amplification of fragments other than the

target sequence. Although no evidence for the presence of *Wolbachia* in the *U. alpinalis* group was found in the present screening, the possibility of *Wolbachia* infection and its biological implications for the hosts should be kept in mind for future studies.

The analyses of the two nuclear markers (Figs 2–3) show partially contradictory results: in the wingless gene tree (Fig. 3), a relatively long terminal branch leads to the *U. donzelalis* clade, whereas in the EF1a gene tree (Fig. 2), the branch of the same clade is much shorter. Further, in the EF1a phylogram *U. uliginosalis* and *U. alpinalis* form separate clades, whereas in the wingless phylogram the specimens of those two species share a common clade. This indicates that more nuclear markers are required to reliably reconstruct this contradictory and insufficiently supported part of phylogenetic inference.

These results shed further light on the *U. alpinalis* species group, but more material is needed from the mountain systems of Central and West Asia to study the morphology and genetics of the species found there, to bring them in phylogenetic context with European species of the *U. alpinalis* group and to investigate the biogeography of the species group. Additional morphological investigations are encouraged regarding the status of the genetic clades. In a similar case, new techniques, like the eversion of the phallus vesica, allowed the morphological differentiation of hitherto exclusively genetic clades (Zlatkov and Huemer 2017).

Other *Udea* species groups require revisionary work, like the Palaearctic *U. numeralis* group that needs the most systematic attention in this region. Mally et al. (2016) described a new species from Crete and placed it in the *U. numeralis* group, based on a phylogenetic analysis, but several problematic taxa remain to be revised in this group, e.g. the *U. fimbriatralis* complex and the *U. numeralis* complex as well as *U. praepetalis* (Lederer, 1869) and *U. bipunctalis* (Herrich-Schäffer, 1848). Future systematic studies on this group should include material from the Middle East and the East Palaearctic, especially the taxa described by Amsel (1961, 1970). In North America, the majority of the 25 *Udea* species belong to the *U. itysalis* group (Munroe 1966) that requires careful revision with the help of molecular data. A large COI Barcode dataset for Nearctic *Udea* material has been accumulated, but analysis of the data is pending (pers. comm. Jean-François Landry). Apart from these studies, *Udea* is poorly investigated in systematic terms. With this integrative revision, the number of *Udea* species is raised from 217 to 221 species, with 40 of them occurring in Europe.

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RESEARCH ARTICLE

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Poecilia vivipara Bloch & Schneider, 1801 (Cyprinodontiformes, Poeciliidae), a guppy in an oceanic archipelago: from where did it come?

Waldir Miron Berbel-Filho[1,2], Luciano Freitas Barros-Neto[1], Ricardo Marques Dias[3], Liana Figueiredo Mendes[4], Carlos Augusto Assumpção Figueiredo[5], Rodrigo Augusto Torres[6], Sergio Maia Queiroz Lima[1]

1 *Laboratório de Ictiologia Sistemática e Evolutiva, Departamento de Botânica e Zoologia, Universidade Federal do Rio Grande do Norte, Av. Senador Salgado Filho 3000, 56078-970, Natal, RN, Brazil* **2** *Department of BioSciences, College of Science, Swansea University, SA2 8PP, Swansea, Wales (present address)* **3** *Museu Nacional do Rio de Janeiro/UFRJ, Setor de Ictiologia, Departamento de Vertebrados, Quinta da Boa vista, s/n, 20940-040, Rio de Janeiro, RJ, Brasil* **4** *Laboratório do Oceano, Departamento de Ecologia, Av. Senador Salgado Filho 3000, 56078-970, Natal, RN, Brazil* **5** *Universidade Federal do Estado do Rio de Janeiro, Instituto de Biociências, Departamento de Ciências do Ambiente. Av. Pasteur, 458, sala 512-F, 22290-240, Rio de Janeiro, RJ, Brazil* **6** *Laboratório de Genômica Evolutiva e Ambiental, Departamento de Zoologia, Universidade Federal de Pernambuco, 50570-420, Recife, PE, Brazil*

Corresponding author: *Waldir Miron Berbel-Filho* (waldirmbf@gmail.com)

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Abstract

Poecilia vivipara, a small euryhaline guppy is reported at the Maceió River micro-basin in the Fernando de Noronha oceanic archipelago, northeast Brazil. However, the origin (human-mediated or natural dispersal) of this insular population is still controversial. The present study investigates how this population is phylogenetically related to the surrounding continental populations using the cytochrome oxidase I mitochondrial gene from eleven river basins in South America. Our phylogenetic reconstruction showed a clear geographical distribution arrangement of *P. vivipara* lineages. The Fernando de Noronha haplotype fell within the 'north' clade, closely related to a shared haplotype between the Paraíba do Norte and Potengi basins; the geographically closest continental drainages. Our phylogenetic reconstruction also showed highly divergent lineages, suggesting that *P. vivipara* may represent a species complex along its wide distribution. Regarding to the insular population,

P. vivipara may have been intentionally introduced to the archipelago for the purpose of mosquito larvae control during the occupation of a U.S. military base following World War II. However, given the euryhaline capacity of *P. vivipara*, a potential scenario of natural (passive or active) dispersal cannot be ruled out.

Keywords
Fernando de Noronha arquipelago, Human-mediated dispersal, Mitochondrial DNA, Mosquitofish, Mosquito larvae control, Natural dispersal

Introduction

The origin of terrestrial and freshwater organisms on oceanic islands has historically been a topic of intrigue within the field of biogeography. Oceanic islands are created by volcanic or coralline processes (de Queiroz 2005), making them isolated from the continent. As a result, these islands typically exhibit depauperate freshwater ichthyofauna, as such species are physiological incapable of dispersing across salt water (McDowall 2004). Thus, excluding introduced species, only secondary freshwater fishes (which can tolerate salinity and occasionally cross small marine barriers) or peripheral fishes (freshwater species with a recent marine origin) can transverse this biogeographic barrier and are naturally found on those oceanic islands (Bianco and Nordlie 2008; Walter et al. 2012).

There are four oceanic archipelagos in the Brazilian territory: Rocas Atoll, Fernando de Noronha, São Pedro and São Paulo, and Trindade and Martin Vaz (Serafini et al. 2010). The Fernando de Noronha archipelago is a Brazilian Protected Area composed of 21 volcanic islands, in an area of c. 26 km^2 (Barcellos et al. 2011), located 345 km off the northeast Brazilian coast (Rangel and Mendes 2009). There are reports of freshwater fish species across the Fernando de Noronha archipelago, including species used as alternative food sources, such as the tambaqui *Colossoma macropomum* (Curvier 1816) and the tilapia *Oreochromis niloticus* (Linneaus, 1718) (Soto 2001; 2009). Other species, such as the guppy (or mosquitofish) *Poecilia vivipara* Bloch & Schneider, 1801, were supposedly introduced for mosquito larvae control. (Soto 2001; 2009).

Originally described from Suriname, *P. vivipara* is a small poeciliid species found mainly in lentic waters, ranging in salinity from freshwater to hypersaline conditions (Gomes-Jr and Monteiro 2007). The known distribution of *P. vivipara* spans from the delta of the Orinoco River (Venezuela) to Uruguay. The species may have potentially been introduced to Puerto Rico (Lucinda 2003) and Martinique (Lim et al. 2002), both of which are oceanic islands in the Caribbean Sea. It is thought that *P. vivipara* was intentionally introduced to the Fernando de Noronha archipelago (Brazil) to control mosquito larvae during the installation of the World War II military bases (Soto 2009). However, there are no studies investigating whether this is indeed an introduced or a native species, and therefore the full geographic range of this species remains unknown (Soto 2001).

Molecular approaches have been used to identify the source regions of introduced species, particularly in cases where the species in question has a wide geographical range and introduction events are not well documented (Roux and Wieczorek 2009).

Given that *P. vivipara* is a continental euryhaline fish species, the present study aims to shed light onto the presence of *P. vivipara* in the Fernando de Noronha oceanic islands using phylogenetic analysis of mitochondrial DNA. The overall objective is to identify how this isolated population is phylogenetically related to its continental conspecifics.

Materials and methods

During a field trip to sample *Bathygobius soporator* (Valenciennes 1837) at the Fernando de Noronha archipelago, we unexpectally found *P. vivipara* (Fig. 1). More specifically, in the estuary of the Maceió River micro basin (Fig. 2). A total of of 43 *P. vivipara* individuals (5 males and 38 females, 9.0–42.9 mm SL) were collected using hand nets and plastic bags. The site of species occurrence (03°51'57.80"S, 32°25'32.79"W) is the only oceanic mangrove in the South Atlantic (Serafini et al. 2010), and is comprised of a single mangrove tree species, *Laguncularia racemosa* (L.) Gaertn (Batistella 1996) (Fig. 2) covering an area ca. 0.01 km^2. Five individuals were fixed and stored in 100 % ethanol for molecular analysis. These samples were deposited at the fish collection of the Universidade Federal do Rio Grande do Norte (UFRN 0225 and UFRN 0822). Sampling was conducted under an ICMBio/MMA permit (10806-4/2011).

DNA extraction was performed using the DNA easy Tissue Kit (Qiagen). Cytochrome Oxidase I (COI) mitochondrial DNA gene was amplified, using the prim-

Figure 1. Live male of *Poecilia vivipara*, UFRN 0225, 25.2 mm SL. Maceió River microbasin, Fernando de Noronha Archipelago, Pernambuco, Brazil.

Figure 2. Sampling site of *Poecilia vivipara* in the border of the mangrove at Maceió River microbasin, Fernando de Noronha Archipelago, Pernambuco, Brazil.

ers FISH-BCH2 (5' ACTTCYGGGTGRCCRAARAATCA 3') and FISH-BCL (5' TCAACYAATCAYAAAGATATYGGCAC 3') (Tornabene et al. 2010). PCR reactions (25 µL of final volume) were performed using 10–30 ng of DNA template, 0.10 ng/uL of each primer, 12.5 µL of 2x Taq Master Mix Vivantis^{M}, and 10.2 µL of ultrapure water. Amplification consisted of an initial cycle at 95 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 35 s, 72 °C for 70 s, a final extension step of 72 °C for 7 min, and 2 min at 20 °C. PCR product was examined using a1 % agarose gel and purified using the QIAquick PCR Purification Kit (Qiagen). All sequencing reactions were performed using Big Dye v3.1 (Applied Biosystems) and screened using ABI PRISM 3500 Genetic Analyzer (Applied Biosystems). All sequences obtained in this study were deposited in GenBank (Table 1).

In order to determine how *P. vivipara* collected from Fernando de Noronha relate to their continental conspecifics, specimens from eight basins across northeast Brazil (including the closest coastal drainages to Fernando de Noronha archipelago), two basins from south and southwest Brazil, and one continental island in Venezuela (Isla Margarita), were included in the phylogenetic analysis (Fig. 3; Table 1). Original sequences were edited using Geneious version 6.1 software (http://www.geneious.com/), imported into MEGA v. 5.1 (Tamura et al. 2011), and aligned using the ClustalW algorithm. A total of 30 *P. vivipara* sequences were obtained. Nine *P. vivipara* sequences were retrieved from GenBank. A final alignment of 50 sequences with 610bp (including 11 sequences as outgroups) were used for the molecular analysis (Table 1).

Table 1. List of species, sampling sites (basin, municipality, state, country, and number in the map), geographical coordinates, catalogue number and GenBank access number used in the phylogenetic analysis. Abbreviations: N, number of individuals; *, sequences from GenBank; CE, Ceará State; PB, Paraíba State; MG, Minas Gerais State; PE, Pernambuco State; PR, Paraná State, RJ; Rio de Janeiro State; RN, Rio Grande do Norte State; SE, Sergipe State.

Species	N	Sampling site	Latitude	Longitude	Voucher N°	GenBank N°
*Poecilia vivipara**	3	Macanao, Margarita island, Nueva Esparta, Venezuela (1)	10,988	-64,164	–	KP761881–KP761883
*Poecilia vivipara**	2	Tubores, Margarita island, Nueva Esparta , Venezuela (1)	10,905	-64,107	–	KP761884–KP761885
Poecilia vivipara	4	Maceió, Fernando de Noronha, PE, Brazil (2)	-3,865	-32,425	UFRN0822	KU684422–KU684425
Poecilia vivipara	1	Jaguaribe, Saboeiro, CE, Brazil (3)	-6,541	-39,910	UFRN0337	KU684421
Poecilia vivipara	5	Piranhas-Açu, Serra Negra do Norte, RN, Brazil (4)	-6,579	-37,255	UFRN0289	KU684426–KU684430
Poecilia vivipara	4	Potengi, Macaíba, RN, Brazil (5)	-5,881	-35,369	UFRN2694	KU684417–KU684420
Poecilia vivipara	4	Paraíba do Norte, Barra de Santana, PB, Brazil (6)	-7,529	-35,998	UFRN0431	KU684431–KU684434
Poecilia vivipara	3	Ipojuca, Ipojuca, PE, Brazil (7)	-8,583	-35,043	UFRN1072	KU684414–KU684416
Poecilia vivipara	3	São Francisco, Serra Talhada, PE, Brazil (8)	-8,211	-38,534	UFRN0529	KU684441–KU684443
Poecilia vivipara	3	Piauí, Estância, SE, Brazil (9)	-11,209	-37,282	UFRN0823	KU684438–KU684440
Poecilia vivipara	3	São João, São João da Barra, RJ, Brazil (10)	-22,523	-42,559	UFRN1074	KU684435–KU684437
*Poecilia vivipara**	1	Paraná, Piraguaçu, MG, Brazil (11)	-22,613	-45,514	–	GU701911
*Poecilia vivipara**	2	Paraná, Califórnia, PR, Brazil (11)	-23,675	-51,313	–	GU70190; GU701908
*Poecilia vivipara**	1	Paraná, Campo Mourão, PR, Brazil (11)	-24,078	-52,296	–	GU701904
*Poecilia hondurensis**	2	Aguán, Honduras	–	–	–	JX968669–JX968670
*Poecilia mexicana**	1	Lempa, El Salvador	–	–	–	JX968662–JX968663
*Poecilia reticulata**	2	Pernadeles, Dominican Replubic	–	–	–	X968695–X968696
*Poecilia sphenops**	2	Nahualate, Guatemala	–	–	–	JX968660–JX968661
Pamphorichthys hollandi	4	Sergipe, Aracaju, SE, Brazil	-10,926	-37,102	UFRN3663	KU484444–KU684447

Phylogenetic analysis was carried out using only the haplotype data (one representative per haplotype) via Bayesian Coalescent constant size tree reconstruction using Beast v.1.75 (Drummond et al. 2012). The HKY85 + G was used as the substitution model, as defined by the AIC criterion in Modeltest v. 3.7 software (Posada and Crandall 1998). A total of 10^6 MCMC runs was performed, saving one tree every 1000 runs, resulting in a

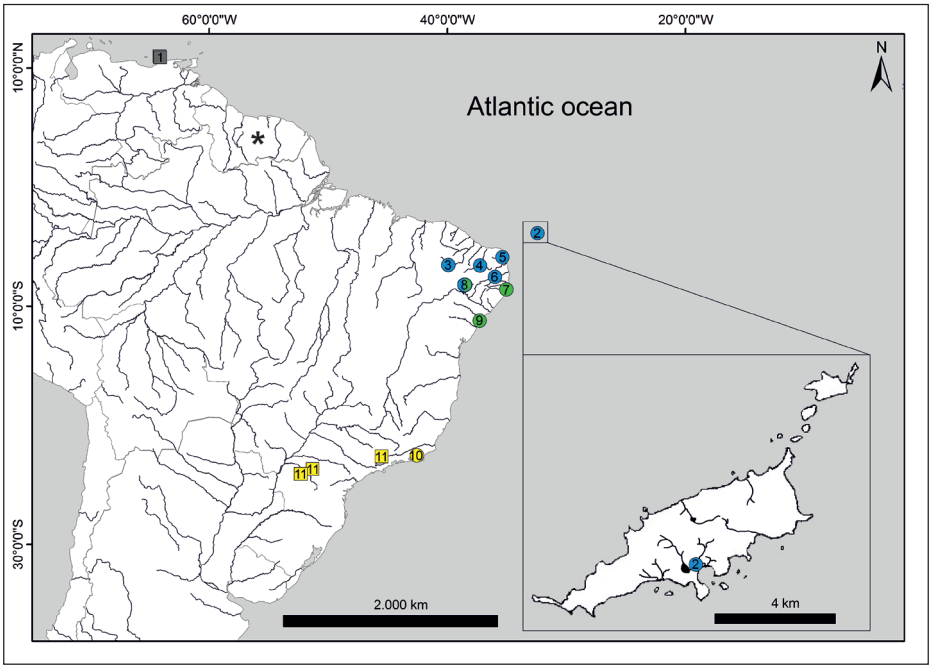

Figure 3. Map with the sampling sites of individuals used on genetic analyses. Circles represent sampled sites. Squares represent sequences retrieved from GenBank. An asterisk represents the type locality of *Poecilia vivipara*. Different colours represent different phylogenetic clades. Sampling sites: **1** Margarita island **2** Maceió **3** Jaguaribe **4** Piranhas-Açu **5** Potengi **6** Paraíba do Norte **7** Ipojuca **8** São Francisco **9** Piauí **10** São João **11** Paraná.

total of 1000 trees. The MCMC parameters were checked using Tracer v. 1.6 (Rambaut et al. 2014). The first 15 % of the trees were removed in order to account for the burn-in period of the analysis. A consensus tree accessing the posteriori probability values of each clade was constructed using TreeAnnotator v1.6.1 (Drummond et al. 2012).

To visualize the relationships among intraspecific haplotypes, a haplotype network was generated using PopART v. 1.7 (Leigh and Bryant 2015) with a 95 % statistical probability that multiple mutation had not occurred. Additionally, a pairwise matrix of K2P genetic distances between sampling localities was performed in order to enable comparison of the intraspecific genetic distance between and within basins. This analysis was conducted in MEGA v. 5.1.

Results

The phylogenetic reconstruction revealed 12 haplotypes in *P. vivipara*. In most cases, each haplotype was restricted to a single drainage. In fact, only three out of eleven drainages sampled (Paraná, Piauí, and São Francisco) presented more than one haplotype. Four major clades were revealed in the phylogenetic analysis, hereafter named

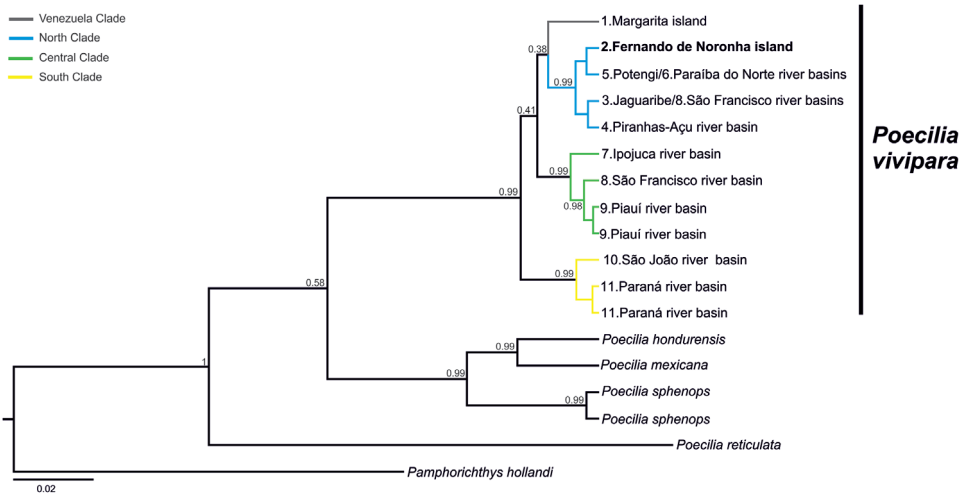

Figure 4. Rooted Bayesian phylogenetic reconstruction tree of Cytochrome Oxidase I mitochondrial gene of *Poecilia vivipara*. Number in nodes represents the value of posterior probability.

the 'Venezuela', 'north', 'central', and 'south' clades. The Venezuelan specimens represented one haplotype which was closely related to the samples from northeast Brazil, although with low posterior probability. The Fernando de Noronha individuals exhibited a unique haplotype, which was closely related to the shared haplotype found in Potengi and Paraíba do Norte drainages. Thus, the insular oceanic individuals fell within the 'north clade', comprised of lineages of northeast Brazil (Jaguaribe, Piranhas-Açu, Potengi, Paraíba do Norte, and the haplotype 3 from São Francisco). The 'central clade' was composed of haplotypes south of Paraíba do Norte river basin (Ipojuca and Piauí), including the São Francisco river basin (haplotype 7), located in northeast Brazil. Haplotypes found within the southernmost drainages comprised the 'south clade' (São João and Paraná basins) (Figs 3–5).

Our haplotype network reconstruction also showed a clear geographic pattern, within the Brazilian clades: north, central, and south clades grouping within haplogroups. The only exceptions to this pattern were the haplotypes from the São Francisco river basin. Two distantly related haplotypes (3 and 7) were found at the same sampling site; however, haplotype 3 fell within the north clade, while haplotype 7 fell within the central clade. The Fernando de Noronha haplotype is placed within the north clade, separated by two mutational steps from the haplotype shared between the Potengi and Paraíba do Norte rivers (Fig. 5).

The phylogenetic reconstruction and haplotype network clearly showed that lineage distribution followed a geographic pattern, and this pattern was corroborated by the genetic distance among drainages. The pairwise K2P distances were zero among drainages with shared haplotypes (Piranhas-Açu and Paraíba do Norte, and Jaguaribe and São Francisco), while the furthest apart sites exhibited higher genetic distance (2.6 % between Piranhas-Açu and Paraná River basins) (Fig. 4; Table 2). When com-

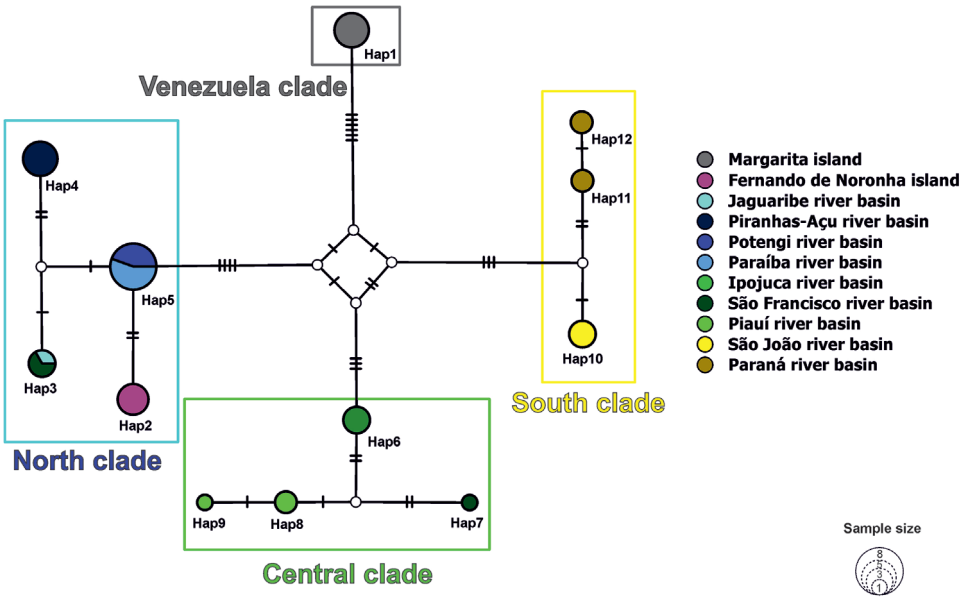

Figure 5. Haplotype network showing intraspecific relationships among *Poecilia vivipara* haplotypes. Empty circles represent non-sampled haplotypes. Thin bars on branches represent mutational steps.

Table 2. K2P distance of *Poecilia vivipara* among basins (lower diagonal), and within basins in bold (main diagonal). River basins are MAR = Isla Margarita; MAC= Maceió; JAG= Jaguaribe; PAC= Piranhas-Açu; POT= Potengi; PBN= Paraíba do Norte; IPO= Ipojuca; SFR= São Francisco; PIA= Piauí; SAO= São João; PAR= Paraná.

	1	2	3	4	5	6	7	8	9	10	11
1.MAR	**0**										
2.MAC	0.022	**0**									
3.JAG	0.022	0.007	–								
4.PAC	0.023	0.008	0.005	**0**							
5.POT	0.018	0.003	0.003	0.005	**0**						
6.PBN	0.018	0.003	0.003	0.005	0	**0**					
7.IPO	0.018	0.017	0.017	0.018	0.013	0.013	**0**				
8.SFR	0.023	0.012	0.007	0.012	0.009	0.009	0.013	**0.013**			
9.PIA	0.024	0.022	0.022	0.024	0.019	0.019	0.005	0.017	**0.001**		
10.SAO	0.018	0.022	0.022	0.023	0.018	0.018	0.015	0.022	0.021	**0**	
11.PAR	0.021	0.024	0.024	0.026	0.021	0.021	0.017	0.024	0.021	0.006	**0.001**

pared among clades, the lowest K2P distance (1.9 %) was found between the clades that were geographically closer (north and central). The highest distance (2.2 %) was found between the most distant clades (north and south).

Discussion

Darwin (1859) argued that oceanic islands are most likely to be colonized by individuals from a neighbouring continent. Our results suggest that *Poecilia vivipara* from Fernando de Noronha may have derived from a genetic lineage that is closely related to the current lineage present at the closest continental drainages (north Clade, Figs 4–5). This suggests that a natural dispersal event or human assisted introduction may have occurred by fish originating from this clade. The oceanic island lineage is closely related to the shared haplotype from the Potengi and Paraíba do Norte River basins, which are geographically the closest drainages to Fernando de Noronha. The low genetic (0.3 %) differentiation observed between *P. vivipara* individuals from Fernando de Noronha and those from Potengi/Paraíba do Norte (two mutational steps) suggests that, if natural, this colonisation occurred relatively recently. Despite evidence of an exclusive lineage within the north clade, we cannot rule out the possibility that the oceanic haplotype could be originated from a non-sampled in the Rio Grande do Norte and Paraíba coastal basins. Indeed, we acknowledge that subsampling and small sample sizes are the two most common limitations when investigating source populations of biological invasions (Muirehad et al. 2008). Therefore, there are still two possible explanations for the presence of *P. vivipara* in Fernando de Noronha archipelago: human-mediated introduction and natural dispersal. The former may have occurred during United States (U. S.) military occupation of the area and after the World War II (Soto 2001). During this period of time the introduction of poeciliids was recommended to limit the spread tropical epidemic diseases by controlling mosquito larvae populations on the archipelago (Soto 2009). The closest major U. S. military base to Fernando de Noronha was located in Natal, the capital of Rio Grande do Norte State, which is by Potengi River basin (Calkins 2011). Since the 1940s, excessive amounts of DDT were spread across Fernando de Noronha island to avoid mosquito-transmitted diseases. Chemical control in addition to the intentional introduction of *P. vivipara* may have been used to control mosquito larvae population in freshwater reservoirs such as the Xaréu Reservoir on Maceió River (Serafini et al. 2010). However, the possibility of natural colonization cannot be completely ruled out.

Dispersal routes across marine barriers and the subsequent colonization events of oceanic islands by unlikely organisms have been extensively described using DNA-based methods (de Queiroz 2005). According to this author, oceanic dispersal might occur at a higher frequency or across greater distances than expected. The two major cyprinodontiform families, Cynolebiidae (previously Rivulidae) and Poeciliidae, are considered secondary freshwater fishes that are able to support and disperse through saltwater barriers (Bianco and Nordlie 2008; Albert and Reis 2011), and there is extensive evidence of saltwater dispersal among cyprinodontiform fishes, including colonization of oceanic islands (Jowers et al. 2007; de León et al. 2014).

Several factors can facilitate marine dispersal of freshwater species during particular stages of life (eggs, larvae or adults), including transport by birds, storms, rafting, ocean currents, sea level fluctuations, and decreases in superficial water salinity (Darwin

1859; Jowers et al. 2007; Measey et al. 2007). Like the vast majority of poeciliids, *P. vivipara* undertakes internal fertilization. As suggested by its specific epithet, *P. vivipara* is also viviparous, meaning offspring are released during the juvenile phase (Thibault and Schultz 1978). This removes the possibility of egg dispersal via birds or floating rafts. Storms are also an unlikely dispersal agent as they typically maintain strength for short distances (Measey et al. 2007). The ocean current passing through the Fernando de Noronha archipelago moves towards the coastal line, although some studies report drastic changes in current direction during the Pleistocene (Lumpkin and Garzoli 2005; Nunes and Norris 2006; Ludt and Rocha 2015). These historic changes in the oceanic current are corroborated by the presence of an endemic worm lizard, *Amphisbaena ridleyi* Boulenger, 1890, in the Fernando de Noronha archipelago, which is closely related to the South American group (Laguna et al. 2010); suggesting a passive natural colonization in a direction reverse to the present current direction. However, there is no record of freshwater fishes being carried by rafting (Thiel and Gutow 2005). Although *P. vivipara* is a salt-tolerant species (Gomes-Jr and Monteiro 2007), our molecular data suggests population structuring amongst continental drainages, indicating a low capacity for long-distance dispersal across continental drainages during sea level fluctuation events. Therefore, the distance between the Brazilian coast and the Fernando de Noronha archipelago is likely to be too far for a small fish such as *P. vivipara*.

Although it was not the main aim of the study, our results revealed a deep genetic structure within on *P. vivipara*. Usually, K2P distances above 2 % for COI have been considered as high intraspecific divergences, or a threshold value for species delimitation in freshwater fishes (Pereira et al. 2013). Recent studies using morphological (Lucinda 2008) and multi-locus phylogenetic data (Bagley et al. 2015) have revealed cryptic lineages within putative poeciliid species with broad geographic distribution. Our analysis revealed values above over 2 %, specifically between the lineage from Venezuela and the other clades, as well as between north and south clades As Venezuela is the geographically closest sampled site to Suriname (type locality) included herein, these high K2P distances suggest potential cryptic species of *P. vivipara* in Brazil. The high number of nominal species under the synonymy of *P. vivipara* (see Eschmeyer and Fong 2017) and its wide distribution, ranging from Venezuela to Uruguay coastal water habitats, support the possibility that *P. vivipara* may be a species complex. A further multi-locus phylogeographic study is required in order to test the evolutionary and taxonomic cohesiveness of *P. vivipara* along its wide geographic distribution.

Conclusions

The present study represents a preliminary phylogeographical survey of *Poecilia vivipara*, a widely distributed South American guppy. Particularly, looking into the insular population of the Fernando de Noronha oceanic archipelago, which has been controversially reported as an introduced species to the archipelago. Our phylogenetic reconstruction showed a clear geographical arrangement within the distribution of *P. vivipara* lineages,

and deep genetic divergence among clades. These findings indicate *P. vivipara* as a potential species complex; however, this possibility requires further investigation. The Fernando de Noronha population possibly represents an exclusive lineage which is phylogenetically related to the closest continental river basins. *Poecilia vivipara* may have been intentionally introduced into the archipelago for the purpose of mosquito larvae control during the occupation of a U.S. military base. However, given the euryhaline capacity of *P. vivipara*, a potential scenario of natural (passive or active) dispersal scenario cannot be completely disregarded. Although the origin of the archipelago lineage is still uncertain, this population may represent an interesting biological system for studies on biogeography, ecology, and evolution of isolated populations.

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RESEARCH ARTICLE

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Re-evaluation of the discriminatory power of DNA barcoding on some specimens of African Cyprinidae (subfamilies Cyprininae and Danioninae)

Mariam I. Adeoba[1], Ronny Kabongo[2],
Herman Van der Bank[1], Kowiyou Yessoufou[3]

1 *Department of Zoology, University of Johannesburg, Kingsway Campus PO Box 524, Auckland Park 2006, South Africa* **2** *African Centre for DNA Barcoding, University of Johannesburg, Kingsway Campus, PO Box 524, Auckland Park 2006, South Africa* **3** *Department of Geography, Environmental management and Energy studies, University of Johannesburg, Kingsway Campus PO Box 524, Auckland Park 2006, South Africa*

Corresponding author: *Mariam I. Adeoba* (mariamsalami@yahoo.co.uk)

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Abstract

Specimen identification in the absence of diagnostic morphological characters (e.g., larvae) can be problematic even for experts. The goal of the present study was to assess the performance of COI in discriminating specimens of the fish family Cyprinidae in Africa, and to explore whether COI-phylogeny can be reliably used for phylogenetic comparative analysis. The main objective was to analyse a matrix of COI sequences for 315 specimens from 15 genera of African Cyprinidae using various distance-based identification methods alongside multiple tests of DNA barcode efficacy (barcode gap, species monophyly on NJ tree). Some morphological and biological characters were also mapped on a COI-phylogeny reconstructed using Maximum Parsimony. First, the results indicated the existence of barcode gaps, a discriminatory power of COI ranging from 79 % to 92 %, and that most nodes form well-supported monophyletic clades on an NJ tree. Second, it was found that some morphological and biological characters are clustered on the COI-phylogeny, and this indicates the reliability of these characters for taxonomic discrimination within the family. Put together, our results provide not only an additional support for the COI as a good barcode marker for the African Cyprinidae but it also indicate the utility of COI-based phylogenies for a wide spectrum of ecological questions related to African Cyprinidae.

Keywords
BRONX algorithm, character variations, COI, specimen identification

Introduction

Cyprinidae is the most diverse family of freshwater fishes (Nelson et al. 2006; Imoto et al. 2013) with 377 genera and over 3000 described species (Eschmeyer and Fong 2015; Froese and Pauly 2017). Species of this family are mostly found in Africa, Europe, Asia, and North America (Thai et al. 2007). In Africa, recent studies of the family have identified 24 genera and 539 species (Yang et al. 2015; Vreven et al. 2016). Some species of the family are of economic importance in aquaculture, angling, fisheries, aquarium trade and many serve as an essential source of protein for humans in addition to their high values in recreational fisheries (Skelton 2001; Thai et al. 2007; Collins et al. 2012).

Traditionally, external morphological and osteological characteristics have been used to differentiate species within the subfamilies Cyprininae and Danioninae (Zhou 1989; Chen et al. 2009; Yang et al. 2010; Liao et al. 2011; Nelson et al. 2016). For example, diagnostic characters such as a spinous anal-fin ray in some Cyprininae (Yang et al. 2010), interhyal not ossified (Liao et al. 2011) and an extended anal fin in mature males of some Danioninae (Stiassny et al. 2006) are used for specimen identification in both subfamilies. Additionally, there are key morphological features that distinguish the males from the females, including a brighter breeding colour, longer fins and presence of the tubercles on the body and head in some African genera (Skelton 2001). Similarly, morphological features such as the presence/absence of barbels, the number of barbels, as well as barbel type, pattern of innervation, and barbel position have been used to differentiate species within and between genera of the subfamilies Cyprininae sensu lato and Danioninae sensu lato (Howes 1991; Skelton 2001).

The taxonomy of the family has been a topic debated in several studies (e.g., Howes 1991; Cavender and Coburn 1992; Briolay et al. 1998; Zardoya and Doadrio 1999; Gilles et al. 2001; Yang et al. 2015; Ren and Mayden 2016). Some studies have explored the phylogeny of this family at subfamily and genus levels using both mitochondrial and nuclear genes (Zardoya and Doadrio 1999; Simons et al. 2003; Stiassny and Getahun 2007; Tang et al. 2010; Yang et al. 2010; Zheng et al. 2012; Yang et al. 2015). Specifically in Africa, most cyprinid species were previously assigned to the subfamily Cyprininae (Tsigenopoulos et al. 2002). The former genus *Barbus* forms a large polyphyletic group of more than 800 species across the world and 300 species across Africa (Skelton et al. 1991). Early studies used, in addition to morphological characteristics, the ploidy level to reorganise the genus *Barbus* sensu lato in Africa (Agnèse et al. 1990; Oellermann and Skelton 1990; Güegan et al. 1995; Berrebi et al. 1996; Machordom and Doadrio 2001; Tsigenopoulos et al. 2002). As a result, some African *Barbus* from northern and southern Africa have been regrouped into genera such as *Luciobarbus*

and *Pseudobarbus* (Swartz et al. 2009; Tsigenopoulos et al. 2010) and other species now belong to genus *Labeobarbus* (Oellermann and Skelton 1990; Berrebi et al. 1996; Machordom and Doadrio 2001).

Similarly, the recent molecular and morphological work of Yang et al. (2015) on subfamily Cyprininae had led to a major reclassification and name changes in the global Cyprinidae. This reclassification has since been adopted in some recent works (Armbruster et al. 2016; Decru et al. 2016; Skelton 2016; Vreven et al. 2016). As a result, some genera within the African Cyprinidae are now subfamilies (e.g., Cyprininae, Danioninae and Leuscininae) with few species belonging to non-specified subfamilies (Suppl. material 1). Presently, the African Cyprininae is grouped into four tribes including Barbini, Smiliogastrini, Torini and Labeonini (Yang et al. 2015). The tribe Barbini includes genera such as *Luciobarbus*, *Barbopsis*, *Caecobarbus* and *Coptostomabarbus* and the Smiliogastrini includes the genera *Barbodes*, *Barboides*, *Clypeobarbus*, *Enteromius* and *Pseudobarbus*. The former African diploid '*Barbus*' is now reclassified within the genus *Enteromius* (Yang et al. 2015; Armbruster et al. 2016) and the South African tetraploid *Barbus* has been elevated to genus '*Pseudobarbus*' (Yang et al. 2015; Skelton 2016), although Schmidt and Bart (2015) suggested a revision for genus *Pseudobarbus* to clarify those with inverted comma. Additionally, the former African *Varicorhinus* was reassigned to *Labeobarbus* in the tribe Torini (Beshera et al. 2016; Skelton 2016; Vreven et al. 2016). Yang et al. (2015) also suggested *Sanagia velifera* Holly, 1926 to be grouped with the genus *Labeobarbus*. The tribe Labeonini includes the genera *Labeo*, *Garra* and *Prolabeo* (Rainboth et al. 2012; Yang et al. 2012, 2015). In such context of taxonomic debate around the family Cyprinidae (Yang et al. 2015), it becomes necessary to question whether the ongoing global campaign of DNA barcoding can play a role at least in assigning specimen to their corresponding taxa. The DNA barcoding approach has been employed to complement or refine morphological species identification (Kochzius et al. 2010; Pereira et al. 2011; Chen et al. 2015). DNA barcoding is based on the use of a short standardised cytochrome c oxidase subunit I (COI) sequence to distinguish between animal species (Hebert et al. 2003; 2004). It has gained worldwide support because it is rapid, cost-effective (but see Stein et al. 2014), and applicable to species identification across the animal kingdom (e.g., Hebert et al. 2003; Ward et al. 2005; Van der Bank et al. 2013; Sethusa et al. 2014; Decru et al. 2016; Nigro et al. 2016). In particular, Decru et al. (2016) clearly demonstrated, using DNA barcoding, how knowledge of the African fish species diversity can be greatly improved, but they focused only on the Congo Basin region in Central Africa.

The present study uses a broader sampling of the African Cyprinidae and integrates morphology and ploidy data to further assess the effectiveness of DNA barcoding in discriminating specimens within the family. Specifically, the aim was to: (i) test the reliability of COI as a DNA barcode for the African Cyprinidae based on barcode gap, various distance methods, and the Rosenberg test of species monophyly; and (ii) map six traits including five morphological characters and ploidy level onto a COI-based phylogeny of the African Cyprinidae.

Materials and methods

Sample collections

First, 584 COI sequences of the African Cyprinidae specimens were retrieved from the Barcode of Life Database (BOLD; www.boldsystems.org) and GenBank/EBI (www.ncbi.nlm.nih.gov/nuccore). Some of the sequences from BOLD had been generated from our group (African Centre for DNA Barcoding) (Suppl. material 1). Second, for the purpose of the present study, an additional set of 55 new sequences of southern African specimens were generated to create a total DNA matrix of 639 specimens consisting of 15 of the 24 genera of African Cyprinidae. Sequences of the 55 specimens are made available on BOLD and GenBank/EBI. The BOLD identification numbers, voucher information, GenBank accession numbers, and species authorities for all species analysed in this study are presented in Suppl. material 2. Localities, images and additional information are also available on BOLD. It should also be noted that, as a result of the ongoing taxonomic revision and debates around this family, some of the African species names have been altered in FishBase but are yet to be updated on BOLD and GenBank. Therefore, for this study the old names were retained in our analysis (see Suppl. material 2; but new names are adopted in Figures 4 and 5). All the species analysed in the present study are those that have accession numbers in Suppl. material 1.

DNA extraction, amplification, and sequencing of COI

The 55 new COI sequences mentioned above were generated following the manufacturers' recommended protocol developed from NucleoSpin® Tissue kit (Macherey-Nagel). The sequence amplification (PCR) was done in accord with Hajibabaei et al. (2005). Specifically, PCR reactions were done in a total volume of 25 µL. The master mix consisted of 12.5 µL of top taq, 0.8 µL of BSA, 0.3 µL of both primers and 10.1 µL of dH2O. The DNA templates prepared for the PCR amplification ranged from 1–3 µL, depending on the strength and quality of DNA products visualized from the agarose gel. The PCR conditions were as follows: initial melting for 2 mins at 95 oC, denaturation at 94 oC for 0.5 min, annealing at 52 oC for 0.5 min, extension at 72 oC for 1 min followed by a final extension at 72 oC for 10 mins (35 cycles) and a hold at 4 oC (Steinke and Hanner 2011). The primer pair used was COI-Fish. F1 5'-TCAACCAACCACAAAGACATTGGCAC-3' and COI-Fish.R1 3'-TAGAC TCTG GGTGGCCAAAGAATCA-5'.

After the amplification, PCR products were visualised on 1.5% agarose gels. Visible products were cleaned using silica column kits, viewed again on agarose gels, and selected for cycle sequencing. Sequencing of COI region was done following the standard protocols of the Canadian Centre for DNA Barcoding (CCDB). Sequences were aligned using Multiple Sequences Comparison by Log-Expectation (MUSCLE vs. 3.8.31; Edgar 2004) and exported as a NEXUS file.

DNA barcoding analysis

Because some DNA sequences available on public repositories are not reliable (Nilsson et al. 2006), we first used the BRONX algorithm (Barcode Recognition Obtained with Nucleotide eXpose´s; Little 2011) to reanalyse all sequences retrieved from BOLD and GenBank/EBI to refine the dataset prior to our DNA barcoding analysis. Based on the BRONX analysis, we removed from our dataset (of 639 sequences) sequences that are questionable, for a number of reasons, including shared haplotypes between species, shorter sequences, and incomplete identification, etc. Also, species with no duplicates (singletons) were excluded, and as a result, the total samples included in our DNA barcoding analysis comprise 315 sequences for 86 species representing 14 out of the 24 (58 %) recognised genera in Africa (Suppl. material 2).

All barcoding analysis was conducted in the R package SPIDER (species identity and evolution in R) vs. 1.1-1 (Brown et al. 2012) following three criteria: barcoding gap, discriminatory power, and tree based analysis for species monophyly. Two techniques were used in evaluating the "DNA barcode gap" (Meyer and Paulay 2005). Firstly, the mean, median, and range of intraspecific genetic distances were compared against interspecific genetic distance (Meier et al. 2008). Secondly, the approach of Meier et al. (2006) was used to assess barcode gap. This involves matching the lowest interspecific distance against the highest intraspecific distance. Genetic distances were calculated using the Kimura 2-parameter (K2P) model (Kimura 1980).

The discriminatory power of the COI gene was tested with three methods: Best Close Match, Near Neighbour and the BOLD identification (threshID) (Meier et al. 2006; Collins et al. 2012). A good barcode should exhibit a high rate of correct species identification. Prior to the analysis, the optimised threshold for specimen identification was first determined using the R function *localMinima* (Brown et al. 2012) and then applied in the Best Close Match and Near Neighbour identification. The identification success of the traditional 1% threshold of BOLD was additionally tested in comparison to bestCloseMatch (Brown et al. 2012).

To test for species monophyly, a tree based analysis using Rosenberg's (2007) probability of reciprocal monophyly and a Neighbour-Joining (NJ) phylogram was constructed (Rosenberg 2007). For this purpose, our default was set to be false for singletons and our tree rooted on the longest branch with labels corresponding to species vector (Brown et al. 2012).

Phylogenetic reconstruction and character mapping

A DNA matrix of 315 COI aligned sequences and three outgroups (Suppl. material 3) was formed, and this matrix used to assemble a phylogeny based on Maximum Parsimony (MP) using PAUP* v4.0b 10 (Swofford 2002) with heuristic searches and 1,000 random-addition sequence replicates and tree-bisection-reconnection branch swapping. The following outgroups were chosen from similar past studies: *Moxostoma breviceps* (Cope,

Table 1. Tests of barcoding identification accuracy with numbers (N) and percentages (%) of each score Near neighbour, Best Close Match and Bold criteria.

Methods	Near neighbour		Best Close match				Bold criteria			
Scores	False	True	Ambiguous	Correct	Incorrect	No ID	Ambiguous	Correct	Incorrect	No ID
N	25	290	10	278	17	10	57	250	1	7
(%)	7.9	92.1	3.2	88.2	5.4	3.2	18.1	79.4	0.3	2.2

1870) (De Graaf et al. 2007), *Pseudorasbora parva* (Temminck & Schlegel, 1846) and *Gyrinocheilus aymonieri* (Tirant, 1883) (He et al. 2008) (Suppl. material 3).

Information related to morphological characters and ploidy levels were collected from several sources and presented in Suppl. material 3. We selected six characters based on previous studies: number of anal and dorsal fin rays, number of barbels, presence or absence of barbels, length, ploidy levels, and type of lips (Howes 1991, Skelton 2001; Zheng et al. 2010; Yang et al. 2015). Character states were tabulated and mapped using Mesquite 3.04 (Maddison and Maddison 2015) onto the parsimonious molecular phylogenetic tree.

Results

The length of the aligned COI matrix was 652 bp with the following base composition: A: 25.9 %, C: 26.8 %, G: 18.2 % and T: 29.1 %. The interspecific genetic distances (K2P) ranged from 0 to 0.30 (median = 0.15) and are larger than the intraspecific genetic distances (range: 0 – 0.02; median = 0.001; $p < 0.001$; Figure 1). This is indicative of a barcode gap in the COI dataset of the studied Cyprinidae. The existence of a barcode gap is further confirmed when we compared the lowest interspecific versus the furthest intraspecific distance (Figure 2). We found the optimised distance d = 0.015 suitable for species discrimination in the studied African Cyprinidae (Figure 3). Based on this threshold, the performance of COI varies with the method used (Table 1). The near neighbour method shows a discriminatory power of 92.1 %. The other two methods provide a lower performance of 88.2 % for the best close match (278 specimens out of 315) and 79.4 % with the BOLD method.

In addition, the result presented in Figure 4 shows that most nodes form robust monophyletic clades (red-coded nodes in Figure 4). The level of monophyly is further confirmed on Figure 5 which clearly indicates two distinct subfamilies (Cyprininae and Danioninae) and five tribes in the subfamily Cyprininae (Figure 5). The mapping of morphological characters and ploidy level on the phylogeny indicates that some characters are clearly clustered [e.g., number of anal soft rays and presence/absence of barbels for the tribe Smiliogastrini, the fish length (21–40 cm) for the tribe Labeonini and the tetraploidy for Barbinini; fig. 5].

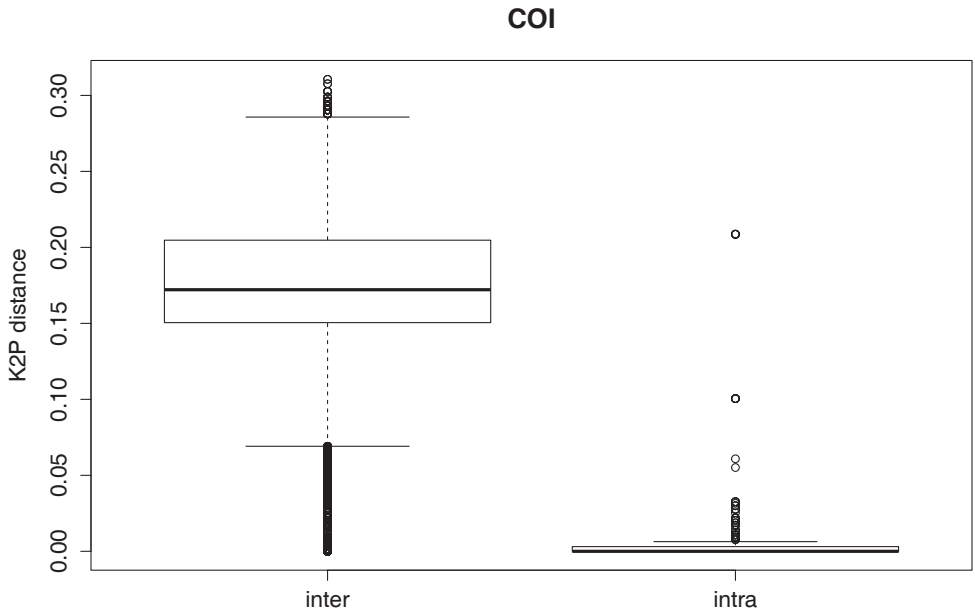

Figure 1. Evaluation of barcode gap in the dataset. Boxplot of the interspecific (inter) and intraspecific genetic (intra) distances, indicating the existence of a barcode gap, i.e., interspecific distance is larger than intraspecific distance. The median is indicated by the horizontal line and the range as the vertical dashed lines and outliers by bold vertical lines.

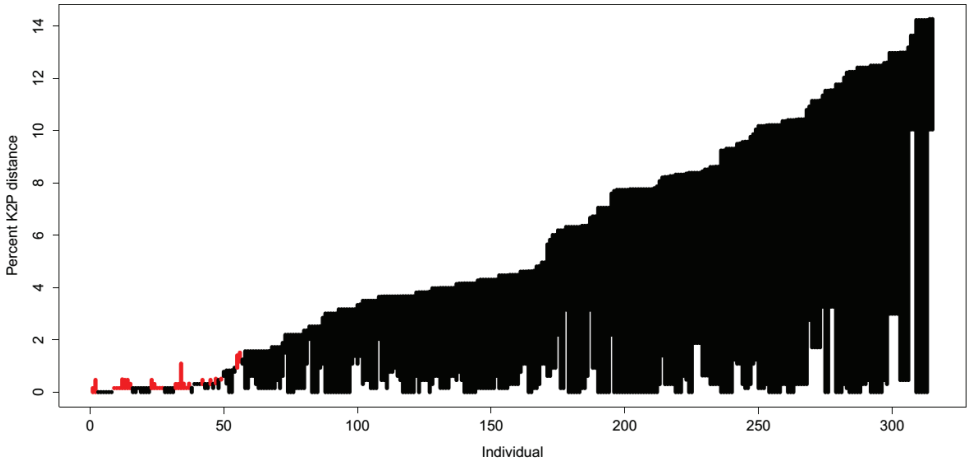

Figure 2. Evaluation of barcode gap in the dataset. Line plot of the barcode gap for the 315 Cyprinidae individuals. The black lines indicate where the smallest interspecific distance is longer than the longest intraspecific distance (bottom of line value), thus showing the existence of a barcode gap. The red lines show where this pattern is reversed.

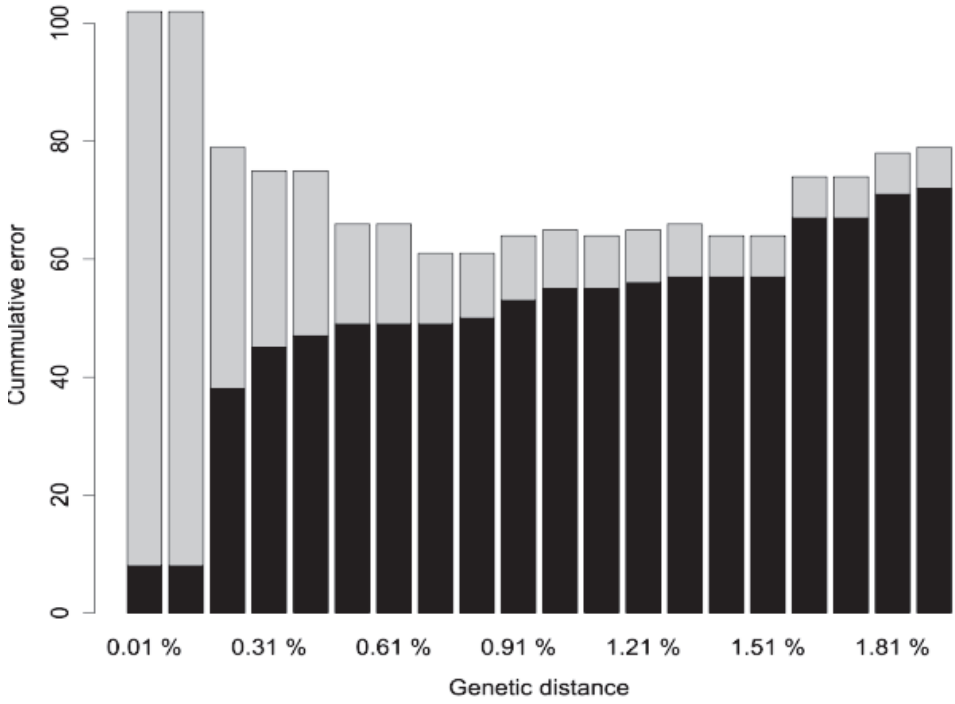

Figure 3. Determination of the threshold genetic distance for species identification. False positive (grey) and false negative (black) identification error rates summed across a range of distance thresholds from 0.01 to 1.9 %. The cumulative error plot indicates the transition between intraspecific and interspecific distances, the genetic distance corresponding to the least cumulative error (1.51 %) showing the appropriate threshold value for the dataset.

Discussion

Although COI is a universally accepted DNA barcode for animal groups (Hebert et al. 2003), its efficacy has also been questioned for some clades (Vences et al. 2005a; Chen et al. 2012; Murphy et al. 2013), and this prompts the need to assess its reliability for any particular group of interest (Collins et al. 2012).

The results presented in this work confirm that COI can be reliably used from a barcode perspective to distinguish between specimens of the African Cyprinidae in a dataset of 315 specimens representing 14 out of the 24 (58 %) recognised genera in Africa. For example, a significant barcode gap was found irrespective of the methods used, and this has also been reported for Cyprinidae of other geographic regions (e.g., Batishchevaa et al. 2011). Our results (79.4 %–92.1 %) from the distance-based method showed a pattern similar to the 90 % to 99 % discriminatory power reported for ornamental cyprinid fish species also mostly from Cyprininae and Danioninae and a catostomid (Collins et al. 2012). Irrespective of some drawbacks associated with the use of DNA barcoding and highlighted by some authors for some taxonomic groups (Vences et al. 2005a, b; Nielsen and Matz 2006; Valentini et al. 2008; Laskar et al.

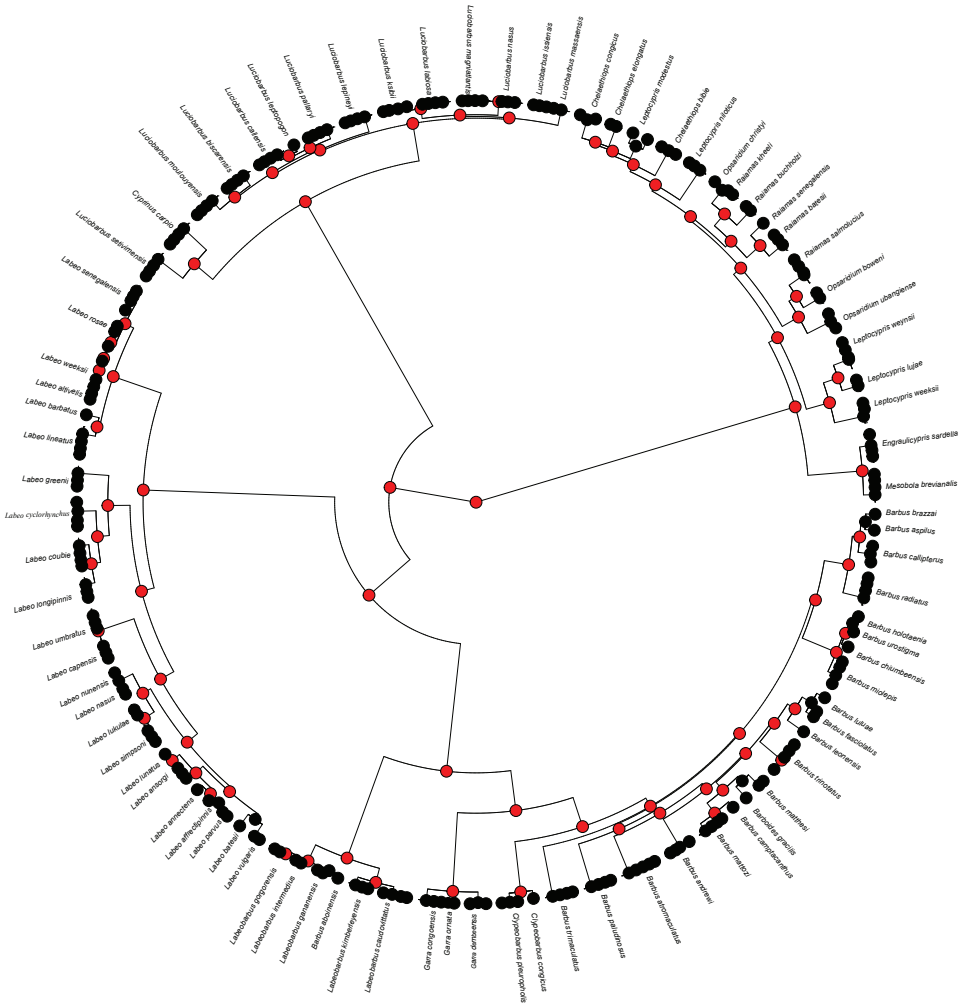

Figure 4. Neighbour-joining tree analysis using Rosenberg's (2007) test. Nodes in red are strongly supported nodes, indicating species monophyly.

2013) as well as the recent development of new generation sequencing techniques (e.g., Taylor and Harris 2012), the marker COI still remains useful for identification purposes (Batishchevaa et al. 2011; Collins et al. 2012; Van der Bank et al. 2013).

For example, the high level of COI discrimination is further supported by the test of species monophyly, a test that resulted in strongly supported clades based on Rosenberg (2007)'s probability of reciprocal monophyly on the NJ tree (see also Collins et al. 2012). Even our Maximum Parsimony tree provides additional support to the COI's power of discriminating between clades of the African Cyprinidae. Specifically, our phylogenetic analysis retrieved 14 monophyletic genera clearly grouped into two subfamilies (Cyprininae and Danioninae). Within the Cyprininae, five tribes are distinctly recovered: Barbini, Cyprinini, Labeonini, Smiliogastrini, and Torini as in

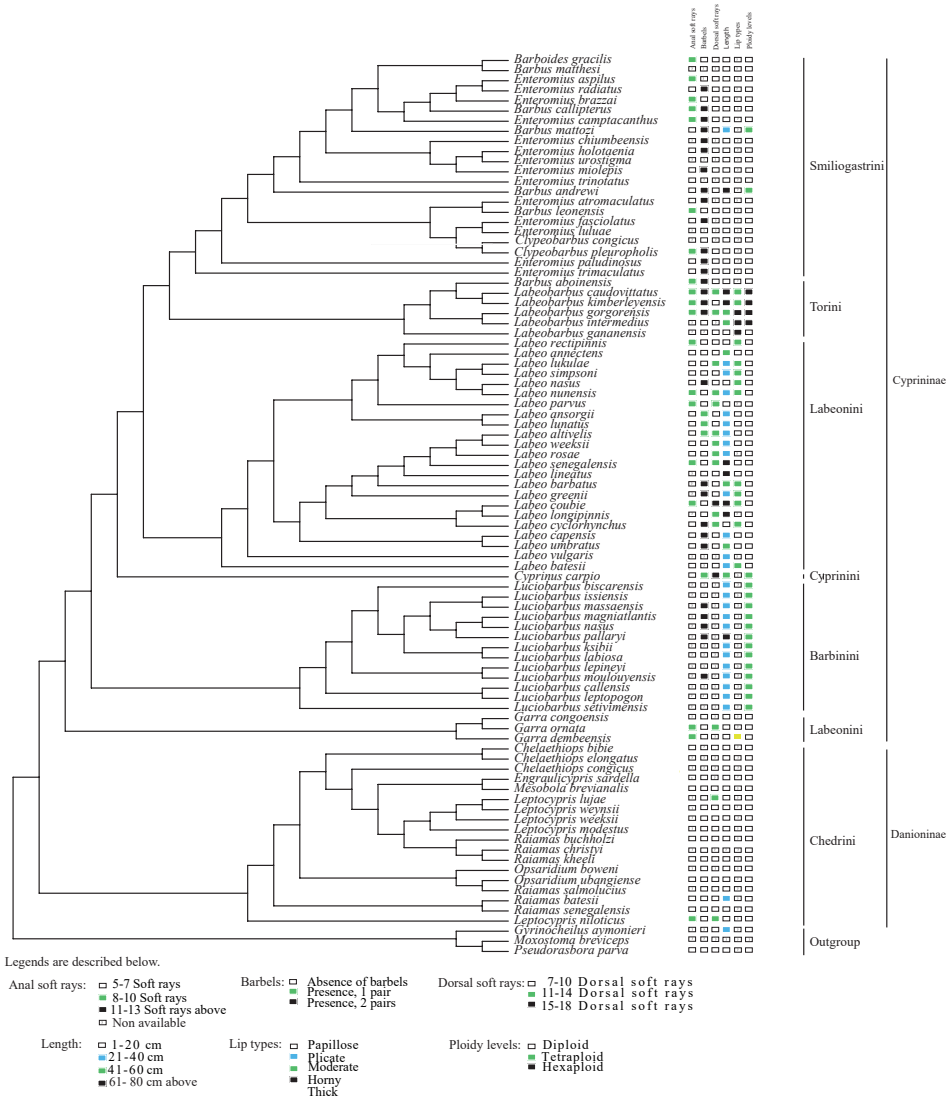

Figure 5. Parsimonious phylogenetic tree showing some plotted morphological characters, such as anal soft rays, barbels, dorsal soft rays, length, lip types, and ploidy status of 86 African Cyprinidae species.

Yang et al. (2015). The subfamily Danioninae was represented in our material by the tribe Chedrini which is well supported and includes *Chelaethiops*, *Engraulicyprus*, *Leptocypris*, *Mesobola*, *Opsaridium*, and *Raiamas* (see also Tang et al. 2010).

This evidence of monophyly accords with the morphology-based taxa delimitation as we found that some morphological characters and ploidy levels clustered within some clades along the phylogeny. Such characters that clustered within clades include, for example, the number of anal soft rays and presence/absence of barbels for the tribe Smiliogastrini, the fish length (21–40 cm) for the tribe Labeonini and the tetraploidy for Barbinini. Such clustering on the COI-phylogeny is evidence not only for COI as DNA barcoding of some

African Cyprinidae 11 a good barcode for the family Cyprinidae but also that COI-phylogeny can be used for a comparative phylogenetic analysis. Only the tribe Labeonini sensu Rainboth 1991 (Yang and Mayden 2010) was retrieved non-monophyletic in our dataset.

Overall, the existence of DNA barcode gap and a high discriminatory power, as well as the high level of monophyly give support to the use of COI as a reliable DNA barcode for African Cyprininae and Danioninae. Several studies have examined the phylogeny of this family at subfamily and genus levels using both mitochondrial and nuclear genes (Simons et al. 2003; Stiassny and Getahun 2007; Swartz et al. 2009; Tsigenopoulos et al. 2010; Yang et al. 2010; Zheng et al. 2012). Our study provides additional evidence for the effectiveness of DNA barcode data as a complementary tool to morphology-based identification of some African Cyprinidae, and our findings indicate that a COI-based phylogenetic tree for the African Cyprinidae can be used in comparative phylogenetic analyses and important applied problems (e.g., conservation) for this group of fish.

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Supplementary material 1

Table S1

Authors: Adeoba MI, Kabongo R, Van der Bank H, Yessoufou K

Data type: occurence

Explanation note: Taxonomy of African Cyprinidae and geographic origins.

Link: https://doi.org/10.3897/zookeys.746.13502.suppl1

Supplementary material 2

Table S2

Authors: Adeoba MI, Kabongo R, Van der Bank H, Yessoufou K

Data type: occurence

Explanation note: List of 315 Cyprinidae specimens analysed in this study. Full names, voucher information, and geographic origins are presented. Accession numbers for ACDB (African Centre for DNA Barcoding at the University of Johannesburg), GenBank, and BOLD are also included.

Link: https://doi.org/10.3897/zookeys.746.13502.suppl2

Supplementary material 3

Table S3

Authors: Adeoba MI, Kabongo R, Van der Bank H, Yessoufou K

Data type: measurement

Explanation note: Matrix of the morphological characters used for character mapping along a phylogeny. Details of the characters (1–6) and sources are provided at the bottom of the table..

Link: https://doi.org/10.3897/zookeys.746.13502.suppl3

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Ugandaltica gen. n., a tiny flea beetle from the forest canopy in Central Africa (Coleoptera, Chrysomelidae, Galerucinae, Alticini)

Paola D'Alessandro[1], Maurizio Biondi[1]

1 *University of L'Aquila, Department of Life, Health and Environmental Sciences, Section of Environmental Sciences, Via Vetoio, 67100 L'Aquila, Italy*

Corresponding author: *Paola D'Alessandro* (paola.dalessandro@univaq.it)

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Abstract

In this contribution, *Ugandaltica wagneri* **gen. n.** and **sp. n.**, collected from the canopies in the Budongo Forest, Uganda, is described. Similarities and affinities with other small-sized and convex-shaped flea beetle genera, occurring in the Afrotropical region, are discussed. Micrographs of diagnostic characters, including male and female genitalia, are supplied. Finally, some considerations on the ecology of canopy flea beetles are also reported.

Keywords

Afrotropical region, canopy, Coleoptera, Chrysomelidae, ecology, morphology, new genus, new species, taxonomy

Introduction

Alticini are a tribe of small to medium sized Coleoptera in the family Chrysomelidae, subfamily Galerucinae, along with the closely related Galerucini (Bouchard et al. 2011). They are named 'flea beetles' because of the presence of a metafemoral extensor tendon that enables them to jump (Furth and Suzuki 1998, Nadein and Betz 2016). Alticini are probably the largest and most diverse tribe of Chrysomelidae, comprising about 600 genera and 8000 species (Nadein 2012, Nadein and Beždek 2014, Insektoid.Info 2017).

Some genera are widespread in more than one zoogeographical region, i.e. *Altica* Geoffroy, *Aphthona* Chevrolat, *Chaetocnema* Stephens, *Longitarsus* Berthold, etc., while others are strictly endemic to very limited areas (Biondi and D'Alessandro 2017). Flea beetles feed on stems, leaves or roots, and rarely flowers, both in the adult and larval stages. Host plants are known from almost all the vascular plant families, generally with high levels of specialization and close relation with the vegetation types (Jolivet and Verma 2002; Biondi and D'Alessandro 2008, Biondi et al. 2015). There is a higher species richness of flea beetles in the tropics of the southern hemisphere, even though our knowledge about this tribe is still incomplete for many of these areas (Biondi and D'Alessandro 2010a, 2012, Nadein and Beždek 2014). Based on our current knowledge, the whole Afrotropical region, including Madagascar, hosts about 1600 known species, ascribed to 101 different genera (Biondi and D'Alessandro 2010a, 2010b, 2012, 2013a, 2013b, 2015, 2016, 2017, 2018, Döberl 2010, D'Alessandro et al. 2014, 2016, 2017, Biondi et al. 2017, Biondi personal data); and sub-Saharan Africa, in particular, hosts 85 flea beetle genera of which about 65% are endemic (Biondi and D'Alessandro 2010a, 2012, Biondi 2017, Biondi et al. 2017). One of the most interesting, but still poorly and/or methodologically incorrectly investigated habitats is the forest canopy (Wagner 2000, Basset et al. 2003a, 2003b, Furth et al. 2003, Charles and Basset 2005, Davis et al. 2011). Studies on the arthropod composition of the forest canopies in sub-Saharan Africa have revealed a high proportion of Alticini when compared to other beetles (Wagner 2001).

In this contribution the small flea beetle, *Ugandaltica wagneri* gen. n. and sp. n., is described from Budongo Forest, a seasonal rain forest in Western Uganda. The similarities and affinities of this new genus with other small, convexly shaped flea beetle genera are discussed. In addition, some considerations on the ecology of canopy flea beetles are reported.

Materials and methods

Material examined consisted of dried pinned specimens, collected by fogging trees during the research activities for the Budongo Forest Project (Wagner 2000, 2001, Budongo Conservation Field Station 2017). Specimens were examined, measured, and dissected using a Leica M205C binocular microscope. Photomicrographs were taken using a Leica DFC500 camera and Zerene Stacker software version 1.04. Scanning electron micrographs were taken using a Hitachi TM-1000 scanning electron microscope. Males and females were measured to determine the mean, standard deviation and range of some morphometric measurements for each sex. The terminology follows D'Alessandro et al. (2016 fig. 10E) for the median lobe of the aedeagus, Döberl (1986), Suzuki (1988), and Furth and Suzuki (1994) for the spermatheca, and Furth (1982), Furth and Suzuki (1998), and Nadein and Betz (2016) for the metafemoral extensor tendon. Geographical coordinates for localities are reported in DDM format; information included in square brackets has been added by the authors. Lines on the same label are separated by "/" and labels on the same specimen are separated by "//". Chorotypes follow Biondi and D'Alessandro (2006).

Abbreviations of depositories:

BAQ collection of M. Biondi, Dipartimento di Medicina clinica, Sanità pubblica, Scienze della Vita e dell'Ambiente, Università dell'Aquila, Italy;
BMNH The Natural History Museum, formerly British Museum (Natural History), London, Great Britain;
NMPC Entomologické oddělení Národního muzea, Praha-Kunratice, Czech Republic.

These internationally recognized codens follow the list on The Insect and Spider Collections of the World Website (Evenhuis 2016). Abbreviations of measurements:

LA numerical sequence proportional to length of each antennomere;
LAED length of aedeagus;
LAN length of antennae;
LB total length of body (from apical margin of head to apex of elytra);
LE length of elytra;
LP medial length of pronotum;
LSPC length of spermathecal capsule;
WE maximum width of elytra together;
WP maximum width of pronotum.

Abbreviations of ecological data, referring to fogged trees and the type of forest, as recorded on the original labels; by courtesy of Thomas Wagner:

C.a.7 *Cynometra alexandri* (Caesalpiniaceae), primary forest;
R.a.7 *Rinorea beniensis* (Violaceae), primary forest;
R.a.22 *Rinorea beniensis*, secondary forest, 8m^2 collecting sheets;
R.a.48 *Rinorea beniensis* secondary forest;
R.a.57 *Rinorea beniensis*, primary forest;
R.a.78N *Rinorea beniensis*, secondary forest, night;
T.r.2, T.r.3, T.r.4, T.r.6, T.r.8 *Trichilia rubescens* (Meliaceae), primary forest.

Taxonomic part

***Ugandaltica* gen. n.**
http://zoobank.org/3118EF6B-DA2C-4F3D-AB9F-BABED77FCC33

Diagnosis. The very small size, convex body, and rather short antennae (Fig. 1A) make the new genus similar to the "moss-inhabiting genera", mainly widespread in the Palaearctic and Oriental Regions (Konstantinov et al. 2013, Damaška and Konstantinov 2016, Ruan et al. 2017). This habitus is, therefore, a clear example of adaptive convergence. The diagnostic characters of the "moss-inhabiting genera" are notably different

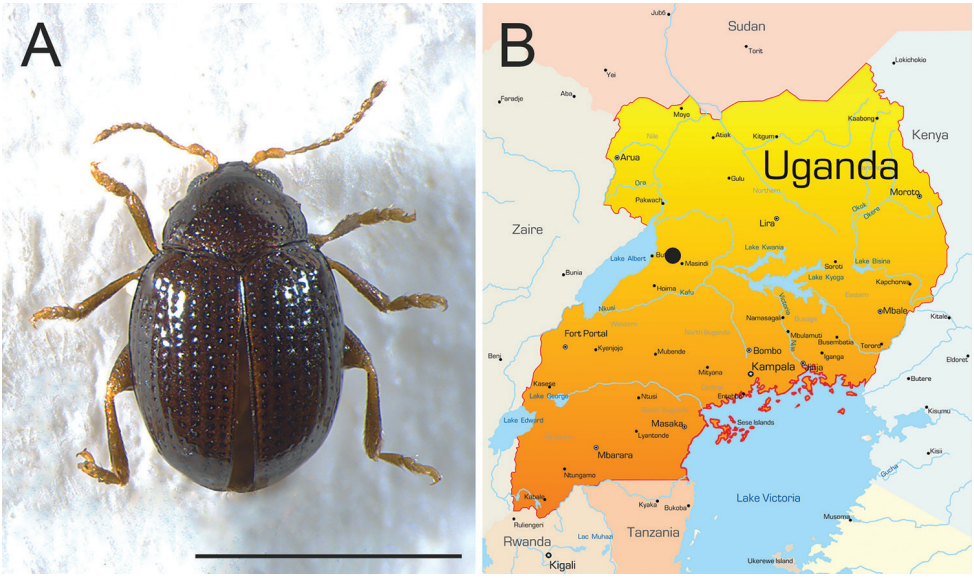

Figure 1. *Ugandaltica wagneri* sp. n. **A** Habitus of male holotype in dorsal view **B** Collecting site (black dot). Scale bar: 1 mm.

from those of *Ugandaltica* gen. n., which is also not associated with mosses (see below). Among the Afrotropical flea beetle fauna, *Bezdekaltica* Döberl from Socotra Island (Döberl 2012), *Jacobyana* Maulik from the Oriental and Afrotropical regions (Biondi and D'Alessandro 2011), and *Stegnaspea* Baly from the Republic of South Africa and Tristan da Cunha (D'Alessandro et al. 2012) show a similar general morphology.

The genus *Stegnaspea* is immediately distinguishable from *Ugandaltica* gen. n. by the lack of a scutellum, along with several other characters, concerning among others the head sculpture, shape of the maxillary palpi, shape of the pronotum and pronotal sculpture, elytral surface, male and female genitalia, and the metafemoral extensor tendon (D'Alessandro et al. 2012).

The genus *Jacobyana* shows some similarities with the new genus in its pronotal shape and sculpture, first two antennomeres, and the metafemoral extensor tendon (Biondi and D'Alessandro 2011). However, it is easily distinguishable from this genus by the shape and sculpture of the head, shape of the distal antennomeres, anterior angles of the pronotum, third and fourth visible tarsomeres, and the rather different male and female genitalia.

Bezdekaltica, a genus known only from the species *B. socotrana* Döberl, shares along with its general shape (Döberl 2012) some aedeagal characteristics with *Ugandaltica* gen. n., such as the absence of sculpture, the presence of rather big pores on the surface, and the peculiar shape of the phallobasis (Figs 3C, 4C). Notwithstanding these similarities in the aedeagus, considered a likely indicator of real affinity, differences in the female genitalia (Figs 3D, 4D), head and pronotal sculpture, the shape of antennomeres, palpi, prosternum, and the tarsi (Döberl 2012), allow us to consider

Ugandaltica gen. n. as a different genus. *Bezdekaltica* specifically differs from *Ugandaltica* gen. n. in having: deeply incised frontal grooves which connect in the middle of the frons (Fig. 4A); an anteriorly and posteriorly bordered pronotum, with short longitudinal lateral impressions touching pronotal base (Fig. 4A); sharp maxillary palpi (Fig. 4B); the third tarsomere larger than the second, and slender fourth visible tarsomeres; antennomeres which are clearly more homogenous in shape; and the prosternum wider anteriorly than the intercoxal prothoracic process (Fig. 4B). In addition, *Bezdekaltica* has closed procoxal cavities, this is contrary to the statement reported by Döberl (2012), which also separates it from *Ugandaltica* gen. n. (Fig. 4B).

Description. The new genus is established on the following set of characters. Body roundish, clearly convex, glabrous (Fig. 1A). Frons and vertex smooth; frontal tubercles absent; frontal grooves distinctly impressed, extending from upper ocular margin to antennal socket on each side; genae moderately elongate; maxillary palpi four-segmented, thickset; labial palpi three-segmented (Fig. 2A, C). Antennae slightly shorter than half the body length (Fig. 1A); antennomeres 1-2 distinctly larger than the rest; antennomere 7 distinctly larger than adjacent antennomeres (Fig. 2A). Pronotum sub-trapezoidal, slightly wider posteriorly, distinctly transverse, and distinctly rounded laterally, as wide as elytral base basally; anterior and basal margin not bordered; basal margin distinctly sinuous; lateral margin moderately expanded; anterior angles distinct and prominent, obliquely bevelled; posterior angles with a slightly prominent setigerous pore; punctation clearly impressed (Fig. 2B). Scutellum slightly elongate, roundish apically (Fig. 2B). Elytra indistinctly elongate, entirely covering pygidium, strongly arcuate laterally, jointly rounded apically; lateral margin moderately expanded up to subapical part of elytra, slightly visible in dorsal view; punctation arranged in 9 (+ 1 scutellar) regular rows, distinctly impressed but becoming shallower towards the elytral apex; humeral callus distinctly prominent; elytral epipleurae horizontally orientated, slightly visible laterally up to apical fourth of elytra (Figs 1A, 2D). Macropterous metathoracic wings. Hind femora clearly swollen; tibiae straight, not channeled, external margin not dentate; apical spur only present on hind tibiae, simple, and moderately elongate; third tarsomere of all legs about as narrow as second; ungual tarsomere of all legs slightly enlarged; and tarsal claws sub-appendiculate (Figs 2D, 3A). Prosternum clearly narrower anteriorly than intercoxal prothoracic process; and procoxal cavities open (Fig. 2C). Metafemoral extensor tendon with extended arm of dorsal lobe moderately elongate and slightly depressed; basal angle of ventral lobe acute; dorsal margin of ventral lobe straight, distinctly oblique; recurved flange distinctly sclerotized; dorsal-basal angle of the tendon almost right angled; ventral-basal angle of tendon widely obtuse; basal edge of tendon slightly curved (Fig. 3B). The metafemoral extensor tendon of this new genus is similar to those of the *Psylliodes* morpho-group (Furth and Suzuki 1998), but most likely constitutes a new morpho-group, which will include *Jacobyana*. Aedeagal surface without any complex sculpture, but with evident pores; phallobasis widely rounded basally; aedeagus distinctly curved in lateral view (Fig. 3C). Spermatheca of the "alticine type" (Type A of Furth and Suzuki 1994), with distal part distinct from basal part, ductus uncoiled, laterally inserted, and not invaginated in the basal part;

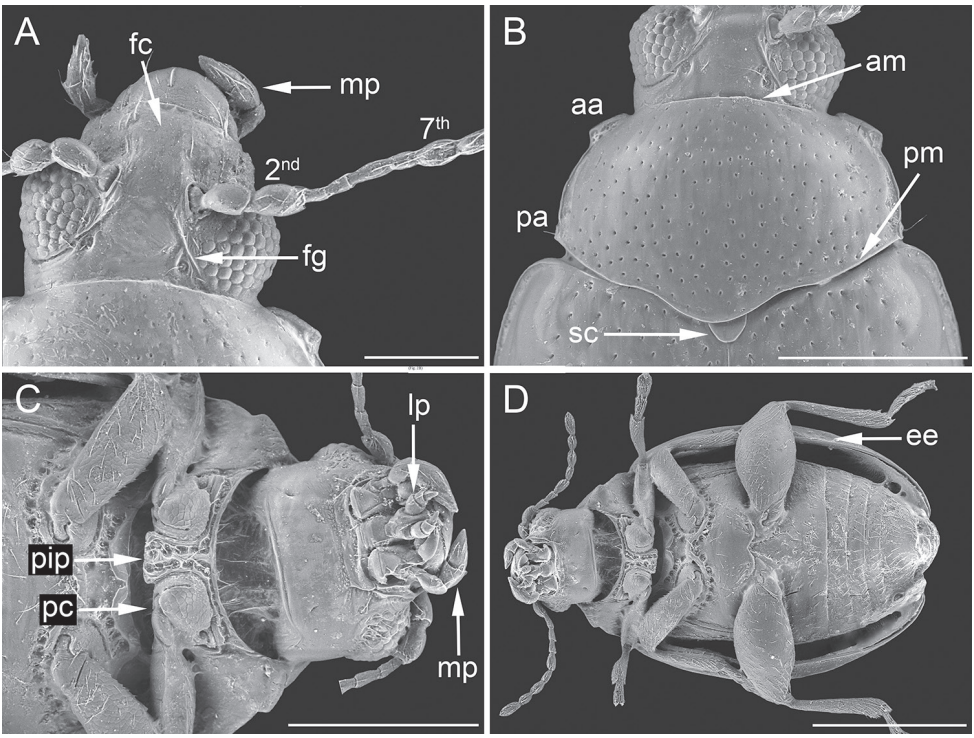

Figure 2. Morphological characters of *Ugandaltica wagneri* sp. n. **A** Head in dorsal view: 2nd = second antennal segment, 7th = seventh antennal segment, fc = frontal carina, fg = frontal groove, mp = maxillary palpus **B** pronotum: aa = anterior angle, am = anterior margin, pa = posterior angle, pm = posterior margin, sc = scutellum **C** Head in ventral view, prosternum and mesosternum: lp = labial palpus, mp = maxillary palpus, pip = prosternal intercoxal process, pc = procoxal cavity **D** Habitus of a female in ventral view: ee = elytral epipleurae. Scale bar: 150 μm (**A**); 300 μm (**B**, **C**); 500 μm (**D**).

neck not distinctly separate from basal part; apical part thicker than neck and distinctly separate (Fig. 3D). Tignum and vaginal palpi as in Fig. 3D.

Etymology. This new genus is named after Uganda, the country in which it was collected. Female gender.

Type species. *Ugandaltica wagneri* sp. n.

Distribution. Central Africa (Uganda) (Fig. 1B).

***Ugandaltica wagneri* sp. n.**
http://zoobank.org/7A52C5A4-EDAF-48BE-A2B0-EDF0C23C893F

Type-specimen. Holotype male, pinned, with genitalia on the same support. Original label: "Uganda, District Masindi / Budongo Forest n. Sonso / 1°45'N, 31°35'E / 15-25.i.1997 / Th. Wagner leg. [white label] // R.a.78N [white label] // HOLOTYPE / *Ugandaltica wagneri* sp. n. / D'Alessandro and Biondi det. 2018 [red label]" (BAQ).

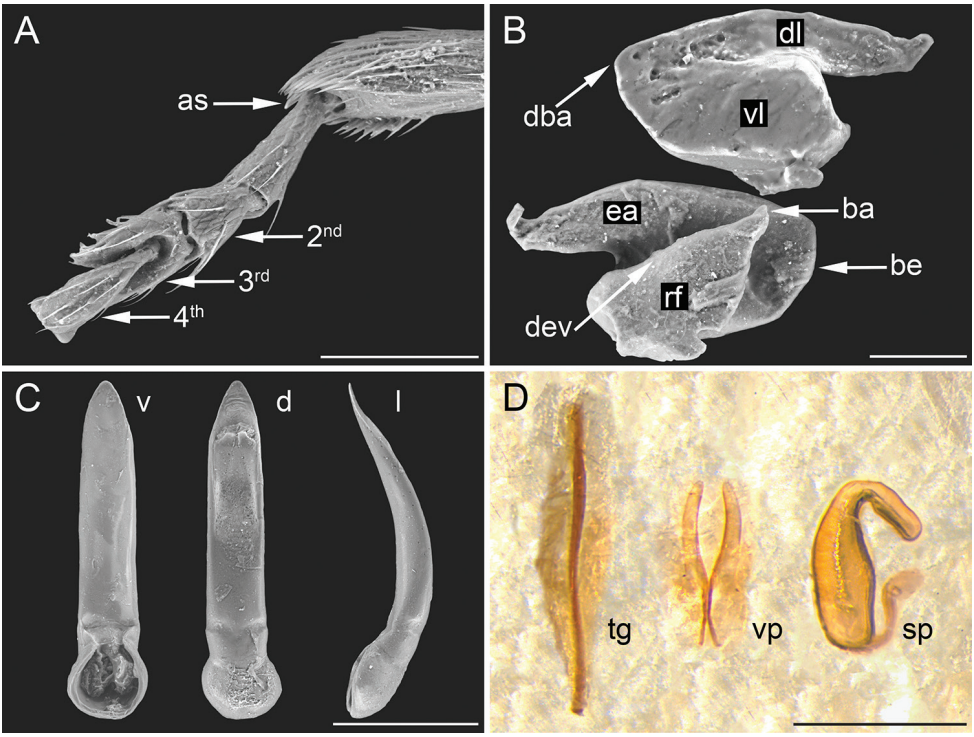

Figure 3. Morphological characters of *Ugandaltica wagneri* sp. n. **A** Distal part of hind tibia, and hind tarsus: 2nd = second tarsomere, 3rd = third tarsomere, 4th = fourth visible tarsomere, as = apical spur of hind tibia **B** Metafemoral extensor tendon: ba = basal angle of ventral lobe, be = basal edge of tendon, dba = dorsal-basal angle of tendon, dl = dorsal lobe, dev = dorsal edge of ventral lobe, ea = extended arm of dorsal lobe, rf = recurve flange, vl = ventral lobe **C** Aedeagus: d = dorsal view, l = lateral view, v = ventral view **D** Female genitalia: sp = spermatheca, tg = tignum, vp = vaginal palpi. Scale bar: 100 µm (**A**); 50 µm (**B**); 200 µm (**C**, **D**)

Paratypes. Uganda, District Masindi / Budongo Forest n. Sonso / 1°45'N, 31°35'E, 19-30.vi.1995 / Th. Wagner leg. // C.a.7, 1 ♂ and 1 ♀ (BAQ); ditto, 1-10.vii.1995 // R.a.7, 1 ♀ (BAQ); ditto, // T.r.2, 1 ♀ (BAQ); ditto, // T.r.3, 1 ♂ (BMNH); ditto, // T.r.4, 1 ♂ (BAQ); ditto, // T.r.6, 1 ♀ (BMNH); ditto, // T.r.8, 1 ♀ (BAQ); ditto, 11-20.vii.1995 // T.r.2, 1 ♀ (BAQ); ditto, 21-30.vii.1995 // R.a.22, 1 ♂ (NMPC); ditto, 5-15.i.1997 // R.a.57, 1 ♀ (NMPC); ditto, 15-25.i.1997 // R.a.48, 2 ♂ and 1 ♀ (BAQ).

Type locality. Uganda, District Masindi, Budongo Forest n. Sonso, 1°45'N, 31°35'E, secondary forest, night, on fogged *Rinorea beniensis*, 15-25.i.1997, Th. Wagner leg.

Description of the holotype. Body small-sized, roundish, distinctly convex (Fig. 1A); LB = 1.20 mm. Maximum pronotal width in basal third (WP = 0.52 mm); maximum elytral width in middle (WE = 0.74 mm). Dorsal integument brownish, shiny, with slightly paler elytral suture. Frons and vertex smooth (Fig. 2A); frontal tubercles absent; frontal grooves distinctly impressed, extending from upper ocular margin to antennal socket on either side; inter-antennal space slightly wider than length of

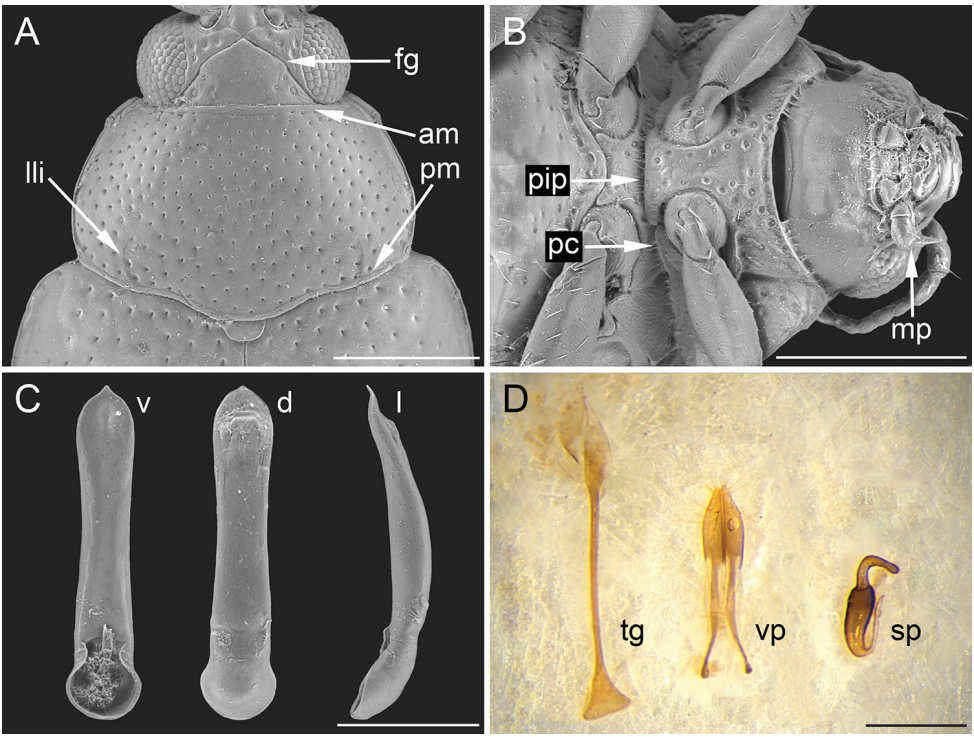

Figure 4. Morphological characters of *Bezdekaltica socotrana* Döberl, paratypes, Yemen, Socotra Island, Dixam plateau, Firmihin, *Dracaena* forest, 12°28.6'N, 54°01.1'E, 490 m, 15–16.xi.2010, J. Bezděk leg. (BAQ). **A** pronotum: am = anterior margin, fg = frontal groove, lli = lateral longitudinal impression, pm = posterior margin **B** Head in ventral view, prosternum and mesosternum: mp = maxillary palpus, pc = procoxal cavity, pip = prosternal intercoxal process **C** Aedeagus: d = dorsal view, l = lateral view, v = ventral view **D** Female genitalia: sp = spermatheca, tg = tignum, vp = vaginal palpi. Scale bar: 250 µm (**A**); 300 µm (**B**); 200 µm (**C**); 150 µm (**D**).

first antennomere; frontal carina wide, flat apically, not delimited laterally, and poorly delimited posteriorly; eye sub-elliptical, rather wide; antennae slightly shorter than half body length (Fig. 1A) (LAN = 0.58 mm; LAN/LB = 0.48); antennomeres (Fig. 2A) 1–2 distinctly wider than the rest, the first sub-conical and the second sub-cylindrical; 7 distinctly wider than the adjacent antennomeres; 1–3 pale in colour, 8–11 brownish; LA: 100:114:43:71:71:71:93:86:100:114:135. Pronotum (Fig. 2B) sub-trapezoidal, slightly wider posteriorly, distinctly transverse (LP = 0.34 mm; WP/LP = 1.51), distinctly rounded laterally, and as wide as elytra basally; not margined anteriorly and basally; basal margin distinctly sinuous; lateral margin moderately expanded; anterior angles distinctly prominent, obliquely bevelled; posterior angles with a slightly prominent setigerous pore; punctation rather dense and uniform; most punctures slightly elongate and deeply impressed on sub-smooth surface. Scutellum (Fig. 2B) slightly elongate, roundish apically, with sub-smooth surface. Elytra (Figs 1A, 2D) slightly elongate (LE = 0.99 mm; WE/LE = 0.75), entirely covering pygidium, strongly arcuate

laterally, jointly rounded apically; lateral margin moderately expanded up to sub-apical part of elytra, slightly visible in dorsal view; punctation arranged in 9 (+ 1 scutellar) regular rows; punctures larger than on pronotum, and distinctly impressed on most of the surface (shallower towards elytral apex); interstriae slightly raised, humeral callus distinct and prominent. Macropterous metathoracic wings. Legs with femora dark brown and tibiae brownish; coxae, apex of femora, base of tibiae, and tarsi yellowish. First tarsomere of anterior and middle tarsi distinctly dilated. Body dark brown ventrally; last visible abdominal sternite without special preapical impressions. Aedeagus (Fig. 3C) (LAED = 0.49 mm; LE/LAED = 2.03) tapering slightly towards the apex in ventral view; sub-triangular apically, acute, without a median tooth; surface smooth, with rather large pores, more numerous laterally; phallobasis widely rounded basally; aedeagus distinctly and evenly curved in lateral view, the apex slightly dorsally oriented; dorsal ligula about half the length of median lobe, wide, formed by an apically acute, triangular, median lobe, and two lateral lobes.

Variations. Paratypes are very similar in size, shape, sculpture and colour to the holotype. Female without the dilated first tarsomere in the anterior and middle tarsi. Spermatheca (Fig. 3D) with sub-cylindrical basal part, narrower towards distal part; neck not distinctly separated from basal part; apical part thicker than neck and distinctly separated from it; ductus thin, as long as half the length of basal part, uncoiled, and laterally inserted.

Male (n = 6; mean and standard deviation; range): LE = 1.01 ± 0.10 mm (0.93 ≤ LE ≤ 1.20 mm); WE = 0.76 ± 0.07 mm (0.71 ≤ WE ≤ 0.89 mm); LP = 0.35 ± 0.02 mm (0.34 ≤ LP ≤ 0.39 mm); WP = 0.53 ± 0.04 mm (0.50 ≤ WP ≤ 0.61 mm); LAN = 0.60 ± 0.03 mm (0.58 ≤ LAN ≤ 0.66 mm); LAED = 0.49 ± 0.04 mm (0.46 ≤ LAED ≤ 0.58 mm); LB = 1.27 ± 0.03 mm (1.18 ≤ LB ≤ 1.53 mm); LE/LP = 2.88 ± 0.12 (2.74 ≤ LE/LP ≤ 3.10); WE/WP = 1.43 ± 0.02 (1.40 ≤ WE/WP ≤ 1.46); WP/LP = 1.51 ± 0.04 (1.48 ≤ WP/LP ≤ 1.58); WE/LE = 0.75 ± 0.01 (0.74 ≤ WE/LE ≤ 0.77); LAN/LB = 0.47 ± 0.02 (0.43 ≤ LAN/LB ≤ 0.50); LE/LAED = 2.05 ± 0.08 (1.90 ≤ LE/ LAED ≤ 2.13). Female (n = 6; mean and standard deviation; range): LE = 1.07 ± 0.04 mm (1.00 ≤ LE ≤ 1.13 mm); WE = 0.82 ± 0.02 mm (0.79 ≤ WE ≤ 0.85 mm); LP = 0.36 ± 0.02 mm (0.34 ≤ LP ≤ 0.39 mm); WP = 0.56 ± 0.01 mm (0.55 ≤ WP ≤ 0.58 mm); LAN = 0.59 ± 0.01 mm (0.58 ≤ LAN ≤ 0.61 mm); LSPC = 0.20 ± 0.01 mm (0.19 ≤ LSPC ≤ 0.21 mm); LB = 1.35 ± 0.04 mm (1.29 ≤ LB ≤ 1.40 mm); LE/LP = 2.97 ± 0.10 (2.83 ≤ LE/LP ≤ 3.13); WE/WP = 1.46 ± 0.02 (1.43 ≤ WE/WP ≤ 1.48); WP/LP = 1.55 ± 0.06 (1.48 ≤ WP/LP ≤ 1.61); WE/LE = 0.77 ± 0.02 (0.73 ≤ WE/LE ≤ 0.79); LAN/LB = 0.44 ± 0.02 (0.42 ≤ LAN/LB ≤ 0.46); LE/LSPC = 5.40 ± 0.28 (4.85 ≤ LE/LSPC ≤ 5.67).

Etymology. The specific epithet is a Latinized noun in the genitive case referring to its collector Thomas Wagner (University of Koblenz-Landau, Germany), renowned specialist of Afrotropical Galerucini.

Distribution. Central Africa (Uganda). Considering both the habitat types and the most common species distributions associated with each chorotype (Biondi and D'Alessandro 2006), *Ugandaltica wagneri* sp. n. possibly falls inside the Northern-Eastern Afrotropical chorotype (NEA).

Ecology. All the specimens were collected in primary and secondary forest, at 1200 m a.s.l., by fogging the following trees: *Trichilia rubescens* (Meliaceae), *Rinorea beniensis* (Violaceae), and *Cynometra alexandri* (Caesalpiniaceae). The species was present during both the wet season, June and July 1995, and dry season, January 1997 (Wagner 1999, 2000, 2001).

Discussion

The general similarities of the new taxon, here described, with those of the "moss-inhabiting genera" seem incidental, and is not due to similar habitat occupancy. In addition, being macropterous is indicative that *Ugandaltica* gen. n. can move easily, a characteristic not found in moss-inhabiting flea beetles (Konstantinov et al. 2013, Damaška and Konstantinov 2016, Ruan et al. 2017). *Ugandaltica wagneri* sp. n. was collected by fogging trees in primary and secondary tropical forest formations (Wagner 1999, 2000, 2001). Wagner (1999, 2001) found that the flea beetle abundance significantly increased in both the primary and the secondary forest during the dry season. The canopies of trees with dense foliage are often the most humid habitats, and small, soft-bodied insects in particular presumably accumulate along a gradient of humidity. Moreover, there is evidence that the abundance of phytophagous insects peaked during leaf flush periods, which happen during the late dry season at the end of January. This is because of their preference for young leaves, and because herbaceous food plants are often no longer available (Wagner 2001). However, Alticini seemed not to feed on trees, as gut dissection of the most abundant species revealed, but rather fed on plants in the surrounding habitat (Wagner 1999). It is interesting that *Ugandaltica wagneri* sp. n. was one of the few flea beetle species also present in the canopy during the wet season. Because of the poor conservation status, and the very small size of the specimens, we preferred not to dissect their gut to investigate whether they were feeding on tree foliage. If they used the canopy as refugium habitat only, then they would only exploit their real host plant during a limited period of the year.

Conclusions

Most studies on the arthropod composition of the canopy have dealt with several different taxa, which is why a morphospecies approach has often been chosen. This implies that the collected specimens often required further taxonomic investigation by a specialist for their determination. In this paper a new genus and species from a tropical forest in Western Uganda are described, providing a contribution to the knowledge of the flea beetle fauna from canopies of Afrotropical forests. Alticini seem to be one of the more representative taxa of the canopy of tropical forests (Basset and Samuelson 1996, Wagner 1999, 2001, Furth 2003, Charles and Basset 2005). However, because of the dynamics of the canopy faunal composition, the correct interpretation of their presence needs more insight on the ecology and biology of the species found there. For this rea-

son, it will be fundamental to understand how they are distributed in the forest habitat as a whole, and not only in canopy habitats (Basset et al. 2003a). In this regard it must be said that further research in forest habitats might reveal that *Ugandaltica wagneri* sp. n. is more closely associated with a specific forest layer, considering the small number of known specimens and the confined habitat from which they were collected.

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RESEARCH ARTICLE

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Mantophasmatodea from the Richtersveld in South Africa with description of two new genera and species

Benjamin Wipfler[1], Tobias Theska[1], Reinhard Predel[2]

1 *Entomology Group, Institut für Zoologie und Evolutionsforschung, Friedrich-Schiller-University Jena, Erbertstr. 1, 07743 Jena, Germany* **2** *Institut für Zoologie, Universität zu Köln, Zülpicher Str. 47b, 50674, Köln, Germany*

Corresponding authors: *Benjamin Wipfler* (benjamin.wipfler@uni-jena.de); *Reinhard Predel* (rpredel@uni-koeln.de)

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Abstract

Two new species and two new genera (*Kuboesphasma*, *Minutophasma*) of Mantophasmatodea that occur in the Richtersveld region of South Africa are described. *Kuboesphasma compactum* **gen. n., sp. n.** was found only in a small area near the village of Kuboes, while *Minutophasma richtersveldense* **gen. n., sp. n.** apparently inhabits a larger area in the Richtersveld. With these two new species, a total of four different mantophasmatodeans are known to live in this area. This is a remarkable exception to the remaining representatives of this order, where even a common occurrence of only two species is rare. We discuss this sympatry in the context of the phylogeny of the group. Additionally, we provide a map of the known distributions and a table with the most important taxonomic features of the mantophasmatodeans in the Richtersveld.

Keywords

heelwalkers, Polyneoptera, lower neoptera, *Kuboesphasma*, *Minutophasma*, taxonomy, South Africa

Introduction

Mantophasmatodea were newly described in 2002 (Klass et al. 2002), which makes it by far the youngest of all insect orders. Since then, a remarkable amount of research was done on the group. Various studies presented detailed information about the mor-

phology of almost all parts of the body (e.g., Dallai et al. 2003; Klass et al. 2003; Baum et al. 2007; Buder and Klass 2013; Wipfler et al. 2015), behavior (e.g., Eberhard and Eberhard 2013; Roth et al. 2014), distribution and biogeography (e.g., Predel et al. 2012; Proches 2014), physiology (e.g., Chown et al. 2006; Eberhard et al. 2010), ecology (e.g., Eberhard and Picker 2008; Roth et al. 2014) and intraordinal phylogeny (e.g., Damgaard et al. 2008; Predel et al. 2012). It was settled without doubt that Grylloblattodea, a very small insect order with a preference for cold habitats in western North America and northern East Asia, is the sistergroup of Mantophasmatodea (Misof et al. 2014; Wipfler et al. 2014). This accumulated research makes Mantophasmatodea one of the best studied insect orders today.

So far 18 species of Mantophasmatodea have been described (Klass et al. 2002, Klass et al. 2003, Zompro et al. 2003, Zompro and Adis 2006, Eberhard et al. 2011, Wipfler et al. 2012). These descriptions encompass 9 species from South Africa, all belonging to a monophyletic group (Austrophasmatidae) that inhabits the winter rainfall region of the Western and Northern Cape. The monotypic genera *Praedatophasma*, *Tyrannophasma*, *Pachyphasma*, and *Striatophasma* can be found in Namibia. The same applies to several representatives of *Mantophasma* and *Sclerophasma*, whose statuses as separate species are poorly resolved (Roth et al. 2014). Members of the genus *Tanzaniophasma* inhabit East Africa, with known populations in South Tanzania (Klass et al. 2002), Malawi (Roth et al. 2014), and Mozambique (Predel, unpublished data). Recent phylogenetic analyses of all known populations of Mantophasmatodea (Predel, unpublished data) revealed two additional taxa of Mantophasmatodea in the Richtersveld. This arid region is located in the far northwest of Southern Africa and thus within the summer- and winter-rain transition zone. It represents the only recognized arid biodiversity hotspot in the world (Myers et al. 2000). Together with *Praedatophasma maraisi* and *Namaquaphasma ookiepense*, which are known to reach their distribution boundaries in this area, these taxa increase the number of species in that relatively small region to four. This is exceptional for Mantophasmatodea, particularly since the different species are not closely related.

Here we describe the two new genera and two new species of Mantophasmatodea from the Richtersveld and present detailed information on how to distinguish the four sympatric species in this area.

Material and methods

The used terminology follows Beutel et al. (2014) and for abdominal structures Klass et al. (2003). If not stated otherwise, the coloration refers to living specimens. Species descriptions are based on a designated holotype, but all available specimens were taken into account in order to assess the intraspecific variation.

The information for the specimens is given in a standard manner, i.e., locality, geographic coordinates, elevation, date of collection (month indicated in lower case

Roman numerals), habitat information, collector, depository, and preparation. Female (♀) and male (♂) symbols indicate the sex.

In accordance with Zompro et al. (2003) and Wipfler et al. (2011), we took the following measurements: total length, length of pronotum, width of pronotum, length of mesonotum, width of mesonotum, length of metanotum, width of metanotum, distance between ventral edge of clypeus and dorsal edge of frontal tubercle (head height of Zompro et al. 2003), width of the head, head width over eyes, width between eyes, length of eye and width of eye. Additionally, we measured the distance between the ventral edge of the labrum and the dorsal-most point of the head capsule in frontal view (total heights of the head). Specimens were examined under a Zeiss Stemi SV11 with a calibrated ocular micrometer. Images of the habitats were taken with a Nikon 3300 camera.

A male (holotype) and a female paratype of each newly described species was critical point dried and subsequently glued to a needle. Then we photographed them with a Keyence VHX-2000 digital microscope. Parts of each species are illustrated in the standard views of dorsal, lateral and ventral. The head is additionally depicted in frontal view (frons being vertically) and the terminalia in caudal view. Subsequent images were edited with Adobe Photoshop and Illustrator (CS6).

The specimens referred below along with the abbreviations used in the text will be deposited in the following collections: SAMC – Iziko South African Museum, Cape Town, South Africa; ZFMK – Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany; ZMBN – University Museum, University of Bergen, Bergen, Norway.

Taxonomy

***Kuboesphasma* gen. n.**
http://zoobank.org/E36A7CC9-55C1-4428-9C64-BBD5EC1D9026

Description and diagnosis. *Kuboesphasma* gen. n. is placed as sistergroup to a clade (*Viridiphasma* + *Namaquaphasma*) + the remaining Austrophasmatidae based on peptide hormone sequences (Predel, unpublished data; Fig. 1).

Kuboesphasma can be distinguished from other mantophasmatodeans, except *Minutophasma*, by the washed-out and indistinct butterfly-shaped spot on the frons: in the South African Austrophasmatidae sensu Klass et al. (2003) except *Viridiphasma clanwilliamense* this spot is clear and dark while *V. clanwilliamense* and the Namibian *Striatophasma*, *Pachyphasma* and Mantophasmatidae sensu Klass et al. (2003) lack it completely. Additionally, all green Austrophasmatidae and *Striatophasma* have a dark dorsal median stripe in males; this median stripe is indistinct in males of *Kuboesphasma*. *Kuboesphasma* can be distinguished from *Minutophasma* by the larger body size (males over 12 mm and females over 13 mm), the absence of the dark dorsal median stripe in males and the absence of black ventrolateral spot on the scapus. On the proti-

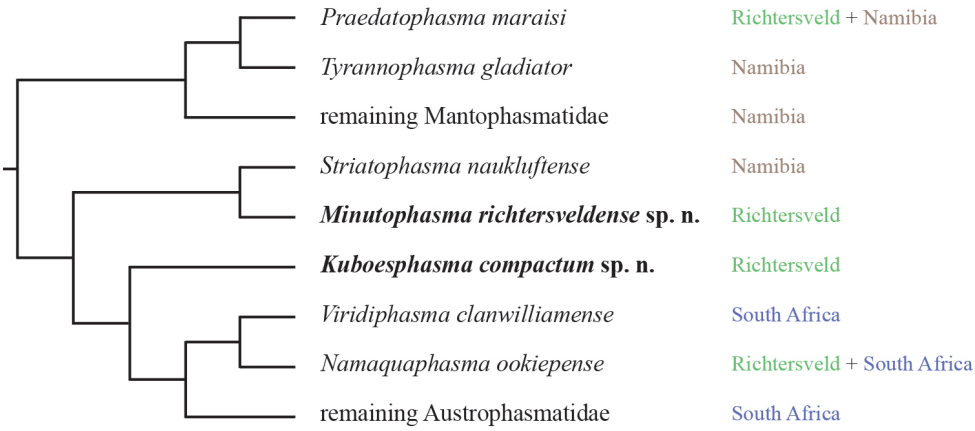


Figure 1. Simplified tree (midpoint-rooted) from a Bayesian phylogenetic analysis of peptide hormone sequences of southern African Mantophasmatodea (adapted from Predel et al., unpublished).

bia, the males of *Kuboesphasma* have nine or more spikes per row (while *Minutophasma* has eight or less). Additionally, the head capsule is distinctly broader on the level of the compound eyes than on the level of the genae in *Minutophasma*, while they are equal in *Kuboesphasma*. Males and females of *Praedatophasma maraisi* are much larger (more than 21 mm) than those of *Kuboesphasma*, grey brown and have distinct spines along the thorax. In contrast to *Namaquaphasma ookiepense*, *Kuboesphasma* has no dark spots on the scapus, no distal dorsal projection on the cercus and an indistinct butterfly shaped spot on the frons. From *Striatophasma naukluftense* the species can be additionally separated by the rounded posterior margin of sternit VIII (pointed in *Striatophasma*) and the equal length of tergum VIII and IX (IX much longer in *Striatophasma*).

Type species. *Kuboesphasma compactum*

Other included species. None thus far.

Etymology. The generic group name *Kuboesphasma* is a composition from the type locality, Kuboes, which is a center of the Nama community in the Richtersveld region and the ending -phasma which is commonly used to term mantophasmatodeans. The gender is neuter.

Kuboesphasma compactum sp. n.

http://zoobank.org/759D3A07-B689-4065-B602-DAB78E0E8AAC

Figs 2–9

Holotype. Male. SOUTH AFRICA: Kuboes, S28°26'25", E16°59'44", 18.viii.2012, 250 m, R. Predel, specimen in ethanol. Table 1 provides an overview of the type material including the collections where it is deposited.

Paratypes. SOUTH AFRICA: Richtersveld S28°26'25", E16°59'44", 18.viii.2012: four males and two females, specimens in ethanol. Table 1 provides an overview of the type material including the collections where it is deposited.

Table 1. Overview of the type material including gender, locality, collection date and museum where it is deposited. Abbreviations: SAMC – Iziko South African Museum, Cape Town, South Africa. ZFMK – Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany; ZMBN – University Museum, University of Bergen, Bergen, Norway.

	Type	Gender	Locality	Collection date	Deposition
Kuboesphasma compactum	Holotype	♂	S28°26'25", E16°59'44"	18.viii.2012	SAMC
	Paratype	♂	S28°26'25", E16°59'44"	18.viii.2012	SAMC
	Paratype	♂	S28°26'25", E16°59'44"	18.viii.2012	ZFMK
	Paratype	♂	S28°26'25", E16°59'44"	18.viii.2012	ZFMK
	Paratype	♂	S28°26'25", E16°59'44"	18.viii.2012	ZMBN
	Paratype	♀	S28°26'25", E16°59'44"	18.viii.2012	SAMC
	Paratype	♀	S28°26'25", E16°59'44"	18.viii.2012	ZMBN
Minutophasma richtersveldense	Holotype	♂	S28°47'26.94", E17°16'20.34"	06.ix.2014	SAMC
	Paratype	♂	S28°47'26.94", E17°16'20.34"	06.ix.2014	SAMC
	Paratype	♂	S28°47'26.94", E17°16'20.34"	06.ix.2014	ZMBN
	Paratype	♂	S28°47'26.94", E17°16'20.34"	06.ix.2014	ZMBN
	Paratype	♂	S28°47'26.94", E17°16'20.34"	06.ix.2014	ZFMK
	Paratype	♀	S28°47'26.94", E17°16'20.34"	06.ix.2014	SAMC
	Paratype	♀	S28°47'26.94", E17°16'20.34"	06.ix.2014	ZMBN
	Paratype	♀	S28°47'26.94", E17°16'20.34"	06.ix.2014	ZMBN
	Paratype	♀	S28°47'26.94", E17°16'20.34"	06.ix.2014	ZFMK
	Paratype	♂	S28°46'31.50", E17°11'12.18"	06.ix.2014	SAMC
	Paratype	♂	S28°46'31.50", E17°11'12.18"	06.ix.2014	ZMBN
	Paratype	♀	S28°46'31.50", E17°11'12.18"	06.ix.2014	SAMC
	Paratype	♀	S28°46'31.50", E17°11'12.18"	06.ix.2014	ZMBN
	Paratype	♀	S28°10'20.80", E17°01'43.60"	07.ix.2014	ZMBN
	Paratype	♀	S28°10'20.80", E17°01'43.60"	07.ix.2014	SAMC

Description male. Measurements (male holotype followed by paratypes in parentheses, in mm): total length: 12.1 (12.5, 12.9, 12.1, 12.4); length of pronotum: 2.5 (2.6, 2.8, 2.6, 2.8); width of pronotum: 2.5 (2.4, 2.6, 2.3, 2.4); length of mesonotum: 2.0 (2.2, 2.1, 2.1, 2.3); width of mesonotum: 2.4 (2.3, 2.4, 2.2, 2.3); length of metanotum: 1.5 (1.9, 1.7, 1.7, 1.7); width of metanotum: 2.2 (2.2, 2.2, 2.1, 2.1); heights of head: 2.3 (2.3, 2.2, 2.2, 2.3); total heights of head: 2.9 (3.0, 2.9, 2.9, 3.0); width of the head: 2.8 (2.9, 3.0, 2.7, 2.9); head width over eyes: 3.0 (3.0, 3.2, 3.0, 3.0); width between eyes: 1.7 (1.7, 1.8, 1.7, 1.6); length of eye: 1.3 (1.2, 1.4, 1.4, 1.4); width of eye: 0.8 (0.8, 0.9, 0.8, 0.8).

Head (Fig. 4): globular, orthognathous, posteriorly covered by pronotum, green without darker stripe; compound eyes whitish with black or brown spots; head slightly wider than prothorax, about twice as wide as long; head capsule covered with setae, setation increasing dorsally. Compound eyes prominent, tapered ventro-mesally, about 1.5 times as long as high; interoccular distance ca. the length of one eye, ocelli absent. Coronal and frontal suture indistinct, pleurostomal ridge well developed. Ventral parts of occipital ridge very prominent; antennal sockets in between eyes, distinct; interantennal distance ca. diameter of one antennal socket; antennifer present; dark spot on lateral corner of scapus absent; dark median butterfly-shaped spot directly below the

Figure 2. Habitus photographies of *Kuboesphasma compactum* sp. n.; copula with smaller ♂ on top of ♀; **A** lateral view from left side **B** dorso-lateral view from right side.

antennal bases washed-out and indistinct; anterior tentorial pits dorso-mesally of anterior mandibular articulation; frons with three bulges, one in between antennal sockets, two ventro-mesal of antennal sockets; frontoclypeal ridge recognizable as an indistinct line. Gena strongly protruding, head capsule on the level of the genae nearly as wide as on the one of the compound eyes; genae have equal heights than the compound eyes. Clypeus trapezoid, with well-developed clypeolabral ridge, no setae present; oval sclerite in between clypeus and labrum present. Labrum flat, anteriorly rounded, with few short setae. Maxilla well developed, green; maxillary palp five segmented, sparsely covered with setae, palpomere one and two as long as wide, palpomere three 2.5 times as long as wide, palpomeres four and five ca. twice as long as wide. Labium green, palp

Figure 3. Type locality of *Kuboesphasma compactum* sp. n., Kuboes, Richtersveld, South Africa.

three segmented. Scape and pedicel bright green; scape as long as wide; pedicle half as wide as scape, twice as long as wide, dilating towards the tip. Flagellum slightly shorter than the entire animal.

Thorax (Fig. 5): bright green, dorso-medially with weak and indistinct longitudinal brown stripe that contains small green areas. Notae covered with setae, much denser than head. Pronotum oval with bulge positioned anterior-laterally; pronotum reaches overhead and mesonotum, ventral border of pronotum arched. Two cervicalia present, second postero-dorsally to first. Pleura subdivided into epimeron and episternum. Coxae large, covered with setae.

Legs: green, spikes in the tibial region black; covered with setae. Prothoracic leg more massive than meso- and metathoracic ones; femur ca. three times as long as wide, with two ventro-median rows of spikes, spikes larger on pro- and mesothoracic leg, smaller on metathoracic one. Tibia green, in pro- and mesothorax ca. 10 times as long as wide, in metathoracic leg between 13 and 15 times as long, with two ventro-median rows of black spikes on pro- and mesothoracic legs, protibia with 9–12 spikes per row, on metathoracic leg only two distal spikes. Tarsus with five tarsomeres, proximal four tarsomeres with euplantulae; arolium very large.

Wings: completely absent.

Abdomen: as long as thorax and head combined; green, meso-dorsal brown longitudinal stripe weak and indistinct. Abdomen covered with setae, tergites stronger than sternits. Abdominal tergum I same width or very slightly thinner as metathorax; terga slightly broadening towards tergum VIII, terga IX and X narrowing again.

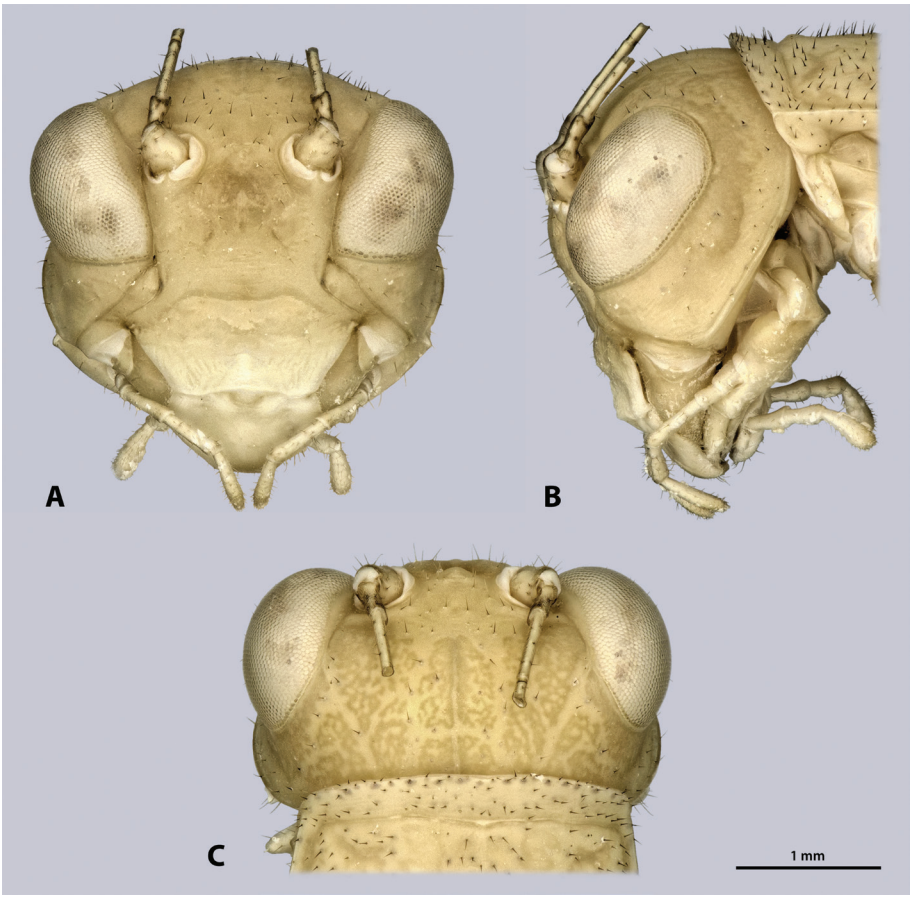


Figure 4. Head of ♂ *Kuboesphasma compactum* sp. n., holotype, photomicrographies **A** frontal view **B** lateral view **C** dorsal view.

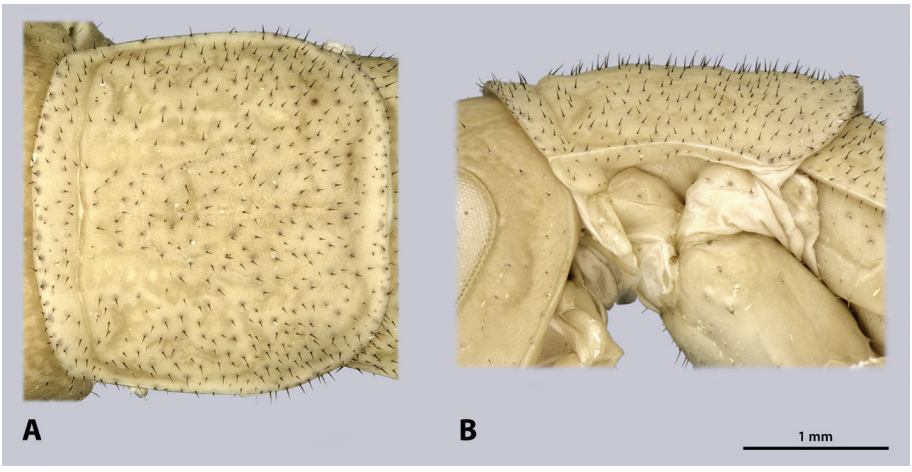


Figure 5. Prothorax of ♂ *Kuboesphasma compactum* sp. n., holotype, photomicrographies; **A** dorsal view **B** lateral view.

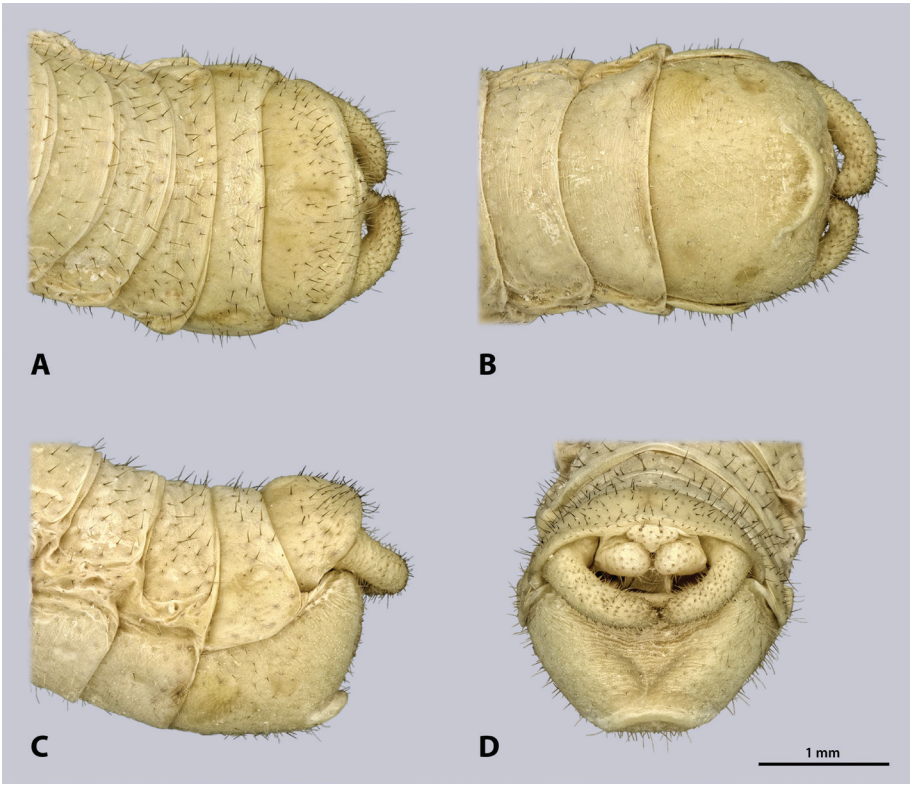


Figure 6. Terminalia of ♂ *Kuboesphasma compactum* sp. n., holotype, photomicrographies; **A** dorsal view **B** ventral view **C** lateral view **D** caudal view.

Male terminalia (Fig. 6): tergum IX green; shorter than tergum VIII. Tergum X green, mesal brown stripe, roof-shaped in lateral view. Subgenital plate (sternite IX) green with brownish areas, posterio-dorsal margin not protruding; process of subgenital plate broad, dorsal slightly arch-shaped when seen from posterior, broadly emarginated dorsally. Cerci one-segmented, densely covered with setae; diameter mesally round, uniformly curved, slightly narrowed towards the apex, with distal dorsal projection; cerci extending towards the middle of the subgenital plate. Paraprocts and epiproct also covered with setae.

Description female. For the female only differences to the male are described. Measurements: total length: 13.5, 15.4; length of pronotum: 3.2, 3.4; width of pronotum: 3.2, 3.7; length of mesonotum: 2.0, 2.5; width of mesonotum: 3.0, 3.5; length of metanotum: 1.5, 2.0; width of metanotum: 2.8, 3.4; heights of head: 2.5, 2.8; total heights of the head: 3.2, 3.9; width of the head: 3.2, 3.8; head width over eyes: 3.4, 3.8; width between eyes: 2.1, 2.3; length of eye: 1.5, 1.7; width of eye: 0.8, 0.9.

Head (Fig. 7): compound eyes slightly smaller than in the male.

Thorax (Fig. 8): dorsal stripe absent. Ventral border of pronotum straight.

Legs: protibia with 9–10 spikes per row.

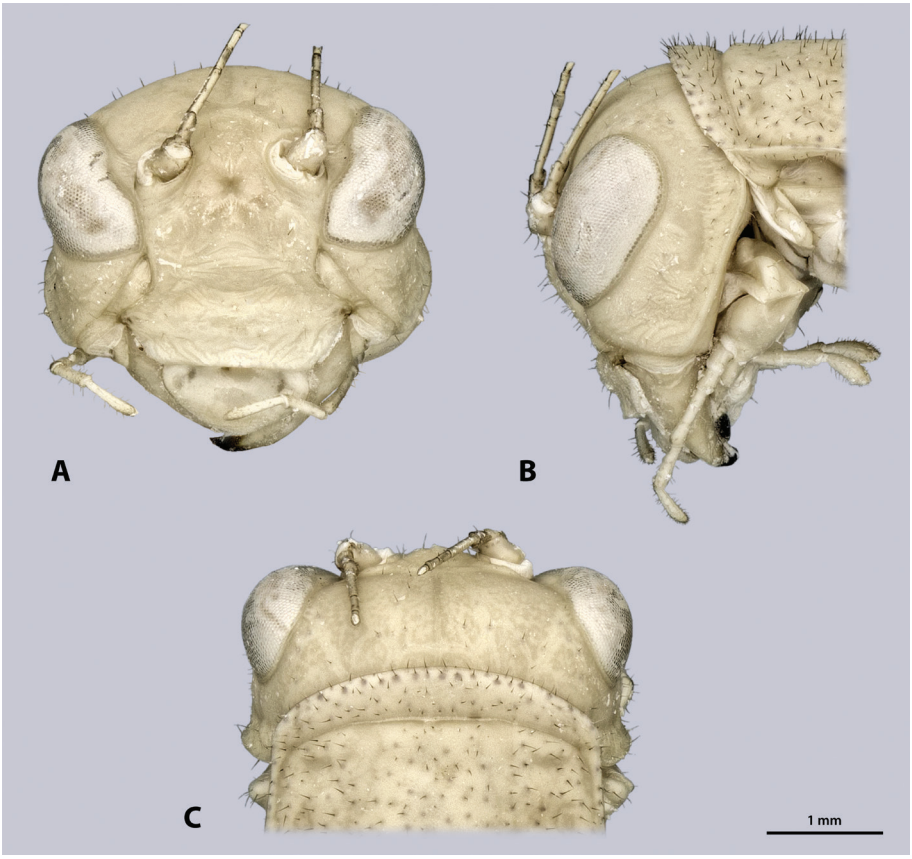


Figure 7. Head of ♀ *Kuboesphasma compactum* sp. n., paratype, photomicrographies; **A** frontal view **B** lateral view **C** dorsal view.

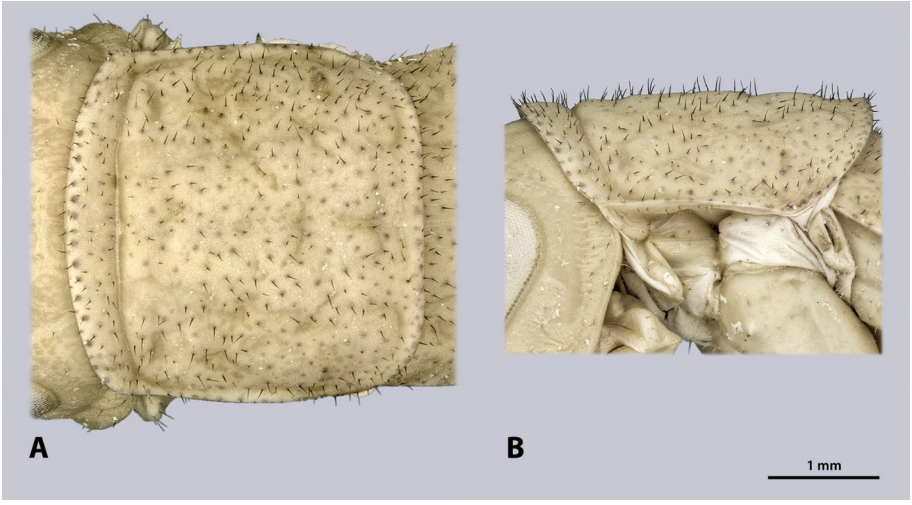


Figure 8. Prothorax of ♀ *Kuboesphasma compactum* sp. n., paratype, photomicrographies; **A** dorsal view **B** lateral view.

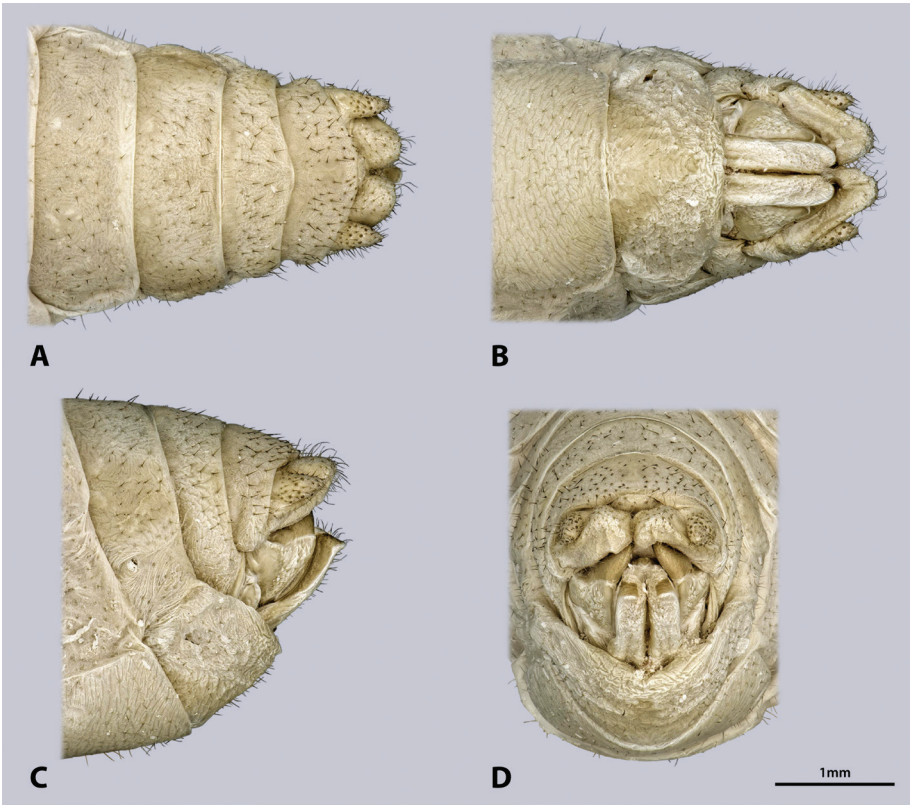


Figure 9. Terminalia of ♀ *Kuboesphasma compactum* sp. n., paratype, photomicrographies; **A** dorsal view **B** ventral view **C** lateral view **D** caudal view.

Abdomen: abdomen slightly longer than head and thorax combined. No dorsal brown stripe. Widest point of abdomen at segments 5 or 6.

Female terminalia (Fig. 9): tergum IX green, approximately as long as tergum VIII, posterior margin with distinct median convexity. Tergum X green, as long as tergum IX; apex rounded posteriorly; all terga setose; epiproct green, very short (ca. 1/5 of the length of tergum X), slightly setose. Paraprocts rounded and densely covered with setae. Cerci slightly shorter than paraprocts, cone shaped and densely covered with setae. Sternite VIII green, posterior margin straight. Gonapophysis VIII long and slender, distally blunt with ventrocaudal process. Gonocoxite IX almost completely concealed in lateral view; gonoplac triangular, heavily sclerotized.

Etymology. The species name *compactum* refers to the compact appearance of that species which distinguishes it easily from the second greenish species in the Richtersveld, *Minutophasma richtersveldense*.

Comments. Specimens were common in a heavily overgrazed area near the settlement of Kuboes; mainly in dense shrubs with small green and succulent leaves (*Suaeda fruticosa*, *Lycium* sp.).

***Minutophasma* gen. n.**
http://zoobank.org/24A52AD5-F6E8-4BA3-B5BF-38E069F1EC59

Description and diagnosis. *Minutophasma* gen. n. is placed as sistergroup to the Namibian genus *Striatophasma*.

Minutophasma can be distinguished from other mantophasmatodeans except *Kuboesphasma* by the washed-out and indistinct butterfly-shaped spot on the frons: in the South African Austrophasmatidae sensu Klass et al. (2003) except *Viridiphasma clanwilliamense* this spot is clear and dark while *V. clanwilliamense* and the Namibian *Striatophasma*, *Pachyphasma* and Mantophasmatidae sensu Klass et al. (2003) lack it completely. Additionally, it can be distinguished from all Austrophasmatidae sensu Klass et al. (2003) except *Viridiphasma* by genae that are similar long in lateral view as the widths of the eyes. *Kuboesphasma* differs from *Minutophasma* by the larger body size (males over 12 mm and females over 13 mm), an indistinct dark dorsal stripe in males and the absence of ventro-lateral spot on the scapus. On the protibia, the males of *Kuboesphasma* have nine or more spikes per row (while *Minutophasma* has eight or less). Additionally, the head capsule is distinctly broader on the level of the compound eyes than on the level of the genae in *Minutophasma* while they are equal in *Kuboesphasma*. Males and females of *Praedatophasma maraisi* are much larger (more than 21 mm) than those of *Kuboesphasma*, grey brown and have distinct spines along the thorax. In contrast to the reddish-brown *Namaquaphasma ookiepense*, specimens of *Minutophasma* are mostly green, much smaller (males of *Namaquaphasma* above 14 mm and females above 16 mm) and have genae that are lower than the eyes are wide. Specimens of *Striatophasma naukluftense* are larger (males 12–15 mm, females 16–27 mm) as those of *Minutophasma* and lack the dorsal distal projection in male cerci. All green species of Austrophasmatidae (*Austrophasma gansbaaiense*, *A. caledonense*, *Viridiphasma*, *Lobatophasma*) and *Striatophasma* lack the ventrolateral black spot on the scapus which is very distinct in *Minutophasma*.

Type species. *Minutophasma richtersveldense*

Other included species. None thus far.

Etymology. The generic group name *Minutophasma* is a composition of the Latin word minutus that refers to the small size of that species which separates it from the other known mantophasmatodeans, and the ending -phasma which is commonly used to term mantophasmatodeans. The gender is neuter.

***Minutophasma richtersveldense* sp. n.**
http://zoobank.org/D4CD7E84-1243-4F1E-87C6-D211A05B3D6B
Figs 10–17

Holotype. Male. SOUTH AFRICA: Northern Cape, north of Eksteenfontein, Richtersveld, S28°47'26.94", E17°16'20.34", 06.ix.2014, 600–700m, R. Predel, specimen in ethanol. Table 1 provides an overview of the type material including the collections where it is deposited.

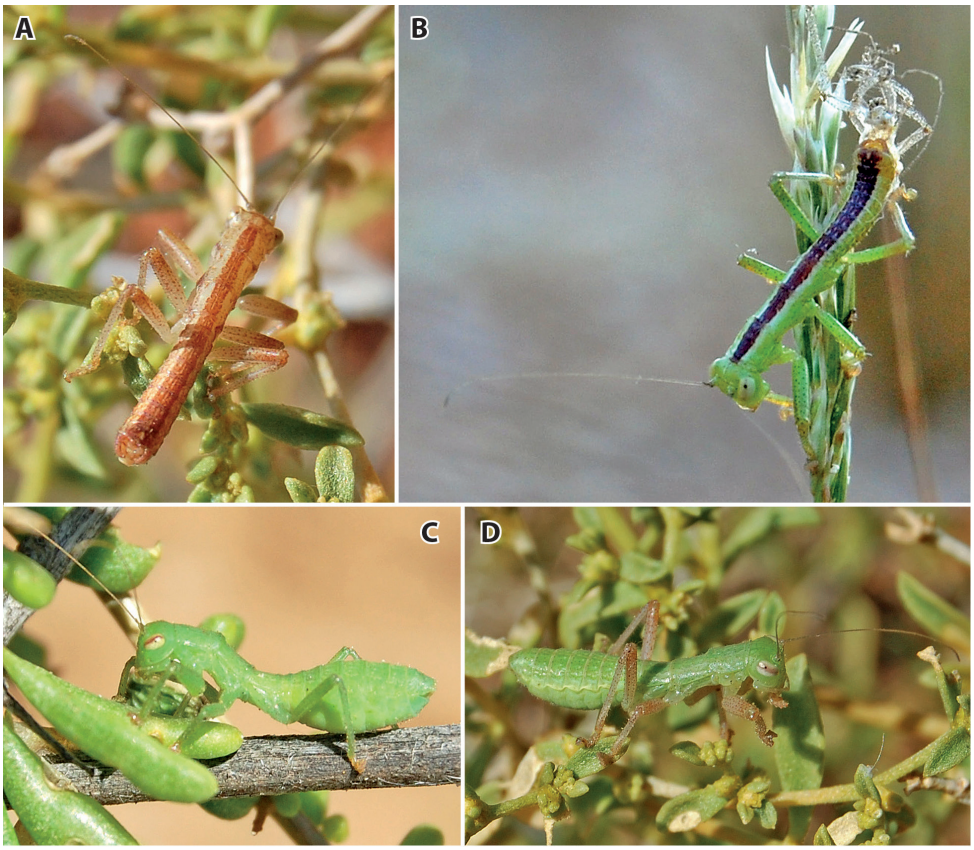


Figure 10. Habitus photographies and color variations of *Minutophasma richtersveldense* sp. n.; **A** ♂ with brown body color **B** ♂ with green body color **C** ♀ with green body color **D** ♀ with green body color and brown legs.

Paratypes. Location 1: SOUTH AFRICA, Northern Cape, north of Eksteenfontein, Richtersveld, S28°47'26.94", E17°16'20.34", 06.ix.2014, 600–700m, R. Predel: 4 males and 4 females, specimens in ethanol. Location 2: SOUTH AFRICA, Northern Cape, west of Eksteenfontein, Richtersveld, S28°46'31.50", E17°11'12.18", 06.ix.2014, 500m, R. Predel: 2 males and 2 females, specimens in ethanol. Location 3: SOUTH AFRICA, Northern Cape, Akkedis pass, Richtersveld, S28°10'20.80", E17°01'43.60", 07.ix.2014, R. Predel: 2 females, specimens in ethanol. Table 1 provides an overview of the type material including the collections where it is deposited.

Description male. Measurements (male holotype followed by paratypes in parentheses, in mm): total length: 9.2 (location 1: 9.1, 10.5, 9.8, 9.9, 10.7) (location 2: 10.6, 10.1); length of pronotum: 1.8 (location 1: 1.9, 1.9, 1.6, 1.9, 1.6) (location 2: 2.0, 2.1); width of pronotum: 1.6 (location 1: 1.7, 1.8, 1.5, 1.8, 1,6) (location 2: 1.9, 1.8); length of mesonotum: 1.7 (location 1: 1.7, 1.7, 1.5, 1.8, 1.5) (location 2: 1.8, 1.8); width of mesonotum: 1.5 (location 1: 1.6, 1.6, 1.4, 1.7, 1.5) (location 2: 1.7,

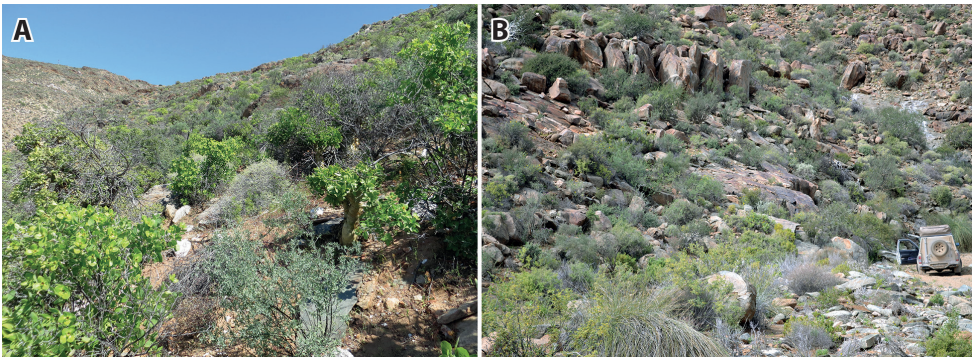


Figure 11. Type locality of *Minutophasma richtersveldense* sp. n., Eksteenfontein, Richtersveld, South Africa.

1.6); length of metanotum: 1.3 (location 1: 1.3, 1.4, 1.4, 1.4, 1.2) (location 2: 1.4, 1.3); width of metanotum: 1.3 (location 1: 1.4, 1.5, 1.3, 1.5, 1.5) (location 2: 1.4, 1.4); heights of head: 1.7 (location 1: 1.6, 1.7, 1.5, 1.6, 1.5) (location 2: 1.7, 1.6); total heights of head: 2.1(location 1: 2.1, 2.1, 1.9, 2.0, 1.9) (location 2: 2.2, 2.2); width of the head: 2.0 (location 1: 2.0, 2.0, 1.8, 2.2, 1.8) (location 2: 2.3, 2.2); head width over eyes: 2.2 (location 1: 2.3, 2.3, 2.1, 2.4, 2.1) (location 2: 2.4, 2.4); width between eyes: 1.3 (location 1: 1.3, 1.4, 1.3, 1.3, 1.2) (location 2: 1.5, 1.5); length of eye: 1.0 (location 1: 1.0, 1.0, 1.0, 1.0, 1.1) (location 2: 1.0, 1.1); width of eye: 0.6 (location 1: 0.6, 0.6, 0.6, 0.6, 0.6) (location 2: 0.6, 0.6).

Coloration (Fig. 10): body color ranges from green to brown to grey-beige (Fig. 10). Distinct and broad dark stripe on dorsal side, stripe in green males with whitish margins.

Head (Fig. 12): globular, orthognathous, posteriorly covered by pronotum, in some specimens the dark stripe is indistinctly visible on the vertex but weaker than on the thorax and abdomen; head slightly wider than prothorax, about twice as wide as long; head capsule sparsely covered with setae. Compound eyes whitish with black or brown spots, prominent, tapered ventro-mesally, about 1.5 times as long as high; interoccular distance ca. the length of one eye, ocelli absent. Coronal and frontal suture indistinct, pleurostomal ridge well developed. Ventral parts of occipital ridge very prominent; antennal sockets in between eyes, distinct; interantennal distance ca. diameter of one antennal socket; antennifer present; dark median butterfly-shaped spot directly below the antennal bases present but washed out and indistinct, size and pigmentation varying between specimens; anterior tentorial pits dorso-mesally of anterior mandibular articulation; frons with three bulges, one in between antennal sockets, two ventro-mesal of antennal sockets; frontoclypeal ridge not recognizable. Gena not strongly protruding, head capsule on the level of the genae distinctly narrower than on the one of the compound eyes; heights of genae lower than heights of compound eyes. Clypeus trapezoid, with well-developed clypeolabral ridge, oval sclerite in between clypeus and labrum present. Labrum flat, anteriorly rounded, with

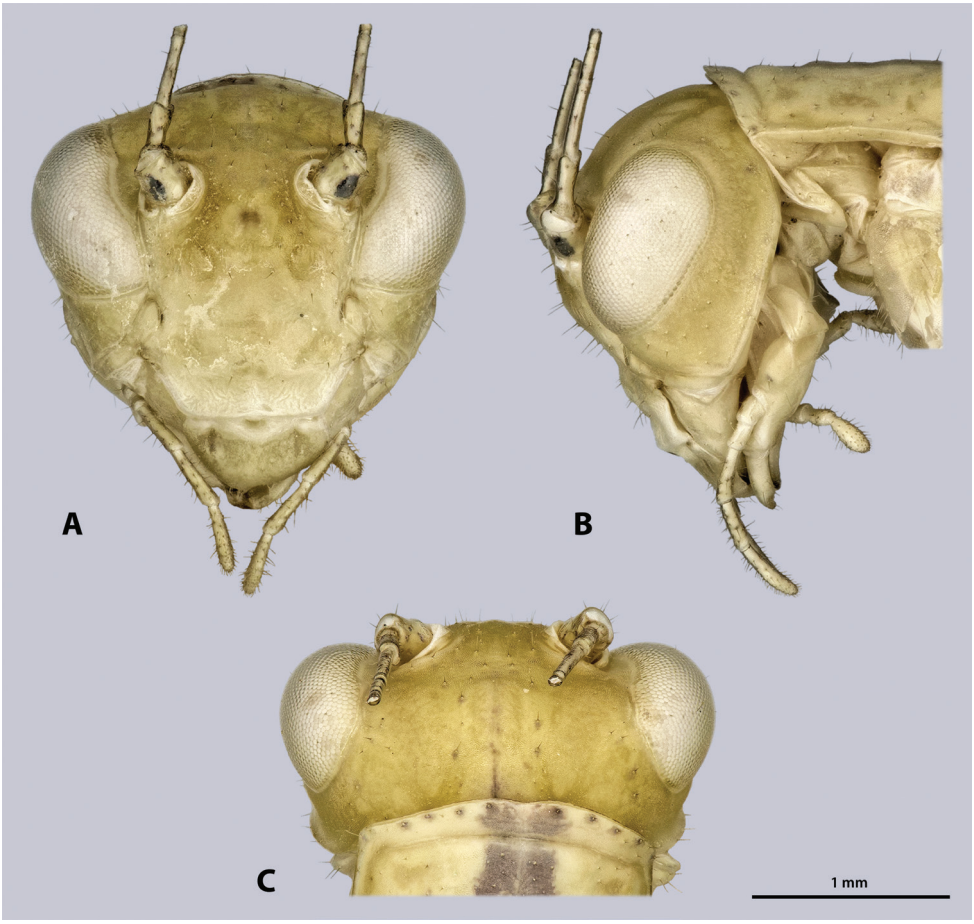


Figure 12. Head of ♂ *Minutophasma richtersveldense* sp. n., holotype, photomicrographies; **A** frontal view **B** lateral view **C** dorsal view.

few short setae. Maxilla well developed; maxillary palp five segmented, sparsely covered with setae, palpomere one and two as long as wide, palpomere three 2.5 times as long as wide, palpomeres four and five ca. twice as long as wide. Labial palp three segmented. Scape as long as wide, with distinct black ventro-lateral spot; pedicle half as wide as scape, twice as long as wide, dilating towards the tip. Flagellum about as long as the entire animal.

Thorax (Fig. 13): dorso-medially with distinct and broad longitudinal dark stripe. Notae sparsely covered with setae. Pronotum oval with bulge positioned anterior-laterally; pronotum reaches over head and mesonotum, ventral boarder of pronotum straight. Two cervicalia present, second located postero-dorsally to the first. Pleura subdivided into epimeron and episternum. Coxae large, covered with setae.

Legs: tibia with black spikes, covered with setae. Prothoracic leg more massive than meso- and metathoracic ones; profemur ca. 4 times, mesofemur ca. 4–5 times and

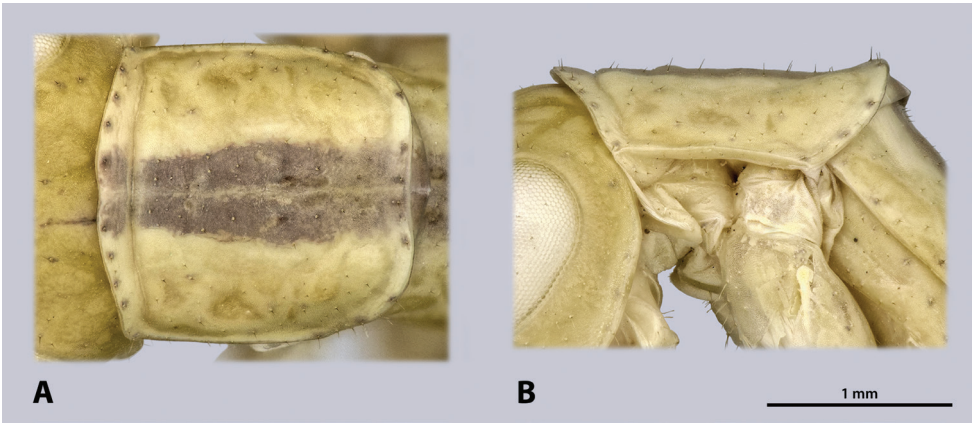


Figure 13. Prothorax of ♂ *Minutophasma richtersveldense* sp. n., holotype, photomicrographies; **A** dorsal view **B** lateral view.

metafemur 6–8 times as long as wide, all legs with two ventro-median rows of spikes, spikes in some specimens larger on pro- and mesothacic leg, smaller on metathoracic one. Tibia, in pro- and mesothorax ca. 8–11 times as long as wide, in metathoracic leg between 14 and 16 times as long, with two ventro-median rows of black spikes on pro- and mesothoracic legs, protibia with 5–8 spikes per row, on metathoracic leg only two distal spikes. Tarsus with five tarsomeres, proximal four tarsomeres with euplantulae; arolium very large.

Wings: completely absent.

Abdomen: as long as thorax and head combined; meso-dorsal dark longitudinal stripe strongly developed. Abdomen covered with setae. Abdominal tergum I same width or very slightly thinner as metathorax; terga slightly broadening towards tergum IX, tergum X narrowing again.

Male terminalia (Fig. 14): tergum IX shorter than tergum VIII, posterior margin concave. Dark stripe on tergum X much broader than on previous segments, almost covering entire tergum, roof-shaped in lateral view. Subgenital plate (sternite IX) large, with strongly protruding posterio-dorsal margin; process of subgenital plate broad, almost straight when seen from posterior, broadly emarginated dorsally. Cerci one segmented, densely covered with setae; diameter round, uniformly curved, narrowed towards the apex; cerci with dorsal projection and extending towards the middle of the subgenital plate. Paraprocts and epiproct also covered with setae.

Description female. For the female only differences to the male are described. Measurements: total length: (location 1: 11.3, 12.6, 12.7, 10.7) (location 2: 11.2, 13.2) (location 3: 12.3, 12.5); length of pronotum: (location 1: 2.2, 2.4, 2.3, 2.0) (location 2: 2.2, 2.1) (location 3: 2.2, 2.3); width of pronotum: (location 1: 2.0, 2.3, 2.2, 1.7) (location 2: 2.1, 2.0) (location 3: 2.0, 2.2); length of mesonotum: (location 1: 1.9, 2.0, 2.0, 1.8) (location 2: 2.0, 1.9) (location 3: 2.0, 1.9); width of mesonotum:

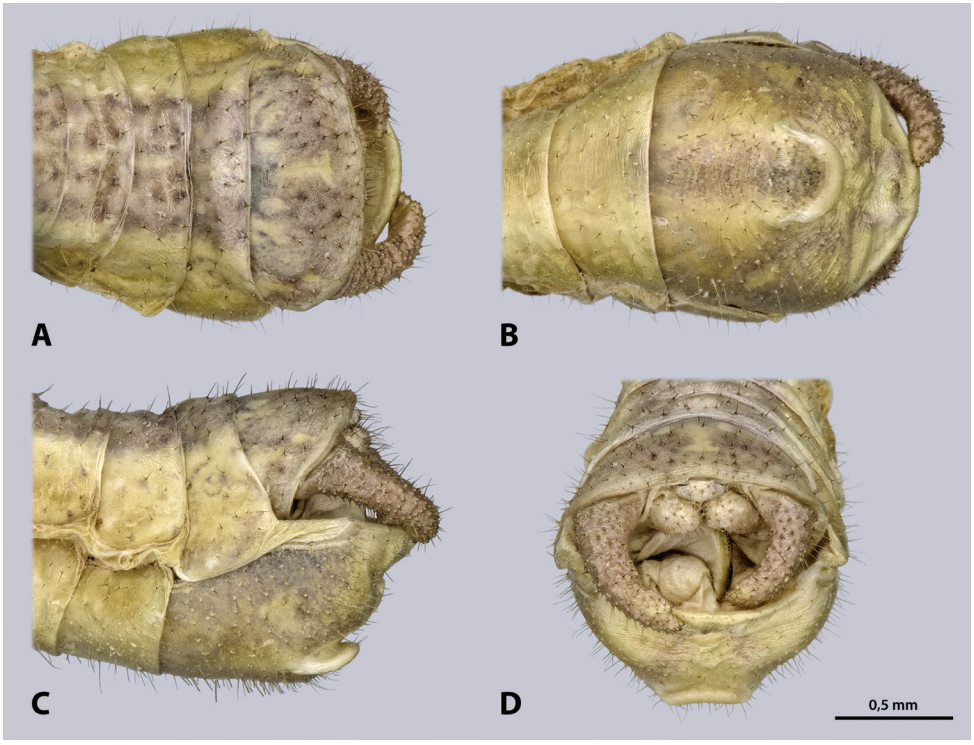


Figure 14. Terminalia of ♂ *Minutophasma richtersveldense* sp. n., holotype, photomicrographies; **A** dorsal view **B** ventral view **C** lateral view **D** caudal view.

(location 1: 1.9, 2.1, 2.0, 1.6) (location 2: 1.9, 1.8) (location 3: 1.8, 2.1); length of metanotum: (location 1: 1.4, 1.6, 1.6, 1.3) (location 2: 1.3, 1.5) (location 3: 1.5, 1.4); width of metanotum: (location 1: 1.8, 2.0, 1.8, 1.6) (location 2: 1.8, 1.8) (location 3: 1.7, 2.0); heights of head: (location 1: 1.6, 2.1, 2.1, 1.8) (location 2: 1.9, 2.1) (location 3: 1.8, 2.1); total heights of the head: (location 1: 2.4, 2.7, 2.7, 2.4) (location 2: 2.6, 2.7) (location 3: 2.4, 2.7); width of the head: (location 1: 2.4, 2.5, 2.4, 2.2) (location 2: 2.4, 2.4) (location 3: 2.3, 2.4); head width over eyes: (location 1: 2.6, 2.7, 2.8, 2.4) (location 2: 2.7, 2.7) (location 3: 2.5, 2.5); width between eyes: (location 1: 1.6, 1.7, 1.6, 1.4) (location 2: 1.7, 1.7) (location 3: 1.7, 1.7); length of eye: (location 1: 1.1, 1.2, 1.1, 1.0) (location 2: 1.2, 1.1) (location 3: 1.1, 1.2); width of eye: (location 1: 0.6, 0.7, 0.6, 0.6) (location 2: 0.7, 0.6) (location 3: 0.7, 0.7).

Coloration: all found females are green, without dorsal longitudinal dark stripe.

Head (Fig. 15): compound eyes slightly smaller than in the male. Head capsule on the level of the genae distinctly wider than on the level of the compound eyes.

Thorax (Fig. 16): notae with slightly denser setation than males. No dorsal dark stripe.

Legs: protibia with 6–9 spikes per row.

Abdomen: no dorsal brown stripe. Widest point of abdomen at segments 5 or 6.

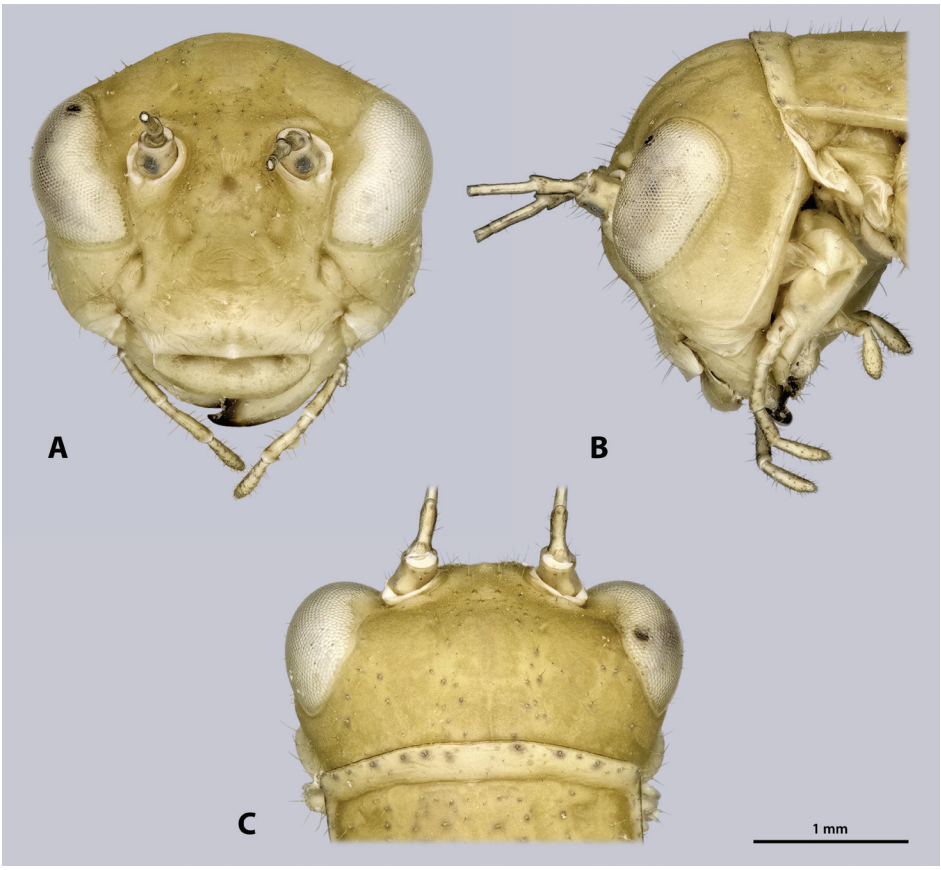


Figure 15. Head of ♀ *Minutophasma richtersveldense* sp. n., paratype, photomicrographies; **A** frontal view **B** lateral view **C** dorsal view.

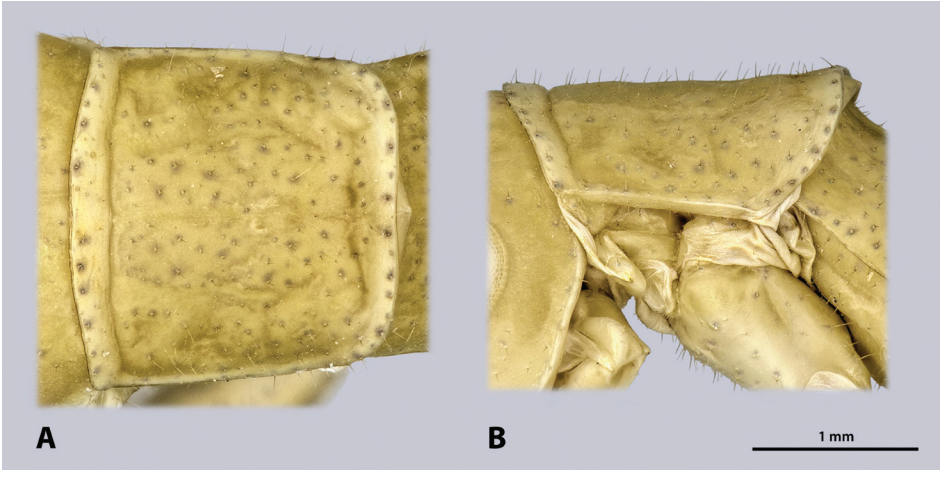


Figure 16. Prothorax of ♀ *Minutophasma richtersveldense* sp. n., paratype, photomicrographies **A** dorsal view **B** lateral view.

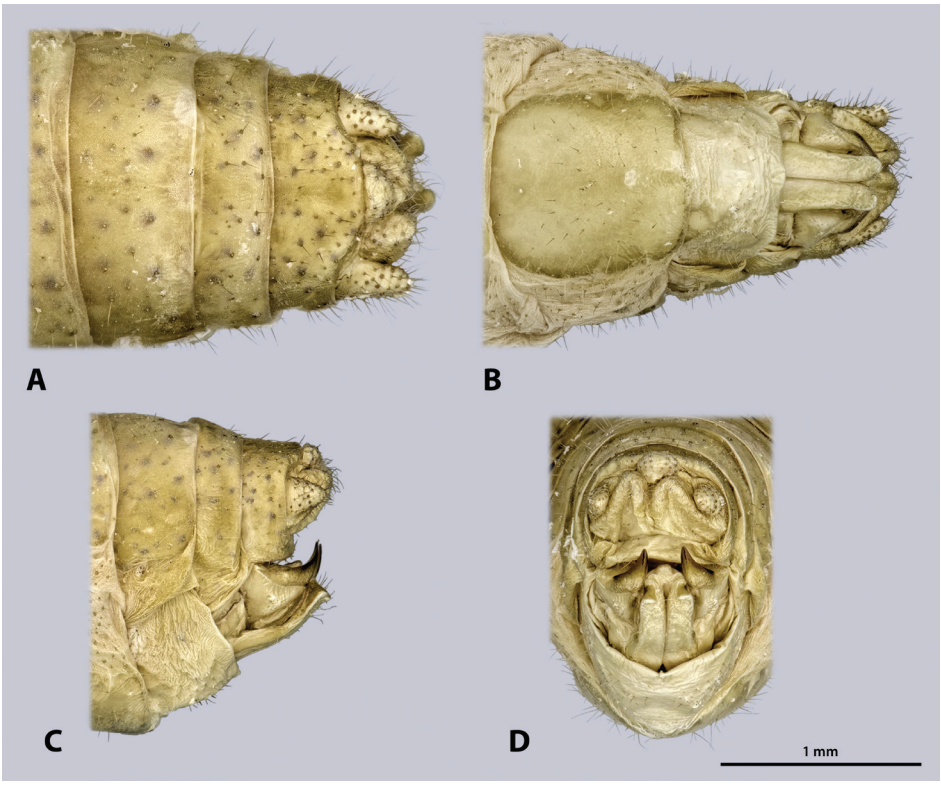


Figure 17. Terminalia of ♀ *Minutophasma richtersveldense* sp. n., paratype, photomicrographies; **A** dorsal view **B** ventral view **C** lateral view **D** caudal view.

Female terminalia (Fig. 17): tergum IX shorter than tergum VIII, posterior margin without distinct convexity. Tergum X slightly longer as tergum IX; apex rounded posteriorly; terga with sparse setation; epiproct half as long as tergum X, setose. Paraprocts rounded and densely covered with setae. Cerci slightly shorter than paraprocts, cone shaped and densely covered with setae. Sternite VIII with straight posterior margin. Gonapophysis VIII long and slender, distally blunt with ventrocaudal process. Gonocoxite IX almost completely hidden in lateral view; gonoplac triangular, heavily sclerotized.

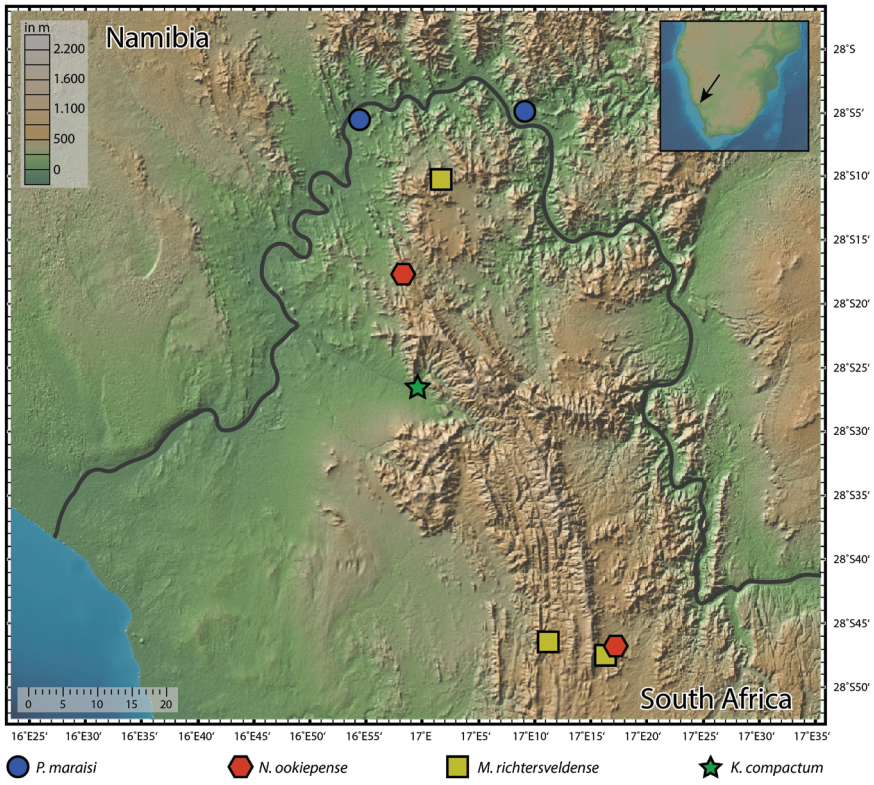
Etymology. The species name *richtersveldense* refers to the currently known area of distribution, the Richtersveld.

Comments. This species was found in a variety of green and grey-green bushes with small leaves; several specimens were also collected from grass stalks. From the most northern population at Akkedis pass, only females or female nymphs (>20) were recorded over a period of three years. The absence of males implies that parthenogenesis might occur; this phenomenon was not reported from mantophasmatodeans so far. The sex ratio in the populations around Eksteenfontein was about 1:1 as usual in Mantophasmatodea.

Discussion

The Richtersveld region is part of the Great Escarpment and an exceptional center of endemism within the Succulent Karoo (Hilton-Taylor 1996). A characteristic feature of the Richtersveld is its location in the transitional area between the summer rain (found north-east of the Richtersveld) and the south-western winter rain region in South Africa, which is accompanied by diverse topographic conditions. Apparently, these circumstances promoted the occurrence of locally distributed taxa and hence, speciation which is also observed in Mantophasmatodea. More precisely, the Richtersveld region is inhabited by not less than four easily distinguishable mantophasmatodean species. Figure 18 provides a table with important taxonomic features to separate these species. Due to a sporadic appearance of these insects, caused by irregular rainfall patterns and the complex topographic features in the Richtersveld, there is only preliminary information about the distribution of these taxa. Even though the data provided in this manuscript result from four field excursions into this comparably small area, *Kuboesphasma compactum* sp. n. was only found in a heavily overgrazed habitat (ca. 2 km^2) near the village of Kuboes (Fig. 18) and therefore seems to have a very limited distribution. This species, which likely represents the sister group of all South African Austrophasmatidae sensu Klass et al. (2003) (Predel, unpublished data), was not yet observed in neighboring areas with less disturbed vegetation. In contrast, *Minutophasma richtersveldense* sp. n. is reported from southern and northern localities in the Richtersveld (see Fig. 18), which confirms a wider distribution in that region. In addition to these two novel taxa, which are endemic to the Richtersveld, members of two other lineages enter this region and reach their currently known distribution boundaries in the Richtersveld. The large *Praedatophasma maraisi* seems to have its southern boundary of distribution just south of the Orange River. This species was described from a single female found at the Namibian-South African border at the mouth of the Fish River into the Orange River (Zompro et al. 2002; Fig. 18). We found another single female and a single male nymph in a very dry and sandy area near Sendelingsdrif at the northern boundary of the Richtersveld in South Africa (Fig. 18), but could not find any further specimens again. However, another population of this species was reported from the very dry Gaab/Fish River region approximately 90 km north-west of the above-mentioned localities (EduVentures Namibia; see Roth et al. 2014). Different from the other species in the Richtersveld, *P. maraisi* is obviously restricted to the vicinity of mostly dried-out riverbeds and its coloration suggests a ground-orientated lifestyle. The fourth species found in the Richtersveld is *Namaquaphasma ookiepense* (Klass et al. 2003). It is a common and widely distributed species in the Northern Cape province of South Africa. The Richtersveld, where this species seems to be rather uncommon, constitutes the northernmost extension of its distribution. Around Eksteenfontein *N. ookiepense* was found in sympatry with *M. richtersveldense*, but size and coloration facilitate a clear distinction of these species in the field (Fig. 18). Towards the south, this species is found to an assumed line from Strandfontein inland to Nuwerus about 400 km

A



B

Species	Namibia	Richtersveld	South Africa excl. Richtersveld	body size male (mm)	body size female (mm)	coloration [1]	destinct dark dorsal medial stripe in male [2]	black spot on scapus	dark butterfly-shaped spot on frons	head width on level of eyes compared to the level of the gena	heights of eyes compared to heights of gena
Praedatophasma maraisi	+	+		22-26	21-30	greyish-brownish	- [3]	-	-	broader	higher
Kuboesphasma compactum		+		12-13	13-15	green	-	-	(+) [4]	equal	equal
Minutophasma richtersveldense		+		9-11	10-14	green (greyish-brownish)	+	+	(+) [4]	broader	higher
Namaquaphasma ookiepense		+	+	12-15	16-17	greyish-brownish	+	+	+	broader	higher

[1]: mantophasmatodeans can have a wide range of browninsh, redish or greyish colors and we are aware that the biological reality and variability cannot be desribed in a single term. However, for taxonomic reasons we summarized all these shades of non-green species as greyish-brownish. [2] All these species have males with a dorsal median stripe. However, it can be almost invisible as it has the same color as the animal. We therefore distinguish here for taxonomic reasons if this stripe is distinctly visible or not. [3] Males of *Praedatophasma* have a stripe on the head and the anterior pronotum but on the rest of the dorsal surface a discontinuous pattern of horizontal stripes. [4] in *Kuboephasma* and *Minutophasma* it is washed-out and almost not distinguishable.

Figure 18. A map of the Richtersveld with the reported findings of the four mantophasmatodean species (scale in km) **B** table comprising the most important features to distinguish the four species of Mantophasmatodea in the Richtersveld.

south of the Richtersveld. There it is replaced by various other species of Austrophasmatidae which are common in the south-westernmost regions of the Northern Cape and the Western Cape (see Roth et al. 2014).

The comparatively high diversity of Mantophasmatodea in the Richtersveld stands in strong contrast to other regions within their currently known distribution, where even sympatry of two species is rare (Eberhard et al. 2011; Predel et al. 2012). This diversity could be interpreted as an indicator of speciation in this area. Yet, the phylogenetic positions of these taxa (Predel, unpublished data; Fig. 1) rather suggest, that the Richtersveld was colonized multiple times independently. *Praedatophasma maraisi*, together with the closely related *Tyrannophasma gladiator* from the Brandberg in northern Namibia, constitute the sistergroup of the remaining mantophasmatids. It therefore probably colonized the Richtersveld independently from the other species in that region. The South African taxa of Mantophasmatodea, including *Kuboesphasma* and *Minutophasma* from the Richtersveld, form a monophyletic clade with the southern Namibian *Striatophasma* (Fig. 1). The Richtersveld thus provided an important gateway in the dispersal of Mantophasmatodea from the summer-rain regions of southern Namibia towards the winter-rain areas of South Africa, where a distinct speciation took place (Klass et al. 2003; Predel et al. 2012; Roth et al. 2014).

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