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Notes on the sinistral helicoid snail *Bertia cambojiensis* (Reeve, 1860) from Vietnam (Eupulmonata, Dyakiidae)

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Abstract

Since the time of the original description there have been no precise locality records in Cambodia of *Bertia cambojiensis* (Reeve, 1860) and it was believed to be extinct. In 2012, a joint Natural History Museum survey with Vietnamese colleagues rediscovered living populations of this huge sinistral helicoid snail in a protected area of southern Vietnam. The genitalia and radula morphology are re-assessed and type specimens of all recognised congeners are figured herein. The unique morphological characters of this species are a small and simple penis, well-developed amatorial organ complex that incorporates four amatorial organ ducts, a short gametolytic organ complex and spiked papilla, and radula morphology with unicuspid teeth. The type locality of *B. cambojiensis*, which has been contentious, is determined here to be in the vicinity of 'Brelum', Vietnam, near the border with Cambodia. In addition, the nucleotide sequences of barcoding genes COI, 16SrRNA and 28S fragments were provided for further comparison.

Keywords

Conservation, DNA barcoding, endangered, left-handed, systematics

Introduction

The Dyakiidae Gude & Woodward, 1921, are a family of helicoid land snails restricted to Southeast Asia. The apomorphic characters of the family are the presence of an amatorial organ complex comprised of amatorial organ glands, amatorial organ ducts and amatorial papilla with a conchiolin spike (Godwin-Austen 1891, Laidlaw 1931, Baker 1941, Schileyko 2003). Of the 12 recognised genera in this family, three are sinistral: *Rhinocochlis* Thiele, 1931, endemic to Borneo; *Dyakia* Godwin-Austen, 1891, comprised of about 20 nominal species distributed in the Greater Sunda Islands and Peninsular Malaysia, and *Bertia* Ancey, 1887, endemic to eastern Indochina with three recognised species (Zilch 1959, Hausdorf 1995, Schileyko 2003, Sutcharit et al. 2012, Thach 2015).

Ancey (1887) described *Bertia* on the basis of the very large and sinistral, helicoid shell, initially as a monotypic genus based on *Helix cambojiensis* Reeve, 1860. Based on the shell characters, Thiele (1931: 1001) placed *Bertia* as a subgenus of *Ariophanta* Des Moulins, 1829, an Indian ariophantid genus that included large sinistral shells. The Bornean endemic species *Helix brookei* Adam & Reeve, 1848 was subsequently included in *Bertia* because of the very similar shell form (Baker 1941, Solem 1964). However, on the basis of the reproductive anatomy of *Helix brookei* (Godwin-Austen 1891, Weigmann 1898, Laidlaw 1932), Baker (1941) subsequently placed *Bertia* within the family Trochomorphidae. Baker placed *Helix brookei* in his new subgenus *Bertia* (*Exrhysota*). Zilch (1959) questioned the placement of *Exrhysota* in *Bertia*. Schileyko (2003) transferred *Bertia* to the Dyakiidae on the basis of characters of the genitalia and erected the new family Ryssotidae including *Exrhysota* as a distinct genus, which was subsequently synonymized with the Chronidae Thiele, 1931 by Bouchet et al. (2017).

Bertia cambojiensis (Reeve, 1860) was originally described under the name *Helix mouhoti* Reeve, 1860 [September], based on specimens collected by the famous French naturalist Henri Mouhot. Since this combination was a junior primary homonym of Pfeiffer (1860a [May]), it was replaced by *Helix cambojiensis* Reeve, 1860 [December] (Reeve 1860a, b). The type locality was given as "Cambojia" [= Cambodia]. Prior to Schileyko (2011), and for over 150 years following Reeve's original description, no specific distribution records of *B. cambojiensis* were published. Schileyko (2003) figured and described the genital anatomy of a museum specimen identified as *B. cambojiensis* which was recorded as being from Cambodia but his figure of a shell was of a different specimen. *Bertia cambojiensis* was widely thought to be extinct (Abbott 1989, Coney 2001). However, examples were seen offered for sale on shell dealers websites based in China in 2014/2015 for over € 400; they are now listed for as low as € 20 but are currently unavailable.

Living populations of *B. cambojiensis* were discovered on a 2012 survey in Cat Tien National Park organized by the Vietnam National Museum of Nature (VNMN) and the Natural History Museum, London (NHM). *Bertia cambojiensis* were listed as Critically Endangered (CR) on the IUCN Red List of Threatened Species (2014) due to their apparent restricted distribution in lowland tropical forest patches. In addition to deforestation and habitat degradation, threats include use as food and as a traditional medicinal resource (Daniel 1869, Phong 2018). Furthermore, *B. cambojiensis* is considered to be at particular risk because it is highly sought after by shell collectors globally due to its strikingly attractive shell and perceived rarity. It may currently be locally abundant but is easily visible at night on the trunks of trees and extremely vulnerable to over-collection (Naggs 2014). To safeguard the survival of *B. cambojiensis*, captive breeding populations were set up at the Vietnamese National Museum of Nature and at the Zoological Society of London and viable cell preparations are stored at the NHM, London. In this study, we present new information on genitalia, anatomy and radula morphology of *B. cambojiensis* based on specimens in the NHM, London. Information on the type specimens of all recognised species in the genus are provided and the systematic position of "*Helix brookei*" is discussed.

Materials and methods

Samples

All voucher specimens deposited in the NHM, London were examined. Two preserved specimens in 70% ethanol (NHMUK 20130833 and 20130874) were dissected for examination of the genitalia, and radulae were extracted and examined under a scanning electron microscope (JEOL, JSM-5410 LV). The radula shape and teeth formula were observed and recorded. Cytochrome c oxidase subunit I (COI), 16S ribosomal RNA (16S) and 28S ribosomal RNA (28S) genes of *B. cambojiensis* samples were sequenced for DNA barcoding. For DNA extraction and PCR amplification conditions and amplified primers see Appendix 1.

Abbreviations

am	amatorial organ;	**ov**	oviduct;
amd	amatorial organ duct;	**p**	penis;
amg	amatorial organ gland;	**pg**	prostate gland;
amp	amatorial organ pilaster;	**pp**	penial pilaster;
at	atrium;	**pr**	penial retractor muscle;
e	epiphallus;	**v**	vagina;
fo	free oviduct;	**vd**	vas deferens;
go	gametolytic organ (duct and sac);	**vp**	vaginal pilaster.

Systematics

Genus *Bertia* Ancey, 1887

Bertia Ancey, 1887: 53. Baker 1941: 320, 321. Zilch 1959: 275, 276. Schileyko 2003: 1362.
Ariophanta (*Bertia*) Thiele, 1931: 1001.

Type species. *Helix cambojiensis* Reeve, 1860, by original designation.

Remarks. Thach (2015) recognised four nominal species in *Bertia*: "*Bertia*" *brookei* (Adam & Reeve, 1848), *B. cambojiensis* (type species), *B. pergrandis* (Smith, 1893) and *B. setzeri* Thach, 2015. Nevertheless, he had overlooked key characters of the genitalia published by Godwin-Austen (1891: pl. 6, fig. 6) and Schileyko (2003: 1345, 1346, fig. 1758). The Bornean endemic species, *Helix brookei* Adam & Reeve, 1848, exhibits a long and cylindrical gametolytic sac, lacks an epiphallus and amatorial organ complex, and a caudal foss and a caudal horn is absent; characters that unequivocally distinguish this species from the Dyakiidae (Godwin-Austen 1891, Laidlaw 1931, Sutcharit et al. 2012). It is clear that *Helix brookei* Adam & Reeve, 1848 does not belong in *Bertia* and we recognise the following three as *Bertia* species.

Bertia cambojiensis (Reeve, 1860)

Figs 1, 2

Helix mouhoti Reeve, 1860a [Sep.]: 203, 204 [not Pfeiffer 1860a [May]: 136, pl. 50 fig. 5]. Type locality: Cambojia [Cambodia]. Pfeiffer 1860b: 173, 174, pl. 47, figs 1, 2. Pfeiffer 1868: 64.
Helix cambojiensis Reeve, 1860b [Dec.]: 455 [new replacement name]. Martens 1867: 76. Pfeiffer 1868: 64. Daniel 1869: 126–128. Morelet 1875: 250. Pfeiffer 1876: 78, 79. Pfeiffer and Kobelt 1881: 604, pl. 176, figs 1, 2. Tryon 1886: 18, pl. 6, fig. 9.
Helix cambodjensis: Mabille and le Mesle 1869: 128 [incorrect subsequent spelling].
Nanina cambodgiensis: Ancey 1887: 53 [incorrect subsequent spelling]. Fischer and Dautzenberg 1904: 393.
Ariophanta (*Rhyssota*) *cambojiensis*: Fischer 1891: 23.
Bertia cambodjiensis: Abbott 1989: 127 with text figure [incorrect subsequent spelling].
Bertia cambojiensis: Schileyko 2003: 1362, fig. 1777. Schileyko 2011: 37. Thach 2016: 142, fig. 271.

Material examined. Two syntypes from the Cuming collection. The specimen figured by Pfeiffer (1860b: pl. 47, figs 1, 2) can be recognised by two broken growth lines; one on the border of penultimate and last whorls, and one on the last whorl close to the apertural lip (seen from umbilical view) and is here designated as the lectotype NMUK

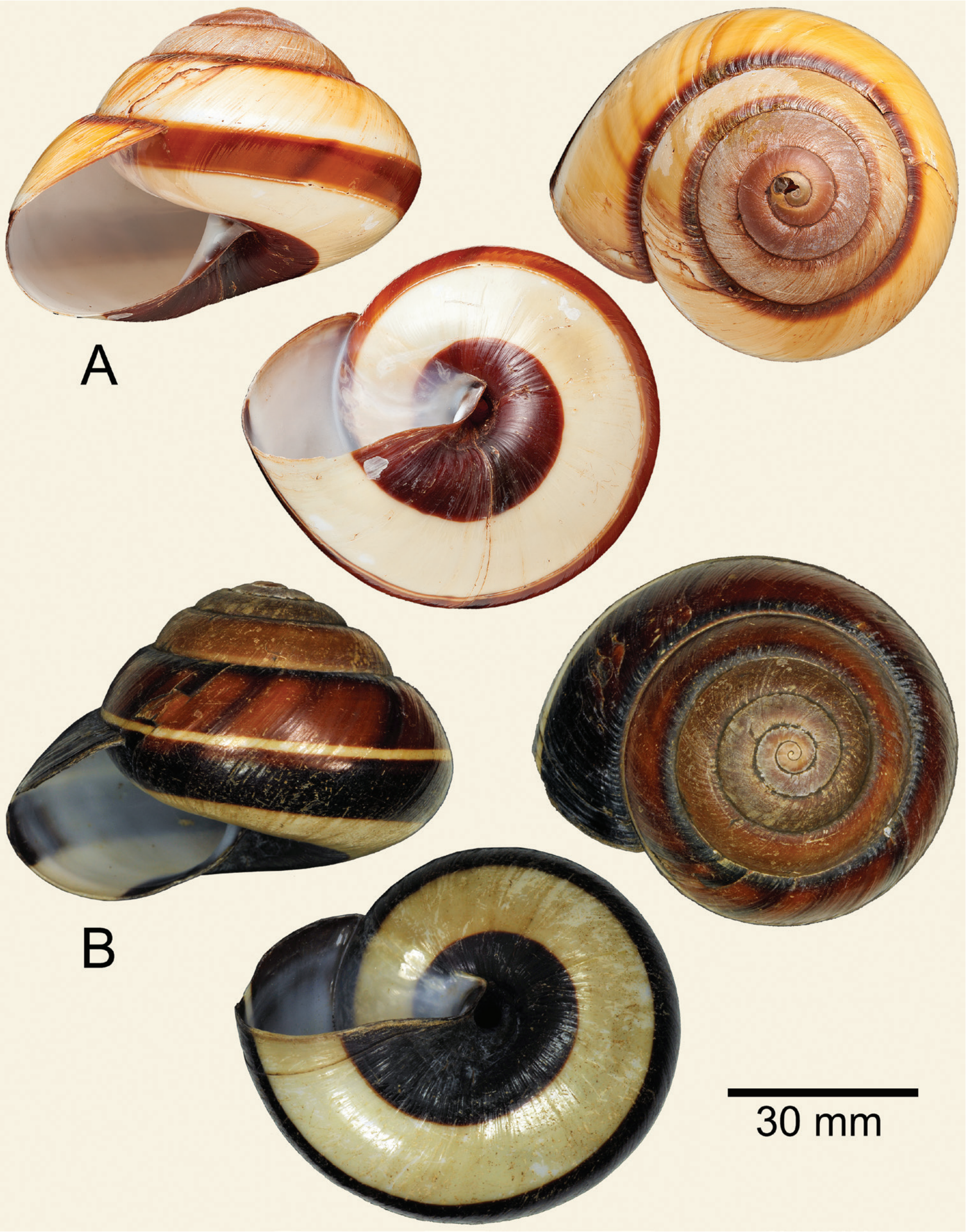

Figure 1. Shells of *Bertia cambojiensis* **A** lectotype NHMUK 20130220 and **B** specimen NHMUK 20130833.

20130220 (height 54.6 mm, width 76.2 mm; Fig. 1A). The remaining paralectotype NHMUK 20130219 measures height 54.4 mm and width 73.6 mm.

All additional specimens were from the area of Cat Tien National Park, Dong Nai Province, Vietnam (11°26.147"N, 107°25.643"E): NHMUK 20130818 from

Lodge Gardens (4 specimens + 2 juveniles); NHMUK 20130833 from Cave Site (7 specimens + 1 juvenile; Fig. 1B; COI accession no. MN296022, 16sRNA accession no. MN296390); NHMUK 20130874 from Near Lake (6 specimens + 3 juveniles; COI accession no. MN296023, 16sRNA accession no. MN296391 and 28S accession no. MN296349). Measurements: shell height 49.9–55.7 mm, average 52.6 mm; shell width 64.4–74.5 mm, average 69.7 mm.

Description. ***Shell.*** Shell sinistral, large, dome shape and thickened. Whorls 6 to 7, increasing regularly, slightly convex, and with wide and shallow suture. Periostracum thin to slightly thickened, corneous. Spire convex, apex obtuse, embryonic shell large with smooth surface, following whorls possess a series of thin nodules on growth lines. Last whorl well rounded. Upper shell surface glossy, varying from rich cream and brownish to blackish bands; narrow pale white to yellowish spiral band on periphery; narrow reddish-brown to dark subsutural band. Below periphery always with reddish-brown to dark colour and with broad white spiral band surrounding umbilicus. Umbilical area usually reddish-brown or darker. Aperture ovate; parietal callus translucent whitish; columella thickened and slightly dilated. Lip simple or slightly thickened in old adults. Umbilicus narrowly perforate to rimate and deep.

Genital organs. Atrium (at) very short ($N = 2$). Penis (p) long cylindrical tube. Penial retractor muscle (pr) short, thickened and attached distally to penis. Epiphallus (e) cylindrical tube, about half of penis length and slightly smaller diameter than distal penis. Vas deferens (vd) relatively smaller diameter and thin tube extending from free oviduct (fo) and entering epiphallus, apically; flagellum absent (Fig. 2A). Internal wall of penis with large penial pilasters (pp) for nearly entire length of chamber; proximally with smooth surface and distally with very thin crenellations on surface; penial verge absent (Fig. 2B).

Gametolytic organ (go; duct and sac undifferentiated) proximal to genital opening about one-fourth of amatorial organ length. Slightly swollen proximally, then tapering to small, long cylindrical tube and attached to ovary with thin connective tissues. Amatorial organ (am) well-developed enlarged cylinder; proximally attached to atrium. Amatorial organ glands (amg) enlarged, composed distally of four major lobes bounded to amatorial organ by thin connective tissue. Each of four major lobes of the amatorial organ gland extend proximally into thick amatorial organ ducts (amd) that are twisted together and bound with thin connective tissue before entering the distal tip of the amatorial organ (Fig. 2A). Internal wall sculpture of amatorial organ: proximally smooth surface for about one-third of chamber; distally consists of smooth surface of enlarged longitudinal amatorial organ pilasters (amp). Amatorial organ papilla small, short and conical, tipped by a large and long blackish spike (Fig. 2C).

Vagina (v) long enlarged cylinder, about the same length as penis. Free oviduct (fo) cylindrical tube; oviduct (ov) long with lobules; prostate gland (pg) bound to oviduct. Most of albumen gland, hermaphroditic duct and gland missing from figured specimen (Fig. 2A). Internal wall of vagina sculptured with uniform scale-like or triangular lingulate pilasters (vp), varying in size from small to large (Fig. 2D).

Radula. Teeth arranged in wide angle V-shaped rows with approximately 245 teeth with formula ((124-118)-1-(120-122)). Central tooth symmetric unicuspid and triangular. Lateral and marginal teeth undifferentiated, slightly curved unicuspid, tri-

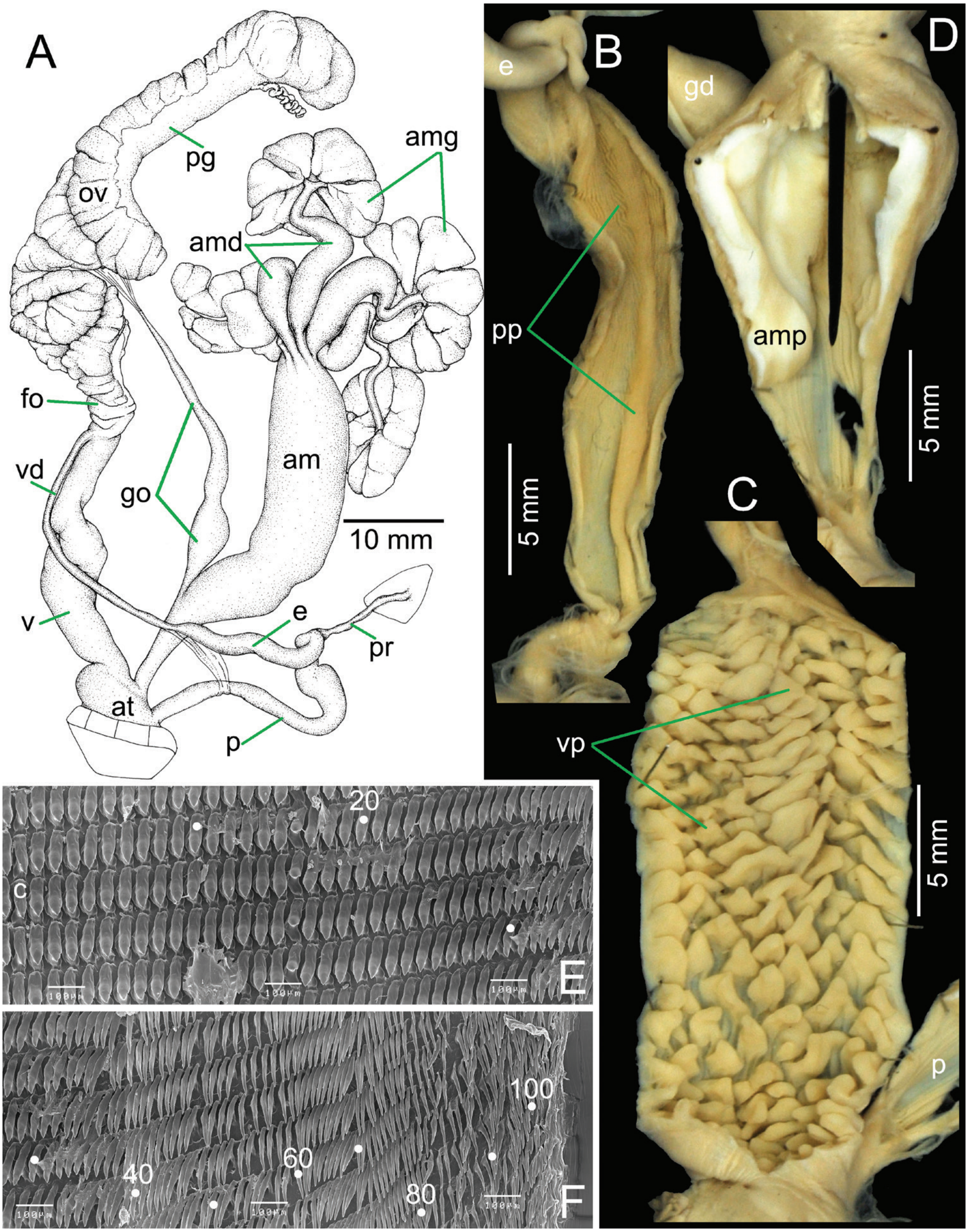

Figure 2. Genitalia and radula of *Bertia cambojiensis*, NHMUK 20130833. **A** overview of genital system **B–D** internal wall sculpture of **B** penis **C** amatorial organ and **D** vagina **E, F** SEM image of radula **E** central and inner latero-marginal teeth and **F** outermost teeth. Numbers indicate tooth order from lateral to marginal end; central tooth indicated by 'C'.

angular, and inclined towards central tooth (Fig. 2E). Inner teeth similar in shape and size to central teeth and then gradually reducing to slender and elongate sword-shaped with pointed cusp; outermost teeth slightly shorter with pointed tip (Fig. 2F).

External features. Living snail with long, blackish-brown tentacles. Skin reticulated brown with black reticulations around head. Foot sole relatively elongated, broad and unipartite. Sole of foot brownish to orange and unspotted; side of body brownish. Tail curved mid-dorsally, tall dome-shaped in cross section. Caudal horn not overhanging; caudal foss long vertical slit in tail above sole margin. Typical aulacopoda with well-defined pedal groove.

Mantle collar large and shell lobes thickened, shell lappet absent. Right dorsal lobe (right side of anus) large and thickened. Left dorsal lobe (left side of anus) composed of thick crescentric anterior left dorsal lobe and thin elongated posterior left dorsal lobe. Pulmonary cavity typically sigmurethran.

Distribution. The range of *B. cambojiensis* is likely to be confined to localised forest patches within Dong Nai, Binh Thuan, Lam Dong and Binh Phuc provinces. Records far outside of this range such as Ba Vi National Park (Schileyko 2011) are very doubtful and to date, no specific localities have been recorded for Cambodia.

Remarks. The specimens from southern Vietnam that we examined exhibit clear differences in genital anatomy from those described by Schileyko (2003: 1362, fig. 1777b, c) that were based on a specimen in the Muséum National d'Histoire Naturelle, Paris, recorded from Cambodia. The Paris specimen exhibits a long, cylindrical gametolytic duct (spermathecal stalk in Schileyko) and a bulbous gametolytic sac (receptaculum seminalis in Schileyko), distally pointed. The amatorial organ contains numerous amatorial organ ducts. In the NHM specimens the gametolytic duct is short and the amatorial organ includes four amatorial organ ducts. On the basis of numerous studies in the reproductive anatomy of pulmonate snails, these character differences strongly suggest species level distinction. The shell of the Paris specimen was not figured by Schileyko, his figured shell of *B. cambojiensis* (fig. 1777a) being from the Naturalis Museum, Leiden, which was also recorded as being from Cambodia.

In December 1858, the French explorer and naturalist Henri Mouhot set off from Bangkok by sea to the port of Komput (Kampot), Cambodia, from where he continued on his destination to Brelum in Annam (Vietnam), He reached the Catholic Mission in Brelum in August 1859. Stuart et al. (2006) state that Brelum was considered to be within the confines of Cambodia at the time of Mouhot's visit. However, Mouhot (1864a: 237) unequivocally described how 'It took us two long days' journey to reach Brelum' after having passed the Cambodian border town of Pump-Ka-Daye. Brelum was Mouhot's collecting base for the following three months before setting off on his return journey in November (Mouhot 1864a). During his stay in Brelum his host, the missionary R.P. Guillon, wanted Mouhot to sample the local escargot, which proved to be *B. cambojiensis*. Staggered by the beauty of this snail Mouhot set about obtaining examples for his collections from the local area, where it was abundant (Daniel 1869). Daniel (1869) went on to state that *B. cambojiensis* had not been found in Cambodia. Historical records may all relate to the mistaken assumption that the type locality was Cambodia. Mouhot was dependant on the sale of his collections to finance his fieldwork and it is likely that he shipped a large number of specimens of *B. cambojiensis* to his agent in London, Samuel Stevens, who would have sold them on to museums and collectors throughout Europe, presumably labelled with the locality 'Cambodia'. The field and travel conditions impacted severely on Mouhot's collections and he clearly had insufficient time to arrange them in good order before they were shipped. Writing to Stevens from Pinhalu, near Phnom Penh, on 20th December, 1859 Mouhot (1864b: 248) commented: '…I have little time to give you any details as to what I despatch from Komput and Singapore'.

According to Ashburton (1864), the location of Brelum, the type locality, is 11°58"N, 107°12"E, which is at an altitude of 460 m (Google Earth). This location is 30 km north of the north-western boundary of Cat Tien National Park and some 94 km north and west of the entrance to Cat Tien National Park. However, there remains some doubt as to the exact location of Brelum. Under the heading of a letter to Stevens, Mouhot (1864b: 241) stated: 'Brelum, among the savage Stiêns, lat, 11°46'30"N, 103°3'W merid. of Paris, 15th October, 1859'. This is clearly wrong because in addition to W (west) it should be E (east) and in the following text he gives exactly the same geo-reference for Pinhalu that he states to be about nine miles from the capital, Phnom Penh 11°46'58"N, 104°22'59"E (Google Earth) whereas Mouhot's reading 11°46'30"N, 103°3'W Paris (= 2°20'14.03" east of Greenwich) = 105°23'14"E, is some 54 km to the east and north of Phnom Penh (Google Earth). Kottelat and Tan (2017) identify Bro Lam Phe, 11°56'N, 106°40'E, in Loc Ninh District, Binh Phuoc Province, Vietnam, as the location of Brelum. This places Brelum at 100 m altitude and only 2 km from the closest boundary with Cambodia, which at this point follows the course of a meandering tributary of the Mekong. It is some 70 km from the boundary of Cat Tien National Park and 100 km from the main park entrance. It seems that Mouhot would have been most unlikely to retrace his steps and cross the river into Cambodia before his return journey and we conclude that he only collected *B. cambojiensis* from the vicinity of Brelum in Vietnam. Nevertheless, being in close proximity to the Cambodian border it does seem likely that *B. cambojiensis* will occur in this area of Cambodia.

Bertia pergrandis (Smith, 1893)

Fig. 3A

Rhyssota pergrandis Smith, 1893: 11, with text figure. Type locality: Annam.
Nanina pergrandis: Fischer and Dautzenberg 1904: 4.
Ariophanta pergrandis: Schileyko 2011: 29.
Bertia pergrandis: Thach 2015: 214, figs 9–12. Thach 2016: 62, pl. 18, fig. 270, pl. 19, fig. 272.

Material examined. Syntype NHMUK 1893.2.26.1 (1 shell, Fig. 3A).

Remarks. *Ariophanta* ranges from India to Indochina (Godwin-Austen 1888, Schileyko 2003) and includes large species with both dextral and sinistral shells. Although the reproductive anatomy of *Ariophanta* and *Bertia* are quite distinct the sinistral shells exhibit a close convergence in shell form and definitive generic attribution requires

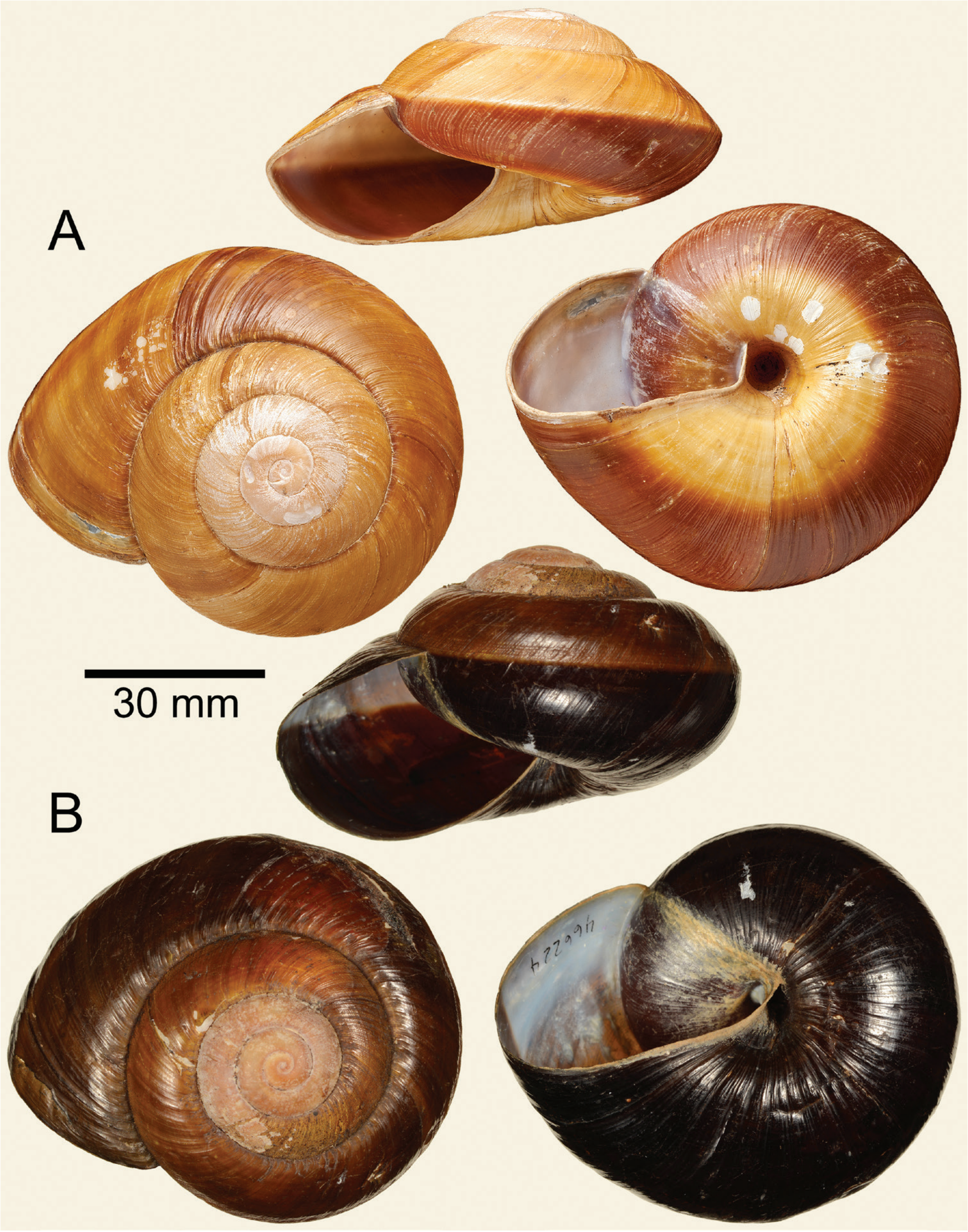

Figure 3. A *Bertia pergrandis*, syntype NHMUK 1893.2.26.1 **B** *Bertia setzeri*, holotype ANSP 466244.

investigation of reproductive anatomy or molecular evidence. We provisionally follow Thach (2015) in attributing this species to *Bertia* based on its very close similarity to the type species in shell form and colour and to the presence of earlier whorls with nodules arranged along the growth lines. *Bertia pergrandis* differs from the type and following species in possessing a stronger peripheral keel and widely open umbilicus; the other species exhibit a rounded last whorl and rimate umbilicus.

Bertia setzeri Thach, 2015
Fig. 3B

Bertia setzeri Thach, 2015: 240, 241, figs 1–4, 17–20. Type locality: Khanh Vinh District and Nha Trang outskirts, Khanh Hoa Province, central Vietnam. Thach 2016: 62, pl. 18, figs 267–269.

Remark. Images of the holotype are shown in Figure 3B. Thach (2015) mentioned that this species differs from the *B. cambojiensis* in possessing a more depressed shell, slightly angular periphery and a monochrome dark colour below the periphery. The living specimen figured by Thach (2015: figs 17, 19) shows a similar aulacopod foot sole with less developed caudal horn than in the type species. An examination of the reproductive organs or molecular data are required to determine its systematic status.

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Appendix 1

Molecular methods

DNA was extracted from small pieces of mantle collar tissue using a NucleoSpin Tissue kit (MACHEREY-NAGEL), following the manufacturer's protocol. The COI gene was amplified using the universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAAT-3') (Folmer et al. 1994). The 16S gene was amplified using the primers 16Sar (5'-CGCCTGTTTATCAAAAACAT-3') and 16Sbr (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi et al. 1991). The 28S gene was amplified using the primers 28SF4 (5'-AGTACCGTGAGGGAAAGTTG-3') and 28SR5 (5'-ACGGGACGGGCCGGTGGTGC-3') (Morgan et al. 2002). The thermal cycling was performed at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 42–52 °C (depending on samples and gene) for 60 s, extension at 72 °C for 90 s, then followed by a final 72 °C for 5 min. All PCR products were bi-directional sequenced by an automated ABI prism 3730XL sequencer at Bio Basic Inc., Canada, with the same PCR primers. The resulting nucleotide sequences of COI, 16SrRNA and 28S fragments are 640 bp, 456 bp and 536 bp long, respectively. Nucleotide sequences and deposited in the GenBank database with accession numbers: MN296022–3 for COI, MN296390–1 for 16S and MN296349 for 28S.

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New records of Lumbricidae and Collembola in anthropogenic soils of East European tundra

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Abstract

The terrestrial environment of the East European tundra consists of a mosaic of habitat types. In addition to the natural habitat diversity, various human-influenced types may occur. In the town of Vorkuta, Komi Republic, Russia the manure-enriched soils near hydrogen sulfide springs were observed. This site represents an unusually nutrient-rich location with considerable development of organic soils, in contrast to the naturally forming soils in East European tundra which are typically thin and nutrient poor. In these organic soils, two species of Lumbricidae and two species of Collembola previously not recorded from the natural ecosystems in the study area of research territory were found. One earthworm species, *Dendrodrilus rubidus tenuis*, is likely to have been introduced. The presence of the three other species (*Eiseniella tetraedra*, *Folsomia fimetaria*, and *Proisotoma minuta*) is quite natural in East European tundra and such anthropogenic soils with high organic content may be a good habitat for them.

Keywords

DNA barcoding, earthworms, springtails

Introduction

Tundra ecosystems are characterized by a low number of species, together with low productivity (Olejniczak et al. 2018; Phillips et al. 2019). Unfortunately, these ecosystems are known to be susceptible to disturbances, particularly if they are of anthropogenic origin (Coulson et al. 2015; Mikola et al. 2014). Extraction of natural resources, air pollution, human settlements, and tourism all affect tundra soils, vegetation, and thus soil organisms (Chapin et al. 2015; Coulson et al. 2013a, 2015; Olejniczak et al. 2018). Human activity is usually accompanied by a reduction in biodiversity, but it can also contribute to increased biodiversity of polar ecosystems by the introduction of non-indigenous species (Coulson et al. 2015). Man-made habitats are often more suitable and successful for colonists than natural habitats (Ødegaard and Tømmerås 2000). More than 65 % (1040 species) of alien arthropod species in Europe are associated with human-made habitats, especially parks and gardens, human settlements, and agricultural lands (Lopez-Vaamonde et al. 2010). Habitats of this type include accumulations of dead plant material and manure heaps of animal origin. These rich organic layers provide climatically stable living spaces and food for different groups of animals. One such habitat is associated with the anthropogenic soils in the Russian mining town of Vorkuta in the East European tundra.

The East European tundra is the territory bound by the Kanin Peninsula on the west and the Kara River Basin in the east between 67–71°N and 50–65°E. This territory is diverse, ranging from polar deserts with extremely low plant cover to moss, dwarf birch, willow, and forest tundra. The heterogeneity is also reflected in the soil animal fauna, where often clear relationships can be observed between vegetation cover and invertebrate species diversity (Coulson et al. 2013b).

The earthworm fauna in this territory is very poor and includes only three species: *Dendrobaena octaedra* (Savigny, 1826), *Lumbricus rubellus* Hoffmeister, 1843, and *Eisenia nordenskioldi nordenskioldi* (Eisen, 1879), which widely distributed in the study area because of their ability to withstand soil freezing (Makarova and Kolesnikova 2019; Shekhovtsov et al. 2018). This fauna can be seen as a marginal element compared to the more diverse earthworm fauna of Fennoscandia, where eight species are found to the north of the 65th parallel north (Terhivuo 1988) and Kola Peninsula, where six species were found beyond the Arctic Circle (Zenkova and Rapoport 2011; Rybalov and Kamaev 2012). Both these territories are heated by the Gulf Stream and were covered by glacial sheets that erased much of the fauna (Hewitt 2000). Therefore, endemic species are lacking here and the species present are post-glacial immigrants, that have invaded either spontaneously or in association with human activities. East European tundra had only limited glaciation (the furthest glacial maximum and last glacial maximum of Quaternary glaciation) but a harsher climate, and some populations of earthworms could survive or only recently colonize this territory (Shekhovtsov et al. 2018). However, the macro-scale distribution of earthworm species shows little connection to the pattern of the last glaciation. The earthworm fauna of the northern Russian plain is composed mainly of peregrine species of European origin (Tiunov et al. 2006).

On the other hand, springtails play an important role in tundra ecosystems as they affect the processes of humification and mineralization of organic matter (Babenko 2012; Coulson et al. 2013a, b, 2015; Olejniczak et al. 2018). A total of 192 collembolan species is registered for the territory of the East European tundra. Among them, 30 species are unique and absent from neighboring regions, due to a number of ecological factors (Babenko et al. 2017). However, there is no information about the invertebrate fauna, native or introduced in anthropogenic soils.

The aim of this study was to assess the distribution of earthworms and springtails that are new in the Eastern European tundra, and test the working hypothesis that these records of these species are confined to anthropogenic soils near hydrogen sulfide sources beyond the Arctic Circle.

Materials and methods

Soil samples were collected from the sides of a gully formed in the organic soils accumulated near hydrogen sulfide brooks (67°29'N, 64°02'E) in Vorkuta in Komi Republic, Russia (Fig. 1). The soil formed layers 10 cm thick which were created from a mixture of discarded poultry factory food stores, city hospital, and railway depot (Getsen 2011). Due to the warm municipal sewage that is discharged into the stream, in winter it does not freeze. Analysis of physical and chemical properties showed that the soils are characterized by a neutral pH (pH = 7.3) with contents of nitrogen (N_{tot} = 1.3 %), carbon (C_{tot} = 21 %) and narrow C:N ratio. A similar picture was obtained for postagrogenic soils in the European Northeast of Russia. On the contrary, an acid reaction, high content of carbon (C_{tot} = 32 %), low content of nitrogen (N_{tot} < 1 %) and wide C:N ratio were recorded in tundra soils, which indicates a low enrichment of soil organic matter with nitrogen and a weak degree of decomposition (Taskaeva et al. 2019).

Twenty soil samples 10 × 10 × 10 cm were taken near hydrogen sulfide springs in Vorkuta on July 2017 and August 2018. The soil samples were immediately returned to the Institute of Biology, Syktyvkar, Komi Republic, and placed in Tullgren soil extractors within 24 h of sampling. The soil fauna was extracted under 40 W light bulbs into 96 % alcohol for seven days until the soil was completely dry. Accounting of earthworms near hydrogen sulfide springs by manual sorting of soil samples 25 × 25 × 10 cm was not carried out because of the small area of the studied plots. Moreover, the recent results showed that the earthworm abundance estimated by the Tullgren funnel extraction method exceeds the values obtained through manual sorting of the samples by an order of magnitude (Makarova and Kolesnikova 2019). The Collembola were identified to species by morphological characters (Fjellberg 2007; Potapov 2001). Identification of Lumbricidae was performed according to morphological characters provided by Vsevolodova-Perel (1997) and Timm (2009), as well as DNA barcoding for immature individuals.

DNA was extracted from several caudal segments using 6 % Chelex 100 DNA extraction kits (Sigma-Aldrich, USA). A fragment of the COI gene was amplified

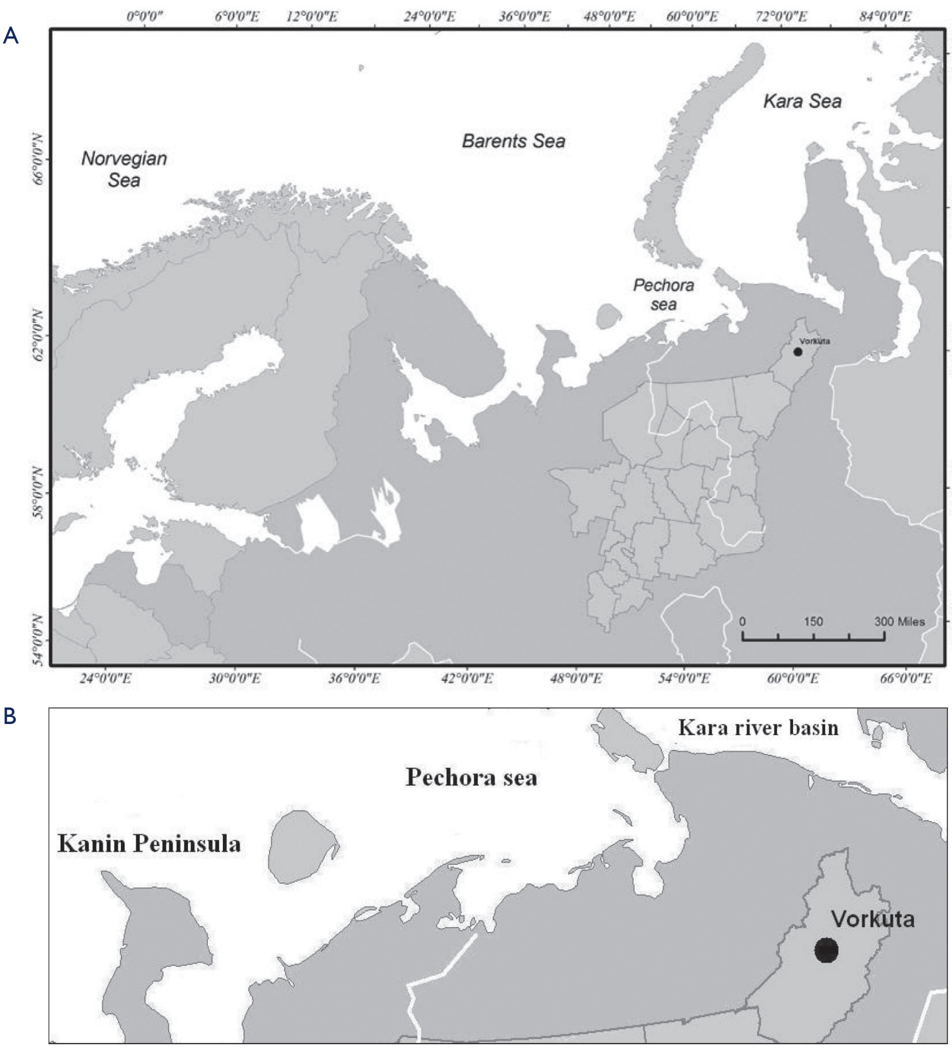

Figure 1. The location of Komi Republic, Russia (**A**) and East European tundra (**B**).

in 50 µl of mixture containing 10 µl ScreenMix (Eurogen, Russia), 10 µl of each primer (0.3 µM) (Eurogen, Russia), 18 µl ddH2O (Ambion, USA), and 2 µl DNA template (1÷100 ng). Two universal primers designed for invertebrate organisms were used to amplify the site of the COI fragment: LCO1490m (5'-TACTC-AACAA-ATCAC-AAAGA-TATTG-G-3'; modified from Folmer et al. 1994) and HCO2198m (5'-TAAAC-TTCAG-GGTGA-CCAAA-AAATC-A-3'; Folmer et al. 1994). Sequencing was performed using the equipment of The Center for Collective Use "Molecular Biology" of the Institute of Biology of the Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences. The GenBank database was used for sequence identification. Sequence alignment (automatic and manual) and choice of an evolutionary model were performed using the MEGA 7.0 program (Kumar et al.

Table 1. Invertebrate species previously unrecorded in East European tundra collected from the anthropogenic soils near a hydrogen sulfide spring in Vorkuta.

Class	Species	Records in East European tundra	Records beyond Arctic Circle in Europe	Distribution	GenBank accession number
Oligochaeta	*Dendrodrilus rubidus tenuis* (Eisen 1874)	Vorkuta (67°29'N, 64°02'E)	Iceland? (Blakemore 2007); Svalbard (Coulson et al. 2013a, b, form is unknown); Fennoscandia (Terhivuo 1988, form is unknown); Kola Peninsula (Zenkova and Rapoport 2011)	Holarctic. Records from the southern hemisphere	
	Eiseniella tetraedra (Savigny, 1826)	Pechora delta (68°11'N, 53°82'E) Pymvashor (67°09'N, 60°51'E) Kharbey lakes (67°58'N, 62°34'E) Vorkuta (67°29'N, 64°02'E)	Iceland (Blakemore 2007); Fennoscandia (Terhivuo 1988); Kola Peninsula (Timm and Abarenkov 2018)	Cosmopolitan	
Collembola	*Folsomia fimetaria* (Linnaeus, 1758)	Vorkuta (67°29'N, 64°02'E)	Greenland (Babenko and Fjellberg 2006) Svalbard (Coulson et al. 2013a, b) Fennoscandia (Fjellberg 2007) Kola Peninsula (Babenko 2012)	Holarctic	–
	Proisotoma minuta (Tullberg, 1871)	Vorkuta (67°29'N, 64°02'E)	Fennoscandia (Fjellberg 2007)	Cosmopolitan	–

2016). We applied Neighbor Joining (NJ) and Maximum Likelihood (ML) estimation methods. Since both methods resulted in a similar outcome, here we present the trees obtained by the NJ method only. The default parameters for tree building were selected. The Tamura-Nei model was selected for ML analysis. To align sequences, we used the ClustalW algorithm and the robustness of the resulting lineages was tested by bootstrap analysis with 1,000 replications. For NJ we used the p-distance model. Sequences were sent to GenBank (accession numbers and BINs are presented in Table 1).

Results and discussion

Two species of lumbricids, *Dendrodrilus rubidus tenuis* (Eisen, 1874) and *Eiseniella tetraedra* (Savigny, 1826), and two species of springtails, *Folsomia fimetaria* (Linnaeus, 1758) and *Proisotoma minuta* (Tullberg, 1871), not previously recorded from East European tundra were collected. Their records on this territory and beyond the Arctic Circle are shown at Table 1.

Both species of Lumbricidae are widespread, including records from the southern hemisphere. *Dendrodrilus rubidus* is found on every continent except Antarctica and inhabits not only continents, but also many islands. It is often found in wet and moist soils by rivers, brooks, and springs, and thrives in compost heaps and in a variety of man-made habitats including rich soils close to settlements (Terhivuo 1988). It was recently found at 70°N (eastern Finmark, Norway) and appears to be common throughout the northern mainland of Norway and in Svalbard cowsheds (Coulson et al. 2013a, 2013b). In our study we found the subspecies *Dendrodrilus rubidus tenuis*. COI sequence of one individual was identified and differed from a sequence of this species from Canada (KM612222) by two substitutions and from Russia, Southern Kuriles (KX400643) by four substitutions (Fig. 2A). This species is abundant in temperate ecosystems of Eastern Europe, cultivated areas in the taiga zone of European North-East, southern part of Siberia, stone birch-forests of Kamchatka (Vsevolodova-Perel 1988, 1997; Shekhovtsov et al. 2014; Akulova et al. 2017) and in greenhouses in northern settlements of Yakutia, Magadan oblast, and Chukotka (Berman et al. 2010). In winter, for example, greenhouses are not heated and the temperature may descend to below -40 °C. Nonetheless, the cocoons are viable at temperatures lower than – 40 °C (Berman et al. 2010), but adults cannot survive exposure below -4 °C (Meshcheryakova and Bulakhova 2014). Considering the harsh winter conditions in Vorkuta, it will probably be restricted to areas with local enrichments of organic matter to provide protection against low air temperatures, such as anthropogenic soils. In our study it was found only in built-up areas, where the soil does not freeze in winter due to the warming action of some shelters (warm municipal wastes, poultry factory storages, etc.). Previously, beyond the Arctic Circle, *D. rubidus tenuis* was registered only in Khibiny Mountains (Kola Peninsula), where it was found in soils with a pH > 5 and high organic matter content (Zenkova and Rapoport 2011).

Eiseniella tetraedra is a cosmopolitan earthworm widely distributed in the Old and New World countries (Terhivuo et al. 2011). COI sequences of two *E. tetraedra* individuals were identified and differed from sequences of this species by only one substitution (Fig. 2B) from the most closely related GenBank entries, i.e. from France (MF121728), Austria (KT073959), Switzerland (LN810249), and Canada (HM400424). It is abundant in wet and moist alluvial shore soils, fucoid wrack beds, the banks of rivers and brooks, and it can be found in waterlogged or even limnic habitats such as the bottoms of rivers, brooks, and springs (Terhivuo 1988). Among all species of lumbricids it is the most resistant to flooding (Plum 2005). It is not directly linked with human culture and is not intentionally transported by human activity (Terhivuo et al. 2011), but it is a “key” species in anthropogenic habitats, the activity of which determines the character of soil dynamics (Barne and Striganova 2005). Considering its widespread distribution and parthenogenic reproduction, allowing it to settle and colonize quickly, *E. tetraedra* is an interesting object for studying biogeography and genetic diversity in soil, aquatic, and ecotone systems (de Sosa et al. 2017). In natural habitats, these worms live in large colonies (Barne and Striganova 2005) and can reach up to 1000 ind./m^2 (Malevich 1959). Despite insignificant resistance to cold, its dispersal is determined by its amphibiotic nature: it can successfully overwinter only in talik or waterlogged soils, which only freeze in winter (Meshcheryakova and Berman 2014). It was previously found in Pechora River delta (Baturina 2018) and other aquatic ecosystems in East European tundra (Table 1).

Two species of Collembola previously unrecorded in East European tundra were found, *Folsomia fimetaria* and *Proisotoma minuta*. Both of these are typical members of a fauna associated with soils having a high organic content such as compost, garden soil, fucoid wrack beds along seashores, and stream banks. They are very seldom found in

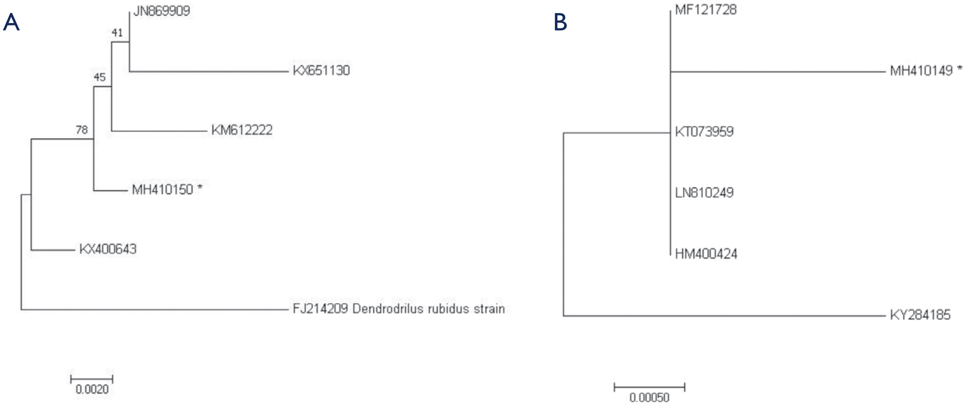

Figure 2. Phylogenetic tree constructed by the NJ method on the basis of a comparison of the nucleotide sequences of the COI gene of *Dendrodrilus rubidus tenuis* (**A**) and *Eiseniella tetraedra* (**B**) species from other regions (Austria JN869909, KT073959; USA KX651130; Canada KM612222, HM400424; Russia, Southern Kuriles KX400643; Finland FJ214209; France MF121728; Switzerland LN810249; Spain KY284185). * the numbers of our data sequences from Komi Republic.

temperate forests in the European part of Russia. Due to their ecological preferences, they could be artificially introduced (Potapov 2001). For example, in Iceland and Fennoscandia *F. fimetaria* is often found in organic soils along seashores (Fjellberg 2007); in Kola Peninsula it was found in anthropogenic stations heavily enriched with organic matter (Babenko 2012). This species was recently found in cowsheds soils in Svalbard (Coulson et al. 2013a). It is a good test subject for chemical studies (Holmstrup and Krogh 1996). *Proisotoma minuta* is also very tolerant of unfavorable conditions; it prefers roots infected by several phytopathogenic fungi, but the preference does depend on the species of fungi (Potapov 2001). The preliminary list of Collembola is presented in Appendix 1.

Three of the four new species records for East European tundra observed here appear to be not currently invasive. However, our molecular genetic analysis of the earthworm *D. rubidus tenuis* that was found in Vorkuta suggests that our sample of this species potentially invasive in nutrient-high habitats. In contrast to the natural soils, the anthropogenic soils provide a nutrient-rich, organic soil (contents of N = 1.3 % and C = 21 %) with excellent water-holding properties and with a cool moist environment during the summer but beneficially altered via human activities. The brook Vodny, in the area of which the studies were conducted, does not freeze as it depends on warm municipal and domestic waste waters flowing into it. Thus, soils with high organic content can stay warm throughout the season as a result of a continuous fermentation process. Consequently, species preferring warm soils probably do not suffer from chilling injuries (Bale 1993) during the winter in Vorkuta, despite the fact that their temperature tolerances are adapted to a warmer climate (Ødegaard and Tømmerås 2000). However, both species of lumbricids *D. rubidus tenuis* and *E. tetraedra* are cold tolerant only in the cocoon stage, while adult worms die at – 1 °C to – 3 °C (Berman et al. 2010; Meshcheryakova and Berman 2014). This means that they have adapted to complete their life cycle in the short warm period. Both species of springtails *F. fimetaria* and *P. minuta* cannot be called aliens, because their presence was also noted in anthropogenic soils in tundra of other regions. To conclude, it is evident that despite not tolerating the cold, all four newly recorded species are still capable of living in anthropogenic environments in cold climates.

Acknowledgments

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Appendix I

Preliminary list of Collembola.

Order	Family	Species
Poduromorpha	Onychiuridae	*Protaphorura* sp.
	Hypogastruridae	*Ceratophysella denticulata* (Bagnall, 1941)
	Neanuridae	*Friesea truncata* Cassagnau, 1958
		Pseudachorutes subcrassus Tullberg, 1871
Entomobryomorpha	Isotomidae	*Desoria blufusata* (Fjellberg, 1978)
		Desoria breviseta Potapov, 2017
		Desoria sp.
		Folsomia amplissima Potapov & Babenko, 2000
		Folsomia fimetaria (Linnaeus, 1758)
		Folsomia sp.
		Isotoma anglicana Lubbock, 1873
		Isotoma viridis Bourlet, 1839
		Isotomiella minor (Schäffer, 1896)
		Parisotoma notabilis (Schäffer, 1896)
		Proisotoma minuta (Tullberg, 1871)
Symphypleona	Sminthurididae	*Sminthurides aquaticus* (Bourlet, 1842)
		Sphaeridia pumilis (Krausbauer, 1898)
	Katiannidae	*Sminthurinus aureus* (Lubbock, 1862)

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A new singular species of *Croscherichia* Pardo Alcaide, 1950 (Coleoptera, Meloidae, Mylabrini) from arid zones of eastern Morocco

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Abstract

A new species of blister beetle (Coleoptera, Meloidae, Mylabrini), *Croscherichia armass* Ruiz, François & García-París, **sp. nov.**, is described from the arid steppes of eastern Morocco (Missour, Boulemane Province). The new species presents traits shared with both *Croscherichia* and desert species of the genus *Ammabris*, making it morphologically singular. Conspicuous external similarities (coloration pattern, shape of the mandibles, setation) between *C. armass* **sp. nov.** and *Ammabris* allow the two to be easily confused. However, *C. armass* **sp. nov.** can be readily distinguished from all other *Croscherichia* species by the following traits: reddish-orange legs with dark tarsi; relatively short black antennae with the proximal-most three to four antennomeres of each antenna having a reddish-brown coloration; dense and silvery body setation that lies over most of the body integument; straight and pointed outer mandible margins that protrude from the labrum; a mesosternum with an angulate anterior margin; a short, subcylindrical, and weakly spatulate external metatibial spur that is truncated obliquely at the apex. *Croscherichia armass* **sp. nov.** is only known from three localities in the arid Hammada steppes, which are located within the Quaternary alluvial plains of the Muluya river valley. Live specimens of *C. armass* **sp. nov.** were found in flight and actively feeding on *Atriplex halimus* (Chenopodiaceae) flowers at the end of summer (mid-September). The phenology of *C. armass* **sp. nov.** is exceptional as no other Mylabrini species known from eastern areas of Morocco are active in late summer.

Keywords

Ammabris, arid steppes, *Atriplex*, biodiversity, blister beetle, Hammada, morphology, North Africa, taxonomy

Introduction

Mylabrini Laporte 1840, which is distributed over most of the Old World (Palaearctic, Oriental and Afrotropical regions), is the most diversified tribe within the family Meloidae. Mylabrini is comprised of about 760 species distributed in 12 genera (Bologna 1991, Bologna and Pinto 2002, Pan et al. 2013, Pan and Bologna 2014, Salvi et al. 2018). Morphological and molecular analyses support the monophyly of Mylabrini (Bologna 1991, Bologna and Pinto 2001, Bologna et al. 2005, 2008a, 2008b, Pan et al. 2013, Salvi et al. 2018). However, taxonomic assignments made within the tribe have been problematic, with many issues still unresolved. Most of the species within Mylabrini (82%) are assigned to one of two genera: *Hycleus* Latreille, 1817 or *Mylabris* Fabricius, 1775, with around 450 and 175 species, respectively (e.g., Bologna 1991, 2000, Bologna and Pinto 2002, Bologna et al. 2011, Pan and Bologna 2014). Although the systematics of *Hycleus*, which is likely polyphyletic, have not been adequately addressed, the monophyly of *Mylabris* was recently confirmed using molecular data, and its phylogeny resolved to the subgenus level (Salvi et al. 2018).

In contrast to *Hycleus* and *Mylabris*, *Croscherichia* Pardo Alcaide, 1950 is a medium-sized genus currently comprised of 18 described species, whose internal phylogeny based on morphological traits and a largely undisputed taxonomy is well recognized (Biondi and Bologna 1991, Bologna and Coco 1991). *Croscherichia* is widely distributed in northern Africa, the northern Sahel region, the Middle East, the Arabian Peninsula, and western India (Bologna 1991, Bologna and Coco 1991, Bologna and Pinto 2002, Bologna 2008). The presence of the genus in western Europe (particularly, the Iberian Peninsula and the Balearic Islands), based on old records of *Croscherichia paykulli* (Billberg, 1813), has been rejected by several authors (García-París and Ruiz 2005, García-París et al. 2010), as has its presence in Turkestan (Bologna and Coco 1991, Bologna and Pinto 2002, Bologna 2008).

In its current sense, *Croscherichia* [type species: *Mylabris circumflexa* Chevrolat, 1840 (= *Mylabris paykulli* Billberg, 1813), by monotypy] is monophyletic. This genus, with its sister group *Mimesthes* Marseul, 1872, is characterized by the synapomorphic condition of its external metatibial spur, which is spatulate in both groups (Bologna et al. 2005, Salvi et al. 2018). Some ecological and developmental studies of *Croscherichia* have been reported, including the description of first-instar larvae of a few species (Cros 1917, 1927, 1940, MacSwain 1956, Bologna and Coco 1991). First-instar *Croscherichia* larvae present a few traits typical of phoretic taxa, which are exceptional for Mylabrini; however, their biology is still largely unknown (Bologna and Pinto 2001, Bologna et al. 2005).

Bologna and Coco (1991) established the generic limits of *Croscherichia* by reassigning to it some species previously included in *Hycleus* (= *Gorrizia* Pardo Alcaide, 1954) or in *Mylabris*. The species of *Croscherichia* are characterized by the following morphological diagnostic characters: a medium to large size (8–22 mm); black or reddish-brown integument, without a metallic shine; large convex eyes; antennae with 11 elongate antennomeres (rarely, X and XI almost fused) that are not flabellate or compressed and that do not have a marked distal thickening; mesopleurae that are not bordered along the anterior margins; an angled mesosternal suture; mesosternum without a modified middle-anterior area (*scutum*) or divergent groves; an elytral design consisting of sinuated transverse bands or rounded spots; a cylindrical external metatibial spine that is truncated obliquely at the apex and usually broadened and spatulate (spoon-like) distally; aedeagus with elongate, narrow parameres (=gonoforceps), with straight and equally elongated distal lobes; and a median lobe with two subequal small teeth (harpagae), with the distal tooth far from apex (Pardo Alcaide 1950, 1954a, Bologna and Coco 1991, Salvi et al. 2018). Of the species currently included in *Croscherichia*, the assignment of one is particularly controversial: a morphologically peculiar species from the Arabian Peninsula (Bologna and Pinto 2002) that was originally described as *Mylabris (Gorrizia) sonyae* Kaszab, 1983 but that shares a combination of traits with *Hycleus* and *Croscherichia* (Kaszab 1983, Bologna and Pinto 2002, Bologna and Turco 2007, Batelka and Geishardt 2009). Therefore, its inclusion in *Croscherichia* needs to be re-evaluated (Bologna and Turco 2007).

In this work, a new species of *Croscherichia* is described from arid zones of central-eastern Morocco (Missour region) (Figs 1A, B; 2). The new species presents morphological traits that are different from those of any other *Croscherichia* species, and similar to *C. sonyae* (Kaszab, 1983), singular traits that resemble those in other Mylabrini genera. The shape of the mandibles, the macrosculpture and setation of the pronotum, and the elytral design of the new species (Fig. 1C, D) closely resemble those of some North African species of the genus *Ammabris* Kuzin, 1954 [*Ammabris* was considered a subgenus of *Mylabris* but is now treated as a genus following the proposal by Salvi et al. (2018)].

The fauna of Mylabrini is relatively well known in Morocco (e.g., Martínez de la Escalera 1909, 1914, Pardo Alcaide 1954a, 1954b, 1954c, 1961, 1969, Kocher 1956, 1964, 1969, Ruiz 2000, 2004, Ruiz and García-París 2008, 2014, Bologna 2008); therefore, it is remarkable that the new species remained undescribed for so long, an indication of the ongoing lack of zoological knowledge for the arid and semi-arid eastern regions of the country. The imaginal phenology of the new species is restricted to the end of summer, an unusual period for entomological surveys of North African arid zones, which may explain its late discovery.

Material and methods

A total of 19 dry-preserved (5 males and 14 females) and 4 ethanol-preserved (2 males and 2 females) specimens were used to describe the new species of *Croscherichia*. The specimens were collected from three nearby localities (separated by a maximum distance of 35 km) in the region of Missour (Fès-Boulemane, Morocco) (Fig. 2). Of the 23 studied specimens, 7 were collected in pitfall traps (in 2002) and 16 by hand while they were actively feeding on *Atriplex halimus* L. (in 2015). Samplings were carried out as part of several projects on the entomological diversity of arid areas in eastern Morocco that were sponsored by the Emirates Center for Wildlife Propagation

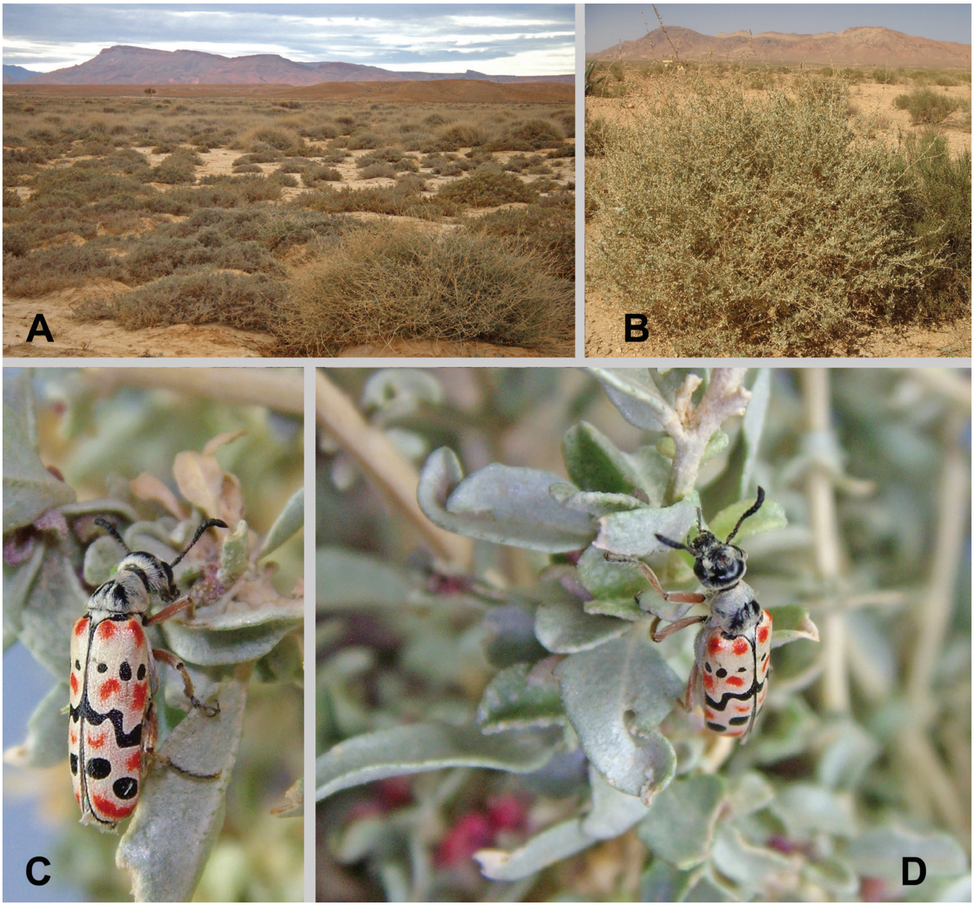
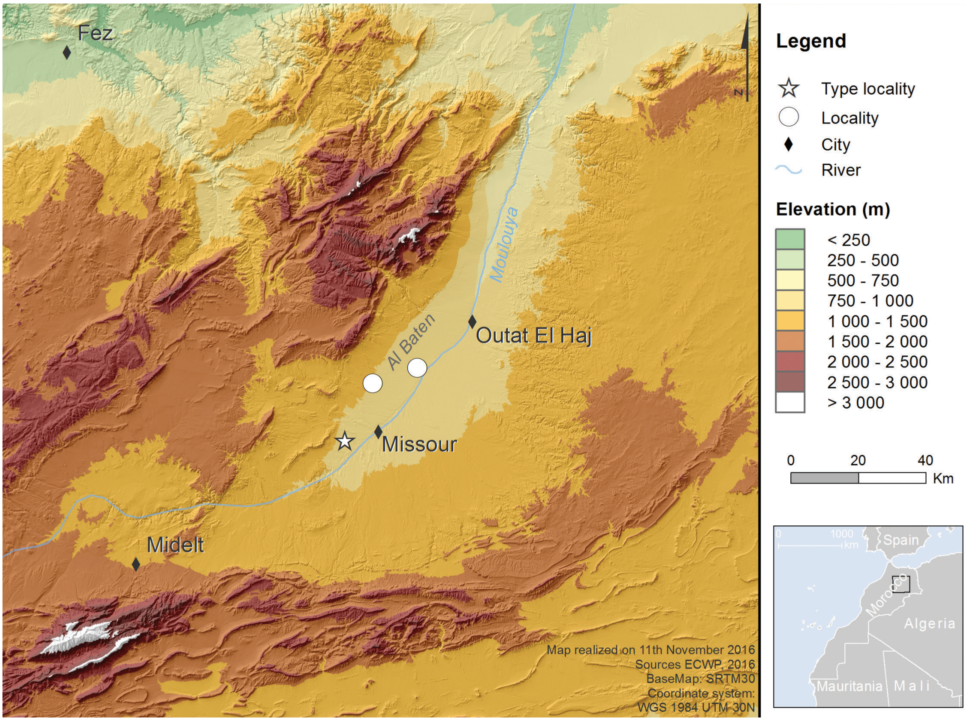


Figure 1. Habitat and live specimens of *Croscherichia armass* sp. nov. **A** thickets of saltbush (*Atriplex halimus*) in an alluvial silt plain of the Al Baten area between Missour and Outat el Haj **B** detail of *Atriplex halimus* L. where the species is usually found. Emirates Center for Wildlife Propagation (ECWP, Missour) **C, D** active specimen on *Atriplex halimus* L. (ECWP, Missour). Photographs by AF.

(ECWP). The type series has been deposited in the following collections: the ECWP collection (Missour, Morocco) (9 paratypes, all dry-preserved); the Museo Nacional de Ciencias Naturales (MNCN-CSIC) (Madrid, Spain) (the holotype and 9 paratypes, 5 dry-preserved and 4 in ethanol); and the J. L. Ruiz collection (JLR) (Ceuta, Spain) (4 paratypes, all dry-preserved).

Interspecific comparisons were performed using the material indicated below (308 specimens), including specimens of all known species of *Croscherichia* except *C. femorata* (Klug, 1845) from Arabia, *C. quadrizonata* (Fairmaire, 1875) from eastern Algeria, Tunisia, and Libya, and the aforementioned problematic *C. sonyae*. The diagnostic morphological traits of these three species were extracted from Kaszab (1983), Bologna and Coco (1991), and Bologna and Turco (2007). Additional acronyms or abbreviations: HNHM, Hungarian Natural History Museum (Budapest, Hungary) and ex./x., exemplar/s.



Map realized on 11th November 2016
Sources ECWP, 2016
BaseMap: SRTM30
Coordinate system:
WGS 1984 UTM 30N

Figure 2. Map of the Missour area in eastern Morocco, showing the type locality of *Croscherichia armass* sp. nov. (star) and the two other localities where the species occurs (circles).

Material examined: ***Croscherichia albilaena*** (Bedel, 1899): ALGERIA: Biskra, L. Bleuse, Mai 1885 (Slg. R. Oberthür, Coll. E. Martin, Eing. Nr. 4 1956, *Croscherichia albilaena* Bedel, Dr Kaszab det. 1957): 1 ex. (HNHM). ***Croscherichia bedeli*** (Bleuse, 1899): ALGERIA: Aïn-Sefra, Mai 1896, A. Chobaut (Coll. Reitter, *Mylabris bedeli* Bleuse, det. Dr Kaszab, *Croscherichia bedeli* Bleuse, Dr Kaszab det. 1957): 4 exx. (HNHM). MOROCCO: Figuig, 29-V-1991, G. Chavanon leg.: 2 exx. (JLR); Figuig, 21-V-1993, G. Chavanon leg.: 3 exx. (JLR); Figuig, erg à *Aristida pungens*, 10-V-1997, G. Chavanon leg.: 15 exx. (JLR); Figuig, 14-V-1999, G. Chavanon leg.: 9 exx. (JLR). ***Croscherichia delarouzei*** (Reiche, 1865): SYRIA: Syrien, Kafa, Reitter (Coll. Reitter, *Croscherichia delarouzei* Reich., Dr Kaszab det. 1957): 1 ex. (HNHM). ***Croscherichia fulgurita*** (Reiche, 1866): ALGERIA: Aïn-Sefra (Oran), L. Bleuse (Coll. Reitter, *Mylabris fulgurita* Reiche, det. Dr Kaszab, *Croscherichia fulgurita* Reiche, Dr Kaszab det. 1957): 1 ex. (HNHM). MOROCCO: Figuig, 14-V-1999, G. Chavanon leg.: 1 ex. (JLR). ***Croscherichia gilvipes*** (Chevrolat, 1840): EGYPT: Le Caire Hénon (Slg. R. Oberthür, Coll. E. Martin, Eing. Nr. 4 1956, *Croscherichia angulata* Klug, Dr Kaszab det. 1959): 1 ex. (HNHM). MOROCCO: Bouârfa, 20-V-1993, G. Chavanon leg.: 3 exx. (JLR); Bouârfa, 23-V-1993, G. Chavanon leg.: 5 exx. (JLR); Figuig, erg à *Aristida pungens*, 10-V-1997, G. Chavanon leg.: 1 ex. (JLR); Route Bouàrfa-Figuig, 32.47915/-1.74761, 1170 m, 24-V-2015, A. François, M. García-

París & J.L. Ruiz leg.: 2 exx. (ECWP & JLR). TUNISIA: Tunisia Reitter (Coll. Reitter, *Croscherichia gilvipes* ab. Dr Kaszab det. 1957): 1 ex. (HNHM). ***Croscherichia goryi*** (Marseul, 1870): IRAN: Midjan, 9-V-1969, Paz-Hasch: 1 ex. (HNHM). PALESTINE: Palestine, Wadi Fukra, IV-1945, leg. Bylinski-Salz (*Mylabris goryi* Mars., det. Kaszab 1956, *Croscherichia goryi* Mars., Dr Kaszab det. 1957): 1 ex. (HNHM). ***Croscherichia litigiosa*** (Chevrolat, 1840): MOROCCO: Bouârfa, 32.53565/-2.55283, 8-V-2008, S. Touil leg.: 7 exx. (ECWP); Bouârfa, 32.40921/-2.59711, 15-V-2008, S. Touil leg.: 8 exx. (ECWP); Bouârfa, 32.40921/-2.59711, 29-V-2008, S. Touil leg.: 5 exx. (ECWP); Tamlelt, Bouârfa, 32.42330/-2.58946, 1056 m, 6-V-2015, S. Touil leg.: 1 ex. (ECWP); Road Bouàrfa-Figuig, 32.25457/-1.71231, 1275 m, 24-V-2015, A. François, M. García-París & J.L. Ruiz leg.: 4 exx. (ECWP & JLR); Mengoub, Road Bouârfa-Bouanane, 32.2695/-2.35015, 1002 m, 26-V-2015, A. François, M. García-París & J.L. Ruiz leg.: 12 exx. (ECWP & JLR). ***Croscherichia mozabita*** (Pic, 1897): MOROCCO: Figuig, 10-V-1997, G. Chavanon leg: 3 exx. (JLR); Anoual, 32.64221/-3.12263, 1365 m, 24-V-2008, A. François leg.: 7 exx. (ECWP); Sud Matarka, 32.62186/-2.85240, 1193 m, 24-V-2008, A. François leg.: 1 ex. (ECWP); Bouârfa, 32.48182/-2.72814, 5-VI-2008, S. Touil leg.: 5 exx. (ECWP); Bouârfa, 32.48212/-2.7282, 12-VI-2008, S. Touil leg.: 6 exx. (ECWP); Bouârfa, 32.40936/-2.59689, 29-V-2008, S. Touil leg.: 2 exx. (ECWP); Bouârfa, 32.26789/-2.19231, 25-VI-2008, S. Touil leg.: 2 exx. (ECWP); Entre Outat el Haj et Matarka, 33.51521/-3.47590, 886 m, 13-VI-2010, A. François leg.: 4 exx. (ECWP); Road Bouàrfa-Figuig, 32.47915/-1.74761, 1170 m, 24-V-2015, A. François, M. García-París & J.L. Ruiz leg.: 14 exx. (ECWP & JLR); Mengoub, Road Bouârfa-Bouanane, 32.2695/-2.35015, 1002 m, 26-V-2015, A. François, M. García-París & J.L. Ruiz leg.: 13 exx. (ECWP & JLR). ***Croscherichia paykulli*** (Billberg, 1813): MOROCCO: Zaio, prov. Nador, V-1975, C. Peláez leg.: 2 exx. (JLR); Tleta de Oued Laou, prov. Tetouan, 9-VI-1991, J.M. Ávila leg.: 19 exx. (JLR); Dar Driouch, Oued Kert, prov. Nador, 12-VI-1991, J.M. Ávila leg.: 2 exx. (JLR); Crrtra. Midelt-Rich, Col du Tarhemt, 1900 m, 26-VI-1992, J.M. Vela leg. 1 ex. (JLR); Ifrane, Medio Atlas, 29-VI-1992, J.M. Ávila leg.: 2 exx. (JLR); Castillo Karia-Arkemane, prov. Nador, 29-VI-1996, J.M. Guzmán leg.: 11 exx. (JLR); Larache, cercanías de Lixus, 10-V-1997, J.L. Ruiz leg.: 4 exx. (JLR); Guefoüt, 26-V-1998, G. Chavanon leg.: 1 ex. (JLR); Oujda, Route de Toussit, 15-VI-2000, G. Chavanon leg.: 13 exx. (JLR); Missour Al Baten, 33.23047/-3.87382, 13-VI-2002, J. Yvernault leg.: 1ex. (ECWP); La Mamora, Kenitra, 23-V-2004, J.L. Ruiz leg.: 13 exx. (JLR); Missour ECWP, 33.00722/-4.09760, 954 m, 21/05/2010, A. François leg.: 3 exx. (ECWP); Massa, Souss-Massa National Park, 13-IV-2009, F.J. Martínez leg.: 5 exx. (JLR); Missour ECWP, 33.00722/-4.09760, 954 m, 17/06/2009, H. Hdidou leg.: 1 ex. (ECWP); Marismas del Oued Lucus, Larache, 4 m, 23-V-2009, J.L. Ruiz leg.: 7 exx. (JLR); Sidi Bou-Ghaba, Mehdia, Kenitra, 5 m, 24-V-2009, 5 exx. (JLR) Missour ECWP, 33.00722/-4.09760, 954 m, 21/05/2010, A. François leg.: 3 exx. (ECWP); Entre Ain Bni Mattar et Maatarka, 34.03349/-2.16300, 967 m, 5-VII-2012, A. François & M. Sbai leg.: 1 ex. (ECWP); Maatarka, Oued Sidi Ali, 33.35562/-2.77507, 1225 m, 13-VI-2013, A. François & L. Castro leg.: 2 exx.

(ECWP); Missour ECWP, 33.00722/-4.09760, 954 m, 12/05/2014, S. Boullenger leg.: 2 exx. (ECWP); Outat Ouled el Haj, 33.34978/-3.66961, 812 m, 21-V-2015, A. François, M. García-París & J.L. Ruiz leg.: 5 exx. (ECWP & JLR); Maatarka, road to Debdou, 33.71822/-3.03126, 1338 m, 22-V-2015, A. François, M. García-París & J.L. Ruiz leg.: 1 ex. (ECWP & JLR); Entre El Ateuf et Debdou, 33.85486/-3.03628, 1484 m, 22-V-2015, A. François, M. García-París & J.L. Ruiz leg.: 7 exx. (ECWP & JLR); Missour ECWP, 33.00467/-4.09993, 965 m, 23-VI-2015, A. François leg.: 2 exx. (ECWP). ***Croscherichia richteri*** Kaszab, 1957: IRAN: Belutschistan Jranshar Dünen Nordwest Rig Ispakeh 2-IV-1954 Richter ü Sehäuffele leg. // **Paratypus** female *Croscherichia richteri*, m., det. Dr Kaszab, 1956 // *Croscherichia richteri* Kaszab, Dr Kaszab det. 1957: 2 exx. (HNHM). ***Croscherichia salavatiani*** Kaszab, 1968: IRAN: 62 km 300 S Iranshar 14-IV-1965 // Museum Paris Mission Franco-Iranienne 1965 // **Paratypus** 1967 *Croscherichia salavatiani* Kaszab // *Croscherichia salavatiani* Kaszab, Dr Kaszab det. 1957: 1 ex. (HNHM); Iran Bandar Abbas 29-III-1965 25 à l'ouest près de la cote // Museum Paris Mission Franco-Iranienne 1965 // **Paratypus** 1967 *Croscherichia salavatiani* Kaszab // *Croscherichia salavatiani* Kaszab, Dr Kaszab det. 1957: 1 ex. (HNHM). ***Croscherichia sanguinolenta sanguinolenta*** (Olivier, 1811): EGYPT: Aegyptus Reitter (Coll. Reitter, *Croscherichia sanguinolenta* Ol., Dr Kaszab det. 1957): 1 ex. (HNHM). MOROCCO: Erfoud, Tafilalet, V-1998, Bouraada leg.: 2 exx. (JLR); Figuig, 10-V-1997, G. Chavanon leg.: 7 exx. (JLR); Figuig, Defilia, 11-V-1997, G. Chavanon leg.: 2 exx. (JLR); Figuig, 14-V-1999, G. Chavanon leg.: 2 exx. (JLR); Road Bouârfa-Tamlelt, 32.39393/-2.18189, 1055 m, 25-V-2015, A. François, M. García-París & J.L. Ruiz leg.: 10 exx. (ECWP & JLR). ***Croscherichia tigrinipennis*** (Latreille, 1827): EGYPT: Aegypte (Coll. Reitter, *Croscherichia tigrinipennis* Latr., Dr Kaszab det. 1957): 1 ex. (HNHM). MOROCCO: Merzouga, 15-V-1998, Bouraada leg.: 1 ex. (JLR); Road N-12 Alnif-Rissani, 31.230172/-4.736069, 815 m, 12/04/2017, J.M. Vela & G. Bastazo leg.: 5 exx. (JLR). ***Croscherichia vigintipunctata*** (Olivier, 1811): EGYPT: Le Caire, Hénon (Slg. R. Oberthür, Coll. E. Martin, Eing. Nr. 4 1956, *Croscherichia vigintipunctata* Ol., Dr Kaszab det. 1957): 4 exx. (HNHM). ***Croscherichia wartmanni*** (Pic, 1896): ALGERIA: Ain Sefra Coll. Reitter // **Paratypus** 1896 *Zonabris wartmanni* Pic. // *Croscherichia wartmanni* Pic, Dr Kaszab det. 1957: 1 ex. (HNHM); Aïn-Sefra (Slg. R. Oberthür, Coll. E. Martin, Eing. Nr. 4 1956): 1 ex. (HNHM). MOROCCO: Figuig, 16-V-1995, G. Chavanon leg: 1 ex. (JLR); Figuig, 10-V-1997, G. Chavanon leg: 4 exx. (JLR); Figuig, dunes à *Aristida*, 18-IV-1998, G. Chavanon leg: 3 exx. (JLR).

The morphological study was carried out on dry-mounted specimens using stereomicroscopy. Male specimens were rehydrated prior to the extraction of their genital structures, which were subsequently mounted on cardboard with dimethylhydantoin formaldehyde resin and pinned adjacent to their respective specimen. Measurements were taken using a micrometre coupled to one of the eyepieces, and camera lucida drawings were made of the structures. The ethanol-preserved paratypes were not measured to prevent possible tissue deterioration. Total specimen length was measured along the dorsal side from the anterior end of the labrum (with the head extended) to the elytral apex. Maximum width was measured as the width between the outer edges of the elytra at approximately three-fourths of the elytral length, also in dorsal vision. Photographs of live and recently dry-mounted specimens were taken with a digital camera. The terminology suggested by Pardo Alcaide (1948, 1950, 1954a, 1954c) and Bologna (1991) was used to describe the various parts of the male genitalia.

In the present study, the evolutionary species concept (as modified by Wiley 1978, 1981 and Wiley and Mayden 2000, see Ruiz and García-París 2015) was adopted to define species. Under this concept, a species can be defined as a unique lineage that maintains its identity with respect to other lineages and that presents its own evolutionary tendencies and historical fate. This concept combines the basic methodological implications of the phylogenetic species concept, in which lineages are defined objectively by the existence of reciprocal monophyly, with subjective aspects that allow for the characterization of the phyletic line, or species, as an independent unit. These aspects, evaluated in light of an informed hypothesis about the evolutionary future of the lineage, are critical for determining whether an independent lineage can be considered as a species (García-París et al. 2008).

For practical purposes and to separate groups of morphologically similar species of *Croscherichia*, the taxonomic scheme in the species key of Bologna and Coco (1991) was used. However, the resulting clusters may not correspond to monophyletic entities. The specific and subspecific composition of the genus proposed by Bologna and Coco (1991) and Bologna (2008) was also followed in the present study.

Results

Croscherichia armass Ruiz, François & García-París, sp. nov

http://zoobank.org/46F4A3E2-5A2D-451E-B059-FC39A8D9094E
Figs 1A–D, 3A–D, 4, 5A, B, 6A–D, 7

Type material. ***Holotype***: 1 male (dry-preserved) (Fig. 3), labelled: "10/09/2015, Missour ECWP, 33.00722/-4.0977600, A. François" / "954 m, Steppe à *Hammada scoparia*, Sur fleurs d'*Atriplex halimus*" (ivory labels, printed); "Holotypus, *Croscherichia armass* Ruiz, François et García-París des. 2018" (red label, printed). ***Paratypes***: 2 males, 4 females (dry-preserved), labelled: "12/09/2002, Missour Al Baten, 33.16433/-4.01064, J. Yvernault" / "032P1-2 BD019, Surface dépandage, *Salsola vermiculata*" (ivory labels, printed); 1 female, labelled: "13/09/2002, Missour Al Baten, 33.20696/-3.8697, J. Yvernault" / "Piège Barber 09p4-3, Surface d'epandage, *Salsola sieberi* et *Peganum hamala*, B032AB" (ivory labels, printed); 4 males, 11 females (2 males and 9 females dry-preserved, 2 males and 2 females preserved in ethanol), labelled: "10/09/2015, Missour ECWP, 33.00722/-4.0977600, A. François" / "954 m, Steppe à *Hammada scoparia*, Sur fleurs d'*Atriplex halimus*" (ivory labels, printed). All paratypes labelled: "Paratypus, *Croscherichia armass* Ruiz, François et García-París des. 2018" (red labels, printed).

Description of holotype (male): ***Total length***: 9.1 mm. Maximum width: 2.85 mm. General appearance elongated, stylized (Fig. 3). General coloration of the

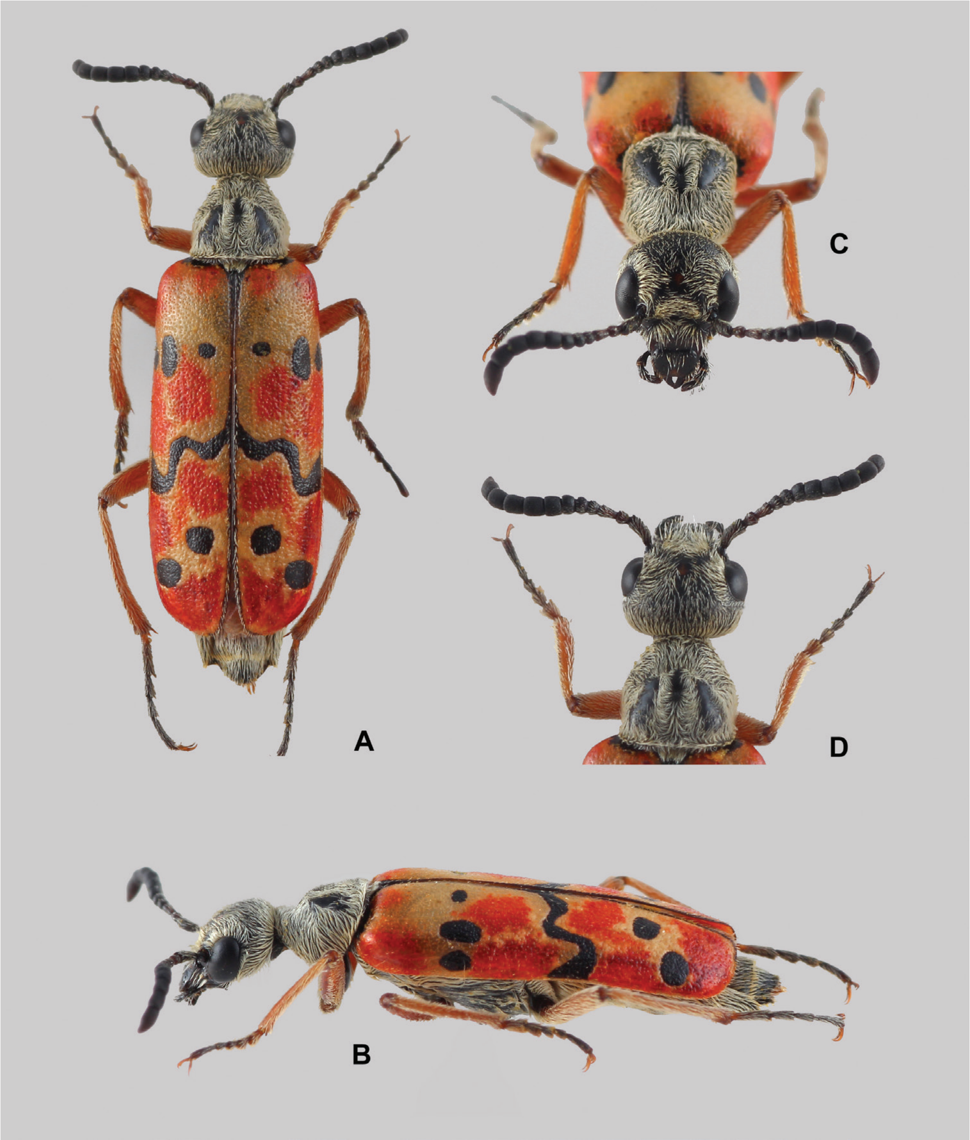


Figure 3. Holotype of *Croscherichia armass* sp. nov. **A** dorsal view **B** lateral view **C** frontal view **D** dorsal view of head and pronotum. Photographs by A. Sánchez-Vialas.

body tegument black, with orange legs, except tarsi that are chestnut brown, almost black. Antennae black, with antennomeres II, III, and the central area of I brownish. Elytra tricolored, reddish orange, with bands and black spots widely fringed with a halo of yellowish-ivory coloration lighter than the rest of the elytral integument; the contrast in coloration is especially noticeable *in vivo*. Very dense, greyish-silver body pubescence applied against the integument and mostly masking it, giving the body (except the elytra) a silvery grey appearance; pubescence on the elytra very short, with very fine and erect hairs.

Head (Fig. 3B, C, D) widely rounded and broad in frontal view, without grooves or depressions, regular and weakly arched at vertex level, relatively narrow and short in lateral view; maximum width in front view: 1.75 mm; vertex to clypeo-frontal suture length: 1.26 mm; minimum width between the eyes (at the level of the front): 1.1 mm; short and broadly rounded temples, with a "maximum eye width (smaller diameter) to temple length" ratio of 1.02. Tegumentary surface glossy black with very thin microreticulation, presenting a small circular red spot that is diffuse but highly visible in the centre of the forehead at the level of the upper lobes of the eyes. Eyes convex but not very protuberant, slightly projecting from the natural convexity of the head, weakly notched at the level of the antennal insertions and with a very fine perimeter rim; larger diameter: 0.87 mm, smaller diameter: 0.51 mm. Forehead flat, except for a very small and diffuse central gibbosity at the level of the red spot, with a broad central zone, smooth and shiny, with a triangular outline, at the posterior end of which is the red spot. Vertex broadly arched, without a central longitudinal groove. Net, deep, arched clypeo-frontal suture. Clypeus transverse, 1.91 times wider than long, black, with the anterior half semimembranous and impunctate. Labrum relatively elongated, 1.4 times wider than long, with arcuate sides and the anterior margin slightly notched in the middle, with a narrow, weak, and diffuse central longitudinal line; black, except for an antero-central triangular zone with a semimembranous brownish-grey appearance, located just behind the notch. Cephalic capsule with fine but net and deep, dense punctures that are spaced apart by 0.5 to 1.0 times their diameter, except in the smooth disc region of the forehead; the highest density of punctures appears between the smooth zone and the inner margins of the eyes, as well as behind them and in a transverse band between the clypeo-frontal suture and the posterior margin of the antennal insertions; the lowest density occurs in the posterior region of the temples and vertex. Punctures of the clypeus restricted to the posterior half, puncture points slightly thicker and denser than those on the forehead, contiguous, almost masked by the dense setation; punctures of the labrum similar to that of the clypeus in a narrow basal region but thicker and scattered on the rest of the surface. Setation of the head greyish silver, relatively dense and semi-erect or stretched (according to zones), following the pattern of the punctures in which it is inserted; longer and denser setation around the eyes, side areas of the temples, and in a transverse strip located between the clypeo-frontal suture and the posterior margin of the antennal insertions; shorter and less dense setation on the sides and posterior regions of the frons and vertex; setation of the vertex and the posterior area of the frons, stretched and forward-facing; setation of the sides of the forehead and the bands around the eyes semi-erect and directed towards the smooth disc; the setation inserted into the anterior transverse band of the forehead (between the antennal insertions and the clypeo-frontal suture), semi-erect and directed symmetrically toward the centre (of each half); setation of the temples mostly lying on the integument and directed forward, except for some long erect hairs located along the latero-ventral region that are directed downwards. Setation of the clypeus restricted to the posterior half, following the pattern of the punctures in which it is inserted, dense, semi-erected, and directed forward, with longer hairs on the sides and shorter hairs in the centre. Setation of the labrum very scarce and constituted by some hairs that are dispersed, fine and semi-erect, similar to those of the clypeal sides. Ventral region of the head, except the sides, smooth and hairless. Mandibles black, shiny, narrow, with their outer margins almost straight, except at the apical end where they are slightly curved; almost entirely hidden by the labrum in dorsal view. Maxillary palps short and black, with scarce setation similar to that of the labrum and subcylindrical segments slightly widened toward the extremity, the basal segment very short and the distal one truncated at the apex. Labial palps short and black with subconical palpomeres, the basal palpomere very small and the distal one truncated at the apex and slightly thickened towards the end. Dark brown ligule, notched at its anterior margin and with a deep longitudinal groove.

Antennae (Fig. 3C, D) with 11 antennomeres: antennomere I black, with a dark brown coloration in the central zone; antennomeres II, III, and the basal zone of IV dark chestnut, bright; antennomeres IV (except in a basal ring) to X, black; antennomere XI black, with a dark brown area in the apex; antennomeres IV and V shiny, antennomeres VI to XI less shiny. Antennae relatively long, and when extended backwards, they reach the third quarter of the pronotum, weakly and gradually thickening toward the end from antennomere V. Length of the right antenna (extended): 2.92 mm; antennomere I elongated (length, l = 0.56 mm; maximum width, w = 0.19 mm; l/w: 2.94), slightly curved in the middle and slightly thickened at the end, with semi-lying, whitish, setation similar to that of the labrum but shorter; antennomere II short (l = 0.18 mm; w = 0.17 mm; l/w = 1.05), subcylindrical in the basal half and globose in the distal half, with setation similar to antennomere I but shorter; antennomere III narrow, elongated (l = 0.27 mm; w = 0.18 mm; l/w = 1.5), subconical, with pubescence similar to antennomere II; antennomeres IV (1 = 0.17 mm; w = 0.2 mm; l/w = 0.85) and V (1 = 0.18 mm; w = 0.24 mm; l/w = 0.75), short and wide, transverse, with setation similar to antennomere III but with scarcer and shorter hairs; antennomere VI transverse, wide (1 = 0.21 mm; w = 0.26 mm; l/w = 0.8), with very few hairs similar to those of antennomere V; antennomere VII subcylindrical, slightly wider at the distal end (1 = 0.24 mm; w = 0.26 mm; l/w = 0.92); antennomeres VIII (1 = 0.26 mm; w = 0.28 mm; l/w = 0.92), IX (1 = 0.28 mm; w = 0.26 mm; l/w = 1.07) and X (1 = 0.3 mm; w = 0.29 mm; l/w = 1.03) cylindrical and subequal; antennomere XI (1 = 0.5 mm; w = 0.27 mm; l/w = 1.85) cylindrical in the basal half and conical in the distal half, with a blunt tip; setation of antennomeres VI to XI hardly noticeable, whitish yellow, and applied to the tegument surface, with a few hairs longer, thin and erect.

Pronotum (Fig. 3A, B, D) with glossy black tegument, with hardly noticeable fine microrreticulation; with a predominantly silver appearance due to the dense setation that covers the pronotal surface, except for a central depression and two smooth zones at its sides; almost as long as wide; length along the mid-line: 1.62 mm; maximum width, measured between the second and third pronotal fifth (at the level of the lateral angles): 1.65 mm; shape subquadrangular to trapezoidal in dorsal view, weakly truncated anteriorly; in lateral view, markedly sloping from lateral angles forward, backwards smoothly convex; lateral margins converging in the anterior-most two fifths and sinuated in the posterior-most three fifths; lateral angles well marked, protruding in dorsal view and rounded, like the anterior ones; posterior angles obtuse and rounded; basal region very weakly notched in the centre, directly facing the scutellum, with a very fine rim, masked by a line of dense, short, and semi-erect hairs directed towards the centre. Pronotal macrosculpture constituted by a central depression (located approximately in the pronotal third fifth) that appears as a longitudinal fossa with fuzzy borders, smooth, with a length of approximately 0.34 mm and a maximum width of 0.14 mm, and two broad slightly elevated symmetrical areas, smooth and hairless, ovoid in outline, widest in the posterior area, obliquely located on both sides of the discal depression, almost reaching the pronotal basis. Pronotal sculpture barely visible, mostly hidden by dense setation, consisting of thin, net punctures similar to those of the forehead, dense, subconfluent, uniformly distributed throughout the surface except for the central depression and the two smooth areas. Setation silvery, dense, relatively long and thick, mostly applied against the pronotal surface, and distributed according to the pattern of punctures in which it is inserted; setation directed backwards in the anterior-most two fifths, towards the centre on the sides, and forward in the posterior fifth; the setation surrounding the central depression is semi-erect and radially and outwardly directed; the setation located along the inner margins of the smooth areas is semi-eccentric and directed forward; the ends of the hairs from these two areas (central depression and smooth areas) overlap, giving rise to two bands of hairs with the appearance of a long tuft.

Scutellum hemi-elliptic, rounded along its posterior margin, with black and shiny integument, very densely and finely punctured, with a setation that completely covers the surface, similar to that of the pronotum, giving it a silvery appearance.

Elytra (Fig. 3A, B) elongate, subparallel, with the humeral region slightly protruding and broadly rounded; length from the base of the pronotum to the apical end: 6.15 mm; maximum width, measuring both elytra together at the third quarter: 2.9 mm; semi-glossy surface; background coloration reddish orange, with a band and several black spots with well-defined contours surrounded by a broad, diffuse, yellowish-ivory halo; with the following design: (1) a small basal hemi-elliptic black spot contiguous to the humeral (prehumeral) region, which is almost completely covered by the posterior angular margins of the pronotum, that continues toward the centre as a thick line that borders the scutellum and, from the end of it, extends towards the elytral suture; (2) an anterior transverse series of three elliptic spots, clearly separated, aligned and located approximately in the posterior area of the anterior third of the elytron; the inner spot almost rounded and well separated from the suture, with its major axis (Ma) positioned transversely (Ma = 0.32 mm; minor axis, ma = 0.24 mm, measured on the left elytron); the central spot, closer to the external spot, is the largest, with its Ma positioned longitudinally (Ma = 0.70 mm, ma = 0.40 mm); the external spot, similar to but smaller than the central one (Ma = 0.50 mm, ma = 0.36 mm), is located in the lateral declivity of the elytron, barely visible dorsally and well separated from the external edge of the elytron; (3) a narrow central zigzagging band (width in the middle = 0.30 mm, maximum width in the contact zone with the elytral edge =

0.68 mm) located slightly behind the centre of the elytron; this band joins the elytral suture and external edge; (4) two large posterior blotches, obliquely arranged, located in the posterior third of the elytron; the internal elliptical blotch (Ma = 0.62 mm, ma = 0.48 mm) is positioned anterior to the external, almost rounded one (Ma = 0.49 mm, ma = 0.46 mm) and well separated from the elytral edge; and (5) a thin black longitudinal line running through the entire suture and extending through the inner half of the elytral apex, its widest point is just below the scutellum in the area of attachment to the median band. Elytral punctures relatively coarse, subconfluent, dense and uniformly distributed over most of the elytral surface except in the periscutellar and humeral region where they are less dense and smaller. Setation of the elytral surface scarcely perceptible but deciduous and composed of very short yellowish-white hairs, directed vertically or subvertically; suture and elytral margin with a line of longer hairs (approximately two to three times the length of surface hairs), lying backwards.

Mesopleurae without a careniform fold or rim along the anterior margin; setation dense and directed backwards, covering most of the mesopleural surface except along the central zones contiguous to the mesosternum, which are smooth and almost hairless. Mesosternum (Fig. 4) black, shiny and very finely microreticulated, without a modified anterior medial area (*scutum* or "central shield" sensu Pardo Alcaide 1950, 1954a); slightly elevated at the centre of the anterior edge, with the anterior margins rimmed, forming an open angle; lateral branches of the mesosternum relatively short and narrow; central posterior projection in blunt point between mesocoxae; discal and anterior areas of mesosternum smooth and hairless. Mesosternal punctures fine, scarce, and restricted to mainly the lateral branches and the posterior projection. Setation long, similar to that of the mesopleurae, lying backwards, following the pattern of the punctures in which they are inserted.

Ventral region of the body with black integument, shiny, finely microreticulated, with fine and very dense punctures, subconfluent, hidden under the setation; setation very dense, evenly distributed and longer than that of the pronotum, giving the body a silver appearance except along the narrow mid-longitudinal band of the metasternum, which is smooth and hairless. Last abdominal ventrite with a deep and wide V-shaped central notch in its posterior margin, with a setation much less dense than the rest of the abdominal ventrites.

Legs thin and narrow, with reddish-orange femora and tibiae, somewhat obscured distal ends, brownish-red trochanters that are slightly orange at the ends, black coxae that turn brownish red towards the apex; tarsi, dark brown almost black, except for the first protarsomere and the basal half of the first meso- and metatarsomeres, which are reddish brown; relatively short protarsi (length excluding claws = 1.39 mm), with protarsomere I short and wide, subconical in dorsal view (length, l = 0.30 mm; maximum width, w = 0.22 mm; l/w = 1.36), protarsomeres II (l = 0.22 mm; w = 0.16 mm; l/w = 1.37), III (l = 0.20 mm; w = 0.16 mm; l/w = 1.25) and IV (l = 0.18 mm; w = 0.12 mm; l/w = 1.5) subequal to protarsomere I, although gradually becoming smaller; protarsomere V (l = 0.5 mm; w = 0.14 mm; l/w = 3.57) subcylindrical, narrow and elongated; mesotarsi similar to but slightly more elongated (l = 1.58 mm) than protarsi

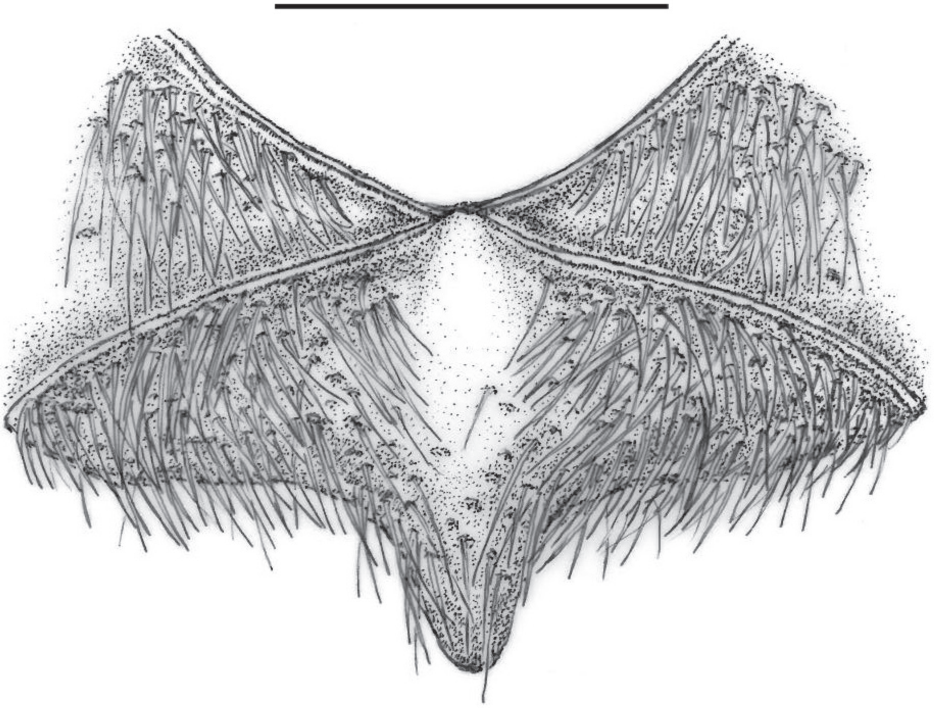


Figure 4. Mesosternum of *Croscherichia armass* sp. nov. Note the absence of a modified anterior medial area (*scutum* sensu Pardo Alcaide 1950). Scale bar: 0.5 mm. Drawing by JLR.

and with slightly narrower tarsomeres; metatarsi longer and narrower than mesotarsi (l = 2.02 mm), with narrower tarsomeres. Leg punctures very dense and thin, slightly smaller than those on the ventral region of the body; setation whitish yellow, dense and thinner and shorter than that of the ventral region, almost lying on the surface, with the highest density on the tibiae and the inferior side of the femora; internal side of the anterior tibiae with a band of whitish hairs shorter and slightly denser than on the rest of the protibiae, hairs on the distal half of the outer carina slightly more erect and denser than the rest, uniform in size, without longer hairs. External apical end of the protibiae terminates in a small or narrow digitiform expansion almost covered entirely by setation. Tarsi with tarsomeres I to V bearing a small hirsute brush on the underside; on tarsomere V, the brush only occupies the proximal half; hairs on the protarsomeres are exclusively white, while those on the meso- and metatarsomeres are a mixture of white and dark brown. Apical spines of pro- and mesotibiae very small and narrow, subequal, with a blunt tip; apical internal spine of the metatibiae similar to that of the pro- and mesotibiae (length = 0.21 mm), the external one slightly longer and thicker (length = 0.22 mm), subcylindrical, obliquely truncated at the apex, weakly spatulate but slightly widened distally (Fig. 5). Brown to orange claws, curved along the apical two-thirds, with a weak lower basal tooth; upper and lower lobes of similar length, the lower lobe narrower.

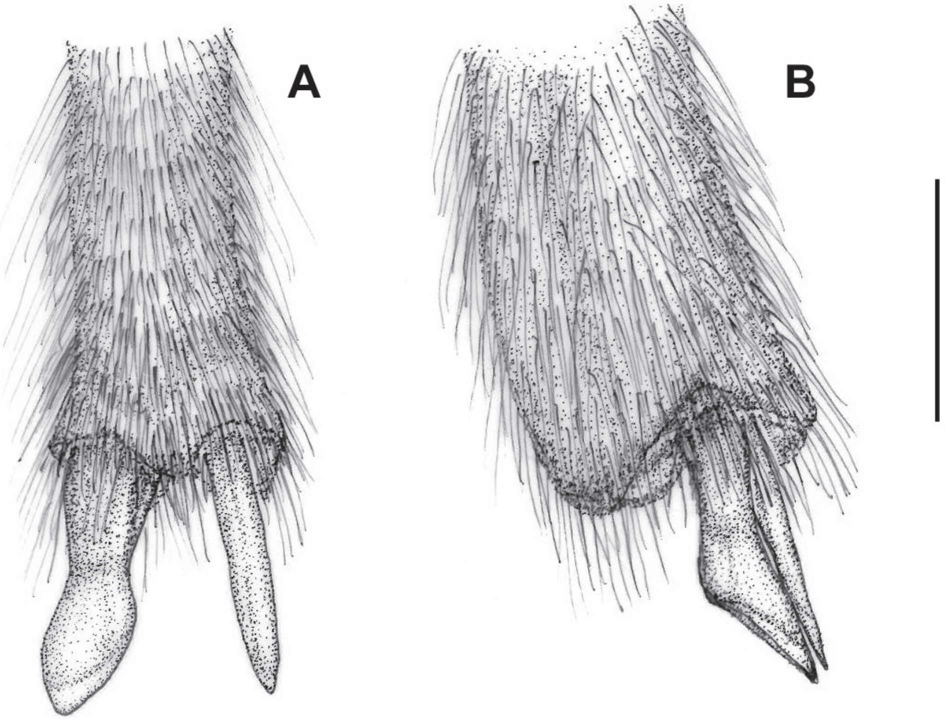


Figure 5. Metatibial spurs of *Croscherichia armass* sp. nov. **A** ventral view **B** lateral view. Scale bar: 0.5 mm. Drawing by JLR.

Aedeagus (Fig. 6A–C) with narrow and elongated parameres, 1.55 times longer than the phalobase, with a deep and narrow central longitudinal cleft in the apical half in dorsal view; parameral lobes slightly curved, having a digitiform aspect in lateral view and narrow, laminar and acuminate towards the apex in dorsal view. Phalobase slightly widened in dorsal view and narrow in lateral view. Middle lobe narrow, with rounded apex, obliquely truncated in its dorsal-apical region and curved in its proximal half, with two evident teeth in the ventral region, subequal and clearly separated (type *isoharpagae* sensu Pardo Alcaide 1948, 1950), near but well distanced from the apex. Dorsal-apical hook (*uncus* sensu Pardo Alcaide 1948, 1950), small, curved at the end, and with a sharp tip. Spiculum gastrale as in Figure 6D.

Female: Similar to the male but differing in the following features: protibiae with a small sharp tooth at the external apical end, with long and fine whitish semi-erect hairs along the outer edge, standing out from the short and lying setation of the surface; external side of the first four protarsomeres with long erect hairs, directed forward, similar to those on the outer edge of the protibiae, which also stand out from the short lying setation; last abdominal ventrite with a posterior margin that is not notched in the middle. Valvifer and stylus as in Figure 7.

Variability. Not very marked but present in the following characters: total length, 7.3 to 10.1 mm, mean 8.6 mm (*N* = 19), males 7.6 to 9.1 mm, mean 8.2 mm (*N* = 5),

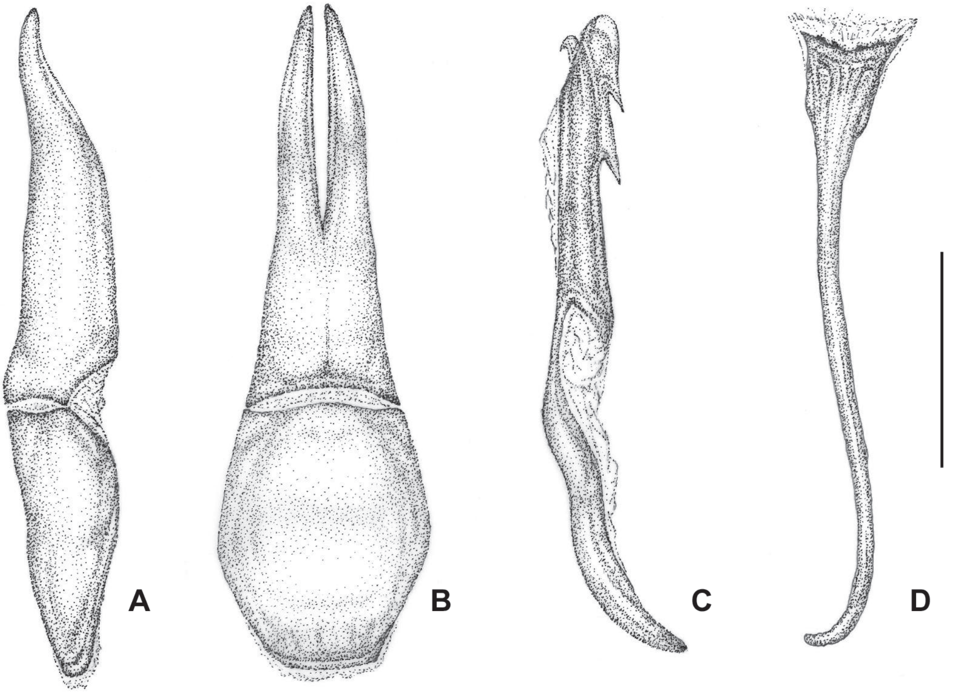


Figure 6. Aedeagus of *Croscherichia armass* sp. nov. **A** tegmen, lateral view **B** tegmen, dorsal view **C** median lobe, lateral view **D** spiculum gastrale. Note the narrow and elongate parameres and the middle lobe with two evident teeth in the ventral region that are subequal and clearly separated (type *isoharpagae* sensu Pardo Alcaide 1948). Scale bar: 0.5 mm. Drawing by JLR.

females 7.3 to 10.1 mm, mean 8.7 mm (*N* = 14); maximum width, 2.2 to 3.7 mm, mean 2.8 mm (*N* = 19), males 2.2 to 2.8 mm, mean 2.6 mm (*N* = 5); females 2.3 to 3.7 mm, mean 2.9 mm (*N* = 14); frontal red spot with little variation in diameter and intensity; chromatic pattern of the antennae variable, particularly in the amplitude of the brown coloration: between antennomeres I and V, both inclusive: 26.3% (*N* = 19) (antennomere I presents a very dark basal region), between antennomeres II and V: 52.6% (sometimes the apical third of antennomere V is black), between II and IV: 5.3%, and between II and III: 15.8%, including the holotype; antennomeres VI–XI always black, although some specimens present a narrow basal brown ring on antennomeres VI and XI; punctures and setation of the head slightly variable in density, especially in the posterior area of the frons and in the vertex, with larger individuals showing greater density; smooth and glabrous pronotal areas that vary to a slight extent; elytral design generally constant, although there is a certain variation in the size of the spots of the anterior and posterior series (in the latter, the two rounded spots are joined in some specimens) and in the thickness of the sinuous median band, which, in some specimens, is interrupted in the middle; the external apical spine of metatibiae in larger specimens is visibly widened in the distal half. No variation is observed in the aedeagus of the studied males.

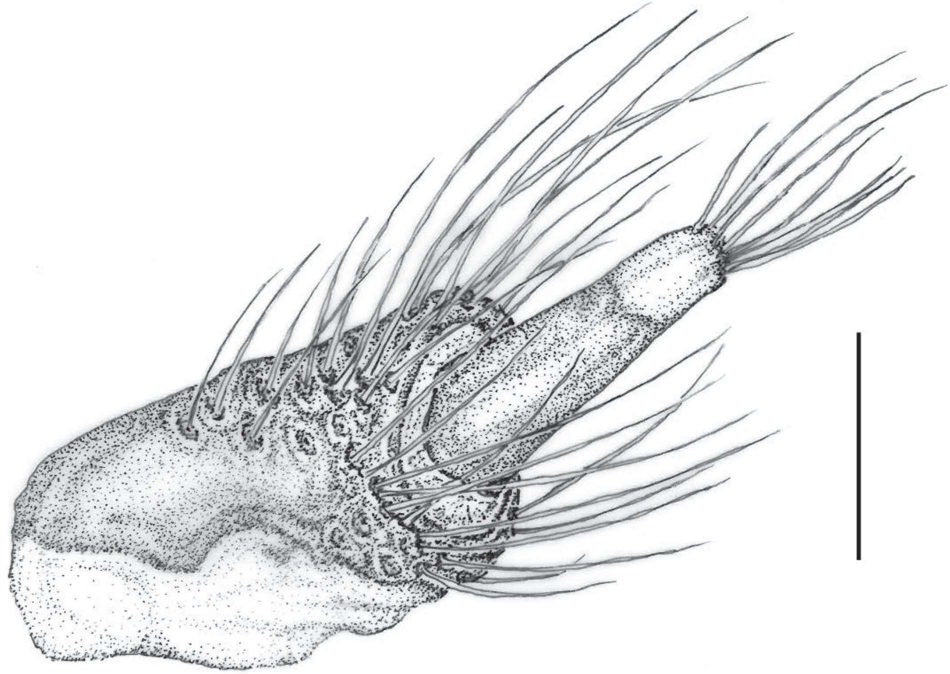


Figure 7. Valvifer and stylus of *Croscherichia armass* sp. nov. Scale bar: 0.5 mm. Drawing by JLR.

The coloration of live specimens varies markedly with that of the preserved specimens (Fig. 1C, D). Contrasts in the elytral coloration pattern are much more evident in live specimens, especially the ivory tones of the background and the reddish-orange coloration, which remains in only a few of the preserved specimens. In the specimens collected in 2002, the reddish coloration has been completely lost, and the elytra only retain a bicolored appearance with a uniform pale background and black spots and bands.

Etymology. The word "armass" refers to the Tamazight (Bereber) name of the plant on which *C. armass* is usually found (*Atriplex halimus*).

Distribution and autoecological notes. *Croscherichia armass* is only known from three nearby localities (separated by a maximum distance of about 35 km) in the region of Missour (Boulemane Province, Fès-Boulemane Administrative Region), east of the Eastern Middle Atlas in the valley or middle section of the Muluya River (Oued Moulouya) (Fig. 2): 1. Interior area of ECWP (Missour), 954 m, 33.01191°N, 4.09693°W. 2. Al Baten 1 (between Missour and Outat el Haj), 830 m, 33.20696°N, 3.8697°W. 3. Al Baten 2 (between Missour and Outat el Haj), 980 m, 33.16433°N, 4.01064°W.

The region is dominated by calcareous sedimentary materials: Quaternary alluvial plains with superficial soils. The area is within the mesomediterranean bioclimatic zone (Michalet 1991) and presents a lower arid ombroclime (mean annual rainfall: 159 mm in Missour, and 153 mm in Outat el Haj) and a marked continentality, particularly during cold winters (Le Houerou 1989). The habitat of the species' area of occupation (*sensu* IUCN 2001) is, in general, steppes with little vegetation. The interior area of the ECWP-Missour) includes mostly open formations known as the Hammada steppes. Typical species occurring in this arid environment include *Hammada scoparia* (Pomel) Iljin, *Launaea arborescens* (Batt.) Murb., *Atriplex halimus* L., *Acanthorrhinum ramosissimum* (Coss. & Durieu) Rothm., and *Echinops spinosissimus* Turra, *Salsola* L. In addition, the species *Retama sphaerocarpa* (L.) Boiss., *Ziziphus lotus* (L.) Lam., and *Tamarix* L. (Quézel et al. 1994) are found along the wadi courses. The vegetation of the ECWP interior area is in an excellent state of conservation and presents a high level of diversity, mainly due to a perimeter enclosure that isolates it from anthropogenic activities, including livestock grazing. This space can, therefore, be considered as an authentic reserve for the region's flora and fauna. The Al Baten area (both localities 1 and 2) (Fig. 1A) has lower vegetation cover and floristic diversity, and is in a poorer state of conservation, compared with the ECWP, a likely consequence of livestock (sheep and goats) grazing. This area is a steppe on an alluvial flood zone comprised of clay substrates (alluvial plain silt) that, depending on precipitation conditions, is used as a cereal field. Two species predominantly dominate the vegetation in Al Baten: *Atriplex halimus* L. and *Salsola gaetula* (Maire) Botsch. The conservation state of the Al Baten 2 vegetation (Fig. 1A) is better than that of Al Baten 1, particularly due to the presence of *Ziziphus lotus* and *Halogeton sativus* (L.) Moq. bushes.

Live *C. armass* specimens can be found in flight and actively feeding on flowers of the Chenopodiaceae *A. halimus* roughly between the hours of 10:00 and 16:00 (UTC). The coloration of *C. armass* blends in with the inflorescences of *A. halimus*, which can make locating specimens difficult. Bologna and Coco (1991) did not report *Croscherichia* feeding on any species of Chenopodiaceae. According to the scarce data available, the active period of *C. armass* adults is restricted to the end of summer, specifically to mid-September, which coincides with the blooming of *A. halimus*. Although the phenology of *C. armass* is not unique for *Croscherichia*, it is exceptional as only one other species, *C. delarouzei*, has been recorded as active in September (Bologna and Coco 1991). Moreover, to date, no other Mylabrini species have been reported as active in late summer in the Missour region. In fact, adults of Mylabrini in this region are only active during spring or, at most, until early summer, coinciding with the main plant flowering period (François, Ruiz and García-París, pers. obs. and in prep.).

Discussion

Croscherichia armass has a very particular combination of morphological characters that distinguishes it from all other species of the genus. It has a relatively small size (mean = 8.3 mm, range: 7.3–10.1 mm); a black head, pronotum, and ventral region; reddish-orange legs with darkened tarsi; black antennae except for the proximal-most three to four antennomeres, which have a dark reddish-brown colour; and a dense and silvery body setation lying over most of the body integument. The broad head has a red spot on the frons and a dense and relatively thick setation on the integument. The outer margins of the mandibles are straight, pointed, and protrude from the labrum (at approximately one third of its length). The antennae are relatively short and black except for antennomeres II or III to V (rarely including antennomere I and the base of VI), which are dark or reddish brown. The pronotum is as broad as it is long and is neither elongated or narrowed at the anterior margin. It is characterized by a weak depression in its anterior third, a marked central longitudinal depression, and two well-defined smooth and glabrous areas lateral to the mid-line. The pronotal setation is silvery, thick, and dense against the integument, masking it for most part. The mesosternum has an angulate anterior margin and is covered with setation lying along its lateral branches. The external metatibial spur is short, subcylindrical, and not spatulate; it is also slightly widened at its distal end and truncated obliquely at the apex. The leg claws have upper and lower lobes of equal length that are markedly curved. The last abdominal sternum of the male has a deep cleft in its posterior margin. The elytral design of *C. armass* is unique within *Croscherichia*: tricolored, with a small prehumeral black spot, a narrow black zigzagging central band, and two rows of black rounded spots. The anterior row is comprised of three spots and the posterior one of two spots that neither touch the suture nor the external margin of the elytron.

The general appearance of *C. armass* resembles that of some North African species of *Ammabris*, particularly *Ammabris boghariensis* (Raffray, 1873) and, to a lesser extent, *A. avilai* (Ruiz & García-París, 2008). The three species are similar in the following features: elytral coloration, mandible morphology (straight and pointed outer margin), the presence of a red spot on the frons, pronotal sculpture (a central longitudinal groove with smooth areas lateral to it), and the overall silvery setation on the integument (see Ruiz and García-París 2008). However, the new species can be assigned to *Croscherichia*, and distinguished from *Ammabris*, by the following characters: the morphology of the external metatibial spur (subcylindrical, a little broadened distally, and truncated at the apex); the lack of a modified antero-central area in the mesosternum ("*mesosternal scutum*" *sensu* Pardo Alcaide 1948, 1950, 1954a, 1954c) that presents an angulate anterior margin; male genitalia with narrow parameres but without longitudinal groves along the parameral lobes; and the presence of two subequal, clearly separated teeth in the ventral region of the median lobe that are close to, but well distanced from, the apex.

According to the identification key of Bologna and Coco (1991), *Croscherichia* species with a black pronotum [the only species with a reddish-orange pronotum is *C. tigrinipennis* (Latreille, 1827)] can be divided into one of two groups based on antennal coloration. *Croscherichia armass* can be included in the group characterized by black or dark brown antennae [i.e., *C. delarouzei* (Reiche, 1866), *C. gilvipes* (Chevrolat, 1840), *C. goryi* (Marseul, 1870), *C. litigiosa* (Chevrolat, 1840), *C. mozabita* (Pic, 1897), *C. paykulli* (Billberg, 1813), *C. quadrizonata* (Fairmaire, 1875), *C. sanguinolenta* (Olivier, 1811), and *C. wartmanni* (Pic, 1896)] versus the one characterized by orange antennal antennomeres [i.e., *C. albilaena* (Bedel, 1899), *C. bedeli* (Bleuse, 1899), *C. femorata* (Klug, 1845), *C. fulgurita* (Reiche, 1866), *C. richteri* Kaszab, 1957, *C. salavatiani* Kaszab, 1968, and *C. vigintipunctata* (Olivier, 1811)].

Croscherichia armass can be further grouped with species having claws with upper and lower lobes of similar length, which includes *C. paykulli*, from the Maghreb (from Morocco to Libya); *C. delarouzei*, from Palestine, Israel, Jordan, and Lebanon; two subspecies of *C. sanguinolenta*, *C. s. sanguinolenta*, widely distributed throughout arid and semi-arid zones of North Africa, Senegal, and the Near East to Iran, and *C. s. arabica* Bologna & Coco, 1991, only known from Saudi Arabia; and *C. gilvipes*, distributed throughout arid regions of North Africa and the Near East (Israel, Jordan, and Syria) (Bologna and Coco 1991, Bologna 2008). *Croscherichia paykulli*, *C. delarouzei*, and the two subspecies of *C. sanguinolenta* can be easily distinguished from *C. armass* as these species have black or dark brown legs, black and hirsute setation (head, pronotum, and ventral region), distinct pronotal macrosculptures that lack smooth and glabrous lateral areas, clearly spatulate external metatibial spurs, and different elytral designs (see Bologna and Coco 1991). *Croscherichia armass* and *C. gilvipes* both have orange coloration on their legs, but the two species differ markedly in many other traits. Compared with *C. armass*, *C. gilvipes* has shorter antennae, jaws with curved external margins, and a whitish body setation that is thinner, slightly denser, and more lanuginose, and that does not conceal the tegument (Bologna and Coco 1991). In addition, the pronotal sculpture of *C. gilvipes* does not have smooth lateral areas, and the external metatibial spur is spatulate. Finally, its elytral setation is longer and denser, and the elytral chromatic design consists of distinctive internal spots that contact the suture (Bologna and Coco 1991).

Croscherichia sonyae, assigned with reservations to *Croscherichia* (Bologna and Pinto 2002), is endemic to Saudi Arabia and the United Arab Emirates (Bologna and Turco 2007, Bologna 2008, Batelka and Geishardt 2009). This species differs markedly from *C. armass*: it has orange antennae in which antennomeres V to VII are subtrapezoidal, making them stand out from the other antennomeres, protarsi with short and wide tarsomeres, overall scarce setation (head, pronotum, and ventral region), and a clearly banded elytral pattern (Kaszab 1983, Bologna and Pinto 2002, Bologna and Turco 2007, Batelka and Geishardt 2009).

Bologna and Coco (1991) based phylogenetic relationships within *Croscherichia* on an analysis of 26 morphological characters, which recovered 12 poorly supported groups. On the basis of morphology, *C. armass* most resembles the *C. delarouzei* and the *C. femorata*-*C. salavatiani* groups. *Croscherichia delarouzei* and *C. armass* share the derived condition of having elongated and pointed mandibles that extend beyond the labrum. *Croscherichia femorata*, *C. salavatiani* and *C. armass* share the derived state of having an unspatulated external metatibial spur. *Croscherichia armass* differs from *C. femorata*, only known from Saudi Arabia, and from *C. salavatiani*, widely distributed from the Arabian Peninsula to Pakistan (Bologna and Coco 2001, Bologna and Turco 2007, Bologna 2008), as these species present orange antennae, a whitish body setation that is much less dense, mandibles with curved outer margins, and a clearly banded elytral design (Bologna and Coco 1991, Batelka and Geishardt 2009).

Prior to the present study, nine *Croscherichia* species (i.e., *C. bedeli*, *C. fulgurita*, *C. gilvipes*, *C. litigiosa*, *C. mozabita*, *C. paykulli*, *C. sanguinolenta*, *C. tigrinipennis*, and *C. wartmanni*) were known for Morocco. None of these species are endemic to the country and, with the exception of *C. paykulli* (widely distributed throughout Morocco), all are restricted to arid or semi-arid zones in the south or the east (Pardo Alcaide 1954a, 1961, Kocher 1956, Bologna and Coco 1991, Ruiz and López-

Colón 1996, Bologna 2008). With the discovery of *C. armass*, the only Moroccan endemic species of *Croscherichia* reported thus far, the number of species present in Morocco increases to 10, and the number of currently recognized species of *Croscherichia* increases to 19.

With the recent discoveries of new blister beetle species endemic to Morocco (Ruiz and García-París 2008, 2009, 2015, Černý and Bologna 2018, this work), the proportion of endemism has increased in the country. Given the large number of currently recognized species and the relatively high rate of endemism within the family, this trend is only likely to increase, making Morocco a hotspot for Meloidae diversity.

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Remarkable confusion in some Western Palearctic *Clepsis* leads to a revised taxonomic concept (Lepidoptera, Tortricidae)

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Abstract

The taxonomy of some Palearctic species of the genus *Clepsis* Guenée, 1845 (Lepidoptera, Tortricidae), in particular *C. neglectana* sensu auctt. and *C. consimilana* sensu auctt., is revised based on combined characters of external and internal adult morphology, including everted vesicae in male genitalia, and DNA barcodes. *Clepsis striolana* (Ragonot, 1879), **stat. rev.**, *C. acclivana* (Zerny, 1933), *C. trivia* (Meyrick, 1913), **stat rev.**, and *C. xylotoma* (Meyrick, 1891), **stat. rev.** are resurrected from synonymy with *C. neglectana* (Herrich-Schäffer, 1851), *C. semiana* (Chrétien, 1915), **stat. nov.** is considered as a valid species; *C. eatoniana* (Ragonot, 1881), **stat. rev.**, is resurrected from synonymy with *C. consimilana* (Hübner, 1817), and *C. razowskii* Gastón, Vives & Revilla, 2017, **syn. nov.** is synonymised with *C. eatoniana*.

Keywords

DNA barcoding, *Clepsis consimilana*, *C. neglectana*, genitalia, vesica

Introduction

Clepsis Guenée, 1845 is a large genus in the tribe Archipini with 163 valid species described worldwide (Gilligan et al. 2014) and 32 species present in Europe (Aarvik 2013). It is likely that these numbers will change in the future because a recent phylogenetic study revealed that the genus is paraphyletic and its position within the tribe is not completely resolved (Dombroskie and Sperling 2013). As such, the genus is in need of revision and the status of some taxa assigned to the genus should be evaluated. As with many groups of Lepidoptera, characters of the male genitalia have been used almost exclusively to define the present-day species boundaries of the taxa comprising *Clepsis*. Many taxa were synonymised in the past decades because of their similarity in male genitalia, resulting in long synonymic lists for some species. Two of the most problematic species are *C. consimilana* (Hübner, 1817) and *C. neglectana* (Herrich-Schäffer, 1851), each with many synonymic taxa: 13 for *C. consimilana* and eight for *C. neglectana* (Gilligan et al. 2014). These species are widely distributed in the Western Palaearctic and parts of Asia (Razowski 2002), and appeared to be variable in wing pattern, size, and even genital morphology. DNA barcode sequences of these species from different parts of Europe revealed that there is a considerable genetic variability within each of the species, and the DNA sequences cluster into compact groups. These groups appear to correspond well with certain wing patterns, secondary sex characters (costal fold in males), and genital morphology. It appears that some previously described taxa in this group were erroneously synonymised because of an incorrect assessment of variability in the genitalia, and that these names should be reinstated. A few taxa (also previously synonymised) were described from a single male specimen and no further material has been collected. Their present status is unknown and these taxa are in need of thorough revision, which is currently not feasible because of difficulties in obtaining fresh material from their type localities. The purpose of the present paper is to revise some of the taxa previously in synonymy with *C. neglectana* and *C. consimilana*, and to discuss some questionable taxa.

Materials and methods

We examined ca. 150 specimens from various European collections. Data for the specimens and their repository are given in the results for each taxon treated. The genitalia were dissected and mounted following the methods of Robinson (1976) and Zlatkov (2011). The everted vesicae were drawn before mounting on a slide as described by Zlatkov and Huemer (2017). The female genitalia were submerged in euparal essence without deformation and drawn in a manner similar to the male genitalia. A three-dimensional perception of the studied structures was achieved by modifying a compound microscope Amplival (Carl Zeiss Jena) after Hammond (1996). Certain structures whose shape and size are not affected by preparation were measured. Angles were measured using a photograph of a protractor aligned digitally to photos, and the linear measurements were performed using ocular scales. Forewing length was preferred rather than wingspan, as the latter depends too much on the condition of the specimen. The terminology of the wing pattern and genitalia follows Kuznetzov (1978) and Razowski (2002). The classification of cornuti and microstructures of the signa are after Anzaldo et al. (2014) and Lincango et al. (2013), respectively.

DNA barcode sequences of the mitochondrial COI gene, a 658 base-pair long segment of the 5' terminus of the mitochondrial COI gene (*cytochrome c oxidase 1*), were obtained from 15 specimens, supplemented by 23 publicly available sequences in BOLD (Table 1). DNA samples from dried legs were prepared according to prescribed standards using the high-throughput protocol of deWaard et al. (2008). Samples were processed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph). DNA sequencing resulted in a full barcode for 36 specimens supplemented by nine sequences longer than 550 bp. Thirty-one of the sequences belong to individuals in the *Clepsis consimilana* species group and 14 of the sequences represent individuals in the *Clepsis neglectana* species group. Details of successfully sequenced voucher specimens (Table 1), including complete geographic data and images, can be accessed in the Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert 2007) in the public dataset "*Clepsis consimilana – Clepsis neglectana* species groups" https://doi.org/10.5883/DS-Clepsis. All newly generated sequences were submitted to GenBank.

Degrees of intra- and interspecific variation in the DNA barcode fragments were calculated under the Kimura 2 parameter (K2P) model of nucleotide substitution using analytical tools in BOLD Systems v. 4.0 (http://www.boldsystems.org). A neighbour-joining tree of DNA barcode data of selected taxa was constructed using Mega 6 (Tamura et al. 2013) (Fig. 16).

Institutional acronyms:

BMNH	Natural History Museum, London, United Kingdom
CJJ	Private collection of Jari Junnilainen, Vantaa, Finland
CWK	Private collection of Wojciech Kubasik, Poznań, Poland
MFN	Museum für Naturkunde, Berlin, Germany
MNHN	Muséum National d'Histoire Naturelle, Paris, France
MTD	Senckenberg Museum für Tierkunde, Dresden, Germany
NHMW	Naturhistorisches Museum Wien, Austria
NMNHS	National Museum of Natural History, Sofia, Bulgaria
SDEI	Senckenberg Deutsche Entomologisches Institut, Müncheberg, Germany
TLMF	Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria
ZMUO	Zoological Museum, University of Oulu, Finland
ZSM	Zoologische Staatsammlung München, Germany

Results

Morphology of genitalia

The male genitalia of the studied species, especially the valva, are relatively complex. The female genitalia are simple, but there is some discrepancy in the terms used in literature. For the purpose of unification, we provide general schemes of male and female genitalia of the studied taxa.

Male (Fig. 1). The uncus is a large wide plate, convex dorsally, densely setose on both dorsal and ventral surfaces. In some species its shape can vary depending on the amount of pressure applied during preparation of the slide, and thus should be interpreted with caution. The gnathos is large, with a relatively large medial part, plough-shaped in all taxa. Because it projects posteriorly, it is often bent to the left or right in microscope slides. The socius is small, membranous, setose, and teardrop-shaped. The valva is complex, with largely sclerotised basal half and membranous distal half. The costa is enlarged, swollen, with a large more or less z-shaped, tubular costal sclerite. The sclerite protrudes medially into a subspherical or elongated spinulate structure called labis (plural labides) (Razowski 1979) or processus basalis (Horak 1984). The labides are probably derivatives of the transtilla but since they are not connected with a median sclerite, the term transtilla is less appropriate in this case. They are covered with large sclerotised acanthae. The middle part of the costal sclerite medially transforms into a setose membrane. The ventral part of the costal sclerite ends into a hemispherical transparent protrusion on the medial surface of the valva surrounded by a wrinkled furrow. The sacculus is large, medially concave, sclerotised, with a large triangular basal half, an elongated apical half and a ventrally pointed process located at the transition between the two halves. The distal part of the valva is membranous and terminates into a rounded, more or less extended plate referred to as a brachiola. The medial surface of the membranous part of the valva is densely covered by setae and scales forming a tuft on a small protuberance in some species. Most of the scales can be easily removed during cleaning of the macerated genitalia, but they should not be confused with a tuft of firmly attached scales and setae in some species which is well preserved after such manipulations. There can be a row of several very large modified setae on the median surface of valvae. The setae are elliptical in cross-section, falcate, with curved tips and can be broken during preparation of genitalia, and perhaps during copulation (males with setae broken off were found), but their sockets still remain observable (Fig. 7F). Apart from the medial scales and setae, there is a large, membranous pad on the lateral ("external") surface of the valva bearing a tuft of long deciduous scales (possibly androconia), which are usually lost during dissection. The juxta is a wide nearly semi-circular plate with a median incision for articulation with the caulis. The phallus is slightly sinuate, pointed, predominantly membranous dorsally, with a long stout lateral process on the left side. The anterior portion of the phallus is bent at a large ventral angle. The vesica is comparatively simple, tubular, with a small basal widening and a thumb-like diverticulum apically. A few long aciculate deciduous cornuti are attached near the base of the diverticulum, adjacent to the gonopore.

Female (Fig. 8). The papillae anales and the apophyses are not specialised and they are similar in all the taxa studied. The sterigma is more-or-less broadly funnel-shaped, dorso-ventrally flattened, sclerotised, with a large excavation on the dorsal wall. It ends into a narrow, funnel-shaped colliculum comprising areas of sclerotised tanned plicate and thickened transparent cuticle. This part is referred to as antrum by some authors (Kuznetzov 1978), but the latter term is used mainly for the sclerotised ring or collar in Cochylini (Razowski 1970). Both structures are localised in the same region and are probably homologous (Horak 1984). The ductus seminalis is inserted at the transition

Table 1. List of successfully sequenced specimens of the *C. consimilana* and *C. neglectana* species-groups.

Species	Country/Ocean	Sample ID	Process ID	BIN
C. consimilana	Austria	TLMF Lep 22912	LEAST314-17	BOLD:AAC4212
	Austria	TLMF Lep 18827	LEATJ967-15	BOLD:AAC4212
	Austria	TLMF Lep 17661	LEATI276-15	BOLD:AAC4212
	Austria	TLMF Lep 09912	PHLAW115-13	BOLD:AAC4212
	Bulgaria	CCDB-11133-B10	BTLBP212-11	BOLD:AAC4212
	Bulgaria	CCDB-11133-B08	BTLBP210-11	BOLD:AAC4212
	Canada	PFC-2006-1202	LPVIA896-08	BOLD:AAC4212
	Canada	PFC-2006-1201	LPVIA895-08	BOLD:AAC4212
	Canada	PFC-2006-0914	LPVIA672-08	BOLD:AAC4212
	Canada	PFC-2006-0917	LPVIA675-08	BOLD:AAC4212
	Canada	PFC-2006-2539	LPVIB987-08	BOLD:AAC4212
	Canada	PFC-2006-1560	LPVIB188-08	BOLD:AAC4212
	Canada	PFC-2006-1559	LPVIB187-08	BOLD:AAC4212
	Canada	PFC-2006-1203	LPVIA897-08	BOLD:AAC4212
	Croatia	TLMF Lep 23752	LEAST1249-17	BOLD:AAC4212
	Denmark	MM19630	LEEUA222-11	BOLD:AAC4212
	Germany	BC ZSM Lep 27847	FBLMU817-09	BOLD:AAC4212
	Germany	BC ZSM Lep 25760	FBLMU250-09	BOLD:AAC4212
	Germany	BC ZSM Lep 25551	FBLMU041-09	BOLD:AAC4212
	Germany	BC ZSM Lep 23362	FBLMT132-09	BOLD:AAC4212
	Italy	TLMF Lep 15577	LEATH365-14	BOLD:AAC4212
	Italy	TLMF Lep 10273	LEATB096-13	BOLD:AAC4212
	Italy	TLMF Lep 02195	PHLAC160-10	BOLD:AAC4212
	United Kingdom	UKLB40C05	CGUKD697-09	BOLD:AAC4212
	United Kingdom	UKLB34D07	CGUKD152-09	BOLD:AAC4212
	United Kingdom	UKLB30C02	CGUKC754-09	BOLD:AAC4212
	United Kingdom	UKLB22A10	CGUKB985-09	BOLD:AAC4212
	United Kingdom	UKLB20D10	CGUKB831-09	BOLD:AAC4212
	United States	RWWA-3675	RWWC1018-12	BOLD:AAC4212
C. eatoniana	Spain	TLMF Lep 03187	PHLSA012-11	BOLD:AAJ1025
	Spain	TLMF Lep 03186	PHLSA011-11	BOLD:AAJ1025
C. neglectana	Finland	MM18257	LEFIK682-10	BOLD:AAM0282
	Finland	MM15659	LEFIG795-10	BOLD:AAM0282
C. striolana	Italy	TLMF Lep 18324	LEATJ749-15	BOLD:AAM0282
C. trivia	Greece	TLMF Lep 16958	LECRT048-15	BOLD:ACT3810
	Greece	TLMF Lep 16957	LECRT047-15	BOLD:ACT3810
	Greece	TLMF Lep 16956	LECRT046-15	BOLD:ACT3810
	Greece	TLMF Lep 16953	LECRT043-15	BOLD:ACT3810

between the colliculum and the ductus bursae. The ductus bursae is relatively long, with a smooth elongate sclerite referred to as cestum extending on most of its length and sometimes expanding onto the corpus bursae as well. The corpus bursae is ovoid with two types of signa: one plate-like consisting of sclerotised papillae, and a hook-shaped one that is more typical of most Archipini with longitudinal rows of teeth and a large capitulum.

Taxonomy

The *Clepsis neglectana* species group

The species in this group externally are similar to taxa in the *C. consimilana* group but are distinguished by the absence of large modified setae on the median surface of the valvae (Fig. 1).

Clepsis neglectana (Herrich-Schäffer, 1851)

neglectana Herrich-Schäffer, 1847 (uninominal): pl. 9, fig. 59
Tortrix (*Lozotaenia*) *neglectana* Herrich-Schäffer, 1851: 167 (Germany)
Tortrix (*Heterognomon*) *betulifoliana* Lederer, 1859: 248 (Poland)
Cacoecia delibatana Rotschild, 1912: 27 (Hungary)
Tortrix dorana Kennel, 1919: 60 (Kazakhstan: Ili)
Tortrix flavana Duponchel, 1834: 87, pl. 239, fig. 6 (France)
non *Tortrix striolana* Ragonot, 1879
non *Tortrix xylotoma* Meyrick, 1891
non *Tortrix trivia* Meyrick, 1913
non *Cacoecia acclivana* Zerny, 1933

Material examined. Lectotype ♂ (here designated): pinned, genitalia on a slide, with 7 labels: “Typus!” [handwritten] “Origin.” [red printed] “ex coll. 1/1 / Staudinger” [printed] “Dresden / n. sp.” [handwritten] “Genital-Unters. / Nr. 0024” “H.-Sch.” [both printed] “Lectotype / *Tortrix neglectana* / Herrich-Schäffer, 1851 / des. Zlatkov & Huemer 2019” [red printed].

GERMANY • 1 ♂; Dresden; Staudinger leg.; GS (genitalia slide) 0024; MFN.

Other material: GERMANY •1 ♂; Disque leg.; GS M.044; ZSM • 1 ♂; Southern Germany; Disque leg.; GS 1/2.11.2018; ZSM • 1 ♂; Dresden; Schmidt leg.; GS 1/5.12.2018; MTD • 1 ♀; Dresden, Loschwitz; GS 3/4.12.2018; MTD • 1 ♀; Dresden [?];6 Jul. 1897; GS 4/4.12.2018; MTD • POLAND • 1 ♂; Szczecin, Dabie; 30 Jul.; unknown leg.; GS 1/5.11.2018; ZSM • FINLAND • 2 ♂♂; Valkeala; 28 Jul.–4 Aug. 1999; T. Mutanen leg.; GS 1/2.2.2018, 2/9.2.2018; ZMUO • 1 ♂; Valkeala; 22–27 Jul. 1999; GS 1/7.11.2018; ZMUO • 2 ♂♂; Valkeala; 20–24 Jul. 1998; P. Sundell & T. Mutanen leg.; GS 1/5.2.2018, 1/9.2.2018; ZMUO • 1 ♂; Haapasaari; 15 Jul. 1973; J. Jalava leg.; GS 1/3.2.2018; ZMUO • 2 ♀♀; Haapasaari; 6–7 Aug. 2004; J. Junnilainen leg.; GS 1/14.12.2018, 2/14.12.2018; CJJ.

Diagnosis. Externally, *C. neglectana* is similar to the other species in the *C. neglectana* species group (apart from *C. striolana*) and *C. consimilana*, but the markings are darker and the costal fold is rudimentary. The male genitalia are very close to *C. striolana*, with the most obvious difference in the shape of labis; additionally, the setal tuft of the valva is less compact and smaller, the sacculus is straighter, and the vesica

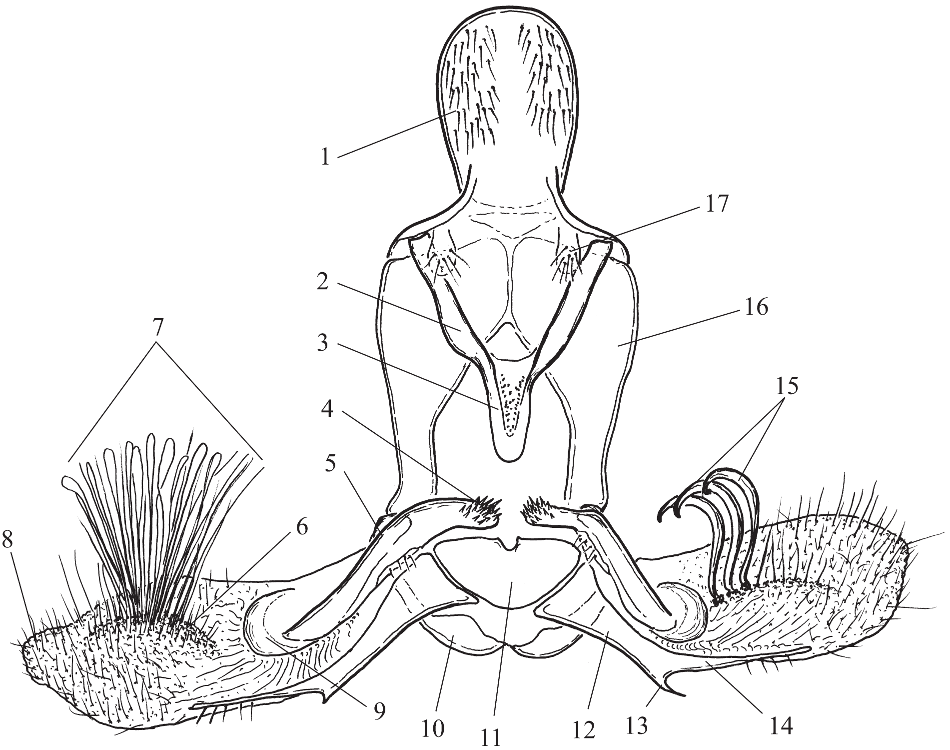

Figure 1. Scheme of male genitalia of *Clepsis neglectana* and *C. consimilana* species groups **1** uncus **2** arm of gnathos **3** medial part of gnathos **4** labis **5** costal sclerite **6** membranous medial protuberance **7** tuft of scales and setae **8** brachiola **9** hemispherical protrusion **10** vinculum **11** juxta **12** basal part of sacculus **13** saccular process **14** distal part of sacculus **15** modified setae **16** tegumen **17** socius.

most often bears a single cornutus. *Clepsis neglectana* differs from *C. acclivana* and *C. trivia* by the shape of the uncus and numerous characters on the valvae and phallus. Apart from the forewing pattern, the female differs from *C. striolana* by the presence of a transparent protrusion of the colliculum on the right side, and from *C. trivia* by the length and shape of the colliculum. In contrast to *C. trivia*, both *C. neglectana* and *C. striolana* lack a plate-like signum.

Description. Adult (Fig. 2A–C). Sexual dimorphism not detected. Head. Vertex, frons, palps and antennae monochrome, covered with ochreous scales. Sensilla trichodea (often referred to as "cilia") on antennae denser and longer in males. Thorax dorsally, legs and tegula ochreous, thorax ventrally creamy. Forewing length in males 6.3–7.4 mm (mean 6.9, $N = 10$), in females 6.5–8.2 mm (mean 7.3, $N = 4$). Forewing elongated, with costa convex basally and slightly concave apically, costal fold rudimentary (Fig. 3A). Upperside background ochreous to ferruginous with darker transverse or reticulate pattern. Markings brown to grey brown: basal blotch usually ill-defined, expressed mainly at costa and dorsum; median fascia widened at the middle. Subapical blotch triangular, ill-defined, sometimes connected with the median fascia. Cilia

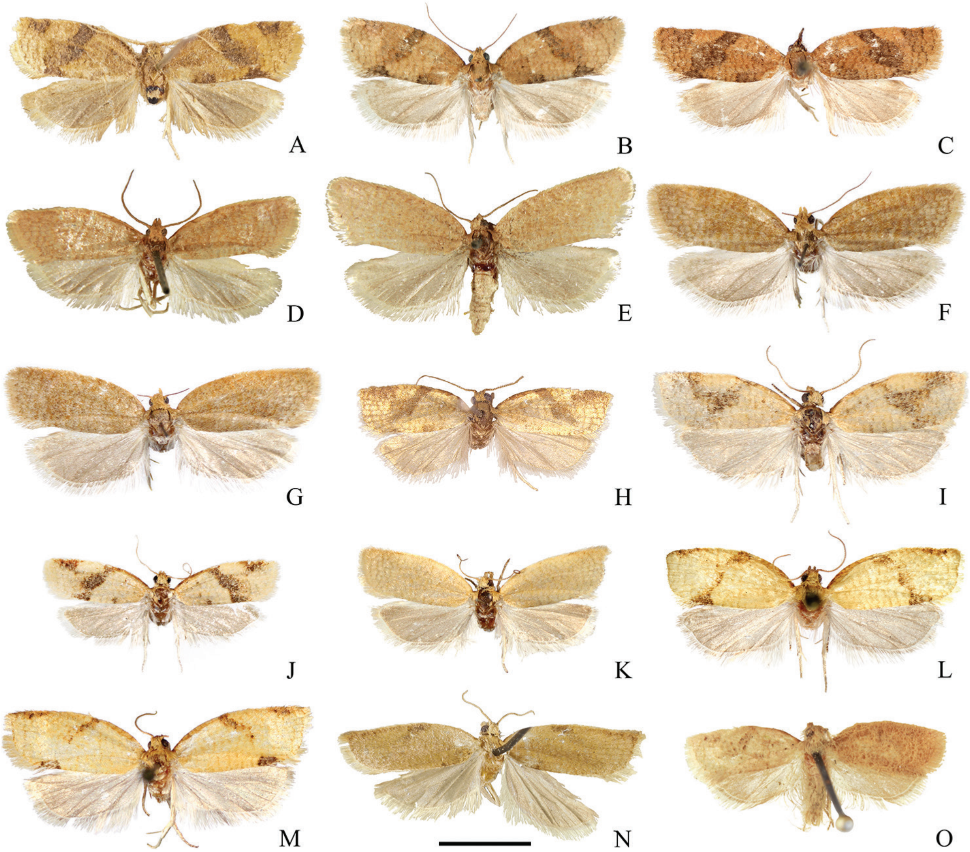

Figure 2. Adults of *Clepsis neglectana* species group **A–C** *C. neglectana*: **A** lectotype of *Tortrix neglectana* **B** male, Southern Germany **C** Female, Germany **D–G** *C. striolana*: **D** lectotype of *T. striolana* **E** paralectotype of *T. striolana* **F** male, Austria, South Tyrol **G** female, Austria, South Tyrol **H–K** *C. trivia*: **H** holotype of *T. trivia* **I–J** males, Crete **K** female, Crete **L–M** *C. acclivana*: **L** lectotype of *Cacoecia acclivana* **M** paralectotype of *C. acclivana* **N** *T. severana* holotype **O** *Cacoecia unifasciana* var. *semiana* holotype. Scale bar: 5 mm, all to scale.

concolourous or paler than background. Underside pale grey-brown, sometimes with ill-defined reticulate pattern and creamy longitudinal blotch in the distal half of costa. Hindwing grey on both sides with underside paler, cilia whitish with grey line. Abdomen grey. Male genitalia (Fig. 4A, B). Uncus ovoid, widening apically, rounded, gnathos relatively large, socius membranous. Valvae pointed dorsolaterally when mounted on slide. Costal sclerite of valva relatively narrow, with short elliptic labis covered with small acanthae and extended into triangular pointed medial process (Fig. 5A, B). Apical part of sacculus ca. 1.4× longer than basal part, both forming angle of 145–155°, saccular process pointed. Membranous part of valva with protuberance bearing tuft of firmly attached, relatively sparse scales and setae; its terminal part with concave dorsal and convex ventral margin, brachiola ill-defined, pointed dorsolaterally. Posterior part of phallus slightly bent dorsally, with lateral process as long as 0.23× distance between

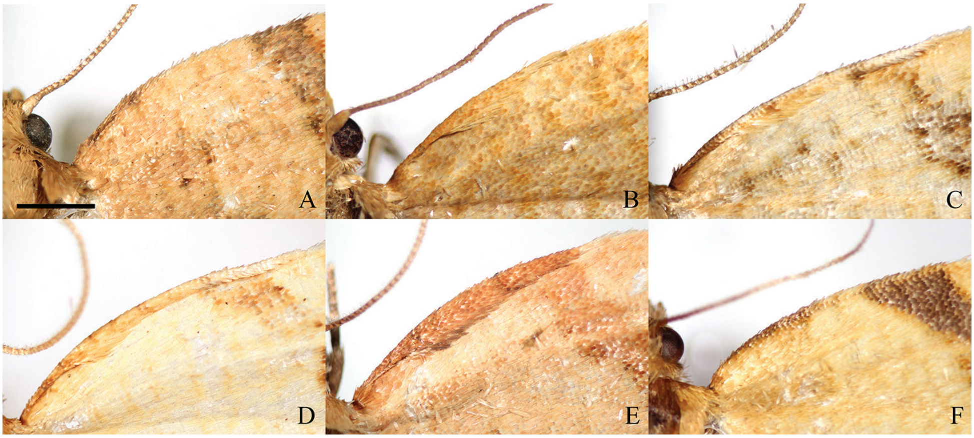

Figure 3. Costal folds of males of *Clepsis* spp. **A** *C. neglectana*, Germany **B** *C. striolana*, Austria **C** *C. trivia*, Crete **D** *C. acclivana*, lectotype of *Cacoecia acclivana* **E** *C. consimilana*, neotype of *Tortrix consimilana* **F** *C. eatoniana*, Spain. Scale bar: 1 mm, all to scale.

anterior opening and tip of phallus, straight, in single specimen apically bent dorsally. Anterior and posterior part of phallus form angle of 130–140°. Caulis large, L-shaped, parallel to coecum. Vesica bent at ca. 110° dorsally, with small basal widening and terminal diverticulum mediodorsally, slightly pointed to right (Fig. 6A). One long, slightly curved deciduous cornutus attached ventroterminally adjacent to gonopore (Fig. 7A); single specimen with two cornuti. Female genitalia (Fig. 8A) with papillae anales not modified. Apophyses anteriores 1.3× longer than apophyses posteriores. Sterigma widened caudad, with shallow lateral sclerotised pockets cephalad and large excavation on the dorsal wall. Colliculum short, with length 0.14× length of ductus bursae, straight, funnel-shaped, with plicate longitudinal sclerotisation and lateral protrusion at right cranial end consisting of colourless thick cuticle. Ductus bursae long and narrow, emerging at left to cuticular protrusion, with cestum extending along cranial 0.9× of its length and expanding for short distance on corpus bursae. Ductus seminalis inserted dorsally at caudal end of ductus bursae. Corpus bursae ovoid, with large falcate signum with capitulum (Fig. 9A).

Preimaginal stages are unknown.

Molecular data (Fig. 16). BIN: BOLD:AAM0282. DNA barcodes identical ($N = 2$). The minimum distance to the nearest BIN-sharing neighbour, *C. striolana*, is 1.53%.

Distribution (Fig. 17). Central and Northern Europe. Previous records from other parts of the Palaearctic (e.g., Wang et al. 2003) need reconsideration.

Ecology. Moths were collected in July and the beginning of August. The larval host plant is stated as *Fragaria* (Razowski 2002) but due to repeated misidentifications this record as well as published habitats need verification.

Remarks. This is the oldest described taxon from the group. Many other taxa were subsequently synonymised, but at least three of them are *species bona* and three others are *incertae sedis* (see below).

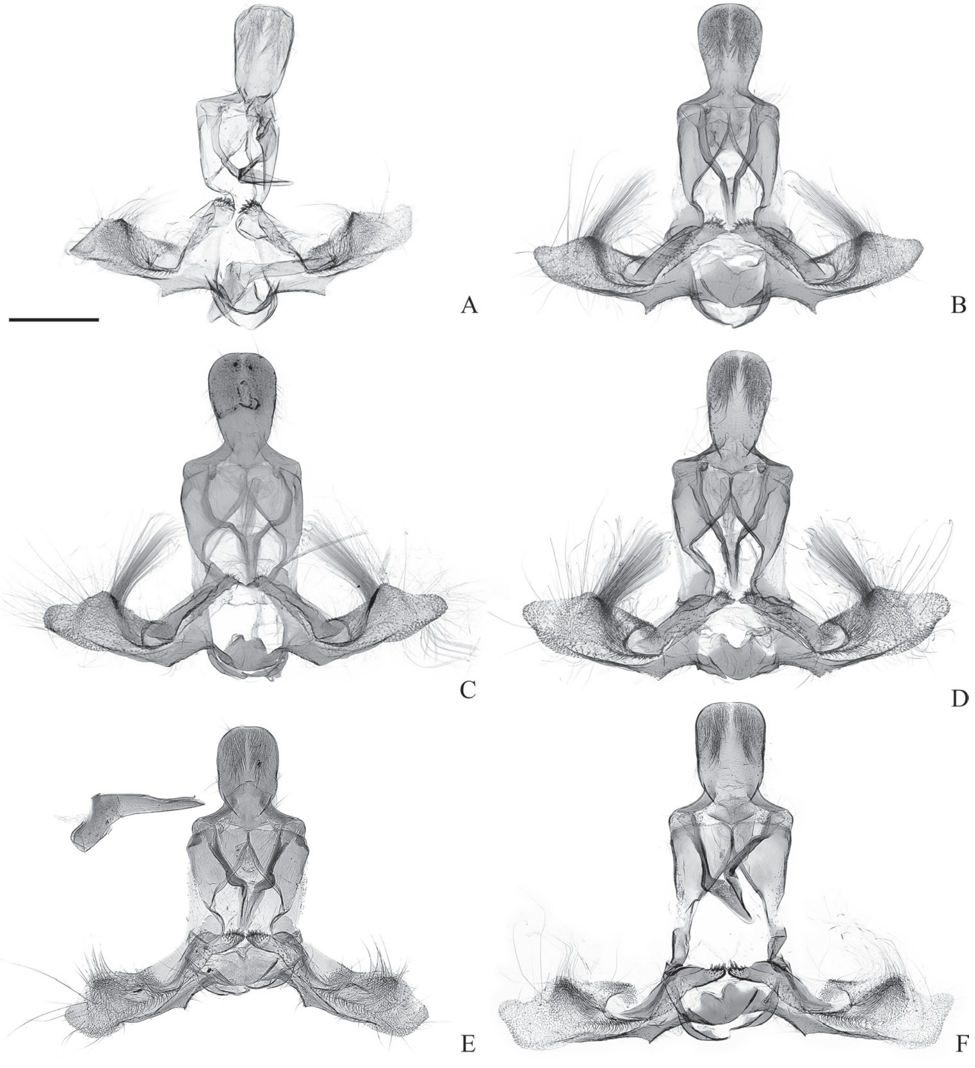

Figure 4. Male genitalia of the *Clepsis neglectana* species group (some without phallus) **A, B** *C. neglectana*: **A** *Tortrix neglectana* lectotype **B** *C. neglectana*, Southern Germany **C, D** *C. striolana*: **C** *T. striolana* lectotype **D** *C. striolana*, Austria, North Tyrol **E, F** *C. trivia*: **E** *T. trivia* holotype **F** *C. trivia*, Crete. Scale bar: 500 µm, all to scale.

Clepsis striolana (Ragonot, 1879), stat. rev.

Tortrix striolana Ragonot, 1879: 132 (Switzerland)

Material examined. Lectotype ♂ by designation of Razowski (1979), pinned, with 5 labels: "*Tortrix* / *striolana* Rag. / Bull. Soc. ent. Fr., / 1879, p. 132." [handwritten] "striolatana [sic] / Rag. Helv." [handwritten] "Type" [printed red] "1901 / coll. E. L. Ragonot / Muséum Paris" "Zool. Mus. Berlin / Genit. – Unters. / Nr. 287." [printed];

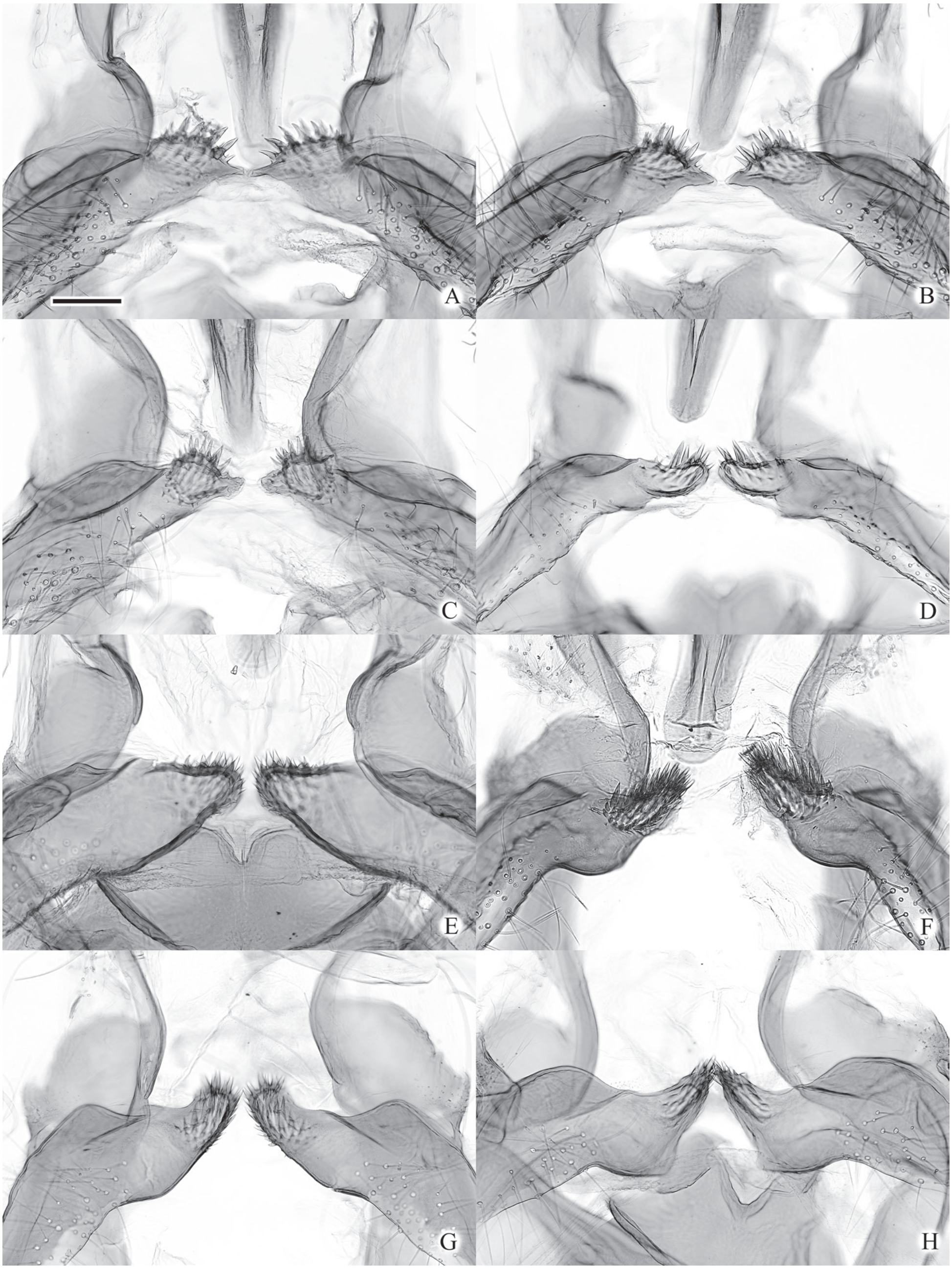

Figure 5. Labides of *Clepsis* spp. **A, B** *C. neglectana*: **A** Southern Germany **B** Finland **C** *C. striolana*, Austria, North Tyrol **D** *C. trivia*, Crete **E** *C. acclivana*, paralectotype of *Cacoecia acclivana* **F, G** *C. consimilana*: **F** Germany, neotype of *Tortrix consimilana* **G** Italy **H** *C. eatoniana*, Spain. Scale bar: 100 µm, all to scale.

male genitalia on a slide with two labels: "287. / Clepsis / striolana / Ragonot / Type." "287. Clepsis / striolana Rag. / Helv. / Bull. Soc. Ent. / Fr. 1879 p. 132 / Type." [both handwritten, with red border].

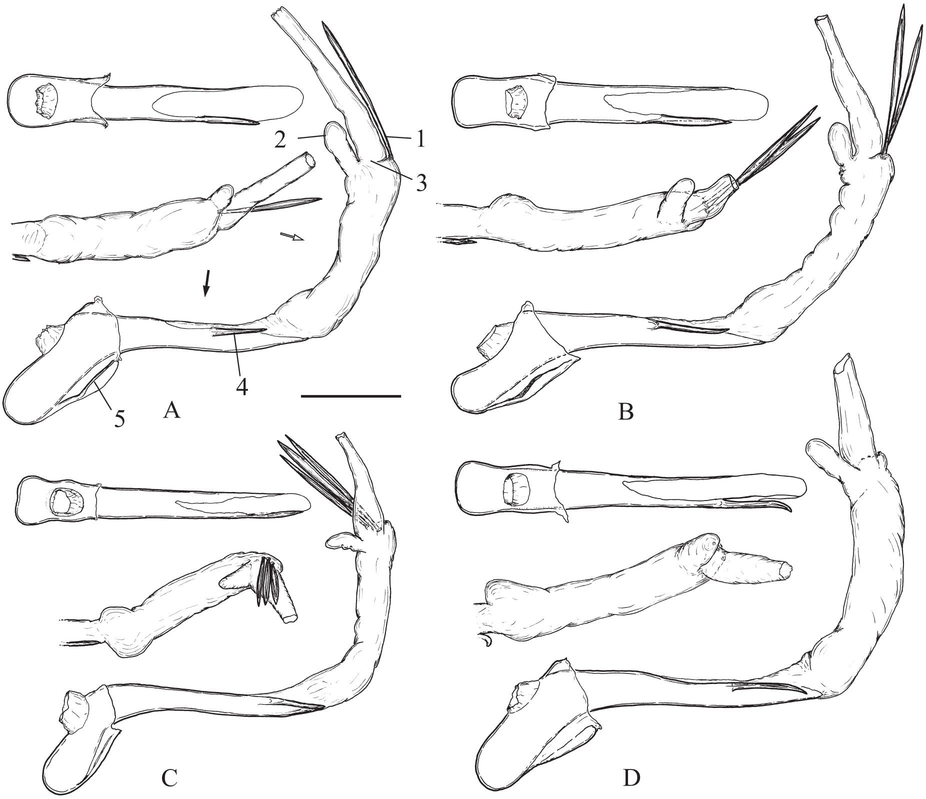

Figure 6. Phalli with vesicae everted of the *Clepsis neglectana* species group **A** *C. neglectana*, Germany **B** *C. striolana*, Austria **C** *C. trivia*, Crete **D** *C. acclivana*, paralectotype of *Cacoecia acclivana*. The phallus of each species is shown in three aspects from top to bottom: sclerotised phallic tube (without vesica) in dorsal view (viewpoint marked with black arrow), vesica in dorsal view (viewpoint marked with white arrow) and whole phallus in left view. 1 cornutus, 2 diverticulum, 3 location of gonopore, 4 phallic process, 5 caulis. Scale bar: 250 µm.

SWITZERLAND • 1 ♂; E. L. Ragonot leg.; GS 287; MNHN.

Paralectotype ♀ pinned, with three labels: "Striolatana [sic] / Rag. Helv." [handwritten] "1901 / coll. E. L. Ragonot / Muséum Paris" [printed] 'Allotype' [handwritten red].

SWITZERLAND • 1 ♀; E. L. Ragonot leg.; MNHN.

Other material: AUSTRIA • 2 ♂♂; North Tyrol, Stanzach, Blockau; alt. 920 m; 5 Jul. 1989; P. Huemer leg.; GS TOR 6; TLMF • 3 ♂♂, 1 ♀; same collection data; 16 Jul. 1989; P. Huemer leg.; GS 1/11.10.2017, 2/11.10.2017; TLMF • 6 ♂♂, 2 ♀♀; same collection data; 26 Jul. 1989; P. Huemer leg.; GS 3/11.10.2017; TLMF • ITALY • 3 ♂♂; Südtirol, Prad, Praderfeld; alt. 900 m; 9 Jul. 1991; P. Huemer leg.; TLMF • 1 ♂; same collection data; 20. Jun. 1998; T. Mayr leg.; TLMF; • 1 ♂, 1 ♀; prov. Torino, Val Chisone, Fenestrelle; alt. 1150 m; Aug. 1928; G. Della-Beffa leg.; NHMW.

Diagnosis. This is the only species of the *C. neglectana* group with a uniform wing pattern in both sexes. Unlike the males of *C. neglectana*, the costal fold is developed.

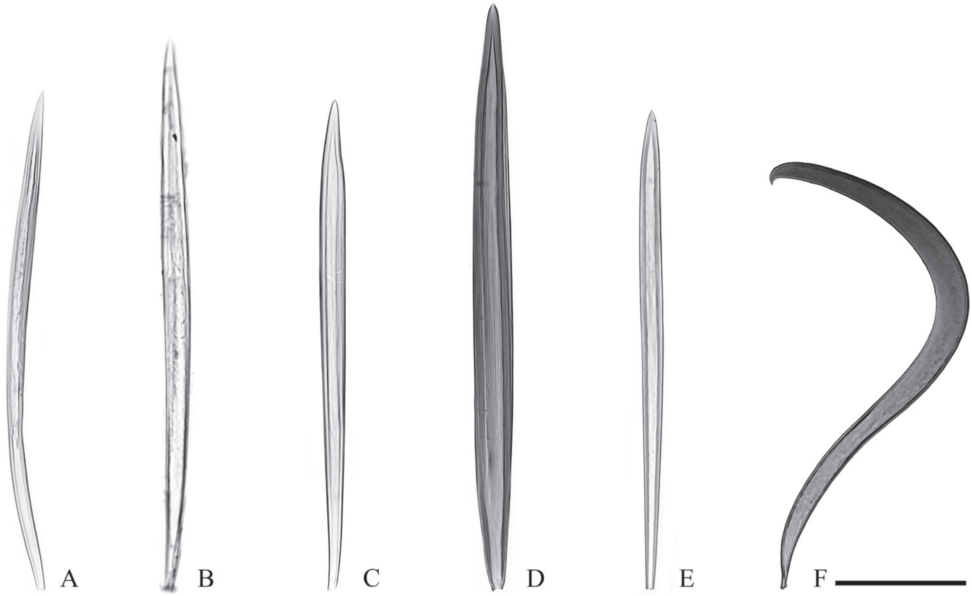

Figure 7. Specialised male setae in *Clepsis* spp. **A–E** cornuti: **A** *C. neglectana*, Finland **B** *C. striolana*, Austria **C** *C. trivia*, Crete **D** *C. consimilana*, neotype of *Tortrix consimilana* **E** *C. eatoniana*, Spain **F** modified large seta from the medial surface of valva of *C. consimilana*, Germany. Scale bar: 100 µm, all to scale.

The male genitalia are very similar to *C. neglectana* but the setal tuft of the valva is better developed, the uncus is slightly longer, the sacculus is more angled, the vesica bears two cornuti instead of one, and the shape of the labis is different. The female genitalia are also similar to *C. neglectana*, although some differences in the colliculum (the transparent cuticular protrusions) are present. The colliculum is much shorter and straighter in *C. striolana* than in *C. trivia*.

Description. Adult (Fig. 2D–G). Sexual dimorphism not detected. Head. Vertex, frons, palps and antennae monochrome, covered with ochreous scales. Sensilla trichodea on antennae denser and longer in males. Thorax dorsally, legs and tegula ochreous, thorax ventrally creamy. Forewing with length 8.0–8.2 mm (mean 8.1, $N = 3$), relatively wide, with costa convex basally and straight apically, costal fold small, extending from base to 0.4× length of costal margin (Fig. 3B). Upperside background ochreous with fulvous reticulate pattern, underside pale grey-brown with creamy margins, cilia pale ochreous. Hindwing upperside pale grey, underside whitish, cilia whitish with grey line. Abdomen grey. Male genitalia (Fig. 4C, D). Uncus ovoid, widening dorsally, rounded, gnathos relatively large, socius membranous. Valvae pointed dorsolaterally when mounted on slide. Costal sclerite of valva relatively narrow, with short round labis covered with small acanthae and extended into pointed medial process (Fig. 5C). Apical part of sacculus ca. 1.4× longer than basal part, both forming an angle of 135–140°, saccular process nearly right angled. Membranous part of valva with protuberance bearing compact tuft of dense, firmly attached scales and setae; its terminal part with

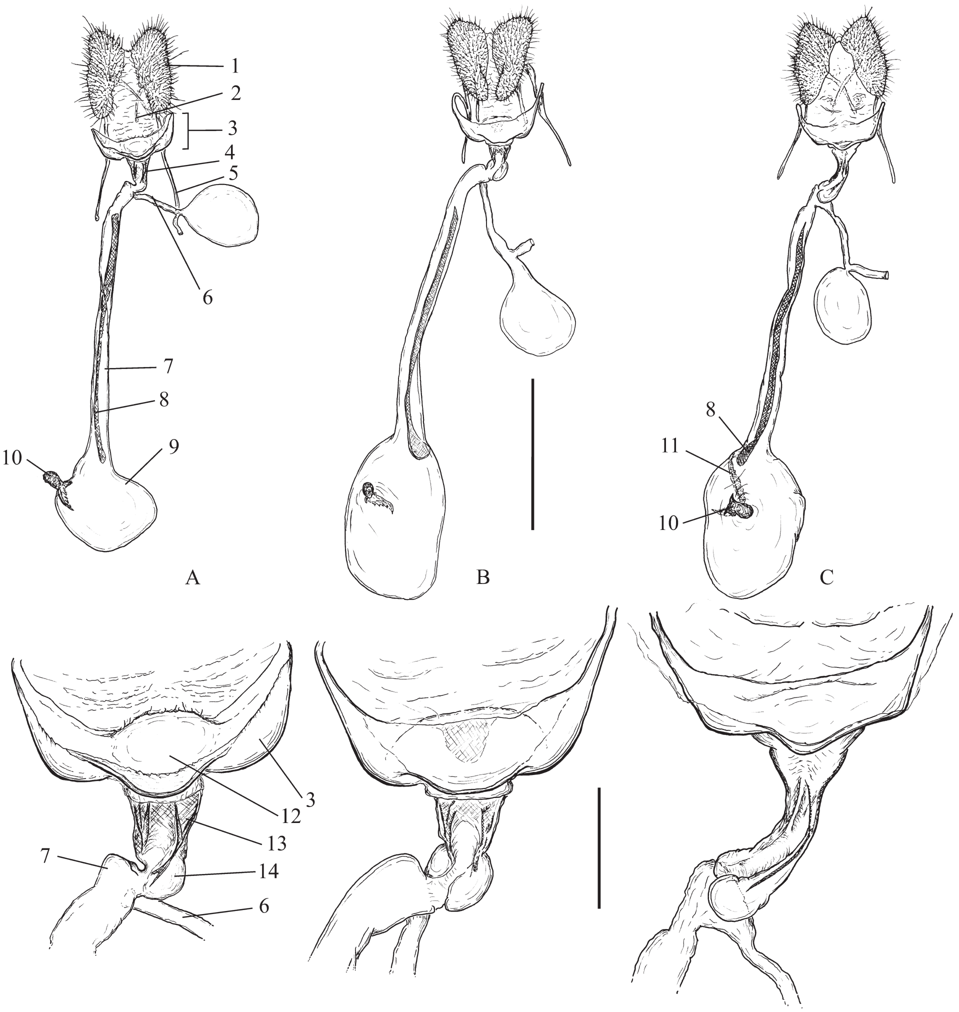

Figure 8. Female genitalia of the *Clepsis neglectana* species group **A** *C. neglectana*, Finland **B** *C. striolana*, Austria **C** *C. trivia*, Crete. The top row shows the whole genitalia, the bottom row shows the sterigma and colliculum enlarged. 1 papilla analis, 2 apophysis posterior, 3 sterigma, 4 colliculum, 5 apophysis anterior, 6 ductus seminalis, 7 ductus bursae, 8 cestum, 9 corpus bursae, 10 falcate signum, 11 plate shaped signum, 12 ostium, 13 sclerotised plicae, 14 protrusion. Scale bars: 1 mm (top row), 250 µm (bottom row).

concave dorsal and convex ventral margin, brachiola large, pointed laterally. Posterior part of phallus straight, with lateral process as long as 0.28× distance between anterior opening and tip of phallus, apically bent dorsally. Anterior and posterior part of phallus form angle of 135–145°. Caulis large, Z-shaped, parallel to coecum. Vesica bent at ca. 130° dorsally, with small basal widening and terminal diverticulum dorsolaterally, pointed to right (Fig. 6B). Two long, slightly curved deciduous cornuti attached ventroterminally adjacent to gonopore (Fig. 7B). Female genitalia (Fig. 8B) with papillae anales not modified. Apophyses anteriores 1.7× longer than apophyses posteriores.

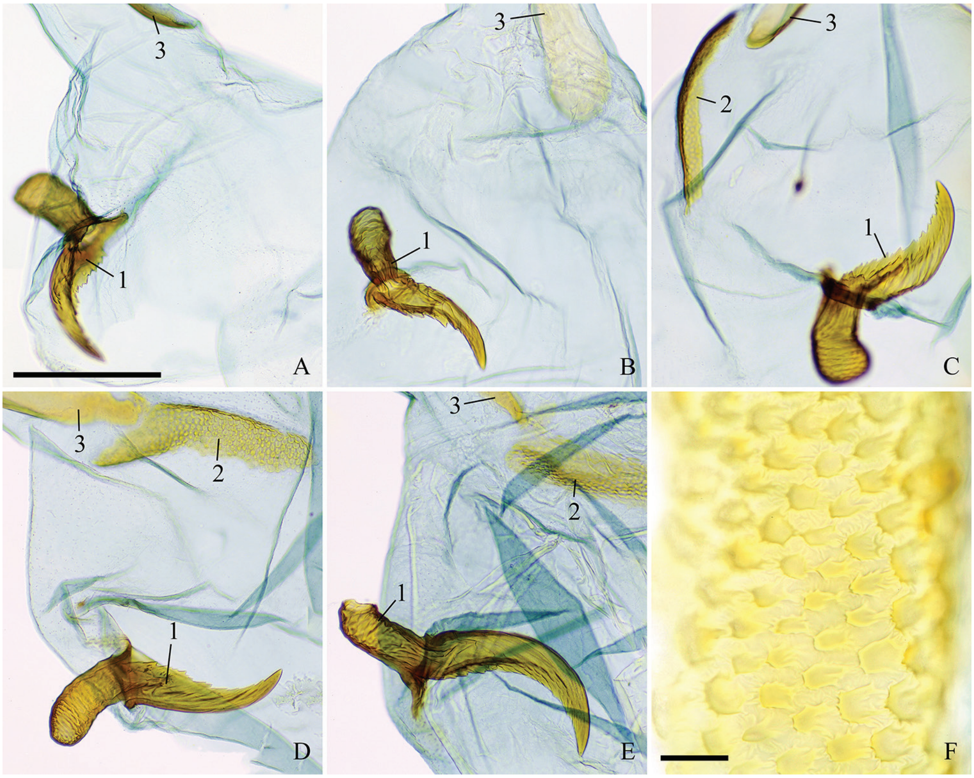

Figure 9. Signa of bursa copulatrix in *Clepsis* spp. **A** *C. neglectana* **B** *C. striolana* **C** *C. trivia* **D** *C. consimilana* **E** *C. eatoniana* **F** A detail of plate shaped signum of *C. consimilana* consisting of sclerotised papillae. 1 falcate signum, 2 plate shaped signum, 3 cestum. Scale bars: 250 µm (**A–E**, all to scale), 25 µm (**F**).

Sterigma widened caudad, with shallow lateral sclerotised pockets cephalad and large excavation on the dorsal wall. Colliculum short, with length 0.15× length of ductus bursae, straight, funnel-shaped, with plicate longitudinal sclerotisation, larger right and smaller left lateral protrusions at cranial end consisting of colourless thick cuticle. Ductus bursae long and narrow, emerging at left between cuticular protrusions, with cestum extending along cranial 0.9× of its length and expanding for short distance on corpus bursae. Ductus seminalis inserted dorsally at caudal end of ductus bursae. Corpus bursae ovoid, with large falcate signum with capitulum (Fig. 9B).

Preimaginal stages are unknown.

Molecular data (Fig. 16). BIN: BOLD:AAM0282. The intraspecific average and maximum distances of the barcode region is unknown ($N = 1$). The minimum distance to the nearest BIN-sharing neighbour, *C. neglectana*, is 1.53%.

Distribution (Fig. 17). This species seems to be limited to the Alps: Switzerland, Austria and Italy.

Ecology. Moths were collected from late June to August on sparse scree along alpine rivers. The larval host plant is unknown.

Remarks. The genitalia of this species resemble those of *C. neglectana*, only a few details in certain structures are different. The genetic distance (though small) and wing pattern both support the existence of two separate taxa.

Clepsis trivia (Meyrick, 1913), stat rev.

Tortrix trivia Meyrick, 1913: 297 (Tunisia)

Material examined. Holotype ♂, pinned, with 6 labels: "Tunis / 27.5 / Coll. O. Leonhard" "T / 29" "Tortrix / trivia Meyr. / type" "Holotypus" [red label] "DEI Müncheberg / Lep-00335" [green label] "Gen.-Präp. / 3145 / präp. Karisch, 2014".

TUNISIA • 1 ♂; 27 May; Leonhard leg.; GS 3145; SDEI Lep-00335.

Other material: GREECE • 1 ♀; Crete, Lassithi region, Agios Joannis; alt.250 m; 28 Apr. 2003; W. Ruckdeschel leg.; GS 1/12.10.2017; TLMF • 1 ♀; same collection data; 1 May 2003; GS 2/12.10.2017; TLMF • 2 ♂♂; Crete, Lassithi region, Koutsounari; alt. 100 m; 1 May 2003; W. Ruckdeschel leg.; GS 1/13.10.2017, 2/13.10.2017; TLMF • 1 ♀; Crete, Lassithi region, Achlia at Koutsouras; alt. 30 m; 5 Nov. 2004; W. Ruckdeschel leg.; GS 3/13.10.2017; TLMF • 2 ♂♂, 1 ♀; same collection data; 7 Nov. 2004; GS 4/13.10.2017, 5/13.10.2017, 6/13.10.2017; TLMF • 1 ♀; same collection data; 9 Apr. 2008; GS 1/14.10.2017; TLMF • 3 ♂♂; same collection data; 12 Apr. 2008; GS 1/15.10.2017, 2/15.10.2017, 3/15.10.2017; TLMF.

Diagnosis. The species is most similar to *C. acclivana*. Externally the males differ in their wing markings, which are better defined in *C. acclivana*. The wings in *C. trivia* are also more elongated. The gnathos of *C. trivia* has angled arms, the sacculus is straighter, the brachiola is displaced dorsally, the phallic process has a different orientation but the vesica is very similar to *C. acclivana*. The wing pattern of *C. trivia* females is similar to those of both the *C. consimilana* and *C. neglectana* groups, but is *C. trivia* is paler. *Clepsis trivia* has the longest colliculum among the known females of the *C. neglectana* group.

Description. Adult. Sexual dimorphism prominent. Male (Fig. 2H–J). Head. Vertex pale fulvous, frons, palps and antennae with ochreous scales. Antennae with numerous sensilla trichodea as long as width of flagellomeres. Thorax dorsally fulvous, ventrally creamy, legs pale brown, tegula creamy with fulvous anterior part. Forewing relatively elongate, with length 6.1–7.9 mm (mean 7.3, $N = 7$). Costa convex basally and straight apically, with small costal fold extending from base to 0.4–0.5× length of costal margin (Fig. 3C). Background pale yellowish with ill-defined reticulate pattern. Basal blotch ill-defined, consisting of small transverse fulvous markings. Median fascia brown or fulvous, widened at middle. Subapical blotch triangular, ill-defined, connected with median fascia. Underside pale grey-brown with creamy area in distal half of costa. Cilia concolourous with background. Hindwing upperside pale grey, underside whitish, cilia white. Abdomen pale grey. Male genitalia (Fig. 4E, F). Uncus wide, more or less round apically, with parallel lateral margins, gnathos with relatively large median part and angled arms, socius membranous. Valvae pointed laterally or slightly dorsolaterally when mounted on slide. Costal sclerite wide, with elongated labis covered with large acanthae (Fig. 5D). Apical part of sacculus 1.6× longer than basal part, both forming an angle of 150–160°, saccular process pointed, relatively small. Membranous part of valva with protuberance lacking tuft of setae but has deciduous scales, terminal part with small dorsal and large ventral curvature, brachiola prominent, pointed dorsally. Posterior part of phallus slightly sinuate, with lateral process 0.24× distance between anterior opening and tip of phallus, weakly curved dorsad or rarely parallel to tip. Anterior and posterior part of phallus form angle of 130°. Caulis small, diverging from coecum. Vesica bent at 110–130° dorsad, with basal widening and terminal diverticulum dorsally, slightly pointed to right (Fig. 6C). Three ventroapically located deciduous cornuti adjacent to gonopore (Fig. 7C). Female more unicolourous than male (Fig. 2K). Head as in male but sensilla trichodea less numerous and shorter. Thorax as in male but tegula fulvous. Forewing length 6.6–7.9 mm (mean 7.3, *N* = 5). Costa convex basally and slightly concave apically. Upperside background pale yellowish with pale fulvous reticulate pattern, without markings, underside pale grey-brown with creamy area in distal half of costa, cilia concolourous with upperside. Hindwings upperside pale grey, underside and cilia whitish. Abdomen grey. Female genitalia (Fig. 8C). Papillae anales not modified. Apophyses anteriores slightly longer (1.1×) than apophyses posteriores. Sterigma widened caudad, with small shallow lateral pockets cephalad and large excavation on dorsal wall, lamella antevaginalis narrow. Colliculum asymmetrical, with length 0.2× length of ductus bursae, funnel-shaped, bent to left, with plicate longitudinal sclerotisation, large elongated protrusion at right and a small one at left both consisting of colourless thick cuticle. Ductus bursae long and narrow, emerging dorsally of cuticular protrusions. Cestum expanding for short distance on corpus bursae and extending along cranial part of ductus bursae for 0.9× of its length. Ductus seminalis inserted dorsolaterally at caudal end of ductus bursae. Corpus bursae ovoid, with large falcate signum with capitulum and flat signum located near end of cestum consisting of sclerotised papillae (Fig. 9C).

Molecular data (Fig. 16). BIN: BOLD:ACT3810. The intraspecific average distance of the barcode region is 0.08%, the maximum distance 0.16% (p-dist) (*N* = 4). The minimum distance to the nearest neighbour, *C. eatoniana*, is 3.34%.

Distribution (Fig. 17). Known from Tunisia (type locality) and Crete.

Ecology. The moths fly in the middle of April to late May and in early November, which may indicate two generations per year.

Remarks. Meyrick (1913) described *C. trivia* from a single male. The name remained valid until Karisch and Blackstein (2014) dissected the holotype (by monotypy) and synonymised it with *C. neglectana*. Despite of proposed synonymy, they explicitly stated that *C. acclivana* and *C. trivia* are very similar to each other but differ from *C. neglectana*. We find very little support for synonymy between *C. neglectana*, *C. trivia*, and *C. acclivana*. The holotype of *C. trivia* has very similar wing pattern to the males collected in Crete, and the male genitalia (apart from the unstudied vesica of the holotype) appear identical, therefore the Cretan population can be assigned to *C. trivia* despite lacking barcode data for the holotype. There is a considerable DNA barcode gap between *C. trivia* (from Crete) and *C. neglectana* (from Europe) which also supports existence of two taxa.

Clepsis acclivana (Zerny, 1933), stat. rev.

Cacoecia acclivana Zerny, 1933:108, pl. 1, fig. 11 (Lebanon)

Material examined. Lectotype ♂ by designation of Razowski (1979), pinned, with 6 labels: "Nord-Libanon / Becharré, 1400 m / 21.–28.vi.[19]31. Zerny" "Cacoecia / acclivana / Zerny Type!" [handwritten] "Cacoecia" ♂ / acclivana Zerny / N. Obraztsov det. 1965 / prep. No. V. 49" [handwritten and printed] "Lectotype" [green label] "Nat. hist. Mus. / Wien / Gen. Praep. / MV 2533" [blue label] "NHMW / Type fot / 2013"; male genitalia on a slide with two labels: "Cacoecia" / acclivana / Zerny / Nord-Libanon / Becharré / 1400 m 21.–28.vi / 1931 Zerny" "♂ / V. 49 / Mus. Vind. 2533 / Lectotypus" [both handwritten].

LEBANON • 1 ♂; Bsharri; alt. 1400 m; 21–28 Jun. 1931; Zerny leg.; GS V. 49; NHMW 2533.

Paralectotype ♂, pinned, with four labels: "Nord-Libanon / Becharré, 1400 m / 11.–20.vi.[19]31. Zerny" "Lectoparatype" [green label] "Cacoecia / acclivana / Zerny Type!" [handwritten] "NHMW / Gen. Prep. ♂ / No. 1/15.2.2018"; male genitalia on a slide with two labels: "Paralectotype / *Cacoecia acclivana* / Zerny, 1933 / Nord-Libanon, Becharré, 1400 / m, 11.–20.vi.[19]31, Zerny" [red label] "NHMW / Gen. prep. / ♂ / No. 1/15.2.2018 / B. Zlatkov 2018 Euparal".

LEBANON • 1 ♂; Bsharri; alt. 1400 m; 11–20 Jun. 1931; Zerny leg.; GS 1/15.2.2018; NHMW.

Diagnosis. *Clepsis acclivana* is most similar to *C. trivia* but the forewings are paler and wider with more distinct markings, the uncus is narrower, the median part of the gnathos is smaller and its arms are not angled, the sacculus is more curved, the labis is more massive and with shorter acanthae, and the apex of the phallic process is curved ventrolaterally instead of dorsally.

Description. Adult. Sexual dimorphism unknown. Male (Fig. 2L, M). Head. Vertex pale fulvous, frons, palps and antennae with ochreous scales. Antennae with numerous sensilla trichodea as long as width of flagellomeres. Thorax dorsally pale fulvous, ventrally creamy, legs pale brown, tegula pale fulvous. Forewing with length of 7.8 mm (in both specimens), costa basally convex, apically straight, with costal fold extending from base to 0.4× length of costal margin (Fig. 3D). Upperside background pale yellowish with fulvous reticulate pattern. Markings ill-defined, consisting of brown and ferruginous scales: basal blotch faint, with remnants only at costa as dark line; median fascia narrow, more prominent at dorsum; subapical blotch reduced, dash-like. Cilia concolourous with background. Underside pale grey-brown, costal and terminal areas creamy with some reticulate pattern. Hindwings upperside monochrome pale grey, cilia concolourous, underside whitish. Abdomen pale grey. Male genitalia (Fig. 10A, B). Uncus round apically, with parallel lateral margins, gnathos relatively small, socius membranous. Valvae pointed laterally or ventrolaterally when mounted on slide. Costal sclerite of valva very wide, with short wide labis covered with small acanthae (Fig. 5E). Basal and apical parts of sacculus with equal length forming

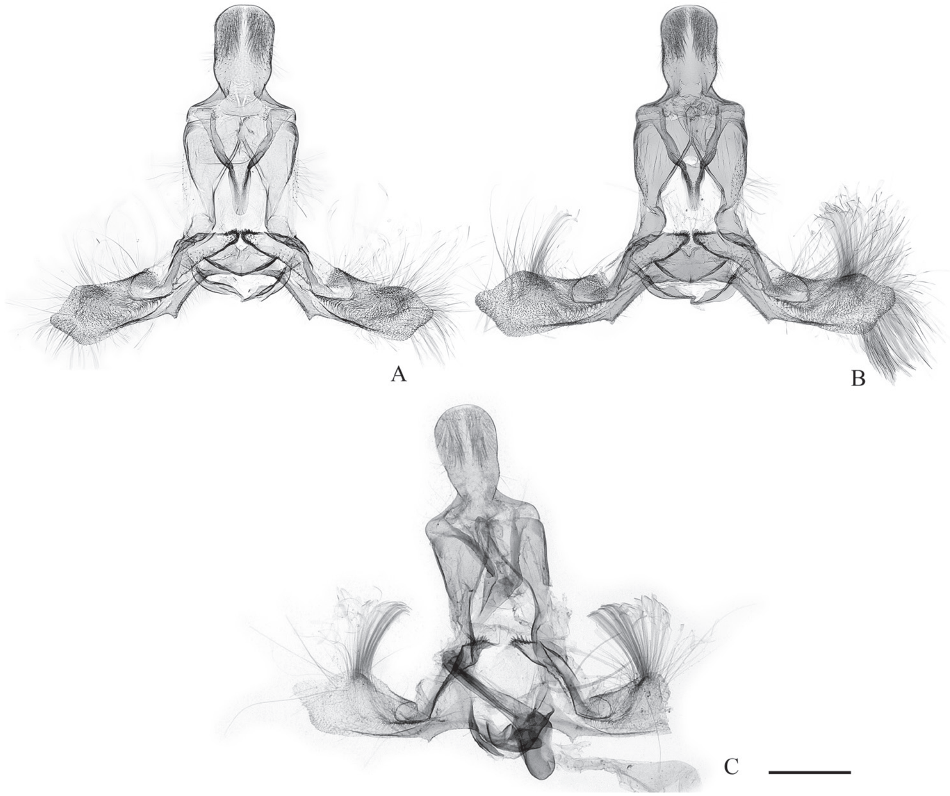

Figure 10. Male genitalia of the *Clepsis neglectana* species group (some without phallus) **A–B** *C. acclivana*: **A** *Cacoecia acclivana* lectotype **B** *C. acclivana* paralectotype, right valva intentionally not cleaned from scales **C** *Clepsis semiana*, holotype of *Cacoecia unifasciana* var. *semiana*. Scale bar: 500 µm, all to scale.

angle of ca. 140°, saccular process almost right-angled, relatively large. Membranous part of valva with protuberance devoid of tuft of setae but has deciduous scales; its terminal part with nearly symmetrical dorsal and ventral curvature, brachiola prominent, pointed laterally. Posterior part of phallus slightly sinuate, with lateral process as long as 0.29× distance between anterior opening and tip of phallus, apically bent ventrolaterally. Anterior and posterior part of phallus form angle of 130°. Caulis large, diverging widely from coecum. Vesica bent at ca. 110° dorsad, with basal widening and terminal diverticulum dorsally, slightly pointed to right. Three sockets of ventroapically located deciduous cornuti adjacent to gonopore are detectable (Fig. 6D).

Female unknown.

Preimaginal stages unknown.

Molecular data. Unknown.

Distribution. Known from the type locality only: Lebanon (Fig. 17).

Ecology. Not known.

Remarks. Comparison of the wing pattern and genitalia of the lectotype and paralectotype of *C. acclivana* with the species considered above confirmed the assumption that it is a distinct species. The barcoding distance to the other species was not studied because of lack of fresh material, but the great similarity in all morphological characters of the two specimens convinced us that they can represent a distinct species, therefore we resurrect the initial status of the taxon *acclivana* synonymised by Razowski (1979) with *C. neglectana*.

Taxa incertae sedis. Without further morphological or genetic support the following species cannot be interpreted with certainty, but the synonymy with *C. neglectana*, *C. acclivana* or *C. trivia* does not seem justified for now. They demonstrate some morphological gap; at least the differences between them are not smaller than the differences with the above mentioned species of the group. Additional material and genetic study is necessary to solve their real status.

Clepsis severana (Kennel, 1901), stat. rev.

Tortrix severana Kennel, 1901: 227 (Algeria)

Remarks. The species was synonymised with *C. neglectana* by Razowski (1979) based on examination of the genitalia of the female holotype (Fig. 2N). The slide subsequently was lost and we were unable to re-examine the genitalia. Regarding the type locality (Algeria) it seems unlikely that this taxon is conspecific with *C. neglectana*.

Clepsis semiana (Chrétien, 1915), stat. nov.

Cacoecia unifasciana var. *semiana* Chrétien, 1915: 296 (Tunisia)

Material examined. Holotype ♂: pinned, with 4 labels: “Cacoecia / unifasciana / v. semiana” [handwritten] “Type” [red, printed] “8.6” [handwritten] “Holotype / *Clepsis semiana* / (Chrétien, 1915) / Zlatkov & Huemer, 2019 des.” [red, printed].

TUNISIA • 1 ♂; MNHN.

Remarks. The male genitalia of *C. semiana* (Fig. 10C) are very similar to those of *C. striolana*. However, there is a clear difference in the wing pattern. Though the lectotype of *C. unifasciana* var. *semiana* is poorly preserved, some poorly defined markings can be detected (Fig. 2O). The physical distance between the populations of this taxon and *C. striolana* is considerable and it is very unlikely that var. *semiana* is conspecific. The synonymy between *C. semiana* and *C. consimilana* proposed by Obraztsov (1955) is not justified. Apparently, it is rooted in the initial incorrect assignment of var. *striolana* to *Cacoecia unifasciana*, but the latter turned out to be a junior synonym of *Clepsis consimilana* (see below). The taxon var. *semiana* was mechanically moved to the synonymic list of *C. consimilana* without studying the genitalia of the type specimen.

The *Clepsis consimilana* species group

This species group is characterised by the presence of large modified setae on the median surface of the valvae (Fig 1).

Clepsis consimilana (Hübner, 1817)

[*Tortrix*] *consimilana* Hübner, 1817: pl. 38, fig. 239 (Europe).
Tortrix unifasciana Duponchel, 1842: 135, pl.61, fig. 6 (France).
Tortrix unifasciana f. *cinnamomeana* Dufrane, 1957: 6 (Belgium).
Tortrix fallaciana Fuchs, 1903: 4 (Sicily).
Tortrix unifasciana f. *fuscana* Dufrane, 1957: 6 (Belgium).
Tortrix unifasciana f. *obliterana* Dufrane, 1957: 6 (Belgium).
Paramesia peregrinana Stephens, 1852: 90 (Britain), unavailable, published in synonymy with *Lozotaenia obliquana* Humphreys and Westwood 1845.
Tortrix productana Zeller, 1847: 660 (Sicily).
obliterana Herrich-Schäffer, 1847 (uninominal): pl. 9, fig. 60.
Tortrix (*Lozotaenia*) *obliterana* Herrich-Schäffer, 1851: 164 (Italy).
Siclobola placida Diakonoff, 1948: 25 (Madagascar).
non *Tortrix eatoniana* Ragonot, 1881
non *Cacoecia unifasciana* var. *semiana* Chrétien, 1915
non *Cacoecia acclivana* Zerny, 1933

Material examined. Neotype ♂ (here designated): pinned, with 5 labels: "Gehegs / Dresden / 18.vi.1922 / E. Möbius" "Coll. E. Möbius / Ankauf 1946" "Neotype / *Tortrix consimilana* / Hübner, 1817 / Zlatkov & Huemer, 2019 des." [red printed] "Museum für Tierkunde Dresden / Gen. prep. / ♂ / No. 2/30.11.2018" "*Clepsis consimilana* / (Hübner, 1817) / Zlatkov & Huemer, 2019 det."; genitalia on a slide with two labels: "Neotype / *Tortrix consimilana* / Hübner, 1817 / [Germany] Dresden, Gehegs / 18.vi.1922, E. Möbius / Zlatkov & Huemer, 2019 des." [red printed] "Museum für Tierkunde Dresden / Gen. Prep. / ♂ / No. 2/30.11.2018 / B. Zlatkov 2018 Euparal".

GERMANY • 1 ♂; Dresden, Gehegs; 18 Jun. 1922; E. Möbius leg.; GS 2/30.11.2018; MTD.

Lectotype ♂ of *Tortrix unifasciana* (here designated): pinned, with 5 labels: "unifasciana" [handwritten] "Duponch[el]" [round, handwritten] "713" [round handwritten] "Type" [red printed] "Chr. Gibeaux dét. / prép. génit. n° 4231 ♂ / unifasciana / Dup." [printed and handwritten]; male genitalia on a slide with two labels: "Tortrix / unifasciana / Dup., 1842 / Type" [handwritten with red border] "Chr. Gibeaux dét. / prép. génit. n° 4231 ♂ / Clepsis / consimilana Hb. / 30.x.91 Euparal M. P." [printed and handwritten].

FRANCE • 1 ♂; GS 4231; MNHN.

Holotype ♂ of *Siclobola placida*: pinned, with four labels: “Tananarive” “Type: ♂ / Siclobola / placida / A. Diakonoff 1946” [printed and handwritten] “Holotype” [printed red] “Siclobola / unifasciana / placida / 1959 Diak. / det. A. Diakonoff” [printed and handwritten]; genitalia on glass slide pinned to the holotype, with one label: “536.D.”

MADAGASCAR • 1 ♂; Antananarivo; MNHN 536.D.

Other material: GERMANY • 1 ♂; Dresden, Gehegs; 24 Jun. 1921; E. Möbius leg.; GS 1/30.11.2018; MTD • 1♂; Dresden, Lössaltz; 28 Jun. 1935; E. Möbius leg.; GS 2/3.12.2018; MTD • 1 ♂; same collection data; 3 Aug. 1935; GS 1/3.12.2018; MTD • 1 ♀; Fritzsche leg.; GS 1/4.12.2018; MTD • 1 ♂; Sylt; GS 2/5.11.2018; ZSM • 2 ♂♂, 2 ♀♀; Baden Württemberg, Marbach am Neckar; 29 Jun. 1957; L. Süssner leg.; TLMF • 1 ♂; Bayern, München, Hochmutting; 19 Aug. 1988; H. Kolbeck leg.; TLMF • 1 ♀; Kehlheim; 12 Jun. 1997; H. Kolbeck leg.; TLMF • ITALY • 1 ♂; prov. Verona, Monte; alt. 300 m; 20 Aug. 1994; Franz leg.; GS 1/16.10.2017; TLMF • 1 ♂, same collection data; 7 Jun. 1991; K. Burmann et al. leg.; GS 2/16.10.2017; TLMF • 2 ♀♀; same collection data; 1 Oct. 1994; Burmann et Erlebach leg.; GS 3/16.10.2017, 4/16.10.2017; TLMF • 1 ♂; prov. Verona, Albisano; alt. 450 m; 27 May–3 Jun. 1962; K. Burmann leg.; TLMF • 1 ♂; prov. Trentino, Pietramurata; alt. 250 m; mid Jun. 1978; F. Zürnbauer leg.; TLMF • 1 ♀; Verona, Mte. Maderno; alt. 250 m; mid Sep. 1963; K. Burmann leg.; TLMF • 1 ♀; Südtirol, Auer; 21 Jun. 1957; Hernegger leg.; TLMF • 3 ♂♂, 1 ♀; Südtirol, Eing. Schnalstal; alt. 700 m; mid Jun.1968; K. Burmann leg.; TLMF • 2 ♀♀; Südtirol, S Kalterer See; alt. 230 m; 26 Jun. 1990; P. Huemer leg.; TLMF • 1 ♂; same collection data; 14 Jun. 1991; TLMF • 2 ♂♂; Südtirol, Umg. Bozen, Kampenn; 26 Jun. 1991; P. Huemer leg.; TLMF • 1 ♂; Carrara; alt. 200 m; early Jun. 1979; F. Zürnbauer leg.; TLMF • 1 ♂, 1 ♀; Elba, Porto Ferroio; alt. 40 m; late May 1967; F. Zürnbauer leg.; TLMF • 1 ♂; Sardinia, Umg. Tempio, Mte. Limbara; alt. 1200 m; 27 May1973; Laubmeier, Sommerer & Witt leg.; TLMF • 1 ♂; Sardinia, Umg. Aritzo, Gennargentu; alt. 750 m; 4–5 Jun. 1973; Laubmeier, Sommerer & Witt leg.; TLMF • POLAND • 1 ♂; Poznań, Ogrody; 13 Jun. 2012; W. Kubasik leg; GS 1/15.3.2019; • CWK 1♂; Poznań, Ogrody; 19 Jun. 2012; W. Kubasik leg.; GS 2/15.3.2019; CWK • 1 ♀; Komorniki; 19 Jun. 2002; GS 1/15.3.2002; W. Kubasuk leg.; CWK • AUSTRIA • 2 ♂♂; Wien, Döbling, Cobenzl; alt. 385 m; 15 Jun. 2012; F. Lichtenberger leg.; TLMF • 3 ♂♂; Niederösterreich, 10 km SE Schwechat, Seiherwiese; 1 Jul. 1998; F. Lichtenberger leg.; TLMF • 3 ♂♂; Niederösterreich, Ebreichsdorf, Welscherhalten; 2 Jul. 1999, F. Lichtenberger leg.; TLMF • 1 ♂; Niederösterreich, Rubring; 20 Jun. 1998; F. Hofmann leg.; TLMF • 1 ♂; Niederösterreich, Amstetten, Sonnleiten; 16 Jun. 1997; H. Brandstetter leg.; TLMF • 1 ♂; Niederösterreich, Purgstall/Erlauf; 7 Jul. 1995; F. Ressl leg.; TLMF • 1 ♂; Oberösterreich, Linz, Biologiezentrum; 11 Jun. 2002; J. Wimmer leg.; GS 3771; TLMF • 2 ♂♂, 2 ♀♀; Vorarlberg, Hard, Fußach, NSG Rheindelta-Rohrspitz; alt. 397 m; 29 Jun. 1992; P. Huemer leg.; TLMF • FRANCE • 1 ♂; Alpes Maritimes, Nice, Bd. Tzarewitsch; 26 May 1971; F. Dujardin leg.; TLMF • 1 ♂; Alpes Martitimes, mt. Alban; alt. 200 m; 4 Jun. 1962; F. Dujardin leg.; TLMF • 1 ♂; Bouches du Rhone, La Ciotat; 4 May

1994; J. Nel leg.; GS 9763 TLMF • 1 ♂; Var, Massif du Pradet; J. Nel leg.; TLMF • 1 ♂; Vaucluse, Mormoiron; alt. 20 m; 6 May 2000; J. Nel leg.; TLMF • 2 ♂♂; Corse, Pinarello; alt. 10 m; early Jun. 1972; F. Zürnbauer leg.; TLMF • CROATIA • 2 ♂♂, 1 ♀; Rovinij; alt. 50 m; 10 Sep. 2002; H. Deutsch leg.; TLMF • 1 ♂; Cres isl., Stivian; 14–17 May 1996; F. Lichtenberger leg.; TLMF • GREECE • 1 ♂; Crete, Heraklion prefecture, Fodele; alt. 100 m; 25 May 2000; W. Ruckdeschel leg.; GS 5/16.10.2017; TLMF • BULGARIA • 1 ♂; Petrich district, Rupite area; 41.4620°N, 23.2583°E; alt. 200 m; 15 May 2007; B. Zlatkov leg.; GS 6/16.10.2017; NMNHS • ALBANIA • 1 ♂; Korça Region, Boboshtica Village; 40.5405°N, 20.7918°E; alt. 1220 m; 4 Jun. 2018; S. Beshkov leg.; GS 1/17.10.2018; NMNHS • MACEDONIA (Republic of Northern) • 1 ♂; Vardar River Valley, Demir Kapiya; 41.3826°N, 22.1958°E; alt. 240 m; 10 Jun. 2018; S. Beshkov & A. Nahirnic leg.; GS 2/17.10.2018; NMNHS • SPAIN • 4 ♂♂, 3 ♀♀; Catalonia, Vidreras; 6–15 Jun. 1993; J. Wimmer leg.; TLMF.

Diagnosis. The wing pattern of males resembles that of *C. neglectana* and *C. eatoniana*. The male genitalia are most similar to *C. eatoniana* but the phallic process is smaller, the phallus lacks a keel, the cornuti are smaller, the valvae are more slender, and the labis is different. The females of *C. consimilana* and *C. eatoniana* externally are very similar but the genitalia (colliculum) show considerable difference, it is much larger in *C. eatoniana*, with a long process at the left side.

Description. Adult. Sexual dimorphism prominent. Male (Fig. 11A–F). Head. Vertex, frons and labial palps fulvous. Antennae with scapus and pedicellus ochreous, flagellum with fulvous brown-tipped scales and numerous sensilla trichodea as long as width of flagellomeres. Thorax. Dorsally fulvous, ventrally creamy, legs brownish. Tegula fulvous. Forewing relatively wide, with length 6.3–7.8 mm (mean 7.0, *N* = 13). Costa basally convex, apically straight, costal fold extending from base to ca. 0.4–0.6 of costa (Fig. 3E). Upperside background dark yellow with rusty reticulate pattern. Markings fulvous to brown: basal blotch atrophied, more prominent at dorsum forming dark dot; median fascia often with darker borders; subapical blotch triangular, often ill-defined. Cilia concolourous with background. Underside pale brown with creamy longitudinal blotch in the distal half of costal area. Hindwings upperside monochrome grey with paler cilia, underside with creamy costal half pale grey with scattered pale brown scales and monochrome pale grey dorsal half. Forewings with variable colouration, darker or paler, sometimes with reduced markings similarly to female (Fig. 11F). Abdomen. Grey. Male genitalia (Fig. 12A–E). Uncus with variable shape and median incision depending on preparation, more or less ovoid, widened distally, rounded. Gnathos plough-shaped. Socius small, membranous. Valvae pointed dorsolaterally when mounted on slide. Costal sclerite protruded medially into large triangular labis with elongated tip (Fig. 5F, G). Apical part of sacculus ca. 1.6× longer than basal part, both forming angle of 160–165°, saccular process small, curved, thorn-shaped. Distal part of valva membranous, widened apically and protruding into small brachiola, costal edge slightly convex, with longitudinal fold on the median surface bearing row of 5–8 large modified setae. Phallus smooth, coecum medially concave, basal part curved ventrad at ca. 140°, distal part smoothly curved dorsad, with small

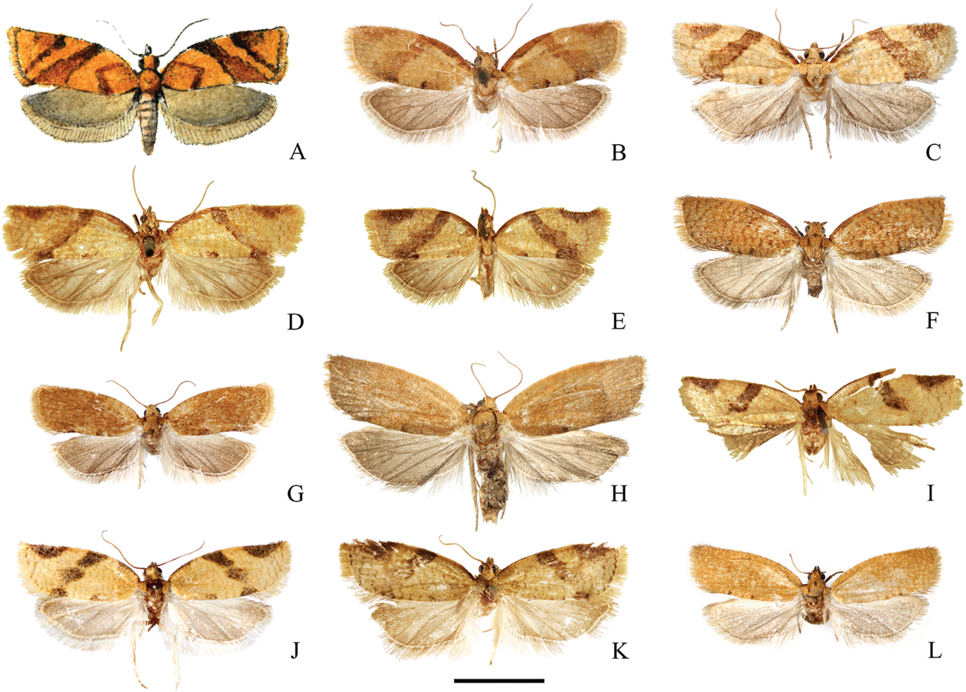

Figure 11. Adults of *Clepsis consimilana* species group **A–H** *C. consimilana*: **A** illustration from the original description of *Tortrix consimilana* **B** neotype of *T. consimilana* **C** male, Bulgaria **D** lectotype of *T. unifasciana* **E** Holotype of *Siclobola placida* **F** male, Poland **G** female, Italy **H** female, Poland **I–L** *C. eatoniana*: **I** Lectotype of *T. eatoniana* **J** male, Spain **K** Lectotype of *Tortrix xylotoma* (courtesy by D. Lees and Trustees of the Natural History Museum, London) **L** female, Spain. Scale bar: 5 mm, all to scale.

sharp tipped lateral process as long as 0.26× distance between anterior opening and tip of phallus, slightly curved laterally and not overpassing tip of phallus. Caulis small, adjoining coecum. Vesica cylindrical, curved at angle of 100–120° to phallus, with expansion basally and finger-like sinistrodorsal diverticulum (Fig. 13A, B). Three to four deciduous aciculate, robust, weakly sinuate, slightly flattened cornuti attached ventroapically (Fig. 7D). Gonopore located at left to cornuti sockets and diverticulum. Female darker than male, with uniform forewings (Fig. 11G, H). Head. Frons, vertex and labial palps fulvous to ochreous, antennae with fulvous brown-tipped scales and sparse sensilla trichodea shorter than width of flagellomeres. Thorax dorsally ochreous, ventrally creamy, legs brownish. Forewing length 6.3–7.6 mm (mean 6.8, $N = 3$). Upperside ground colour ochreous to rusty with darker reticulated pattern and more or less atrophied ill-defined markings. Basal blotch reduced to dark spot at dorsum, median fascia more or less prominent in costal and dorsal area or completely reduced. Cilia paler than background. Underside pale brown, costal and terminal areas paler or creamy with reticulate pattern. Hindwing upperside pale grey, underside with whitish or pale grey reticulate patterned costal half and monochrome pale grey dorsal half. Abdomen. Grey. Female genitalia with papillae anales not modified (Fig. 14A). Apo-

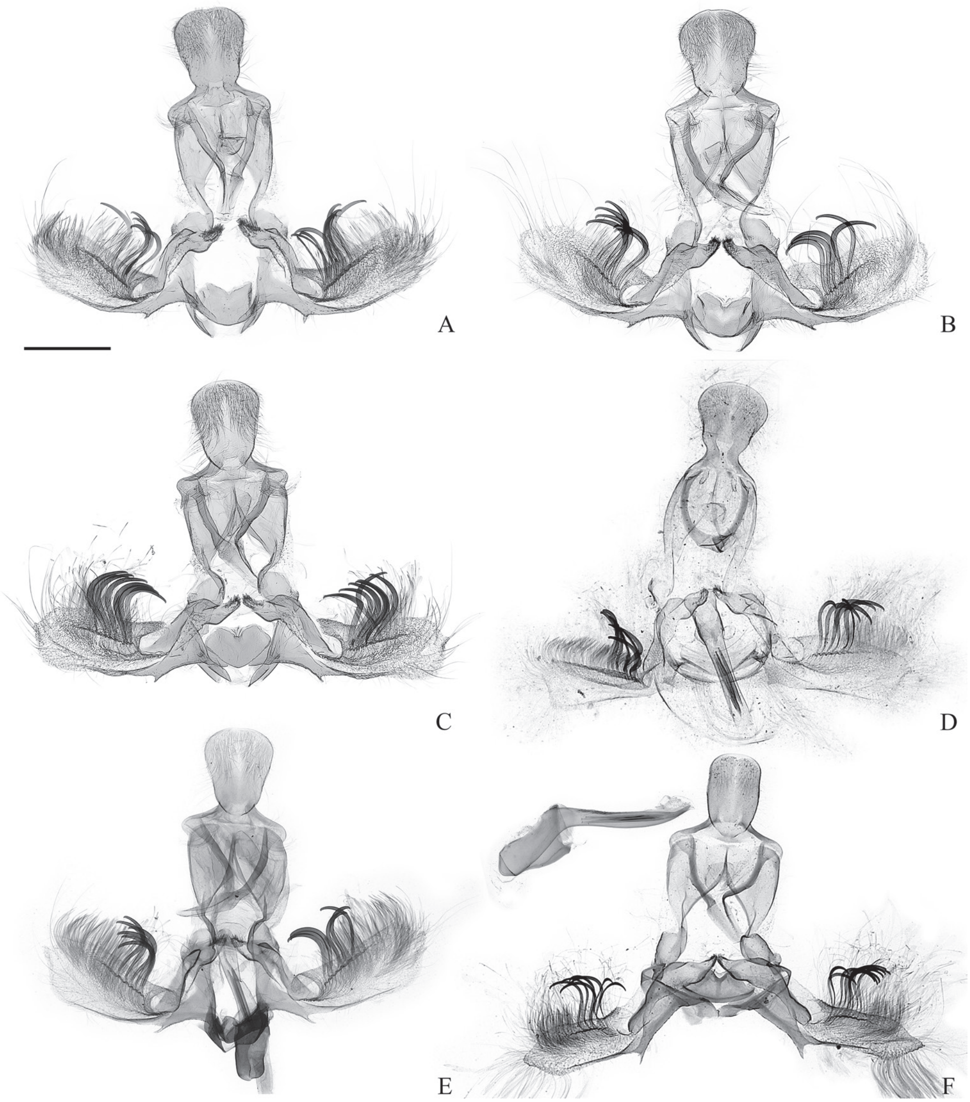

Figure 12. Male genitalia of the *Clepsis consimilana* species group (some without phallus) **A–E** *C. consimilana*: **A** *Tortrix consimilana* neotype **B** *C. consimilana*, Italy **C** *C. consimilana*, Albania **D** *Siclobola placida* holotype **E** *T. unifasciana* lectotype **F** *T. eatoniana* lectotype. Scale bar: 500 µm, all to scale.

physes anteriores 1.5× longer than apophyses posteriores. Sterigma widened caudad, with lateral sclerotised pockets cephalad, large excavation on the dorsal wall and wide v-shaped lamella antevaginalis. Colliculum large, with length 0.17× length of ductus bursae, slightly bent to left, funnel-shaped, with plicate longitudinal sclerotisation forming small process at left, and spherical protrusion at cranial end consisting of colourless thick cuticle. Ductus bursae long and narrow, emerging at left side of cuticular protrusion, with cestum extending along cranial 0.8× of its length. Ductus seminalis

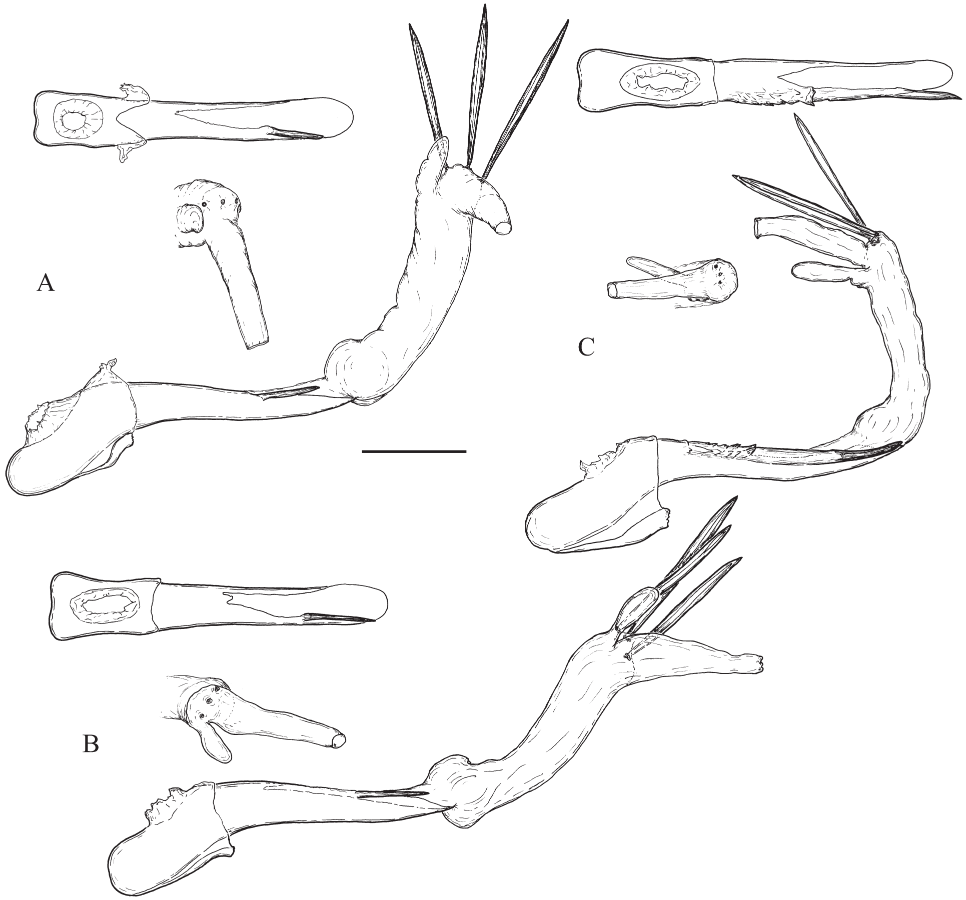

Figure 13. Phalli with vesicae everted of the *Clepsis consimilana* species group **A–B** *C. consimilana*: **A** neotype of *Tortrix consimilana* **B** specimen from Italy **C** *C. eatoniana*, Spain. The phallus of each species is shown in three aspects from top to bottom: sclerotised phallic tube (without vesica) in dorsal view, tip of vesica in dorsal view and whole phallus in left view. Scale bar: 250 µm.

inserted dorsally at caudal end of ductus bursae. Corpus bursae ovoid, with large falcate signum with capitulum and flat signum located near end of cestum consisting of sclerotised papillae (Fig. 9D, F).

Preimaginal stages. Detailed descriptions of the ovum, larvae of all instars, and pupa are provided by Sheldon (1920) and Bradley et al. (1973). The chaetotaxy of the larva is described by Swatschek (1958).

Molecular data (Fig. 16). BIN: BOLD:AAC4212. The intraspecific average of the barcode region is 0.34%, the maximum distance 1.08% (p-dist) (*N* = 29). The minimum distance to the nearest neighbour, *Clepsis eatoniana*, is 2.25%.

Distribution (Fig. 17). Europe, Asia Minor, Syria, European Russia, W Africa to Lebanon, Madagascar (introduced; the data come from the type specimen only), North America (introduced) (Bradley et al. 1973, Razowski 2002).

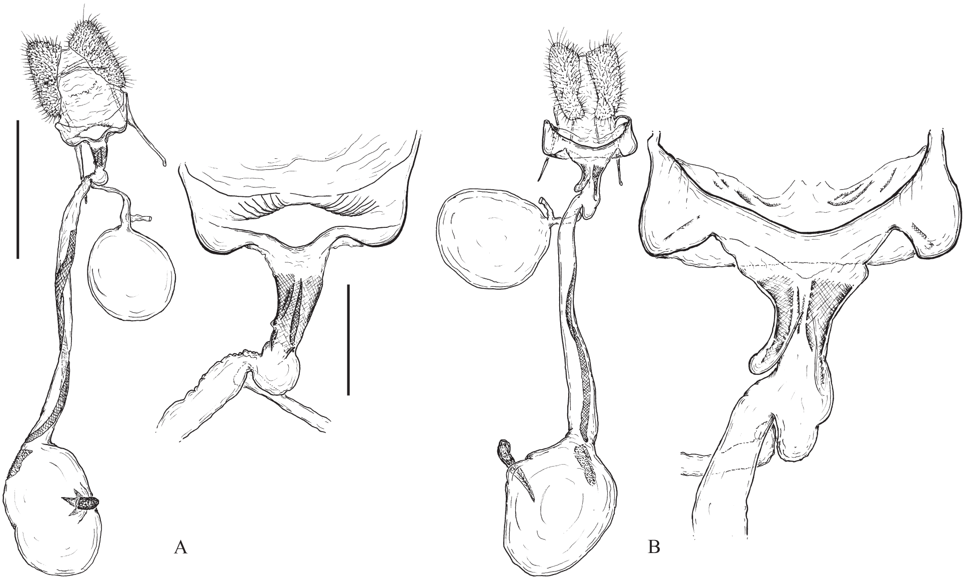

Figure 14. Female genitalia of the *Clepsis consimilana* species group **A** *C. consimilana*, Italy **B** *C. eatoniana*, Spain. The sterigma and colliculum are shown enlarged to the right of the whole genitalia. Scale bars: 1 mm (whole genitalia), 250 µm (sterigma and colliculum).

Ecology. Moths are on the wing from May to October in shrubby habitats. The larvae feed on dead or withered leaves of *Crataegus*, *Malus*, *Carpinus*, *Polygonum*, *Hedera*, *Lonicera*, *Ligustrum*, *Syringa*, and overwinter in the third instar (Bradley et al. 1973).

Remarks. The collection of Jakob Hübner was acquired by Vincenz Abbate Edler von Mazzola in the early part of the 19th Century, and the European material was deposited in the "Hof-Naturalien-Kabinett" in 1823 where most of the material was destroyed by fire during the Vienna Rebellion of 1848 (Calhoun 2003, Gilligan and Wright 2013). Despite a personal search by PH we found no potential type material and conclude that the syntypes are lost or destroyed. As the boundaries of this taxon are revised, designation of a neotype is necessary to preserve the stability of nomenclature. The type locality is presumably Europe, as indicated by the title of the original description (Hübner 1817) and material may have originated from Germany as many other species described by Hübner. Here we designate as neotype a male specimen with an everted vesica and preserved cornuti, and wing pattern resembling the illustration in the work by Hübner (1817: pl. 38, fig. 239). Though not very detailed, this painting demonstrates some important differences with *C. consimilana* sensu auctt., namely the shape of basal fascia and costa, and questions the interpretation of the taxon. The painting either refers to another representative of the tribe Archipini or may be a result of an artistic decision. Without studying the type material any interpretation of the figure may be incorrect, therefore we prefer to preserve the present-day concept for *C. consimilana*. Obraztsov (1955) considered *consimilana* as a probable synonym of *unifasciana*. Bradley and Martin (1956) established the name *consimilana* as having priority over *unifasciana*, though they did not state explicitly this nomenclatorial act. We found support for this synonymy as the lectotype of *Tortrix unifasciana* appears conspecific with the neotype of *C. consimilana*. Razowski (1979) synonymised *Tortrix eatoniana* with *C. consimilana* (see below); this is not justified in our opinion. Some taxa from the synonymic list were not examined by us but, minding the minimal genetic diversity throughout Europe, we consider all of them correctly synonymised with *C. consimilana*. *Cacoecia unifasciana* var. *semiana* was erroneously regarded as a synonym of *Clepsis consimilana* (see above).

Clepsis eatoniana **(Ragonot, 1881), stat. rev.**

Tortrix eatoniana Ragonot, 1881: 231 (Portugal).
Tortrix xylotoma Meyrick, 1891: 13 (Algeria) syn. nov.
Clepsis razowskii Gastón, Vives & Revilla, 2017: 691 (Spain) syn. nov.
non *Clepsis consimilana* (Hübner, 1817)

Material examined. Lectotype ♂ by designation of Razowski (1979), pinned, with 7 labels: "Eatoniana / Rag. n. sp. / Portugal" [handwritten; the same label on backside:] "port Olivaes / Port. [ugal] / 24.4.80" "*Tortrix* / *eatoniana* Rag. / Ent. month. Mag., / 1881, 17, p. 231" [handwritten] "Mus. Paris / coll. Ragonot / 15997" [printed and handwritten] "Type" [red printed] "communiqué / á M. J. Kennel" [handwritten] "Genitalia ♂ / P. Viette / prép. No. 3699" [printed] "1901 / coll. E. L. Ragonot / Muséum Paris" [printed]; male genitalia on a slide with two labels: "♂ / *Tortrix* / *eatoniana* / Rag. / Type" "Prép. P. Viette / # 3699 / Muséum / Paris / Type" [both handwritten, with red border].

PORTUGAL • 1 ♂; Lisbon, Olivais; 24 Apr. 1880; Ragonot leg.; GS 3699; MNHN.

Lectotype ♂ of *Tortrix xylotoma* by designation of Razowski (1979), pinned, with 7 labels: "Bougie / Algeria / 25/4/90" [handwritten] "Tortrix / xylotoma / Meyr[ick] / Type ♂" [handwritten and printed] "Lecto- / type" [circular with violet border] "Type" [circular with red border] "♂ genitalia on / slide 6.IV.1949 / J.F.G.C. 9364" [printed and handwritten] "Meyrick coll. / B. M. 1938–290" "NHMUK010219594 [QR code]".

ALGERIA • 1 ♂; Béjaïa; 25 Apr. 1890; Meyrick leg.; BMNH NHMUK010219594.

Other material: SPAIN • 8 ♂♂, 1 ♀; Valencia, El Saler, Albufera ; 39.3278°N, -0.3078°W; alt. 5 m; 18 May 2004; P. Huemer leg.; GS 1/18.10.2017, 2/18.10.2017, 3/18.10.2017; TLMF • 5 ♂♂, same collection data; 8 Sep. 2005; P. Huemer leg.; TLMF • 1 ♂, 1 ♀; Valencia, 5 km NE Albufera, Sierra de Crevillente; alt. 450 m; 26 May 2004; P. Huemer leg.; TLMF • 1 ♀; Valencia, Santa Pola, Playa del Pinet; 38.1585°N, -0.6256°W; alt. 5 m; 5Sep. 2005; P. Huemer leg.; GS 4/18.10.2017; TLMF.

Diagnosis. The wing pattern in males resembles those of *C. trivia*, *C. acclivana*, and *C. consimilana*. *Clepsis eatoniana* differs from *C. consimilana*, by the more yellowish instead of fulvous forewing ground colour. The most characteristic feature in the external morphology of *C. eatoniana* is the absence of a forewing costal fold, in contrast to *C. consimilana*. The male genitalia are similar to those in *C. consimilana* but the valva is more slender, elongate distally, and the modified large setae are more numerous and more slender. The phallus is adorned with spines, with the keel and terminal process overpassing its tip; in *C. consimilana* the phallus lack lateral spines and a keel, and the phallic process is shorter. The caulis in *C. eatoniana* is larger, the vesica is bent at nearly a right angle to the phallus, its diverticulum has different location, and the cornuti are more slender and smoother. Females of *C. eatoniana* are not distinguishable externally from the females of *C. consimilana* but differ from *C. striolana* by the presence of two brown dots on the forewing. The female genitalia of *C. eatoniana* are similar to those in both the *C. neglectana* and *C. consimilana* species groups, but the protrusion on the right side of the colliculum is much larger and elongated, and the sterigma has larger lateral pockets.

Description. Adult. Sexual dimorphism prominent. Male (Fig. 11I–K). Head. Vertex fulvous, frons and labial palps brown. Antennae with brown scales on scapus and fulvous scales on pedicellus and flagellum, and with numerous sensilla trichodea as long as width of flagellomeres. Thorax. Fulvous dorsally and creamy ventrally, legs brownish. Tegula fulvous with brown costal margin. Forewing with length 6.8–7.4 mm (mean 7.1, $N = 3$), elongated, costa convex at basal half, slightly sinuate apically. Costal fold lacking (Fig. 3F). Upperside background dark yellow with fulvous reticulate pattern more prominent in the paler subterminal area, cilia concolourous or paler. Basal blotch atrophied, with remnants of darker scales at costa and dorsum. Median fascia brown with lead refractive tint, almost interrupted at middle of median cell by yellowish scales, often ceasing at vein CuA. Subapical blotch brown, triangular, more or less well defined. Underside pale grey-brown with creamy longitudinal blotch in distal half of costal area. Specimens with paler and darker forewings and incomplete median fascia observed. Hindwing upperside monochrome pale grey with paler cilia, underside whitish with scattered pale grey-brown scales. Abdomen. Grey. Male genitalia (Fig. 12F, 15). Uncus variably shaped, more or less trapezoidal, slightly widened distally, rounded, with slightly convex distal edge which may look incised if excessive pressure is applied on coverslip. Gnathos plough-shaped. Socius small, membranous. Valvae pointed dorsolaterally when mounted on slide. Costal sclerite wide, protruded medially into large triangular labis with elongated spinulate tip (Fig. 5H). Basal and apical part of sacculus of nearly equal length, both forming angle of 150–160°, saccular process large, flat, triangular. Distal part of valva membranous, comparatively small, narrow, with parallel costal and saccular margins, apically triangular, without distinct brachiola, with longitudinal fold on the median surface bearing row of 8–12 large modified setae. Apical part may look as brachiola due to deformation during preparation. Phallus robust, coecum medially concave, basal part curved ventrad at ca. 140°, distal part smoothly curved dorsad, with wide keel on left side grading into large sharp tipped lateral process as long as 0.23× distance between anterior opening and tip of phallus, slightly curved laterally and dorsally, overpassing phallic tip. Several more or less prominent spines pointed caudad located at basal part of keel. Caulis large, widely separated from coecum. Vesica cylindrical, curved at 80–90° to phallus, with small expansion basally and

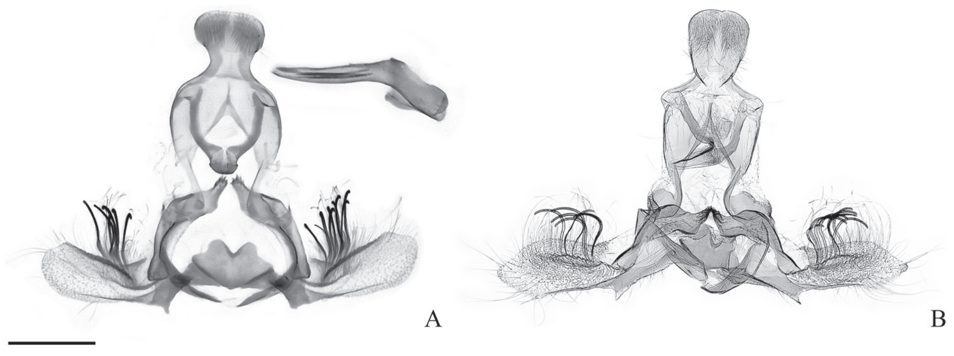

Figure 15. Male genitalia of the *Clepsis consimilana* species group: *C. eatoniana* **A** *Tortrix xylotoma* lectotype (courtesy by D. Lees and Trustees of the Natural History Museum, London) **B** *C. eatoniana*, Spain. Scale bar: 500 µm, all to scale.

fingerlike dorsal diverticulum (Fig. 13C). Three deciduous, slender, straight cornuti attached ventroapically (Fig. 7E). Gonopore located in straight line with cornuti sockets and diverticulum. Female darker than male (Fig. 11L). Head. Vertex rusty, frons and labial palps brown. Whole antennae with brown scales, sensilla trichodea sparse, shorter than width of flagellomeres. Thorax. Rusty dorsally and creamy ventrally, legs brownish. Tegula rusty with brown costal margin. Forewing length 6.7 mm (n = 1), with shape as in males, upperside uniformly rusty with ill-defined reticulate pattern more distinct in distal half and two brown dots at dorsum, underside mainly creamy with scattered pale brown scales, denser in middle of wing. Hindwing upperside grey brown, underside creamy with darker dorsal part. Abdomen grey.

Female genitalia (Fig. 14B). Papillae anales not modified. Apophyses anteriores 1.1× longer than apophyses posteriores. Sterigma relatively wide, widened cephalad, with large lateral sclerotised pockets, large excavation on dorsal wall and wide v-shaped median part of lamella antevaginalis. Colliculum large, with length 0.25× length of ductus bursae, asymmetrical, funnel-shaped, with well-developed sclerotisation and two evaginations: very large one at right and small one at left, both consisting of colourless thick cuticle. Ductus bursae long and narrow, emerging at left between outpocketings, with cestum extending along cranial 0.7× of its length. Ductus seminalis inserted dorsally at caudal end of ductus bursae. Corpus bursae ovoid, with large falcate signum with capitulum and flat signum consisting of sclerotised papillae located near end of cestum (Fig. 9E).

Preimaginal stages unknown.

Molecular data (Fig. 16). BIN: BOLD:AAJ1025. The intraspecific average of the barcode region is 0.09%, the maximum distance 0.15% (p-dist) (*N* = 2). The minimum distance to the nearest neighbour *Clepsis consimilana* is 2.25%.

Distribution (Fig. 17). Europe. Portugal: Lisbon, Olivais; Ponte de Mucela (Ponte de Morcellos) (Ragonot 1881); Lusitania; Spain: Cadiz, Granada, Malaga, Sevilla, Zaragoza; possibly France (Gastón et al. 2017), Valencia. Africa: Algeria.

Ecology. The moths were collected in macchie habitat (Gastón et al. 2017) from April to the first half of September. The larval host plant is unknown.

Figure 16. Neighbour-joining tree of *Clepsis neglectana* and *C. consimilana* species groups (Kimura 2 parameter, built with MEGA6; cf. Tamura et al. 2013). Note: the scale bar only applies to internal branches between species. The width of the triangles represents the sample size, and the depth the relative genetic variation within the cluster (2× scale bar). Source: DNA Barcode data from BOLD (Barcode of Life Database, cf. Ratnasingham and Hebert 2007).

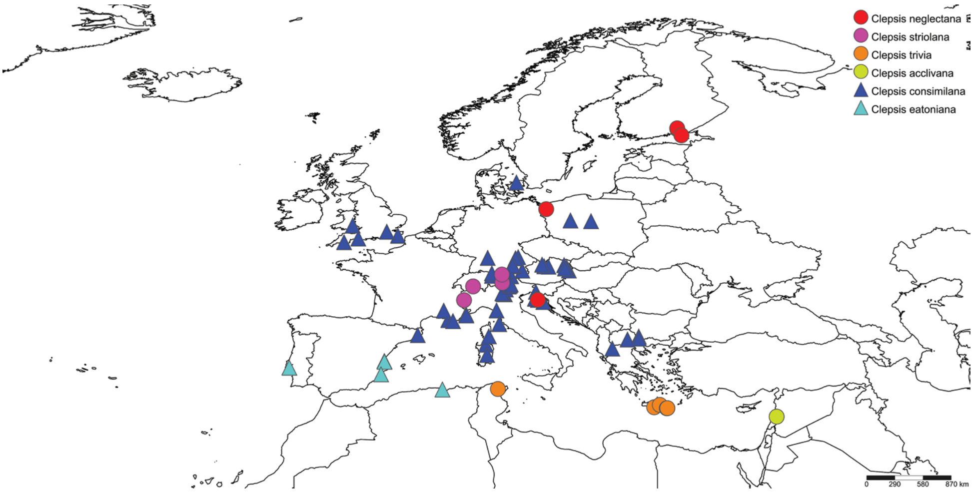

Figure 17. Distribution map of examined material of *Clepsis neglectana* and *C. consimilana* species groups. Map created with SimpleMappr (http://www.simplemappr.net).

Remarks. The species was described by Ragonot (1881) after two males from Portugal. He emphasised its resemblance with *C. consimilana*. Razowski (1979) considered it conspecific with *C. consimilana*, probably relying on the superficial similarity of the modified setae of the valvae of these two taxa. The female remained unstudied until Gastón et al. (2017) described it again under the name *C. razowskii*. *Tortrix xylotoma* was sunk into *C. neglectana* by Razowski (1979). This synonym is not justified as the genitalia of the male lectotype (illustrated also by Clarke 1958: pl. 109) rather resemble *C. eatoniana* regarding the shape of labis, presence of modified setae on the valva, and morphology of the phallus. The preparation of the genitalia is poor, therefore the shape of uncus and valvae looks unusual. The wing pattern fits with *C. eatoniana*. After comparison of material from Spain, the lectotype of *T. eatoniana*, the lectotype of *T. xylotoma*, the original description of *C. razowskii*, and numerous photographs of specimens identified as *C. razowskii* by the authors of the latter taxon, we concluded that all these specimens are conspecific and the present valid name of the species should be *C. eatoniana*.

Discussion

The taxa treated in the present paper are not easily distinguished from each other because of prominent sexual dimorphism in some of them, variability in wing pattern and considerable similarity in the genitalia. The subtle differences in both male and female (when available) genitalia, secondary sex characters (male forewing costal fold) and barcode gap between the populations support presence of more than the two taxa currently recognised. The most defining characters are found in the genitalia. Two groups of taxa can be recognised: those with large modified setae on the median surface of the valvae in males, and those without such setae. Apparently, this was the main criterion for prior synonymisation resulting in only two species: *C. consimilana* (with setae) and *C. neglectana* (without setae). Further scrutinising of the genital characters revealed existence of several taxa, which can be treated as species. Here we propose two species groups named after the oldest described taxon in each of them. They should not be confused with the groups proposed by Razowski (1979) for subgeneric divisions of *Clepsis*, which appear hierarchically superior to our groups.

Since recently collected material for mtDNA sequencing was available for European populations only, the status of some taxa of the *C. neglectana* species group remains unresolved. *Clepsis severana* and *C. semiana* appear to be closely related considering the genital morphology, and their synonymy with some of the remaining species of the group cannot be excluded. They are known only from their holotypes (by monotypy), and moreover, the morphology of the vesica is inaccessible because of the prolonged embedding in microscope slide media (*C. semiana*) or the genitalia slide is missing (*C. severana*). On the other hand, each of them demonstrates some differences with all remaining members of the group, which seemingly are not lesser than those between well separated taxa as *C. neglectana* and *C. trivia*. *Tortrix xylotoma* is certainly a synonym of a member of the *C. consimilana* species group, namely *C. eatoniana*, but not of *C. neglectana* as it was treated up to now. The status of *C. acclivana* is more or less controversial because of lacking DNA barcode sequences, but the two male specimens with practically identical morphology, different to all the other taxa convinced us that they represent a distinct species.

The Cretan population of *C. trivia* is well separated genetically and morphologically from the other European species (*C. neglectana* and *C. striolana*), but demonstrates certain morphological similarity with the other Mediterranean taxon, *C. acclivana*, and is probably related to it. It should be emphasised that the Cretan specimens were assigned to *C. trivia* solely on male genitalia morphology without studying the vesica of the holotype and its DNA barcode and it may still represent a separate taxon.

The barcode distance between *C. neglectana* and *C. striolana* is comparatively small. The genital morphology of these two taxa also demonstrates considerable similarity, but they are well distinguished by wing pattern and costal fold. Apparently, *C. striolana* has a range limited in the region of the Alps. It can be assumed that these species had been isolated recently, maybe only after the last glacial period, and even continuing gene flow cannot be dismissed. Some taxa still in synonymy with *C. neglectana* may turn out to be *species bona* as well, e.g., *Tortrix dorana* from Eastern Kazakhstan.

The *C. consimilana* species group encompasses only two species and is separated from the *C. neglectana* group by considerable morphological and genetic gaps. The species have strikingly disproportional distribution ranges: *C. eatoniana* appears to be a Western-Mediterranean species distributed in the Iberian Peninsula and Algeria, and possibly part of France, but *C. consimilana* is distributed throughout the rest of Europe, part of Asia and has been introduced to the tropics (Madagascar) and North America. Some morphological variability in *C. consimilana* (the position of cornuti and other minor male genitalia characters) somewhat contradicts the genetic invariance within this species in Europe.

The overreliance or underestimation of certain genital characters can cause incorrect taxonomic interpretations, as it is demonstrated by the above mentioned taxonomic problems. Traditionally, the shape of uncus, gnathos and valvae is widely used in taxonomy of Lepidoptera, but our experience with other groups as well (Zlatkov and Huemer 2017) proved that these characters must be treated cautiously. Being complex three-dimensional structures, their visible shape depends on several factors: fixation before embedding in a permanent medium, thickness of the medium layer and pressure applied on the coverslip. The shape of uncus and distal part of valva are especially prone to deformation in the species treated here. Some relatively flat sclerites were found to be less affected and hence more useful for taxonomical purposes, e.g., the sacculus and in lesser extent the costal sclerite, including the labis, of the valva. The morphology of the vesica and phallus is believed to be very informative but must be studied before mounting on a slide. Moreover, these structures are considerably simplified in the taxa considered here and provide a relatively small number of characters. The female genitalia can also provide many characters but only in a comparatively limited region: the colliculum and part of the sterigma. In fact, these are the structures which interact directly with the male genitalia. It can be assumed that the distal part of the phallus is inserted into the colliculum and its lateral process is accommodated into the transparent cuticular extension at the right cephalic end, therefore they should be studied in comparative aspect to obtain additional taxonomical information. Some taxonomic problems in the *C. neglectana* and *C. consimilana* species groups had apparently ensued from ignoring female genitalia morphology or simply the lack of female specimens.

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RESEARCH ARTICLE

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Thitarodes shambalaensis sp. nov. (Lepidoptera, Hepialidae): a new host of the caterpillar fungus *Ophiocordyceps sinensis* supported by genome-wide SNP data

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Abstract

A new species of ghost moth, *Thitarodes shambalaensis* **sp. nov.**, is described from Yanzigou glacier, Mt. Gongga, Sichuan, China. The species is a host of the economically important caterpillar fungus *Ophiocordyceps sinensis*. Establishment of this new species is supported by morphology and genetic differentiation measured in a CO1 phylogeny and in genome-wide SNP coverage. A summary tree from 538 sequences of different genetic markers from *Thitarodes* (including sequences extracted from caterpillar fungus sclerotium samples) support the genus *Thitarodes* as a monophyletic group, and indicate that *Thitarodes* is the host genus for *O. sinensis*. Sampling efforts so far have centered on half of the known phylogenetic diversity of *Thitarodes*, with some species-level clusters (separated by < 2.5% genetic distance) containing 17 described species. Fifteen clusters are known from either a single "orphan taxon" or a single sequence from a caterpillar fungus sclerotium sample. We provide suggestions for building a more robust phylogeny of the genus *Thitarodes* and highlight some of the conservation threats that species from this genus face due to unprecedented habitat exploitation.

Keywords

RAD-Seq, phylogeny, new species, caterpillar fungus, Kangding, Mt. Gongga, Sichuan, China

Introduction

The genus *Thitarodes* Viette, 1968 of the ghost moth family Hepialidae was first established by Viette,1968 to accommodate *Hepialus armoricanus* Oberthür, 1909 found in China and three other new species found in Nepal (*T. danieli* Viette, 1968, *T. eberti* Viette, 1968, and *T. dierli* Viette, 1968). Chu (1965) was the first to show that *T. armoricanus* and other species of this genus are the hosts of caterpillar fungus: the larvae of which are often parasitized by the entomophagous fungus *Ophiocordyceps sinensis* (Berk.) G.H. Sung et al. (family Ophiocordycipitaceae) (see Sung et al. 2007 and review by Shrestha et al. 2014). The infection of *Thitarodes* larvae by *O. sinensis* produces a sclerotium consisting of hyphal threads enclosed within the larval exoskeleton. This sclerotium, known as caterpillar fungus, is coveted in eastern Asia, particularly China, as a traditional herbal medicine (Winkler 2008, 2010) and is in decline due to both overharvesting and climate change (Hopping et al. 2018).

After Chu's (1965) discovery of *T. armoricanus* as the host for caterpillar fungus, many new ghost moth species were subsequently described in the 1980s by workers at the Chinese Academy of Sciences under the genus *Hepialus* Fabricius, 1775 (see review by Wang and Yao 2011). These taxa were transferred to *Thitarodes* in the global revision of infraorder Exoporia by Nielsen et al. (2000). More recently, eight new species have been described by Chu et al. (2004) under *Hepialus*. A total of ten new species has been described by different authors under *Thitarodes* (Ueda 2000; Zhang et al. 2007; Maczey et al. 2010; Zou et al. 2011; Jiang et al. 2016).

The inventory by Ebbe Nielsen et al. (2000) listed 51 species under *Thitarodes* worldwide, while Chu et al. (2004) listed 54 species of this genus in China alone. The review of Wang and Yao (2011) estimated that only 37 of the described *Thitarodes* species are potential hosts of the entomophagous fungus *O. sinensis*. The inconsistencies in the number of species in *Thitarodes* underscores several difficulties in the taxonomy of this group:

1. *Thitarodes* adults are difficult to collect. Both Ueda (2000) and Zou et al. (2011) have mentioned that adults of this genus do not come readily to light and have a short active period around sunset. Ueda (2000) estimated that adults of some species are only active for about a week annually. Tao et al. (2015) conducted laboratory rearing studies where the time to complete the developmental cycle from egg to adult was 494 and 780 days for *T. jianchuanensis* (Yang, 1994) and *T. armoricanus*, respectively. Larvae of the genus are subterranean root-borers. The short window for collecting adults is further hindered logistically by the inaccessibility of the species habitat at high elevation (> 3000 m) valleys of the Himalaya and the Tibetan plateau.
2. Since most *Thitarodes* species have few distinctive wing scale patterns, new species descriptions are based primarily on male genitalia, sometimes coupled with a description of wing venation. Moths of the infraorder Exoporia, which contain the genus *Thitarodes*, are distinguished by the unique configuration of female genitalia:

in female exoporian Lepidoptera, sperm is transferred to the egg via an external seminal gutter. This important feature has been largely ignored in descriptions of the genus *Thitarodes*. After re-examining the holotype of *T. armoricanus*, Ueda (2000) concluded that the male holotype designated by Oberthür (1909) was in fact a female; thus, the identity of *T. armoricanus* is problematic without a male holotype. Although some authors describe *Thitarodes* larvae, pupae and adult leg morphology in detail (Ueda 2000; Chu et al. 2004), these traits have not been included in the classification key proposed by Chu et al. (2004).

3. Several *Thitarodes* species described before 2000s were only known by drawings of wing venation and genitalia structure. Access to the holotypes of these species is limited. Many species are only known from a few samples collected in restricted localities (such as *T. zhongzhiensis* (Liang, 1995) in "the middle of Renzhi snow mountain", *T. anomopterus* (Yang, 1994) on the "north-west slope of Mt. Laojun, Yunnan" , *T. jialangensis* (Yang, 1994) in "Jialang county west of Meili mountain"), and have not been further studied (Yang 1994; Liang 1995). This has made comparative analysis across different species of the genus *Thitarodes* difficult. An effort at revising the genus (Zou et al. 2010) divided *Thitarodes* into four genera, but for this paper, we follow the taxonomy of Nielsen et al. (2000).

In recent decades, molecular evidence has helped resolve difficult problems in taxonomy, including elucidating the backbone of non-ditrysian Lepidoptera including Hepialidae (Regier et al. 2015). Although a few sequences of described species of *Thitarodes* have been contributed to GenBank by different authors (see Suppl. material 1: Table S1 and Suppl. material 3: Table S3), not all sequences have been published, and thus the validity of species identifications cannot be verified. A molecular phylogeny for the genus *Thitarodes* has not yet been published. This is likely due to the difficulty of obtaining genetic material from previously described species, either from museum loans or from the field. Creatively, most of the molecular approaches to resolving the taxonomy of the genus *Thitarodes* has taken the unorthodox route of sequencing genetic material of *Thitarodes* from widely-available samples of caterpillar fungus (Zhang et al. 2014; Quan et al. 2014a, b). This method bypasses the difficult task of sampling adult *Thitarodes* moths at high altitude. Comparing genetic material of *Thitarodes* moths and *Ophiocordyceps* fungi from the same caterpillar fungus sclerotium also provides insight into host-parasite coevolution across a wide geographical range. The drawback of this approach is that the sequences of *Thitarodes* obtained from caterpillar fungus typically have no voucher specimens of either adult moth or mummified caterpillar; unless matched with an identical (or extremely similar) sequence from a known species. It is thus difficult to assign the sequences from caterpillar fungus to a particular moth species.

While molecular work using samples of caterpillar fungus sclerotia has only yielded at most three DNA fragments with which to evaluate each sample, the development of next-generation sequencing techniques has offered researchers new genotyping-by-sequencing methods to obtain large numbers of loci from non-model organisms for phylogenetic studies (Andrews et al. 2016). Restriction site Associated DNA sequencing (RAD-Seq) (Davey and Blaxter 2010) can generate genetic markers across many individuals at a reasonable cost. This is achieved by sequencing genomes of reduced complexity using restriction enzyme digestion. As the technique was first designed to identify SNPs at the within-species level, using RAD-Seq to sequence a population of individuals from multiple species might result in a reduced number of intra-species loci (Cariou et al. 2013; Eaton et al. 2017). This is because if the level of genetic divergence among individuals exceeds what is typical at the population level, this will reduce the number of conserved restriction sites the enzymes can target. We would expect highly diverged species pairs with long branch lengths to share few loci from RAD-Seq. This can often be used to identify samples from distinct lineages (for a visualization tool see de Medeiros and Farrell 2018).

In this paper we describe *T. shambalaensis*, a new species in the genus *Thitarodes* from the Yanzigou glacial valley, Mt. Gongga, Sichuan, China, including analysis of wing venation, male genitalia, labial palps, phenology and habitat. We discuss some of the conservation threats this species is facing due to recent habitat exploitation related to local experimentation on caterpillar fungus-farming. We evaluate the validity of *T. shambalaensis* as a new species by providing data from morphology, CO1 sequences (i.e., constructing a phylogenetic tree with all known CO1 sequences of *Thitarodes*) and genome-wide SNP sequences (i.e., comparing intra and inter-species SNP sequence coverage), and we infer a "summary tree" from all known sequences of different genetic segments of *Thitarodes*, both from sequence deposits with known species names and from sequences of caterpillar fungus sclerotia (Suppl. material 3: Table S3). We show that sequences of sclerotia can be matched with sequences from adult *Thitarodes* samples with identifiable names, and that these sequences together constitute a well-established monophyletic group (the genus *Thitarodes*). We briefly evaluate the taxonomic decision of Zou et al. (2010) to split *Thitarodes* into four different genera, highlight gaps in our knowledge from uneven sampling across the different clades revealed by our phylogeny and provide suggestions for building a more robust phylogeny of the genus *Thitarodes*.

Materials and methods

Collection, preservation, and description

All adult samples described were collected between June and July 2016, at Yangliuping (29°41'2.54"N, 101°53'32.24"E), inside Yanzigou glacier, Mt. Gongga, Sichuan (Fig. 2, Fig. 8). Since *Thitarodes* species do not congregate at light traps and mating flights have not been observed for this particular species, collection was undertaken by thoroughly searching through habitat vegetation with flashlights. Adults can be found hanging at the edge of low vegetation, especially near where pupae molts can be seen. Collection for adults was also attempted at other glacial valleys along eastern

Mt. Gongga from June to July between 2016 and 2018 but were unsuccessful. Pupae and larvae were collected at multiple glacial valleys along eastern Mt. Gongga (Fig. 8, see Suppl. material 2: Table S2 for names of seven valleys and their coordinates) as well as a habitat east of the Kangding-Moxi fault (Yajiageng, 29°53'53.12"N, 102° 0'37.87"E) from May to June, 2016 to 2018, with assistance from the local communities (see Acknowledgments). In each valley, at habitats where caterpillar fungus is harvested by local people, multiple 50 × 50 × 30 cm grassland plots were searched to discover ground-boring *Thitarodes* larvae and pupae. Grass coverage were placed back after sampling. Samples were preserved in 90% ethanol. Photographs of specimens were taken with a Nikon Coolpix 4500 digital camera. Dissections were performed after softening adult genitalia in heated 10% NaOH solution for 10–30 mins and transferring the genitalia on dissection slides with glycerol. Wing venation slides were obtained by softening the wings in 30% dish detergent for 30–60 mins, and gently brushing the scales off the wings. Slides were examined and photographed under a Carl Zeiss Stemi 2000-CS stereoscope system and Carl Zeiss SteREO Discovery V12 stereoscopic microscope system. Although previous authors have used the Comstock-Needham venation nomenclature to describe new species (e.g., Maczey et al. 2010; Zou et al. 2011), Wootton's venation nomenclature (1979) was used in our description to reflect differences in wing homology.

DNA extraction, sequencing, and analysis

Genomic DNA of 134 samples of *Thitarodes* were extracted from leg (adult) or thoracic (larvae, pupae) tissue with Qiagen DNeasy Blood and Tissue Kits (Qiagen Inc.). The COI region of 48 samples was amplified and sequenced with LCO1490 (Folmer et al. 1994) and Nancy (Bogdanowicz et al. 1993) primers, following the protocol for Lepidoptera COI sequencing outlined in Boyle et al. (2014) (with the exception that the annealing temperature was set at 55 °C, repeating for 35 cycles). Samples were sequenced at Thermo Fisher Scientific Inc, Shanghai. These sequences were aligned in Geneious Prime (using the MAFFT algorithm) together with nine other COI sequences of described *Thitarodes* species in China and available on GenBank (five of which are segments of mitogenome sequences). Outgroups were selected according to Grehan (2011, 2012). We used either COI sequences or the COI segment of the mitogenome sequence of the East Asian genus *Napialus* Chu & Wang, 1985, the Neotropical genus *Phassus* Walker, 1856, and the Australian genus *Oxycanus* Walker, 1856 (Hepialidae) (see Suppl. material 1: Table S1 for all sequences used). A Maximum Likelihood tree was inferred using the IQ-Tree algorithm (Nguyen et al. 2015) with a free rate variation model (Kalyaanamoorthy et al. 2017) and ultrafast bootstrap (UFBoot, Hoang et al. 2018) integrated 1000 times and SH-aLRT branch test (Guindon et al. 2010). All 134 extracted DNA samples were digested with EcoRI enzyme and library preparation followed the wet lab protocol of Etter et al. (2012). Samples were sent for 150bp pair-end sequencing on an Illumina HiSeq4000 platform located at 1gene Inc, Hangzhou.

Illumina reads were demultiplexed and processed by STACKS (Rochette and Catchen 2017). For each sample, a locus was identified with a minimum depth coverage of three reads, and a 2bp distance was allowed between each locus. Loci having a distance less than 1bp across individuals were merged into consensus loci. The resulting matrix of all SNPs from all samples was converted into a presence/absence matrix, where missing reads (indicative of divergent enzymatic cut sites) were noted by 0 and otherwise 1, as in de Medeiros and Farrell 2018.The resulting matrix rows were arranged by hierarchical clustering (Ward 1963) to facilitate visualization.

Summary tree

A total of 541 available sequences of *Thitarodes* was used to build a summary tree. These sequences came from three sources: CO1 and mitogenome sequences of known *Thitarodes* species used in generating the Maximum Likelihood CO1 tree in this study (Suppl. material 1: Table S1), 26 Cytb sequences from known *Thitarodes* species as described in Zou et al. (2017), 226 published sequences of different segments extracted from caterpillar fungus sclerotia (Zhang et al. 2014; Quan et al. 2014 a, b). The latter sequences were not associated with clear species names. CO1, Cytb and wg sequences from a single sample in Zhang et al. (2014) were concatenated, and CO1, Cytb and CO2 sequences from a single sample in Quan et al. (2014 a, b) were concatenated. All sequences, after concatenation, were aligned in Geneious Prime (MAFFT algorithm). For mitogenome sequences, only the CO1, CO2 and Cytb segments were used in alignment. The Maximum Likelihood tree was inferred the using the same method CO1 sequence tree was inferred (see previous section). In the resulting phylogeny, each tip within 0.025 distance from each other were clustered and labelled as a single cluster to assess the degree of uneven sampling across the phylogeny.

Taxonomy

***Thitarodes shambalaensis* sp. nov.**
http://zoobank.org/DADDC567-C706-4857-800A-B736C4719774
Figs 1–5

Type material. ***Holotype*****:** CHINA • ♂; Mt. Gongga, Luding county, at the head of Yanzigou valley (燕子沟), Yangliuping habitat (杨柳坪); 29°41'2.54"N, 101°53'32.24"E; alt. 3892 m; 25–30 Jun. 2016; H. Zuo leg.; MK226958; Sichuan Plant Quarantine Station.

Paratypes**:** CHINA • 2 ♂; same collection data as for holotype • 2 ♂; Mt. Gongga, Luding county, at the head of Yanzigou valley (燕子沟), Haizidang habitat (海子凼); 29°40'17.18"N, 101°53'48.25"E; alt. 3977 m; 25–30 Jun. 2016; H. Zuo leg. • 2 ♀; same collection data as for holotype.

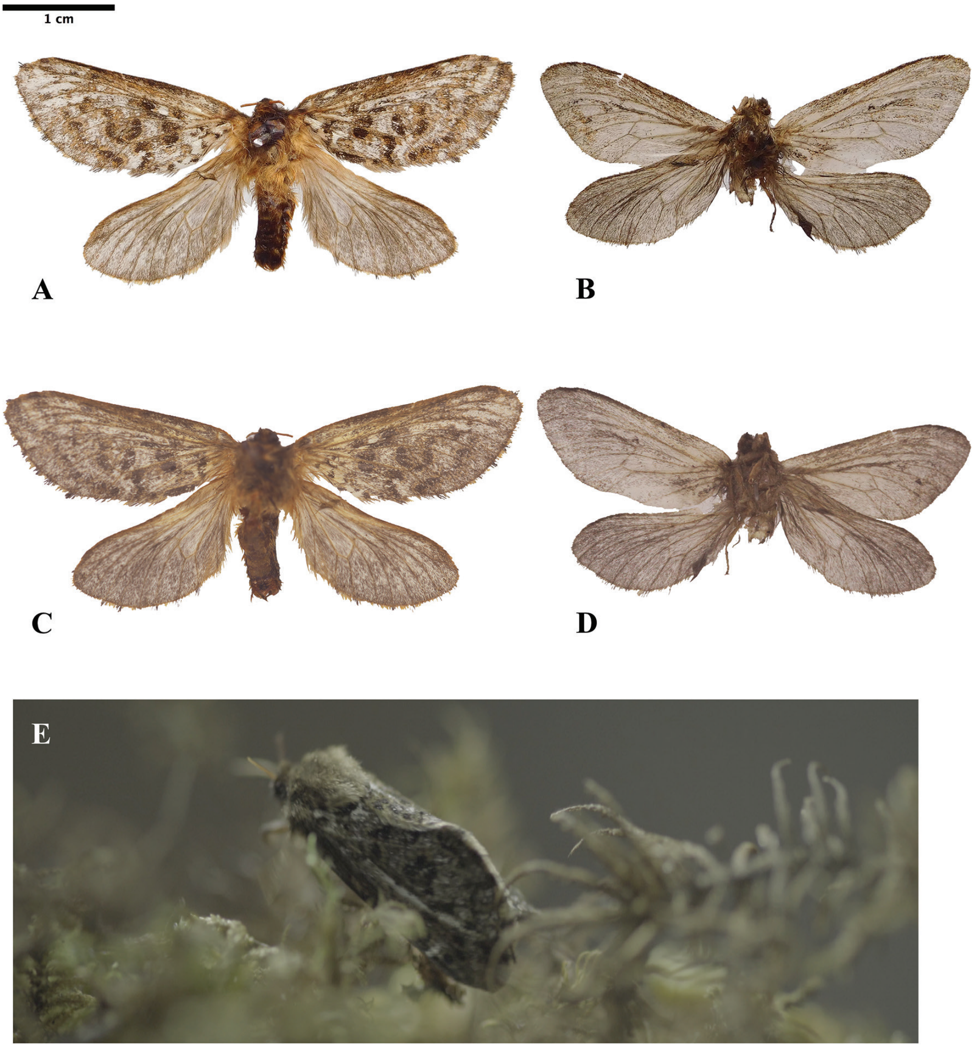


Figure 1. *Thitarodes shambalaensis* sp. nov. **A** holotype, male, dorsal view **B** paratype, female, dorsal view **C** holotype, male, ventral view **D** paratype, female, dorsal view **E** resting position in habitat (photograph credit Hua Zhang). Scale bar: 1 cm.

Etymology. From the Sanskrit word शम्भल (Shambala). In Hindu and Tibetan Buddhist tradition, the term refers to a mythical kingdom hidden in the snow mountains. The name refers to the magnificence of the species' alpine habitat under Mt. Gongga.

Description (based on Holotype). ***General.*** In resting position, forewings fold perpendicular above the sagittal plane of the body and abdomen, completely covering the hindwings. The apex and termen of the two forewings contact each other, while the costa of each forewing extends from the tegula, forming an isosceles. Setae above the thorax form a triangular patch. Wingspan: 44.0 mm (mean = 41.2 mm, SD = 3.5,

Figure 2. A Natural habitat of *Thitarodes shambalaensis* sp. nov. at Yanzigou glacier entrance point, Mt. Gongga, Sichuan, China (photograph credit Meng Li, May 2018) **B** disturbed habitat of *Thitarodes shambalaensis* sp. nov. due to excavation of *T. shambalaensis* pupae, at Haizidang, Yanzigou glacier (photograph credit Wenbin Ju, May 2019).

N = 6). Forewing length: 19.6 mm (mean = 19.2 mm, SD = 1.6, *N* = 6), width: 9.9 mm (mean = 9.9 mm, SD = 0.6, *N* = 6). Hindwing length: 16.9 mm (mean = 16.5 mm, SD = 0.8, *N* = 6), width: 12.25 mm (mean = 11.7 mm, SD = 0.9, *N* = 6). Body length: 15.3 mm (mean = 14.7 mm, SD = 1.1, *N* = 6). Thorax width: 3.7 mm (mean = 3.6 mm, SD = 0.2, *N* = 6).

Head. Antenna (Fig. 3B) with 24 annular segments, rust brown, filiform, apical segment acute, surrounded by ocherous setae at base. Black compound eyes. Labial palps (Fig. 3C) short and hidden under dense setae, two-segmented and fused at the base. Other mouthparts absent.

Body. Red-brown. Dense ocherous and yellow setae on thorax. Red and black setae on lateral, ventral and caudal side of the abdomen.

Legs. Fig. 5. All legs setose. Aerolium present on all legs. Foreleg with tibial epiphysis. Hindleg tibia broad and covered with scent-scales.

Wings. Fig. 3A. Jugum present. Forewing grey, with scattered dark brown spots and pale ocherous border. Alternating dark spots on forewing terminal margin. Costa straight. Sc unbranched. R almost parallel to Rs1 + 2. Apex of curvature occurs between Rs1 and Rs2. Rs1 and Rs2 stalked. Rs3 and Rs4 stalked. First half of Rs3 runs parallel to Rs1 + Rs2. R-M weak but visible, concave towards outer margin, reaching Rs4 distal to the bifurcation of Rs3 and Rs4. On forewing two crossveins between CuP and A with basal crossvein almost opposite CuP-CuA2 crossvein, distal crossvein near terminus of CuP. Single crossvein between CuP and CuA2. Vein A reaches dorsum margin. Hindwing grey to transparent; ocherous color on fringe. Venation similar to forewing except both A and CuP reach dorsum margin.

Male genitalia. Fig. 4A–C. From ventral view, a pair of pseudotegumina form an equilateral triangle, with the apex of the triangle pointing ventrally. Dorsal margins of the pseudotegumina flat. Pseudotegumenal arm is strongly sclerotized, distally forming a fan-shaped sclerotized apex, with minute teeth on the lateral margin. Valvae setose, distally lobate, with a shallow longitudinal groove. Base of valve with prominent hook, heavily sclerotized, projecting disto-medially. Saccus forms a median lobe projecting ventrally.

Female genitalia (based on paratype). Fig. 4D. Dorsal plate (tergum IX) dorsally convex to either side of median, setose. Lateral margins dorsally concave with inner edge, with a triangular projection near base of anal sclerites. Anal sclerites subrectangular with rounded margins. Antevaginalis broad with central lobe subdivided into two dorsally rounded knobs, central lobe setose.

Diagnosis. This species has no distinct external sexual dimorphism. Male pseudotegumen triangular with heavily sclerotized pseudotegumenal arms. Pseudotegumenal arms fan-shaped. Valva densely haired with sclerotized, hook-like apex. Venation of *T. shambalaensis* is similar to that of *T. namnai* Maczey, 2010 in Nepal, but in *T. shambalaensis* both A and CuP reach dorsum margin on the hindwing. *Thitarodes markamensis* (Liang, Li & Shen, 1992) has also reached the degree of heavy sclerotization on the pseudotegumenal arms, but the two species can be distinguished by dif-

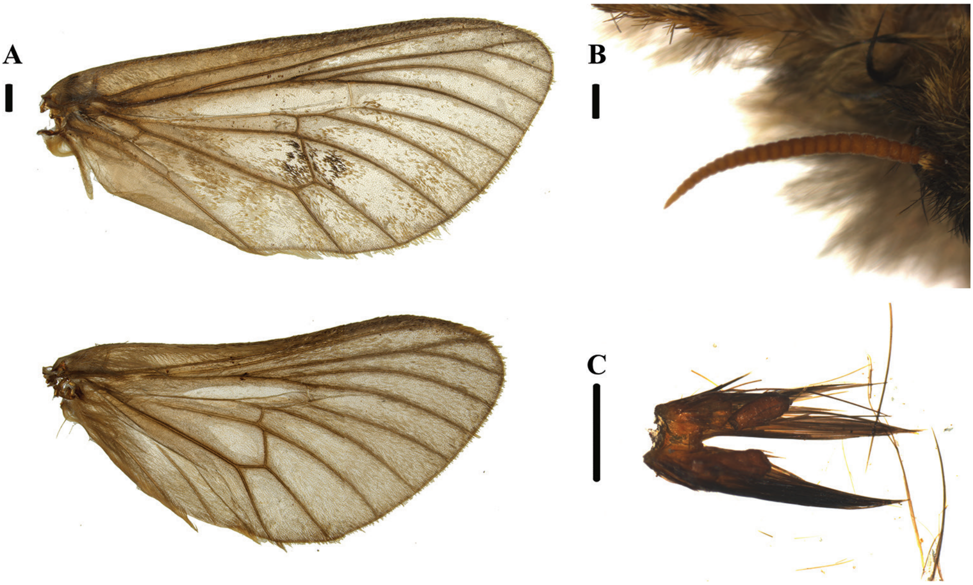
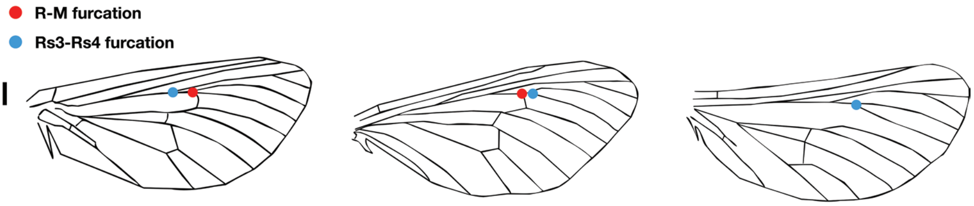


Figure 3. *Thitarodes shambalaensis* sp. nov. **A** forewing (up) and hindwing (bottom) venation from dorsal view **B** antenna, front view **C** labial palp, caudal view, covered under setae. Scale bar: 1 mm.

Figure 4. *Thitarodes shambalaensis* sp. nov. **A** male genitalia from holotype, ventral view **B** valva, caudal view **C** pseudotegumen, lateral view **D** female genitalia from paratype. Scale bar: 1 mm.

ferences in venation (where MR furcation in *T. shambalaensis* is distal to the furcation Rs3 and Rs4) and an inflation on the posterior margin of the saccus. Sclerotization at the ventral base of the valvae with a spinal projection is also present in other species of *Thitarodes*, such as in *T. jiachaensis* Zou, 2011 and *T. sejilaensis* Zou, 2011, but the spinal projection is less curved and the setose lobe of the dorsal side of the valvae is more elongated in *T. shambalaensis*.

Figure 5. *Thitarodes shambalaensis* sp. nov. Foreleg (left), midleg (middle), hindleg (right) with each aerolium enlarged in black rectangles. Scale bar: 1 mm.



T. shambalaensis *T. maquensis* *T. baimaensis*

Figure 6. Diagnostic character of *Thitarodes shambalaensis* sp. nov. in forewing venation in comparison to other species of *Thitarodes*. Scale bar: 1 mm.

Remarks. With the exception of those by Ueda (2000), descriptions of *Thitarodes* species rarely include an illustration of the labial palp. Mouthparts are often difficult to observe and sometimes reduced (or absent) in *Thitarodes*, yet when illustrated, have always shown notable differences between species (Fig. 7A). We encourage in future description of species in *Thitarodes* to include an illustration of the labial palps, as it might serve as a trait useful in resolving clade relationships within the genus.

Phenology. Late instar larvae, sometimes already infected by *O. sinensis*, can be found as early as mid-May, about 30 cm under soil. Pupae can be found starting early June in soil. Adults appear in a week in late June.

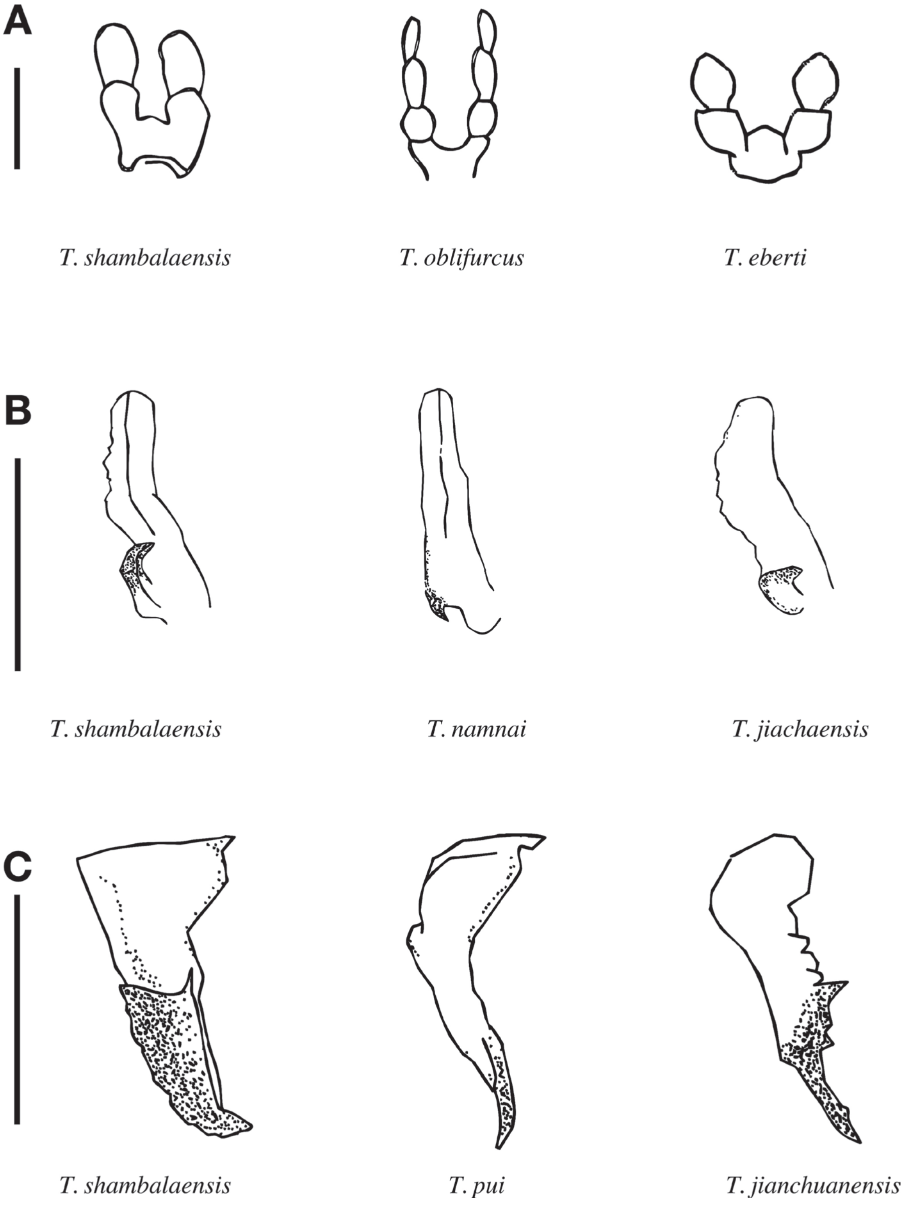


Figure 7. Diagnostic characters of *Thitarodes shambalaensis* sp. nov. in comparison to other species of *Thitarodes* **A** labial palp **B** valva **C** pseudotegumen. Scale bar: 1 mm.

Ecology. The species is found in several high elevation (3400–3800 m) alpine grassland along the glaciers of eastern Mt. Gongga (Fig. 2). Larvae are ground-boring generalist plant root eaters. Common flowering plants in in the grassland include *Saxifraga*, *Rhodiola*, *Polygonum*, *Corydalis*, *Primula*, *Potentilla* and *Anemone*; *Salix* and *Rhododendron* bushes are also present. Adults are not attracted by light and no mating flight has been observed.

Conservation threat. The species is the host of *O. sinensis*. Caterpillar fungus collection has for decades provided income for local people in eastern Mt. Gongga. The traditional method of collection has not had a discernible impact on populations of *T. shambalaensis*, but since 2016, medical pharmaceutical companies have begun buying *T. shambalaensis* pupae from local people for a commercial caterpillar fungus farming project. Many *T. shambalaensis* pupae have been excavated from their habitat each year, sold and transferred to commercial breeding stations. Local people have expressed concern at such habitat exploitation. When we visited the Haizidang habitat in 2019, this alpine grassland had been completely uprooted due to pupae excavation (Fig. 2B).

Results

Validation with CO1 and RAD-Seq

COI sequences of 48 *Thitarodes* samples collected from six glacial valleys were successfully sequenced (Figs 8, 9A, Suppl. material 1: Table S1). The best model for the parsimony tree assessed by the BIC value determined by ModelFinder (Kalyaanamoorthy et al. 2017) is "K3Pu+F+I+G4". All *Thitarodes* sequences form a highly supported monophyletic group, sister to non-*Thitarodes* outgroups. These samples cluster into three highly supported clades in the phylogeny when other available CO1 sequences of *Thitarodes* is taken into account (Fig. 9A): (1) All samples from the Yajiageng valley (GenBank accession number MK226959) form a monophyletic clade that exhibits less than 3% divergence from a deposited sequence of *T. gonggaensis* (Fu & Huang, 1991) in GenBank by Shi et al. (2016). (2) All samples collected at a lower altitude habitat at Gangbogeng valley (GenBank accession number MK226960) formed a highly supported clade with no known sister group that is less than 10% divergent. (3) The rest of the samples, including all samples collected at the habitat of *T. shambalaensis* and other samples collected in six different glacial valleys, form a highly supported clade (GenBank accession number MK226958) with more than 5% divergence from any known sequence of *Thitarodes* species.

A total of 128 out of 134 samples from seven glacial valleys was successfully sequenced according to the RAD-seq protocol (Davey and Blaxter 2010); this generated on average 4,945,132 reads (SD = 2,841,511), and 582,986 SNPs (SD = 357,473) per sample. A total of 3.9 million SNPs was counted (Fig. 9B, Suppl. material 2: Table S2). Hierarchical clustering of reads coverage showed three groups of samples sharing SNPs: (1) All six samples collected from Yajiageng valley share 0.3 of the 3.9 million SNPs. These samples' COI sequences are similar to that of *T. gonggaensis* (GenBank accession number MK226959). (2) All 16 samples from the lower altitude habitat at Gangbogeng valley, collected in both 2016 and 2017, share 0.3 of the 4 million SNPs not present in any other samples. The COI sequences of these samples (GenBank accession number MK226960), form a unique group with no phylogenetically close sister taxon. (3) All 106 samples collected in valleys across the eastern side of Mt. Gongga

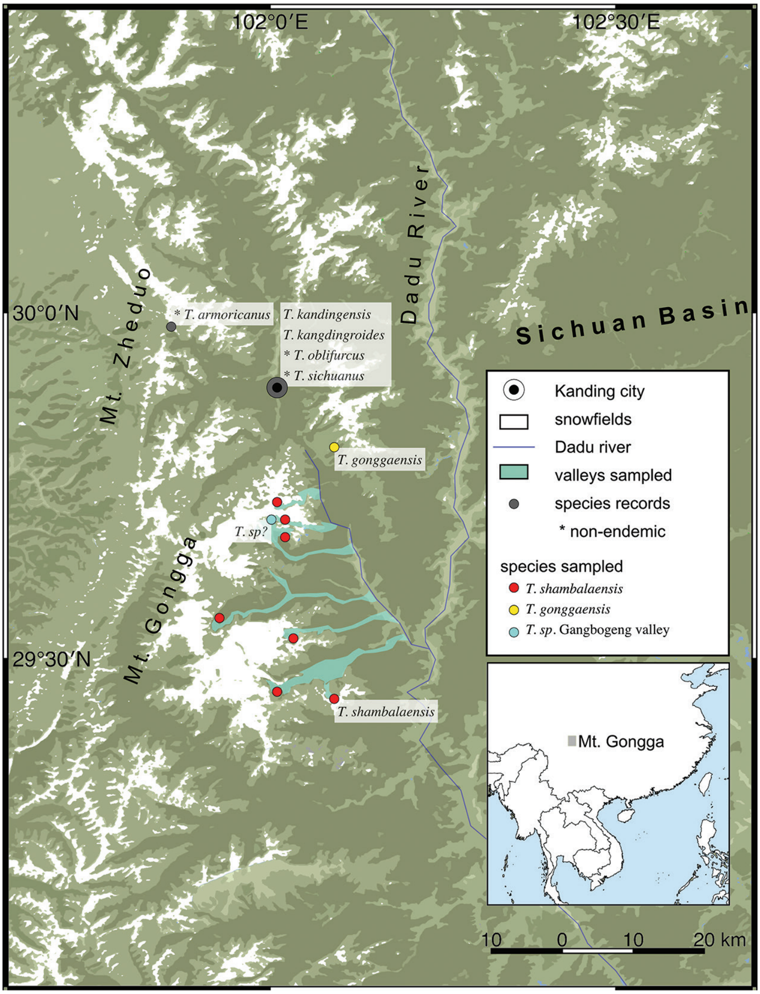


Figure 8. The distribution of *Thitarodes* species sampled in this study and historical records of *Thitarodes* species in the Kangding – Mt. Gongga region.

share the majority of the 4 million SNPS that are not shared by (1) and (2). The COI sequence of this group corresponds to that of *T. shambalaensis* described in this study (GenBank accession number MK226958).

Summary tree

A total of 538 sequences (excluding three sequences from non-*Thitarodes* outgroups) was used to construct a summary phylogenetic tree (Suppl. material 3: Table S3, 142 CO1 sequences,163 Cytb sequences, 33 CO2 sequences, 103 wingless (wg) sequences, 6 mitogenomes, 91 multiple locus haplotypes (MLH) based on CO1).

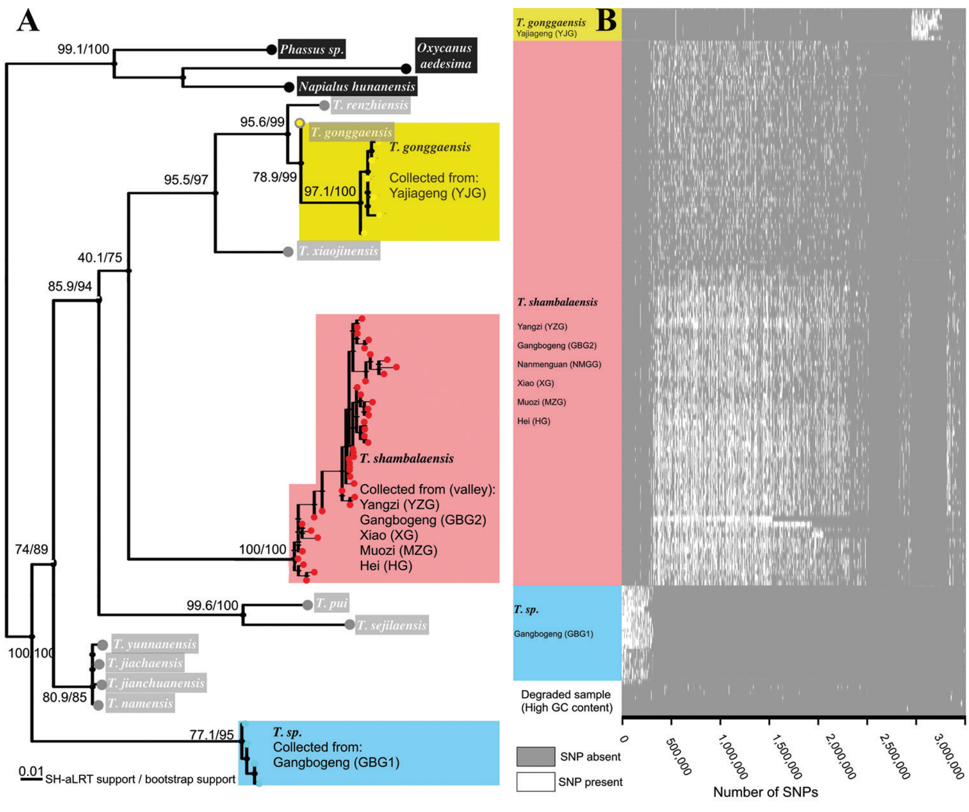


Figure 9. A Maximum Likelihood tree of CO1 sequences of species sampled in this study (yellow, red and blue dots) and other known species of the genus *Thitarodes* (grey dots). Outgroups are in black dots. The corresponding position of the genus *Ahamus* designated by Zou et al. (2010) is labeled **B** RAD-Seq SNP coverage of samples from three species (and degraded samples).

These sequences represent genetic material from at least 175 individuals belonging to the genus *Thitarodes* (excluding MLH sequences). Of the 175 individual samples only 42 have a species name associated, and only eight of which, including two from this study, can be verified in a publication. The best model for parsimony tree by BIC value determined by ModelFinder (Kalyaanamoorthy et al. 2017) is "TIM2+F+I+G4". All sequences from known *Thitarodes* moth individuals and caterpillar fungus sclerotium extractions form one monophyletic group, clearly separated from the other Hepialidae genera *Napialus* Chu & Wang, 1985, *Oxycanus* Walker, 1856 and *Phassus* Walker, 1856. MLH sequences from Quan et al. (2014a) potentially overlap with individual CO1 sequences provided in Quan et al. (2014b). It is unclear whether Quan et al. (2014b) provided individual-level or population-level consensus sequences, but their list of localities is partly identical to Quan et al. (2014a). These MLH sequences (91) were dropped from the analysis. Of the resulting 184 individual samples, 154 have their closest sister group within 0.025 genetic distance from another individual. Cytb sequences of two samples, both labeled

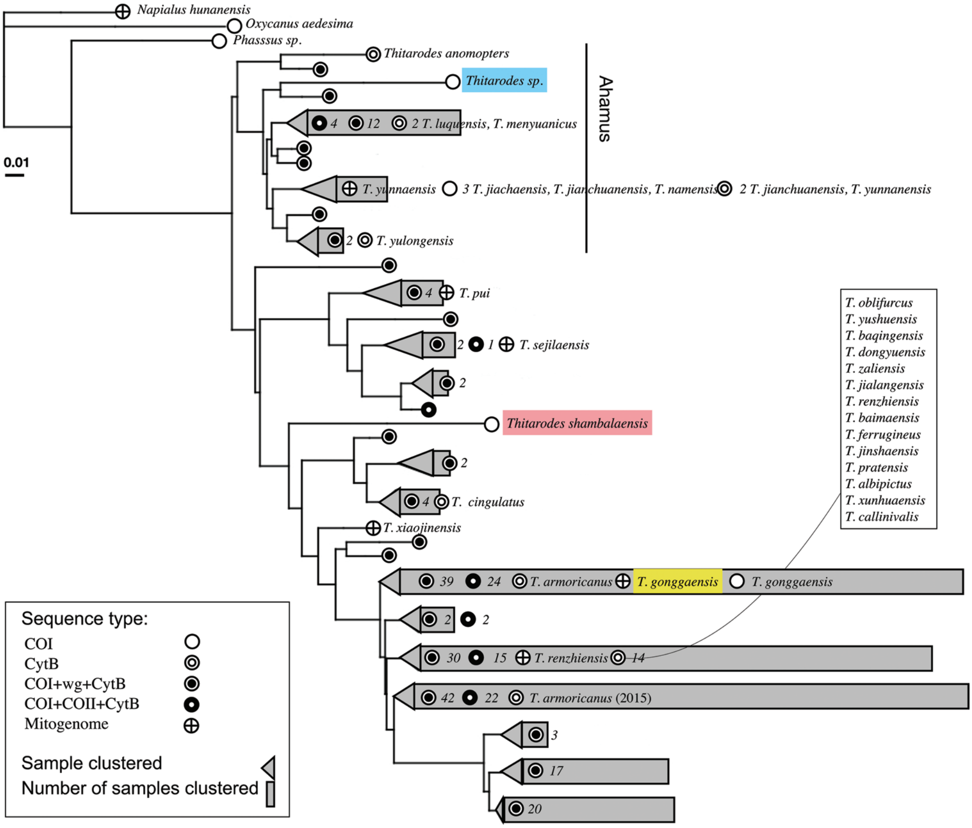


Figure 10. Summary tree for all known species of *Thitarodes*. Samples in this study are labeled in color bars (blue, red and yellow). Each tip represents a cluster of all samples that are within 2.5% genetic distance from each other, the length of the grey bar represents the number of individual samples falling into the cluster. Numbers after the dots enumerate the number of samples that fall into a particular sequence type. All named individual samples are labeled. The genus *Ahamus* as designated by Zou et al. (2010) is labeled.

T. armoricanus, are placed into two distinct clades. The structure of the summary tree is similar to the phylogenetic tree generated from only CO1 sequences, except with the placement of samples collected from Gangbogeng valley.

Discussion

Phylogenetic position of *T. shambalaensis*

Our analysis shows that *T. shambalaensis* is not only morphologically, but also phylogenetically distinct from other known species in the genus *Thitarodes*. Even according to the generic description by Zou et al. (2010), it is still nested amongst other known *Thitarodes* species. Maczey et al. (2010) suggested that based on morphology, it is possible that many currently described species (such as *T. gonggaensis* (Fu & Huang, 1991), *T. jialangensis* (Yang, 1994), *T. namensis* (Chu & Wang, 2004), *T. namlinensis* (Chu & Wang, 2004), and *H. hainanensis* Chu & Wang, 2004) are subspecific variations of a single widespread species ranging from Sichuan to Nepal, and this hypothesized species would include the later described *T. jiachaensis* and *T. shambalaensis*. Our phylogenic analysis shows that these latter two taxa are distinct. Although the genitalic structure and wing venation of *T. shambalaensis* is most similar to *T. jiachaensis*, the two taxa are phylogenetically distant.

Some other *Thitarodes* species also possess triangular pseudotegumina with fan-shaped, indented sclerotization at the pseudotegumenal arms, such as *T. jialangensis* (Yang, 1994), *T. pratensis* (Yang, 1994), *T. callinivalis* (Liang, 1995), *T. litangensis* (Liang, 1995), *T. kangdingensis* (Chu & Wang, 1985), and *T. markamensis* (Liang, Li & Shen, 1992). Although morphologically distinct from *T. shambalaensis* (e.g., *T. jialangensis* Yang, 1994 has a dark forewing without spots; *T. pratensis* has an elongated 23rd antenna segment and orange eyes), it is possible that these species, along with *T. shambalaensis*, are subspecific variations of a single widespread species ranging across the Hengduan Mountains. This hypothesis could not be further tested without a thorough revision of the genus.

Our phylogenies are largely consistent with the subdivision of the genus into *Thitarodes* and *Ahamus* by Zou et al. (2010): the latter genus, containing species such as *T. jianchuanensis* and *T. yunnanensis* (Yang, Li & Shen, 1992), form a monophyletic group in both our CO1 phylogeny and our summary phylogeny. One major inconsistency between our results and Zou et al. 's (2010) treatment is that both *T. jiachaensis* and *T. namensis* in our phylogeny would fall into the clade of *Ahamus*: this is inconsistent because the defining morphological trait for genus *Ahamus* (Latin for "no hook") is the lack of a hook-like structure at the ventral side of the valvae, which *T. jiachaensis* and *T. namensis* clearly possess. We note that since "no hook" is a plesiomorphic trait, it is problematic to use it to justify a monophyletic group within *Thitarodes* group (i.e., *Ahamus*). It is also possible, since CO1 sequences of *T. jiachaensis* and *T. namensis* used in this study are unpublished, that the assignment of the samples to these sequences are in error. We encourage other workers in the field to provide evidence regarding whether *T. jiachaensis* and *T. namensis* phylogenetically fall within the genus *Ahamus* (Zou et al. 2010) and whether there are additional, closely related groups to *T. shambalaensis* described in this study.

An undescribed taxon to test the classification of Zou et al. (2010)

Phylogenetic placement and SNP coverage visualization from our study both suggest that some larvae and pupae samples collected from a lower elevation habitat in Gangbogeng valley across two consecutive years belong to an undescribed taxon (*T. shambalaensis* has been collected along this same valley, but in a habitat at a higher altitude).

Our attempts to collect adults of this unknown taxon from 2016 to 2018 have not been successful, nor is it found in any other valley that we sampled. The limited range of this taxon compared to the parapatric and relatively widespread *T. shambalaensis* and its evolutionary history is intriguing. If the group is indeed a member of the genus *Ahamus*, as defined by Zou et al. (2010), we predict that the adults should not possess a hook-like structure on the ventral sides of its valvae.

Thitarodes around Kangding

CO1 sequences of our samples collected at Yajiageng valley are closely related (within 2% divergence) to known sequences labeled as *T. gonggaensis* in the study of Shi et al. (2016). The type locality of the species is "Kangding" (Fu and Huang 1991); Shi et al. (2016) collected the sample at Gonggashanxiang (29°32'24.0"N, 101°35'24.0"E), 65 km south west of Kangding. The locality of Shi et al. (2016) is also 57 km southwest of the samples of *T. gonggaensis* collected in this study, thus explaining the divergence with samples collected in our studies.

According to Wang et al. (2011), four other species have "Kangding" as the type locality or have been recorded in "Kangding": *T. kangdingroides* (Chu & Wang, 1985), *T. kangdingensis* (Chu & Wang, 1985), *T. oblifurcus* (Chu & Wang, 1985), and *T. sichuanus* (Chu & Wang, 1985). It is unlikely that these samples were collected at Kangding city (30°01'21.1"N, 101°57'27.6"E). Although the city is one of the most prominent trading centers for caterpillar fungus, it is located at a relatively low elevation (2,500 m) and is not a known habitat of caterpillar fungus. These species, like samples of *T. gonggaensis* in this study and in Shi et al. (2016), were most likely collected in the mountains around Kangding city. Specimens of these four other species are difficult to access and no genomic data has been published at this time.

The type species of the genus, *T. armoricanus*, is also recorded as collected (and reared) by Tao et al. (2015), at Mt. Zheduo, 15 km northwest of Kangding, although no specimens have been dissected to verify the identification. This suggests the existence of at least eight species of *Thitarodes* (counting *T. shambalaensis* and the unknown species from Gangbogeng valley sequenced in this study) in the vicinity of Kangding-Mt. Gongga region, six of which are endemic. To our knowledge, much of the caterpillar fungus habitat around the Kangding-Mt. Gongga area, especially toward the western and north-western slope of Mt. Gongga, has not been thoroughly surveyed. We would expect more species to be discovered in this region following a systematic survey. It is hypothesized that vicariance generated by the mountain topology of the Hengduan mountains, of which Kangding is part, contributed to the rate of speciation of this biodiversity hotspot (Xing and Ree 2017). Regarding the genus *Thitarodes*, species diversity and endemism is not unique to the Kangding-Mt. Gongga region: the snow mountains of Yulong, Meili, Laojun in Yunnan, also part of the Hengduan mountains, is habitat to highly endemic species described to be living in close proximity (Yang 1994; Liang 1995).

Species delineation with CO1 and SNP coverage

A common concern in any study of the population genetics of non-model organisms is whether the analyzed samples come from different populations of the same species, or whether multiple species are involved. In our study, we still need to verify whether all *Thitarodes* samples collected across the eastern slope of Mt. Gongga are the same species. Without molecular evidence, this is difficult to do, since adult *Thitarodes* samples are difficult to identify, and most samples are obtained in larval or pupal forms, which are not sufficiently taxonomically informative for species delineation.

CO1 sequences show that our samples cluster into three clades, with inter-clade divergence falling below the 3.78% commonly observed in populations within a species, and intra-group divergence falling within the 11.06 % range of species within the same genus boundary (see review of CO1 sequence divergence by Kartavtsev 2011). We consider this strong evidence supporting for the existence of three distinct species, however, this level of interspecific (intra-population) divergence is not sufficient to distinguish patterns of population evolution history (e.g., evolutionary relationships between population of *T. shambalaensis* in Yanzigou valley and *T. shambalaensis* in Gangbogeng valley in association with the history of glaciation).

RAD-Seq has traditionally been considered useful in population level studies, while species-level divergence would reduce the amount of shared SNP coverage across species (Davey and Blaxter 2010; Cariou et al. 2013; Eaton et al. 2017). Our results suggest that RAD-Seq provides enough SNPs within species to further analyze the population history of each species but is not informative in analysis at the interspecific level, as samples with SNP data form three distinct clusters, but these are not shared among all samples. The clustering of those taxa with SNP data supports the CO1 species delineation indicating three main lineages.

We conclude that in this study, both the phylogeny based on CO1 sequences and the visualization of genome-wide SNP coverage provide evidence for the presence of three species in the eastern slopes of Mt. Gongga: *T. shambalaensis* occupies most valleys in the eastern slopes of Mt. Gongga; *T. gonggaensis* is found in Yajiageng valley; another undescribed species is isolated at a low elevation habitat at Gangbogeng valley.

Thitarodes phylogeny

Our summary tree shows that the genus *Thitarodes* is monophyletic. All known moth sequences extracted from caterpillar fungus sclerotia, despite the difficulty of assigning them to discrete species, nevertheless cluster within the genus *Thitarodes*.

Among the 538 sequences from 184 individuals of the genus *Thitarodes*, only 42 could be associated with a species name, and only eight of these are verified in publications. Of the 54 species summarized by Chu et al. (2004), only 39 have matching genetic information, most of which are unpublished Cytb sequences. 140 of the individuals are caterpillar fungus sclerotia samples, 66 of which are not within 2.5% phylogenetic distance of a known species. This suggests that many additional taxa remain to be identified and described.

In cases where multiple sources of sequences are all labeled as the same species, species identification has shown to be quite consistent: the Cytb sequence of *T. yunnanensis* matches to the mitogenome of *T. yunnanensis*, the Cytb sequence of *T. jianchuanensis* clusters together with CO1 sequence of *T. jianchuanensis* (as both are aligned with other mitogenomes), the CO1 sequence of *T. gonggaensis* matches its mitogenome, and the CO1 sequence of *T. renzhiensis* matches its mitogenome. Slight variations exist between the segment sequences and their corresponding flanking regions on the mitogenome, but we could find no phylogenetic inconsistencies. The only exception is that the two entries of the Cytb sequence of the type species of the genus, *T. armoricanus*, are distinctly different. While we have reason to believe that the type specimen of this sample was collected around Kangding (Chu 1965), Wang and Yao (2011) noted that this species ranges from Gansu, Tibet to Xinjiang. As we discussed in the previous section on the endemism of *Thitarodes* in the Hengduan mountains, we are of the opinion that the range estimate *T. armoricanus* of Wang and Yao (2011) includes undescribed cryptic species that have distinctive Cytb sequences, as revealed in our phylogenetic analysis.

Our summary tree also reveals inadequate sampling in many clades. When clustered by a 2.5% genetic distance (what we consider to be a reasonable threshold for inter-species variation, see Kartavtsev 2011), only 30 genetic clusters were recovered. Out of the 184 individual samples, 154 fall into 15 out of the 30 clusters; the other 15 clusters are known by either a single "orphan taxon" (*T. shambalaensis*, *T. anomopterus*, *T. xiaojinensis* (Tu, Ma & Zhang, 2009), *T.* sp. from Gangbogen in this study) or just a few sequences from a caterpillar fungus sclerotium sample. Additional research will help to elucidate the nature of these lone taxa. It is likely that "close sisters" of these taxa might be found if all known species of *Thitarodes* could be sequenced.

The bulk of the sampling effort so far has focused on only half of the known diversity of *Thitarodes*. This uneven phylogenetic sampling is not simply the result of relying on sequences of caterpillar fungus sclerotia, which have no morphological species description. The uneven sampling problem extends to the phylogeny of named species as well: the reference sequences of 39 out of the 42 named species fall into one of those 30 2.5%-distance clusters. One such cluster (including *T. renzhiensis*, *T. oblifurcus*, etc.) includes 17 known species! Most of these 17 species are known by one Cytb sequence only, which might be the result of sampling a particularly invariant region of the mitogenome. However, this issue has not been brought to attention in previous attempts to summarize genetic data for the genus (Zou 2010, 2017). We noticed that in the comprehensive sampling across known range of caterpillar fungus, Zhang et al. (2014) only recovered seven well-supported clades and two "orphan taxa". It is unclear by what genetic distance Zhang et al. (2014) have considered to be a clade, but their estimate might be a closer estimate of the number of phylogenetically well-established species in *Thitarodes*.

To summarize, our current understanding of the genus *Thitarodes* derives from three major sources:

1. The analysis of samples of caterpillar fungus sclerotia, and sometimes just *Thitarodes* larvae or pupae, has provided crucial information about the habitat and ecology of the species in the genus, but often fails to provide information about the morphology of the *Thitarodes* adult. We encourage workers in this field to include more detailed descriptions of the habitats where samples of caterpillar fungus are collected (including vegetation, climate and soil type), and make an effort to understand adult *Thitarodes* biology in these known habitats.
2. Many described species have types deposited in institutions around China; earlier descriptions of these samples often do not include photos of wing venation and genitalia. Revisions of these taxa with updated photos and molecular data for comparative analysis are critical.
3. New descriptions of species in the group (see Jiang et al. 2016), and NGS-based analysis of several species of the group (see Guo et al. 2016; Li et al. 2016), should provide reference sequences along with genitalia dissections, so that any known species can be linked to its position in the molecular phylogeny.

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Supplementary material 1

Table S1. Sequences used in Fig. 9A

Authors: Zhengyang Wang, Hailing Zhuang, Min Wang, Naomi E. Pierce

Data type: molecular data

Link: https://doi.org/10.3897/zookeys.885.34638.suppl1

Supplementary material 2

Table S2. Samples sequenced shown in Fig. 9B

Authors: Zhengyang Wang, Hailing Zhuang, Min Wang, Naomi E. Pierce

Data type: molecular data

Link: https://doi.org/10.3897/zookeys.885.34638.suppl2

Supplementary material 3

Table S3. Sequences used in Fig. 10

Authors: Zhengyang Wang, Hailing Zhuang, Min Wang, Naomi E. Pierce

Data type: molecular data

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RESEARCH ARTICLE

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Distribution and current conservation status of the Mexican Goodeidae (Actinopterygii, Cyprinodontiformes)

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Abstract

The current distribution and abundance of the 40 species of Goodeidae fishes known from Mexico are described, and a total of 84 Evolutionarily Significant Units (ESUs) is designated within these species. Two species and four ESUs are likely extinct with no captive populations, and three species and eight ESUs are probably extinct in the wild but have at least one captive population in Mexico, the United States, or Europe. Of the 35 extant species, the analyses indicate that nine should be considered as critically endangered, 14 as endangered, nine as vulnerable, and only three as least concern. Twenty-seven of these species have experienced substantial declines in distribution or abundance or both since 2000, and only eight appear to have remained relatively stable. Of the 72 extant ESUs, our analyses indicate that 29 should be considered as critically endangered, 21 as endangered, 18 as vulnerable, and only four as least concern. Brief summaries of the historic and current distributions and abundance of each species are provided, as well as ESU. Three strategies are recommended to conserve Mexican goodeids: protect the best-quality remaining habitats where goodeids still persist, restore degraded habitat and re-introduce species or ESUs where practical, and establish captive populations to ensure continued survival of the many species and ESUs that will almost inevitably go extinct in the coming years. Limited resources require cooperation and collaboration between scientists, conservationists, and aquarium hobbyists for successful captive maintenance.

Keywords
Captive maintenance, Evolutionarily Significant Unit, Goodeinae, Mexico

Introduction

The Goodeidae (Pisces, Cyprinodontiformes) is a family of small-bodied freshwater fishes found in Mexico and the United States. There are two subfamilies, the Empetrichthyinae, with three oviparous species and multiple subspecies found in the Great Basin of the southwestern United States (Campbell and Piller 2017), and the Goodeinae, with ca. 40 viviparous species found in the highlands of central Mexico (Figures 1–3) (Parenti 1981). All Empetrichthyinae species and subspecies are either extinct, endangered, or threatened, and protection and recovery programs have been established for the few remaining populations (Jelks et al. 2008; Minckley and Marsh 2009). The state of the Goodeinae is less clear. A handful of species are clearly extinct or endangered and a few others are believed to be relatively stable and secure, but the current conservation status for many species is undefined and appears to be rapidly changing for the worse (Lyons et al. 1998; Contreras-Balderas et al. 2003; De la Vega-Salazar and Macías-Garcia 2005; Domínguez-Domínguez et al. 2005a, b; Jelks et al. 2008; Ramírez-Carrillo and Macías-García 2015; Gesundheit and Macías-García 2018).

The distribution and abundance of most species within the Goodeidae has declined precipitously during the last 20 years, and the continued survival of many species has become precarious (Lyons et al. 1998; Soto-Galera et al. 1998, 1999; De la Vega-Salazar et al. 2003a; Domínguez-Domínguez et al. 2008a; Helmus et al. 2009; Ramírez-Carrillo and Macías-García 2015). Within some species, unique evolutionary lineages, which are just now being identified and delineated, are on the brink of elimination. There is an urgent need to better document these lineages and their native ranges (De la Vega-Salazar et al. 2003b; Medina-Nava et al. 2005, Domínguez-Domínguez et al. 2006; Helmus et al. 2009; Lyons 2013). For some species and lineages, the situation in the wild is so dire that captive maintenance and breeding programs must be implemented as soon as possible to avoid their imminent extinction (Bailey et al. 2007; Domínguez-Domínguez et al. 2008a; Lyons 2013; Maceda-Veiga et al. 2014).

In this paper, we have assessed the current (2019) conservation status of goodeid fishes in the wild in Mexico (Goodeinae), updating and expanding upon the status and trends surveys from Domínguez-Domínguez et al. (2005a, b), which were published in a book that is not readily available, and the non-peer-reviewed popular summary in Lyons (2013), which was published in an aquarium hobbyist book in German. We have focused on documenting unique evolutionary lineages within populations that

Figure 1. Photos of eight representative goodeid fishes. All photos taken by John Lyons of freshly preserved, wild-caught, adult, male specimens. **A** *Allotoca goslinei*, Potrero Grande Stream, Jalisco, 8 January 2004 **B** *Alloophorus robustus*, Opopeo Lake, Michoacán, 9 January 2011 **C** *Ameca splendens*, Almoloya Springs, Jalisco, 17 January 2008 **D** *Chapalichthys encaustus*, Lake Chapala near Ajijic, Jalisco, 6 January 2005 **E** *Goodea atripinnis*, Tierra Quemada Stream, San Luis Potosí, 15 January 2011 **F** *Skiffia lermae*, Zacapu Lake, Michoacán, 11 January 2005 **G** *Xenotaenia resolanae*, Cuzalapa River, Jalisco, 10 January 2006 **H** *Xenotoca doadrioi*, San Marcos Stream, Jalisco, 9 January 2005.

are worthy of conservation, which we have termed Evolutionarily Significant Units or ESUs. We have followed the definition of ESUs given by Crandall et al. (2000), which incorporates both genetic and ecological distinctiveness and adaptive significance. We propose that this ESU concept will provide a helpful framework for developing protection and restoration plans for wild populations and for compiling priority lists and husbandry guidelines for captive populations. Designation of ESUs may also focus attention on unique populations that may eventually be described as new species. The ESU concept was first applied for fish to Pacific salmon (*Oncorhynchus* species), in which discrete spawning runs were designated as separate ESUs if they met the criteria of being largely reproductively isolated from other potential ESUs and also constituting important components of the evolutionary legacy of the species (Waples 1991), criteria that also apply to the goodeid ESUs we have designated. The ESU concept has since become an important component of the conservation framework for many different fish species (e.g., Hedrick et al. 2001; Stockwell et al. 2013).

Figure 2. Photos of four representative types of goodeid habitats **A** Lake Chapala, near Ajijic, Jalisco, 6 January 2005. The largest natural lake in Mexico and historically the home of at least six species of goodeids. Currently, only *Chapalichthys encaustus* (see Figure 1), *Goodea atripinnis*, and *Zoogoneticus purhepechus* remain **B** 27 de Noviembre Springs, Durango, 11 January 2008, home of *Characodon* species **C** Molino Viejo Reservoir, Jalisco, 17 January 2008, home of *Xenotoca melanosoma* **D** Cuzalapa River, Jalisco, 11 January 2006, home of *Ilyodon furcidens* and *Xenotaenia resolanae* (see Figure 1).

Materials and methods

We have determined the conservation status of the Mexican goodeids based on a combination of the recent scientific literature, museum specimens, communication with other aquatic scientists, and especially our own personal field collections, with emphasis on surveys conducted within the last 20 years. We have generally followed the taxonomy proposed by Miller et al. (2005) but have made some modifications (noted in the text) based on recent genetic analyses by Doadrio and Domínguez-Domínguez (2004) and Webb et al. (2004). We have also included new species described by Radda and Meyer (2003) and Domínguez-Domínguez et al. (2008b, 2016). Collectively, since 2000, we have sampled all known and likely locations for every distinctive population of all of the nominal Mexican goodeid species.

We have defined ESUs based on genetic, morphological, and zoogeographic evidence for distinctive population structure within each goodeid species. All of our proposed ESUs are or were allopatric from each other, occurring within discrete river

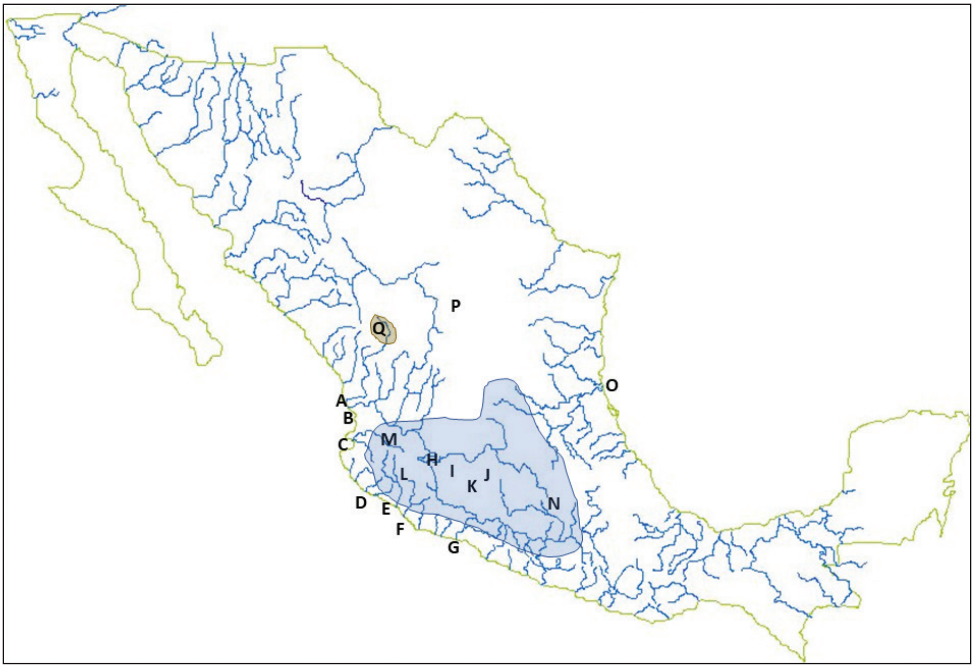

Figure 3. General distribution of goodeid fishes in Mexico, with the location of major river basins and lakes shown. Shaded areas indicate the range of most goodeid species (blue) and the disjunct range of *Characodon* (brown).**A** Mezquital River basin **B** Grande Santiago River Basin **C** Ameca River basin **D** Purificación and Marabasco River basins **E** Armería River basin **F** Coahuayana River basin **G** Balsas River Basin **H** Lake Chapala **I** Lerma River Basin **J** Lake Cuitzeo/Grande de Morelia River basin (endorheic) **K** Lake Pátzcuaro and Lake Zirahuén basins (endorheic) **L** Lake Atotonilco, Lake San Marcos, Lake Sayula, and Lake Zapotlán basins (endorheic) **M** Lake Magdalena basin (endorheic) **N** Valley of Mexico/ Mexico City (endorheic) **O** Pánuco River basin **P** Valley of Parras,(endorheic), *Characodon garmani* collection site **Q** Tunal River drainage (part of Mezquital River basin), home of *Characodon* species.

basins or sub-basins. Genetic evidence for ESUs was based primarily on sequence divergence within the mitochondrial cytochrome b gene, the genetic marker most commonly used thus far in Mexican goodeid studies. We considered sequence divergence of 1% (uncorrected p-distance) or more between populations as sufficient to designate ESUs because many of the species described prior to the inclusion of genetic data are at least 1% divergent from their putative sister species. Where no genetic divergence was evident or where genetic data were lacking, we also relied on morphological analyses to identify ESUs. We required statistically significant multivariate morphological or meristic differences with limited overlap among populations to designate ESUs. Finally, where both genetic and morphological data were lacking, we employed zoogeographic information to designate ESUs, relying heavily on Domínguez-Domínguez et al. (2006a). In this case, we identified ESUs if geological evidence indicated that populations in different basins had likely been isolated for at least 50,000 years and other fish taxa showed clear genetic or morphological differences between the basins. We developed an alphanumeric code to identify ESUs, consisting of three letters from the genus name followed by two letters of the species name followed by a unique number for the ESU. For example, the four ESUs we identified for *Alloophorus robustus* were assigned the codes Alpro1, Alpro2, Alpro3, and Alpro4.

We determined the conservation status of goodeid species and ESUs based on the terminology and criteria of the International Union for the Conservation of Nature (IUCN 2012). Two of the authors of this paper, Omar Domínguez-Domínguez and Michael Köck, participated in an IUCN-sponsored workshop in December 2018 to assess the conservation status of Mexican freshwater fishes. This resulted in most of our status designations for goodeid species being adopted by the IUCN, and these will be published by IUCN in 2019. However, in five cases there were inconsistencies between our and the IUCN's designations, most prominently in that IUCN did not complete assessments for *Girardinichthys ireneae*, *G. turneri*, *Xenotoca doadrioi*, and *X. lyonsi*. The IUCN also considers the re-introduced population of *Zoogoneticus tequila* as established and gave it a status of "Endangered" while we still considered it "Extinct in the Wild" because we felt it was too early to declare the population fully re-established. The IUCN workshop also did not assess potential ESUs.

The IUCN criteria we used are as follows. "Extinct" indicated species or ESUs for which no specimens have been collected despite repeated targeted surveys in appropriate habitats. We distinguished between species or ESUs for which no living specimens existed anywhere on earth ("extinct") and those for which no specimens occurred in nature but for which viable captive populations were still present ("extinct in the wild"). "Critically endangered" was applied to species or ESUs that either persisted in the wild at only 1–3 distinct locations with an estimated combined minimum annual population of fewer than 250 breeding adults or that had experienced overall decreases in distribution and abundance of more than 80% within the last ten years. "Endangered" species or ESUs either occurred at 4–8 distinct locations with a combined minimum annual population of no more than 2,500 breeding adults or had decreased in distribution and abundance by 50–70% within the last ten years. "Vulnerable" species or ESUs either occurred at 9–35 distinct locations with a combined minimum annual population of no more than 10,000 breeding adults or had declined by 30–50% in distribution and abundance within the last ten years. "Nearly threatened" species or ESUs were uncommon and in decline, but they did not quite meet the criteria for designation as vulnerable or endangered. "Least concern" species or ESUs had a broader and more stable distribution and abundance and were not in immediate danger of being designated as vulnerable or endangered, although they may have been declining in distribution or abundance in some areas.

We have also reported the official Mexican government status designation from the federal regulations established to protect rare species ("Norma Oficial Mexicana"; NOM-059-SEMARNAT-2010; NOM 2010 hereafter). Four categories have been applied to the goodeids: "Extinct" (Extinto) – no specimens encountered despite repeated targeted sampling of appropriate habitats; "Endangered" (En Peligro) – species rare and in decline and likely to become extinct within the near future without protection and management; “Threatened” (Amenazado) – species uncommon and in decline and likely to become endangered within the near future without protection and management, and “Under Special Protection” (Sujeta Protección Especial) – species in decline and needing regulation although not qualifying as threatened or endangered.

We have provided population trends for each species based upon our and colleagues’ observations (mostly unpublished) since ca. 2000. If we noted the disappearance of a species from one or more locations or the substantial decline in abundance of one or more populations, we classified the species as declining. If no populations had been eliminated and abundances showed no clear trend, we classified the species as stable. If the species was expanding its range through movement and colonization of new habitats or if one or more populations had grown noticeably, we classified the species as increasing. Note that an increase in the range of a species based on the discovery of a new population that was believed to always have been present did not qualify a species to be classified as increasing.

Results

Summary of goodeid conservation status and population trends

Nearly all Mexican goodeids qualified for a protected conservation status designation under the IUCN criteria (Table 1). Of the 40 species we recognized, two were extinct, three were extinct in the wild, nine were critically endangered, 14 were endangered, nine were vulnerable, none were nearly threatened, and only three were least concern (Figure 4). The Mexican government (NOM 2010), which used a somewhat different taxonomic classification from ours and did not recognize several recently described species, officially listed one species as extinct, 18 as endangered, four as threatened, and one under special protection. Most of the government designations generally agreed with ours with two major exceptions: classifying *Ilyodon furcidens* as threatened whereas we classified it as least concern, and giving no formal protected status to *Allotoca maculata*, *A. meeki*, and *Chapalichthys pardalis*, which we classified as critically endangered, and *Girardinichthys multiradiatus*, which we classified as endangered. Of the 35 species we recognized that were still found in nature, we determined that 27 were declining and only eight were stable. Three species have recently been introduced by humans into new drainage basins, *Chapalichthys encaustus* into the Ameca River basin in the state of Jalisco, *Goodea atripinnis* into the Mezquital River basin in the state of Durango, and *Ilyodon furcidens* into the Citala Reservoir in the Lake Sayula basin in Jalisco, but these expansions have been offset by declines and losses of populations within their native ranges. Therefore, no species qualified as increasing.

For the 40 goodeid species, we have identified 84 ESUs (Table 2). The number of ESUs per species varied substantially, with 20 species with only one ESU, 10 species with two, three species with three, four species with four, two species with five, and one species with nine. Most ESUs qualified for a protected conservation designation.

Table 1. Status and trends of Mexican Goodeidae in the wild as of 2019. "Mexico" refers to the legal classification by the Mexican Federal government (NOM 2010; NC Not Classified; Sp. Protec. Under Special Protection), and "This study" refers to the IUCN designation based on our analyses.

Species	Conservation Status		Trend Since 2000
	Mexico	This Study	
Allodontichthys hubbsi	Endangered	Endangered	Stable
Allodontichthys polylepis	Endangered	Critically Endangered	Declining
Allodontichthys tamazulae	Endangered	Vulnerable	Stable
Allodontichthys zonistius	None	Vulnerable	Stable
Alloophorus robustus	None	Vulnerable	Declining
Allotoca catarinae	Endangered	Vulnerable	Stable
Allotoca diazi	Endangered	Critically Endangered	Declining
Allotoca dugesii	Endangered	Endangered	Declining
Allotoca goslinei	Endangered	Extinct in the wild?	No records since 2004
Allotoca maculata	None	Critically Endangered	Declining
Allotoca meeki	None	Critically Endangered	Declining
Allotoca zacapuensis[1]	NC	Critically Endangered	Stable
Ameca splendens	Endangered	Endangered	Declining
Ataeniobius toweri	Endangered	Endangered	Declining
Chapalichthys encaustus	None	Vulnerable	Declining
Chapalichthys pardalis	None	Critically Endangered	Declining
Characodon species	Endangered	Critically Endangered	Declining
Characodon garmani[1]	NC	Extinct	No records since 1890's
Girardinichthys ireneae[1]	NC	Critically Endangered	Declining
Girardinichthys multiradiatus	None	Endangered	Declining
Girardinichthys turneri	Endangered	Extinct?	No records since 1980's
Girardinichthys viviparus	Endangered	Endangered	Stable
Goodea atripinnis	None	Least Concern	Declining
Ilyodon furcidens	Threatened	Least concern	Declining
Ilyodon whitei	None	Vulnerable	Declining
Neoophorus regalis	Endangered	Critically Endangered	Declining
Neotoca bilineata	Endangered	Endangered	Declining
Skiffia francesae	Extinct	Extinct in the wild	No records since 2008
Skiffia lermae	Threatened	Endangered	Declining
Skiffia multipunctata	Threatened	Endangered	Declining
Xenoophorus captivus	Endangered	Endangered	Declining
Xenotaenia resolanae	None	Vulnerable	Stable
Xenotoca doadrioi[1]	NC	Endangered	Declining
Xenotoca eiseni	Sp. Protec.	Endangered	Declining
Xenotoca lyonsi[1]	NC	Endangered	Declining
Xenotoca melanosoma	Endangered	Vulnerable	Declining
Xenotoca variata	None	Least Concern	Declining
Zoogoneticus purhepechus[1]	NC	Vulnerable	Declining
Zoogoneticus quitzeoensis	Threatened	Endangered	Declining
Zoogoneticus tequila	Endangered	Extinct in the wild[2]	No records since 2008[2]

[1] These six species were not recognized and hence not classified by the Mexican government.

[2] *Zoogoneticus tequila* was considered extinct in the wild as of 2008, but in 2016 it was re-introduced and has successfully reproduced. It is too early to determine whether it will become permanently re-established.

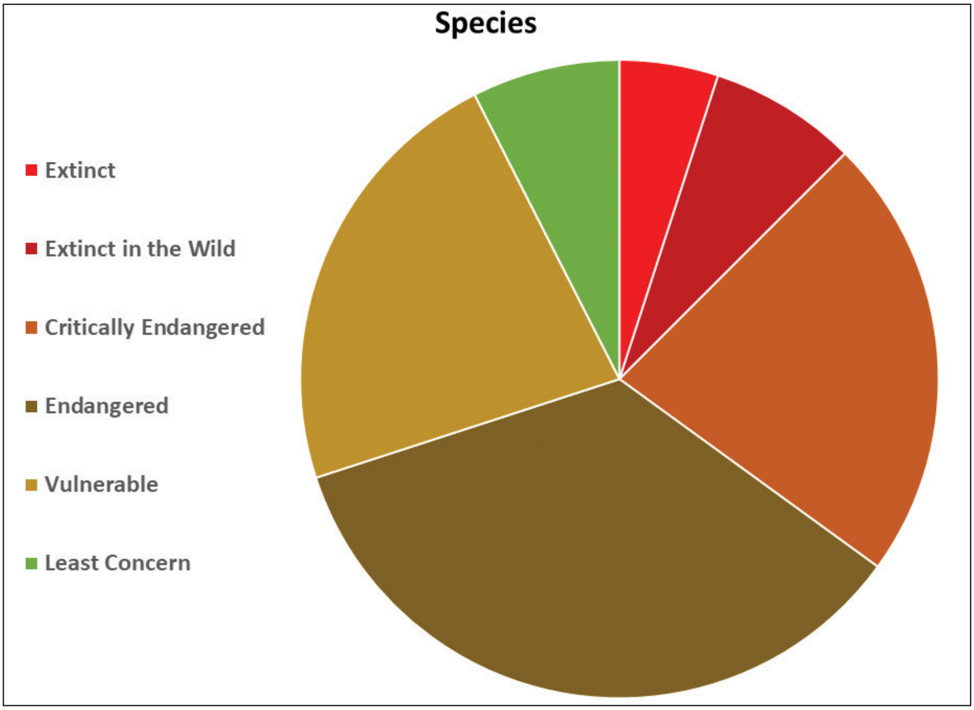

Figure 4. Pie chart of the conservation status of Mexican goodeid species.

Of the 84 ESUs, we determined that four were likely extinct, eight extinct in the wild, 29 critically endangered, 21 endangered, 18 vulnerable, none nearly threatened, and four least concern (Figure 5).

Conservation status and populations trends of species

Allodontichthys: This genus consists of four bottom-dwelling species found in fast-flowing areas of streams and rivers on the Pacific slope of west-central Mexico in the Ameca, Armería, and Coahuayana river basins (Lyons and Mercado-Silva 2000; Webb 2002). It is most closely related to *Ilyodon* and *Xenotaenia* (Webb 2002; Doadrio and Domínguez-Domínguez 2004; Webb et al. 2004).

Allodontichthys hubbsi: Endangered/Stable/2 ESUs (Figure 6) – This species is known from only six areas in the upper Coahuayana River basin (Lyons and Mercado-Silva 2000). We recognized two ESUs based on genetic analyses (Domínguez unpublished data). Aldhu1 is endangered and occupies four areas of the Tamazula River drainage, short segments of the Tamazula River, the San Jerónimo River, a small unnamed tributary of the Tamazula, and the Contla Stream. The Contla Stream had the best population with several hundred adults. Aldhu2 is critically endangered and has small populations in three tributaries of the Coahuayana River that are isolated from

Table 2. Number of proposed Evolutionarily Significant Units (ESUs) per goodeid species and their conservation status (IUCN 2012). Key: EX Extinct, EW extinct in the wild, CE critically endangered, EN endangered, VU vulnerable, NT nearly threatened, and LC least concern.

Species	Number of ESUs	Conservation status						
		EX	EW	CE	EN	VU	NT	LC
Allodontichthys hubbsi	2	0	0	1	1	0	0	0
Allodontichthys polylepis	1	0	0	1	0	0	0	0
Allodontichthys tamazulae	1	0	0	0	0	1	0	0
Allodontichthys zonistius	1	0	0	0	0	1	0	0
Alloophorus robustus	4	0	0	2	0	2	0	0
Allotoca catarinae	1	0	0	0	0	1	0	0
Allotoca diazi	1	0	0	1	0	0	0	0
Allotoca dugesii	4	0	0	3	1	0	0	0
Allotoca goslinei	1	0	1	0	0	0	0	0
Allotoca maculata	2	0	0	2	0	0	0	0
Allotoca meeki	1	0	0	1	0	0	0	0
Allotoca zacapuensis	1	0	0	1	0	0	0	0
Ameca splendens	3	0	1	2	0	0	0	0
Ataeniobius toweri	1	0	0	0	1	0	0	0
Chapalichthys encaustus	1	0	0	0	0	1	0	0
Chapalichthys pardalis	2	0	0	2	0	0	0	0
Characodon species	9	1	2	6	0	0	0	0
Characodon garmani	1	1	0	0	0	0	0	0
Girardinichthys ireneae	1	0	0	1	0	0	0	0
Girardinichthys multiradiatus	2	0	0	0	2	0	0	0
Girardinichthys turneri	1	1	0	0	0	0	0	0
Girardinichthys viviparus	1	0	0	0	1	0	0	0
Goodea atripinnis	2	0	0	0	1	0	0	1
Ilyodon furcidens	1	0	0	0	0	0	0	1
Ilyodon whitei	5	0	0	0	0	5	0	0
Neoophorus regalis	1	0	0	1	0	0	0	0
Neotoca bilineata	2	1	0	0	1	0	0	0
Skiffia francesae	2	0	2	0	0	0	0	0
Skiffia lermae	4	0	0	1	3	0	0	0
Skiffia multipunctata	1	0	0	0	1	0	0	0
Xenoophorus captivus	3	0	1	1	1	0	0	0
Xenotaenia resolanae	2	0	0	0	0	2	0	0
Xenotoca doadrioi	1	0	0	0	1	0	0	0
Xenotoca eiseni	2	0	0	0	2	0	0	0
Xenotoca lyonsi	1	0	0	0	1	0	0	0
Xenotoca melanosoma	4	0	0	1	2	1	0	0
Xenotoca variata	5	0	0	0	0	3	0	2
Zoogoneticus purhepechus	3	0	0	1	1	1	0	0
Zoogoneticus quitzeoensis	2	0	0	1	1	0	0	0
Zoogoneticus tequila	1	0	1	0	0	0	0	0
Totals	84	4	8	29	21	18	0	4

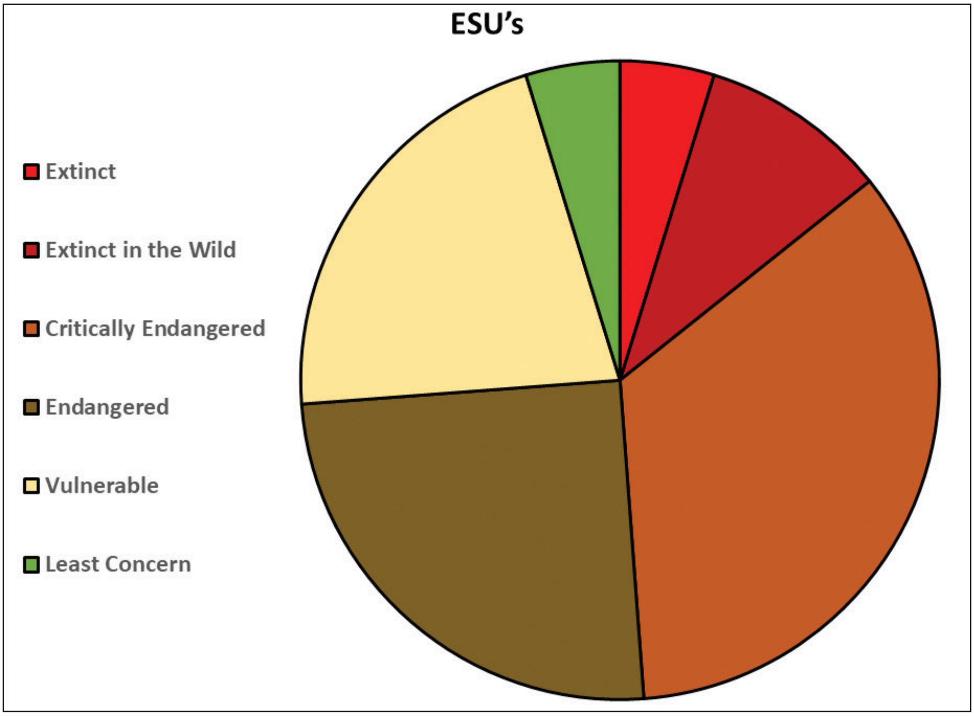

Figure 5. Pie chart of the conservation status of Mexican goodeid evolutionarily significant units (ESUs).

the Tamazula River by waterfalls, San Jose del Tule Stream, El Terrero Stream, and Pihuamo River. A 2019 survey found dozens of individuals in the San Jose del Tule, a single individual in the El Terrero, and none at the one site sampled on the Pihuamo.

Allodontichthys polylepis: Critically Endangered/Declining/1 ESU (Aldpo1) (Figure 6) – As of its initial description in 1988 (Rauchenberger 1988), this species was known from three locations in the upper Ameca River basin. By 2000, the Potrero Grande Stream population had disappeared for unknown reasons, but the populations in the De la Pola River (reported as Bola by some collectors) and its tributary the Dávalos Stream (reported as Diábalos by some collectors) had persisted (Domínguez-Domínguez et al. 2005b). These two populations have declined since then, and an intensive 2016 survey yielded only a single individual from each water body.

Allodontichthys tamazulae: Vulnerable/Stable/1 ESU (Aldta1) (Figure 6) – Historically known from throughout the Upper Coahuayana River basin where it coexisted with *A. hubbsi* (Miller et al. 2005). Pollution from a sugar cane mill near the town of Tamazula has made a portion of the former range of the species in the lower Tamazula River uninhabitable since the 1970s (Lyons and Mercado-Silva 2000). Our recent surveys have encountered *A. tamazulae* at ten locations, several of which had moderately large numbers of fish, and populations appear to be stable.

Allodontichthys zonistius: Vulnerable/Stable/1 ESU (Aldzo1) (Figure 6) – Known from 12 locations in the Armería River basin and two nearby areas in the lower Coa-

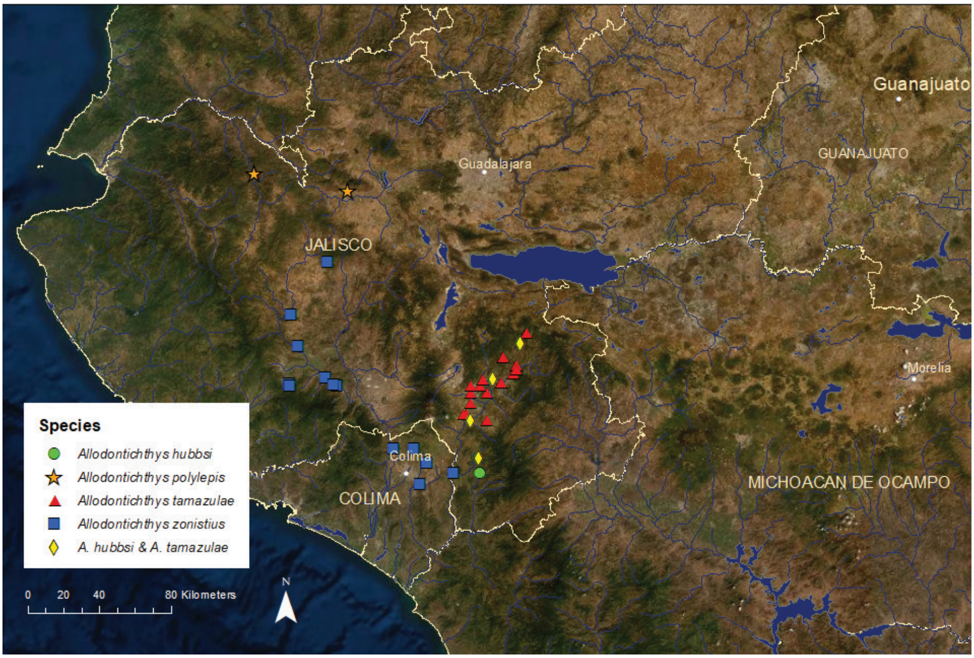

Figure 6. Distribution of the four species of *Allodontichthys*.

huayana River basin, which originated from a relatively recent stream capture (Lyons and Mercado-Silva 2000; Webb 2002). Improved water quality from a sugar mill discharge has led to increases in abundance in the Ayuquila River in the Armería basin near the city of Autlán, but these gains have been offset by population declines further downstream in the Armería River and its tributaries near the city of Colima.

Alloophorus: This genus contains a single species, *A. robustus*, which historically was widespread in the Lerma, upper Santiago (including Lake Chapala), and upper Balsas river basins on the Pacific slope and the endorheic (no outlet) Lakes Pátzcuaro and Zirahuén, and Lake Cuitzeo/Grande de Morelia River basins in central Mexico (Miller et al. 2005). The species has broad habitat tolerances, occurring in lakes, springs, and rivers. As a result of its relatively large body size, in the past, *A. robustus* was harvested for human food in many places, although currently most populations are too small to support a significant fishery. The species is still harvested in Lake Pátzcuaro and Lake Zacapu, Michoacán.

Alloophorus robustus: Vulnerable/Declining/4 ESUs (Figure 7) – Once known from more than 50 different localities, this species now persists at approximately 25 localities. Since 2000, it has disappeared from Lake Chapala and the adjacent Santiago and Lerma rivers, from the De la Laja River, a major Lerma tributary, and from Lake Yuriria. The species has become rare in the Lake Cuitzeo/Grande de Morelia River, Lake Pátzcuaro and Lake Zirahuén basins, persisting mainly in heavily vegetated lake shorelines, spring areas, and small tributaries (Lyons et al. 1998; Soto-Galera et al.

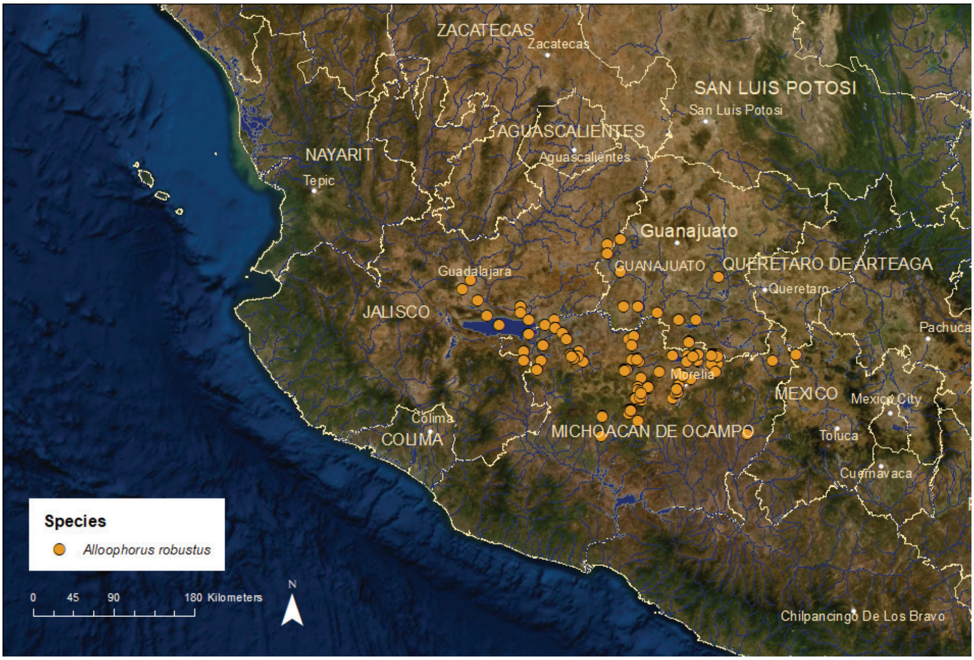

Figure 7. Distribution of *Alloophorus robustus*.

1998, 1999; Mercado-Silva et al. 2006; Domínguez-Domínguez et al. 2008b). Losses have been from a combination of declines in water quality and quantity (e.g., Chapala, Cuitzeo) and predation and competition from introduced non-native species (e.g., *Xiphophorus variatus* (Poeciliidae) in the De la Laja River; *Micropterus salmoides* (Centrarchidae) in Lake Zirahuén). Remaining strongholds include the La Mintzita Springs in the Lake Cuitzeo basin near the city of Morelia, Lake Zacapu in the headwaters of the Angulo River drainage, a Lerma River tributary, and the Duero River drainage, also a Lerma River tributary, including the La Luz and Orandino spring-fed lakes. We recognize four ESUs based on genetic analyses and biogeography. Alpro1 is vulnerable and occupies much of the Lerma River basin (excluding the Turbio River drainage) and the upper Balsas River basin, Alpro2 is vulnerable and occurs in the Lake Cuitzeo/ Grande de Morelia River basin, Alpro3 is critically endangered and is found at only one or two sites in the Turbio River drainage in the Lerma River basin, and Alpro4 is critically endangered and known from the Lake Pátzcuaro and Lake Zirahuén basins. It persists at just one or two locations in small numbers in the Pátzcuaro basin.

***Allotoca*:** This is the most diverse genus of goodeids, with seven currently recognized species and probably one or more additional undescribed species (Doadrio and Domínguez-Domínguez 2004; Webb et al. 2004; Miller et al. 2005). Overall, the genus had a historically wide range in the Ameca, Armería, Magdalena, Lerma, Cuitzeo/ Grande de Morelia, Pátzcuaro, Zirahuén, and upper Balsas river basins on the Pacific slope of central Mexico. However, the individual range of most species is (and might have always been) quite small. Some populations of *Allotoca* that have disappeared may have represented additional undescribed species. For example, a single distinctive *Allotoca* specimen was collected from the upper Armería River basin in the 1930s and a different but also distinctive *Allotoca* specimen was found in Lake Chapala in the 1960s, but no *Allotoca* have been found in either location since. Thus, whatever species these specimens represented, new or otherwise, they have been extirpated (Lyons et al. 1998), and they are not counted in any of our totals or summaries.

Allotoca catarinae: Vulnerable/Stable/1 ESU (Altca1) (Figure 8) – This species is known from approximately ten locations in the upper Cupatitzio River drainage in the upper Balsas River basin near the city of Uruapan and possibly also in the Lake Cuitzeo/Grande de Morelia and Lake Pátzcuaro basins, although the taxonomic status of specimens from outside of the Cupatitzio River drainage is uncertain (Doadrio and Domínguez-Domínguez 2004). None of these populations are particularly large, but all have persisted since 2000. This species is genetically very similar to *A. diazi* and may have reached the upper Balsas basin by a human transfer within the last 1,000 years (Corona-Santiago et al. 2015).

Allotoca diazi: Critically Endangered/Declining/1 ESU (Altdi1) (Figure 8) – Older literature (e.g., Meek 1904; Mendoza 1962) placed this species in the genus *Neoophorus*, but recent morphological and genetic analyses indicate that *Allotoca* is more appropriate (Meyer et al. 2001; Doadrio and Domínguez-Domínguez 2004; Webb et al. 2004). Currently, *A. diazi* is known from just three small areas in the Lake Pátzcuaro basin, the only basin where it occurs. It has declined dramatically in Lake Pátzcuaro proper and persists only as a remnant population there. The largest remaining population is in the Molino de Chapultepec Springs near the town of Pátzcuaro.

Allotoca dugesii (spelled *dugesi* in older literature; e.g., Smith and Miller 1980): Endangered/Declining/4 ESUs (Figure 8) – The widest ranging of the *Allotoca* species, historically known from much of the middle and lower Lerma and upper Santiago river basins on the Pacific slope and the endorheic Lake Pátzcuaro, Lake Zirahuén, and Lake Cuitzeo/Grande de Morelia River basins (Miller et al. 2005). Currently, the species is known from only six or seven locations. A new population was recently discovered in a spring along the Duero River in the Lerma River basin near the town of Etúcuaro, Michoacán, but it is very small. Recent surveys have documented the species' dramatic reduction in the upper Santiago basin and elimination from the Zirahuén basin where it was once widespread and common. Remaining strongholds are the Molino de Chapultepec Springs in the Lake Pátzcuaro basin and La Maiza Springs in the Cuitzeo/Grande de Morelia basin near the city of Morelia. Domínguez-Domínguez et al. (2002) published observations on larval feeding of this species that will be useful in the maintenance of captive populations. We recognized four ESUs based on zoogeography (Domínguez-Domínguez et al. 2006a, b). Altdu1 is critically endangered and is known from the upper Santiago River basin, Lake Chapala, and the lower Lerma River basin. However, perhaps only one viable population remains, in a spring near Lake Chapala. Altdu2 is critically endangered and found at a single site in the Turbio River drainage of the middle Lerma River basin. Atldu3 is endangered and occurs at 3–4 sites

Figure 8. Distribution of the eight species of *Allotoca*.

in the Lake Cuitzeo/Grande de Morelia River basin. Altdu4 is critically endangered and known historically from the Lake Pátzcuaro and Lake Zirahuén basins. Presently, it persists only along at a heavily vegetated shoreline area of southwestern Lake Pátzcuaro and in the Molino de Chapultepec Springs, a tributary of Lake Pátzcuaro.

Allotoca goslinei: Extinct in the Wild/No records since 2004/1 ESU (Altgo1) (Figure 8) – This species was known from only a small tributary of the Ameca River, the Potrero Grande Stream, and the Ameca River itself near the mouth of the stream in the upper Ameca River basin near the city of Ameca (Smith and Miller 1987). The species had been eliminated from the Ameca River by the 1990's by water pollution but was still moderately common in the headwaters of the Potrero Grande Stream. In the early 2000's, the non-native *Xiphophorus helleri* (Poeciliidae) became established in the Potrero Grande Stream. As *X. helleri* numbers increased, the abundance of *A. goslinei* dropped rapidly, presumably from competition or predation on larvae. The last specimen of *A. goslinei* was collected in 2004, and none could be found in targeted surveys in 2005, 2006, 2007, 2016, and 2018 (Helmus et al. 2009; Köck unpublished data). Based on this, *A. goslinei* is feared to be extinct in the wild, although it is possible that a small population persists in an isolated area of the stream not yet invaded by *X. helleri*. Only a few captive populations exist in Mexico, the United States, and Europe, and all are small.

Allotoca maculata: Critically Endangered/Declining/2 ESUs (Figure 8) – This species was described from the endorheic Lake Magdalena basin in west-central Mexico, where it was thought to be endemic (Smith and Miller 1980). Believed extinct by the late 1980s (Miller et al. 1989), this species was rediscovered in the 1990s at two locations, Lake Magdalena and the nearby but hydrologically isolated headwaters of the San Marcos River in the Ameca River basin near the town of Etzatlán. Genetic analyses indicated that the San Marcos populations represented at least a distinct ESU and possibly an undescribed species (Doadrio and Domínguez-Domínguez 2004). We recognize two ESUs. Altma1, the Lake Magdalena ESU, is critically endangered and in decline from water pollution, habitat destruction, and non-native species. It has not been collected in more than four years despite several targeted surveys; and Altma2, the San Marcos ESU, is also critically endangered and in decline from water scarcity, habitat destruction, and non-native species, persisting in small numbers at one or two locations.

Allotoca meeki: Critically Endangered/Declining/1 ESU (Altme1) (Figure 8) – This species is known only from the endorheic Lake Zirahuén basin, where it was once common. The introduction of non-native *Micropterus salmoides* (Centrarchidae), a fish predator, eliminated the species from Lake Zirahuén by the late 1990's (Domínguez-Domínguez et al. 2005b). A population of *A. meeki* persisted in Lake Opopeo in the headwaters of a tributary system, but by the 2000's *M. salmoides* had invaded this lake, and *A. meeki* had become scarce. A small population has persisted in a short segment of the outlet of the lake, which appears to be too narrow and shallow for *M. salmoides*.

Allotoca zacapuensis: Critically Endangered/Stable/1 ESU (Altza1) (Figure 8) – This species was described in 2001 (Meyer et al. 2001) and is known only from Lake Zacapu and a tributary spring, Jesus Maria, which are in the headwaters of the Angulo River drainage in the Lerma River basin. Within the lake it is known from only two spring-fed areas where it is uncommon but apparently relatively stable in numbers (Domínguez-Domínguez et al. 2005b).

Ameca**:** This genus consists of a single species, *A. splendens*, which, until recently was thought to be restricted to the Teuchitlán Springs and their outlet in the upper Ameca River basin on the Pacific slope of west-central Mexico (Miller et al. 2005). However, within the last fifteen years, two new populations have been discovered in nearby basins.

Ameca splendens: Endangered/Declining/3 ESUs (Figure 9) – This species is known from the Teuchitlán Springs and was recently discovered in the Almoloya Springs in the endorheic Lake Magdalena basin and the El Molino Springs in the endorheic Lake Sayula basin, both close to but separate from the Ameca basin. No genetic or morphological analyses of the three populations are available, but we consider each to be a separate ESU based on zoogeography. Amesp1 is endangered and occupies the Teuchitlán Springs and its outlet the Teuchitlán River. Historically, the population extended well down the river to its junction with the Salado River to form the Ameca River. However, it is now limited to the upper part of the Teuchitlán Springs and two small tributary springs that enter the river 2–3 km downstream. The Teuchitlán Springs population probably numbers in the high hundreds or low thousands and appears to be stable (López-López et al. 2004), but the two smaller springs have not been thoroughly assessed. Bailey et al. (2007) provided a detailed analysis of genetic diversity within the

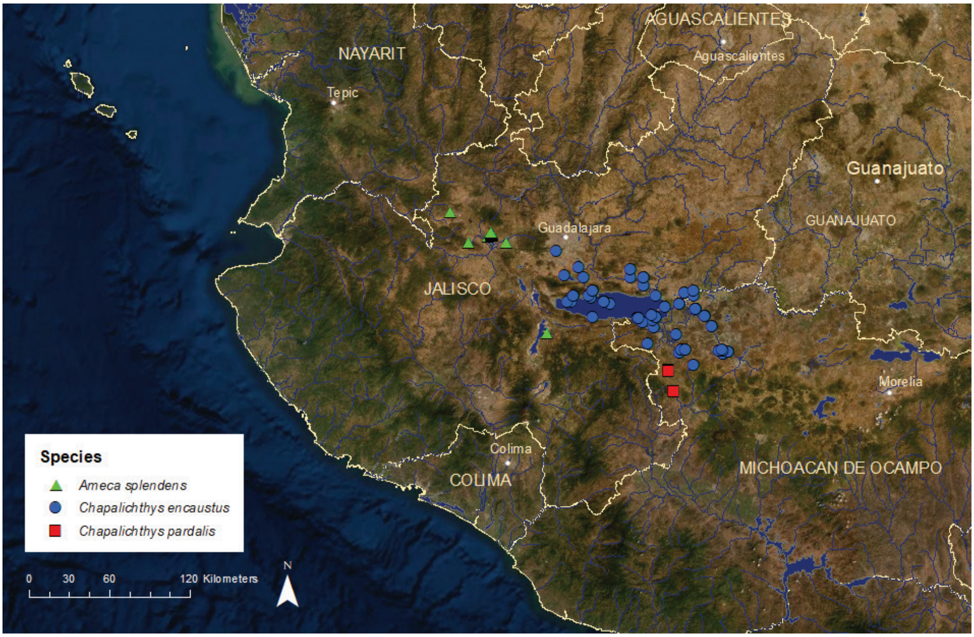

Figure 9. Distribution of *Ameca splendens*, *Chapalichthys encaustus*, and *C. pardalis*.

Teuchitlán Springs population. Amesp2 is critically endangered and known only from the Almoloya Springs in the Magdalena basin ca. 50 km northwest of Teuchitlán. The population has declined steadily since its discovery in the early 2000's. The population decline was associated with the appearance and rapid population growth of non-native *Pseudoxiphophorus bimaculatus* (Poeciliidae), which is known to outcompete and threaten the survival of other goodeid species (Ramírez-Carrillo and Macías-García 2015). No Amesp2 specimens are held in captivity. Amesp3 is possibly extinct and known only from the El Molino Springs near Cuyucapán in the Sayula basin, ca. 80 km southeast of Teuchitlán. The population disappeared when the springs completely dried in 2010 during a drought. No Amspe3 specimens are in captivity. There are unconfirmed reports of populations of *Ameca splendens* from three other springs in the Sayula basin during the 1990's (Rosales-Figueroa 1995), but these springs are now completely dry.

Ataeniobius: This genus is represented by a single species, *A. toweri*, and is limited to the thermal-spring lakes, springs, and streams in the headwaters of the Verde River drainage in the Pánuco River basin on the Atlantic slope of central Mexico (Miller et al. 2005).

Ataeniobius toweri: Endangered/Declining/1 ESU (Atato1) (Figure 10) – This species is known from the Media Luna and Los Anteojitos lakes, adjacent springs, their outlets near the city of Rioverde, and the Villa Juarez stream near the town of the same name (Domínguez-Domínguez et al. 2005b). *Ataeniobius toweri* associates closely with

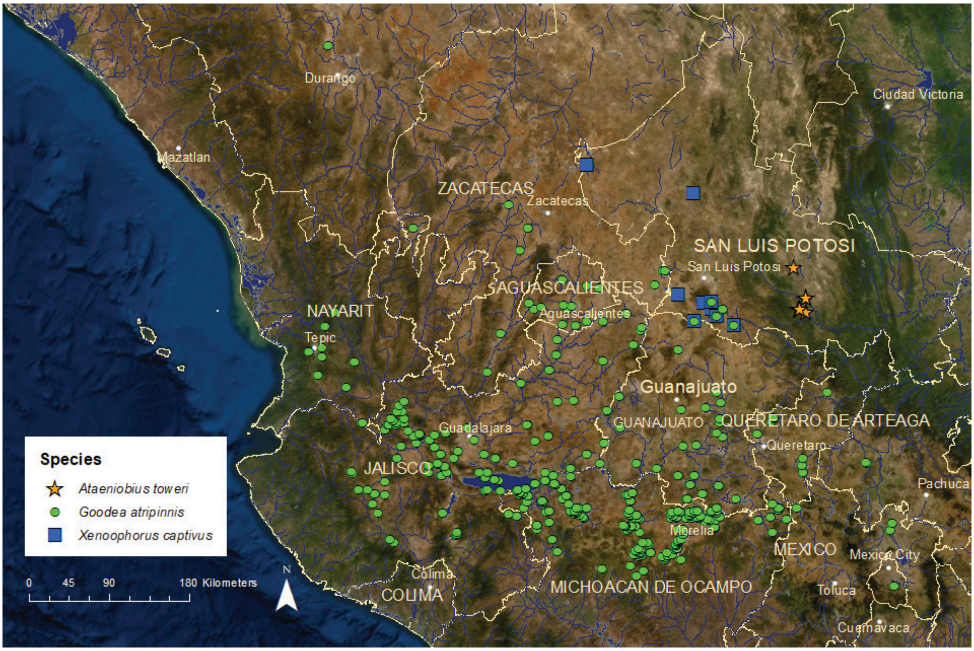

Figure 10. Distribution of *Ataeniobius toweri*, *Goodea atripinnis*, and *Xenoophorus captivus*.

dense aquatic vegetation, and the recent loss of major macrophyte beds in Media Luna and Los Anteojitos have resulted in a substantial decline in the species abundance. At least two small nearby springs, Charco Azul and Los Peroles, maintain good populations. The Villa Juarez population persists but appears to be small.

***Chapalichthys*:** This genus has two currently recognized species, one in the Lake Chapala basin and the other in a nearby portion of the upper Balsas River basin, both on the Pacific slope of central Mexico (Miller et al. 2005).

Chapalichthys encaustus: Vulnerable/Declining/1 ESU (Chaen1) (Figure 9) – This species was formerly abundant throughout nearshore areas of Lake Chapala and was also encountered in adjacent areas of the lower Lerma and upper Santiago rivers and their tributaries (Lyons et al. 1998). Since the late 1990s, *C. encaustus* has disappeared from the mainstem Santiago and Lerma rivers due to pollution and has become much less common in Lake Chapala owing to the invasion of the non-native *Poecilia sphenops* and *Gambusia yucatana* (Poeciliidae) (Becerra-Muñoz et al. 2003). *Chapalichthys encaustus* still persists in the lower portion of the Duero River drainage, a Lerma River tributary, including the La Luz and Orandino lakes, and also in Cajititlán and Los Negritos lakes, both near Lake Chapala. Beginning in 2005, small numbers of individuals have been collected from the La Vega Reservoir and its outlet in the upper Ameca River basin (Mar-Silva in press). These *C. encaustus* were probably introduced accidentally during a stocking of *Oreochromis aureus* (Cichlidae) from Lake Chapala or the Lerma basin.

Chapalichthys pardalis: Critically Endangered/Stable/2 ESUs (Figure 9) – This species is known from only two areas in the upper Balsas River basin, the San Juanico Lake and the Tocumbo Springs, located ca. 25 km downstream along the outlet of the lake (Miller et al. 2005). For many years the San Juanico population was considered a separate species, *C. peraticus* (e.g., Domínguez-Domínguez et al. 2005b), but recent genetic (Piller unpublished data) and morphological analyses (Miller et al. 2005) indicate that there are insufficient differences between the two populations to warrant separate species status, although they do qualify for separate ESU designation based on morphology. Chapa1, from Tocumbo, is critically endangered and possibly extinct in the wild. Formerly it was known only from a small spring system that had been heavily modified as a swimming area. However, none have been observed there since 2015. Chapa2, from San Juanico, is also critically endangered, having a small population occupying the nearshore areas of the lake.

***Characodon*:** The distribution of this genus is separate from that of other Mexican goodeids, encompassing a portion of the Pacific slope of northwestern Mexico in the states of Durango and Coahuila far to the north of the other species (Figure 1). Two or possibly three species exist, one long extinct and the other(s) critically endangered and declining.

Characodon species: Critically Endangered/Declining/9 ESUs (Figure 11) – The taxonomy and relationships of the *Characodon* populations occupying the upper Tunal and Durango river drainages in the upper Mezquital River basin in the state of Durango are currently unresolved. Originally, a single species, *C. lateralis*, was recognized, which occupied a series of semi-isolated spring systems near the city of Durango. However, the locality given for the original collection of the species, “Central America”, was clearly erroneous and the type material could not be attributed to a specific spring system (Miller et al. 2005). In the 1980’s, the population in the springs near the town of El Toboso was described as a separate species, *C. audax*, based on morphology, with the remaining populations considered *C. lateralis* (Smith and Miller 1986). However, more recent genetic analyses revealed little difference between the El Toboso population and other nearby *Characodon* populations (Doadrio and Domínguez-Domínguez 2004; Domínguez-Domínguez et al. 2006b). Instead, these analyses indicated that populations from spring systems located above the El Salto Waterfall on the Tunal River differed from those located below the falls, suggesting that perhaps all populations above the falls could be called *C. audax* and those below the falls *C. lateralis*. However, Artigas-Azas (2014) provided strong circumstantial evidence that the type of *C. lateralis* probably came from somewhere near the city of Durango above the falls, making it an inappropriate name for populations below the falls. Recent morphological analyses have indicated significant differences among ten populations, nine from above the falls and one from below, with the El Toboso population the most distinctive (Tobler and Bertrand 2014). Given uncertainly about which populations the name *C. lateralis* actually refers to and the discordance between the morphological and the genetic distinctiveness of the nominal *C. audax* from the El Toboso Springs, we have chosen to

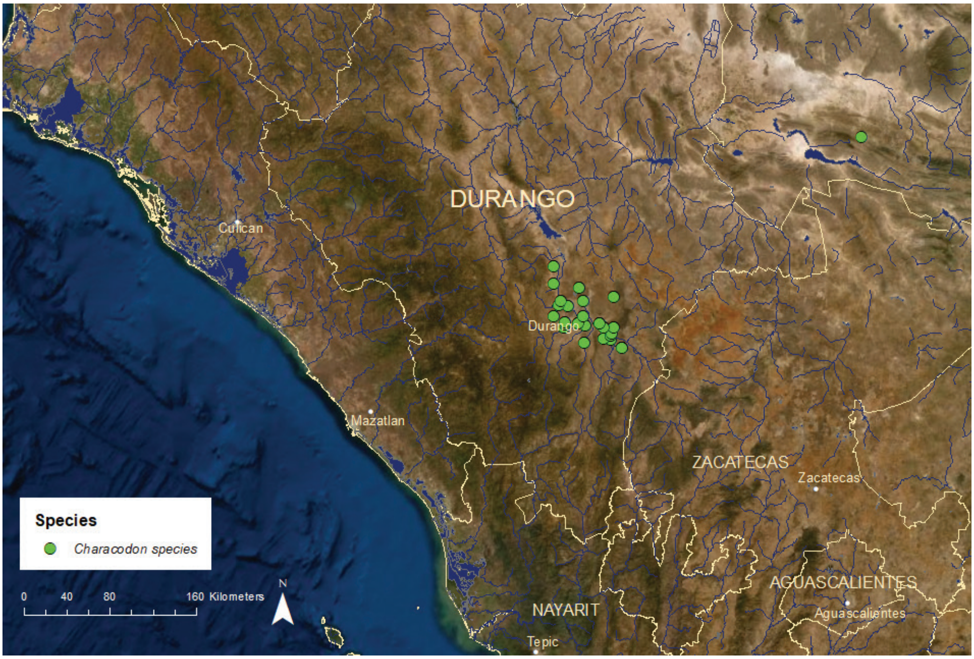

Figure 11. Distribution of *Characodon* species. The single record far to the east represents *C. garmani* and the remainder of points represent *C. audax* and *C. lateralis*.

refer to all populations from the Tunal and Durango River drainages as "*Characodon* species", pending a comprehensive revision of the genus. We have also identified nine ESUs, seven from above the falls and two from below, based on a combination of genetic, morphological, and zoogeographic information.

Regardless of what their taxonomic affinities are, all of the *Characodon* species ESUs are in serious trouble. Three ESUs have gone extinct in the last 20 years and the remaining six have all suffered steep drops in abundance (Artigas-Azas 2002, 2014). Declines have been caused by the drying of springs and streams owing to groundwater pumping and water diversions and by the introductions of non-native fish species. Chrsp1, the nominal *C. audax* from the El Toboso Springs, is critically endangered. Chrsp2, from the Cerro Gordo and El Carmen Springs and from the San Rafael and Las Moras streams, is also critically endangered and persists in small numbers only in the El Carmen Springs and in the Las Moras Stream in the town of San Rafael. Chrsp3, from the Los Pinos Springs and outlet, is extinct in the wild, with the last specimens collected in the late 1990's. There are a few captive populations in Mexico, the United States, and Europe. Chrsp4, from the Guadalupe Aguilera, Laguna Seca, and Aguada de las Mujeres Springs and the Peñon del Aquila Reservoir, is critically endangered and current exists only in the Guadalupe Aguilera Springs. Chrsp5, from the San Vicente de los Chupaderos Springs and the Sauceda River, is extinct with no captive populations. The last collections date from the early 1990's. Chrsp6, from the Abraham Gonzáles, Ojo Garabato, and 27 de Noviembre springs, is critically endangered but is still found in small numbers in all three springs. Chrsp7, from the Puente Pino Suárez Stream, is also critically endangered. Chrsp8, known from the Ojo de Aqua de San Juan, Los Berros, Ojo Nombre de Dios, and La Constancia springs, all located below the El Salto waterfall, is critically endangered and has disappeared from the Ojo Nombre de Dios Springs. Chrsp9, from the Amado Nervo Stream, also located below the El Salto Waterfall is probably extinct in the wild, with the last specimens observed in 2005. A small number of captive populations exists in Mexico, the United States, and Europe.

Characodon garmani: Extinct/No records since 1890's/1 ESU (Chrga1) (Figure 3, 11) – This species is known from only a single female individual thought to have been collected from the endorheic Valley of Parras in Coahuila prior to 1895 (Fitzsimons 1972; Smith and Miller 1986; Miller et al. 2005), although there is circumstantial evidence that it may have come instead from a spring near Durango, making it a member of *Characodon* species rather than a separate taxon (Artigas-Azas, unpublished data). If it did indeed come from the Valley of Parras, it is no longer found there now and must be considered extinct with no captive populations (Miller et al. 1989). The habitat in Parras has been heavily modified during the last 150 years, and no specimens have been encountered during the many fish surveys conducted there from the 1940's to the present.

***Girardinichthys*:** This genus occurs in central Mexico in several different basins. Historically, *Girardinichthys* was believed to have included only two species, *G. multiradiatus* and *G. viviparus*. However, Radda and Meyer (2003) described a new species and combined the genus *Hubbsina* with *Girardinichthys* and relegated *Hubbsina* to a subgenus, which expanded *Girardinichthys* to four species. This change has not been accepted by some ichthyologists (e.g., Domínguez-Domínguez et al. 2005b; Miller et al. 2005).

Girardinichthys ireneae: Critically Endangered/Declining/1 ESU (Figure 12) – Until recently, this species was considered to be part of *Hubbsina turneri* (Domínguez-Domínguez et al. 2005; Miller et al. 2005). When Radda and Meyer (2003) subsumed *Hubbsina* within *Girardinichthys*, they split the former *H. turneri* into two species, *G. ireneae* and *G. turneri*. *Girardinichthys ireneae*, as currently defined, is known only from the upper portion of the Angulo River drainage of the Lerma River basin, primarily in Lake Zacapu and a few smaller spring-fed lakes nearby. It appears to have disappeared from the smaller lakes since 2000 and persists only in spring-fed areas of Lake Zacapu.

Girardinichthys multiradiatus: Endangered/Declining/2 ESUs (Figure 12) – This species was known historically from approximately 16 locations located just northwest, west, and south of greater Mexico City, including 13 streams and wetlands in the upper Lerma River basin and single sites in the headwaters of the Balsas River basin on the Pacific slope, the endorheic Lake Zempoala system, and the Taxingu Reservoir in the upper Pánuco River basin on the Atlantic slope (Domínguez-Domínguez et al. 2005b). Distribution and abundance of *G. multiradiatus* declined substantially in the Lerma basin during the 20th Century, and the seven remaining populations there are

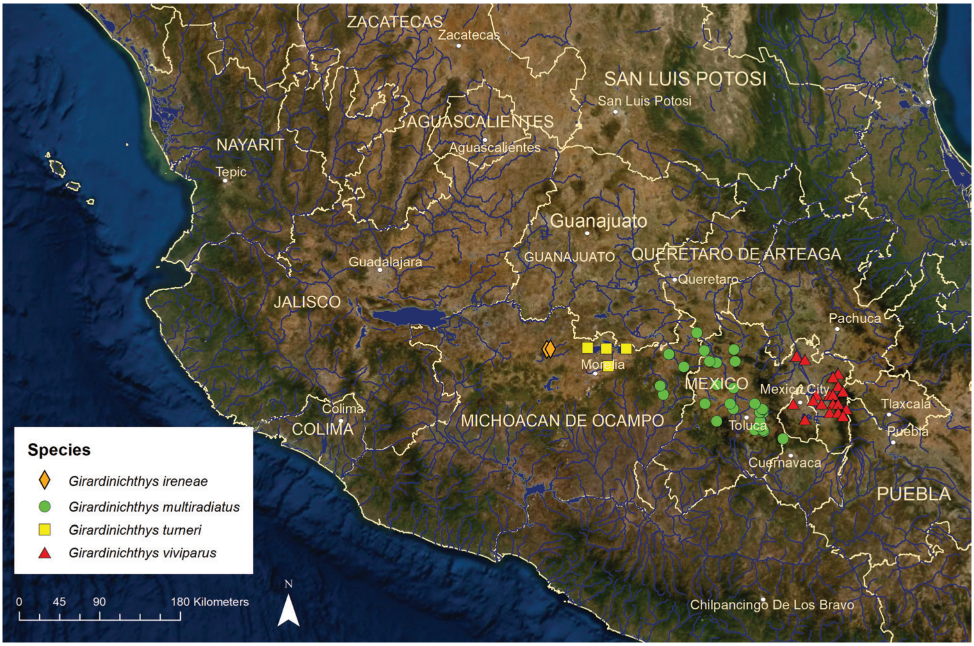

Figure 12. Distribution of the four species *Girardinichthys*.

now low in numbers and isolated, with some in decline and approaching extirpation. The Balsas, Zempoala, and Taxingu populations still persist but are small. Little clear genetic divergence is evident among populations from the Lerma, Balsas, and Pánuco basins (Macias-García et al. 2012). We used zoogeography to identify two ESUs. Girmu1 is limited to Lake Zempoala, which has been long isolated from the upper Lerma and Balsas basins and which experiences colder conditions than other goodeid habitats in Mexico. This ESU is endangered, with a moderate population in the small lake, which is fortunately protected as a National Park. Girmu2 encompasses all other populations and is also endangered.

Girardinichthys (Hubbsina) turneri: Extinct/No records since 1980s/1 ESU (Girtu1) (Figure 12) – As defined by Radda and Meyer (2003), this species was limited to Yuriria Lake in the Lerma River basin and the nearby endorheic Lake Cuitzeo/Grande de Morelia River basin. These two areas have been heavily polluted and modified, and no *G. turneri* have been observed in either area since the late 1980's despite repeated and intensive targeted sampling (Soto-Galera et al. 1999; Domínguez-Domínguez et al. 2005b). Unfortunately, no captive populations exist, so this species appears to be extinct.

Girardinichthys viviparus: Endangered/Stable/1 ESU (Girvi1) (Figure 12) – Historically, this species was endemic to and abundant in the many lakes and wetlands of the endorheic Valley of Mexico, where Mexico City is located (Miller et al. 2005). Construction of a canal in the late 1800's to drain the valley into the upper Tula River allowed the species to colonize Lake Zumpango and Lake Tecocomulco in the headwaters of the Pánuco River basin on the Atlantic slope. Lake Zumpango has poor water quality and the species may no longer exist there. Water quality in Lake Tecocomulco is good and *G. viviparus* persists there in moderate numbers. The drainage of the Valley of Mexico coupled with the rapid expansion of Mexico City led to a drastic decline in the distribution and abundance of *G. viviparus*. During the mid-20th century, the species was eliminated from Lake Texcoco and Lake Chalco and became rare in Lake Xochimilco, all of which have become greatly reduced in size, highly polluted, and dominated by non-native fish species (Domínguez-Domínguez et al. 2005b). Despite poor environmental conditions, the Xochimilco population, which is very small, has managed to survive up to the present. Perhaps the largest remaining population is in the artificial Lake Lago Mayor in Chapultepec Park in downtown Mexico City. This population persists in moderate numbers and seems stable despite very poor water quality, but it is highly vulnerable to drainage of the lake for maintenance.

Goodea**:** This genus is now considered to have a single species (Miller et al. 2005; Domínguez-Domínguez et al. 2010) although in the past two or three species were recognized (e.g., Doadrio and Domínguez-Domínguez 2004). This genus is the most widespread of the Goodeids and occupies the broadest range of habitats, with a native distribution that encompasses most of the highlands of central Mexico including streams, rivers, wetlands, springs, lakes, and reservoirs (Miller et al 2005). This species is also, along with *Alloophorus robustus*, the largest of the goodeids and has been regularly used by humans for food.

Goodea atripinnis: Least Concern/Declining/2 ESUs (Figure 10) – This species has the largest distribution of any goodeid species. Its native range includes the Lerma, upper Santiago (including Lake Chapala), upper Ameca, upper Armería, and upper Balsas river basins on the Pacific slope, the endorheic Lake Zirahuén, Lake Pátzcuaro, and Lake Cuitzeo/Grande de Morelia River basins in central Mexico, and the upper Pánuco River basin on the Atlantic slope. Many years ago, *Goodea* was introduced and became established in the Valley of Mexico. Also, an introduced population was recently discovered in the upper Mezquital River basin within the range of *Characodon* near Durango (Michael Tobler, Kansas State University, Manhattan, Kansas, USA, unpublished data). Some early authors (e.g., Meek 1904; Mendoza 1962) considered the Lake Pátzcuaro population to be a different species, *G. luitpoldi*, but recent genetic and morphological analyses indicate that this population is not distinct from *G. atripinnis* (Doadrio and Domínguez-Domínguez 2004; Webb et al. 2004; Domínguez-Domínguez et al. 2010). Other authors have considered the Pánuco River basin population a distinct species, *G. gracilis* (e.g., Doadrio and Domínguez-Domínguez 2004; Domínguez-Domínguez et al. 2005b). Although there are minor genetic and morphological differences between Pánuco River basin populations and other *Goodea* populations, the Pánuco population is more appropriately considered as a separate ESU rather than a separate species (Domínguez-Domínguez et al. 2010).

Goodea atripinnis remains common in many areas and is probably still the most abundant goodeid species overall, but the distribution and abundance of both ESUs have steadily declined during the last 25 years (Lyons et al. 1998; Soto-Galera et al. 1999; Domínguez-Domínguez et al. 2005b; Mercado-Silva et al. 2006). Despite decreases in distribution and abundance, Gooat1 still qualifies as least concern and remains common in many areas. It appears to be relatively tolerant of poor water quality compared to other goodeids (Rueda-Jasso et al. 2017). Nonetheless, the trends for this ESU are not encouraging. Historically, this ESU supported commercial fisheries in the larger lakes where it occurred, but in recent years it has been eliminated from Lake Zirahuén, reduced to a small remnant population in Lake Pátzcuaro, and greatly decreased in number in Lake Chapala and Lake Cuitzeo, largely owing to predation by and competition with non-native fish species. It is still harvested and eaten in Lake Pátzcuaro and Lake Zacapu, Michoacán. Pollution and habitat modifications have devastated populations in many areas of the Lerma and upper Santiago basins. Gooat2 is endangered, and only four or five small populations persist in the upper Pánuco River basin. Decreases there have been caused primarily by water diversions and groundwater pumping, which have eliminated habitat.

***Ilyodon*:** This genus is native to rocky, fast-flowing streams in the upper Ameca, Armería, Marabasco, Coahuayana, and Balsas basins in the mountains of west-central Mexico. It is most closely related to *Allodontichthys* and *Xenotaenia* (Doadrio and Domínguez-Domínguez 2004; Webb et al. 2004). Substantial morphological and genetic variation exists within and among populations of *Ilyodon* (Kingston 1979), and the taxonomy of the genus has long been confused. Based on recent genetic analyses (Beltrán-López et al. 2017), we recognize only two species, but some ichthyologists and aquarists have recognized as many as five or six.

Ilyodon furcidens: Least Concern/Declining/1 ESU (Ilyfu1) (Figure 13) – As we define it, this species is widely distributed and common in the Armería basin and uncommon in the Marabasco and upper Ameca River basins. Historically, populations in the Coahuayana River basin were also assigned to this species (Miller et al. 2005), but recent genetic analyses indicate that those populations are distinct from Ameca, Marabasco, and Armería basin *I. furcidens* and better assigned to *I. whitei* (Beltrán-López et al. 2017). Populations in the upper Coahuayana basin have *I. whitei* morphology and appearance, whereas populations in the lower Coahuayana basin are more similar to *I. furcidens* in appearance and morphology, but both sets of populations are clearly distinct from *I. furcidens* (as we define it) genetically. Two morphotypes of *I. furcidens* are present in many areas of the Armería and Marabasco river basins (Lyons and Navarro-Pérez 1990), and these were long thought to be two different species, the narrow-mouthed form, *I. furcidens* and the wide-mouthed form, *I. xantusi*. However, work by Turner et al. (1983, 1985) and Grudzien and Turner (1984) demonstrated that narrow-mouthed females could produce both narrow-mouthed and wide-mouthed offspring, as could wide-mouthed females, proving that the two morphotypes were part of the same species. *Ilyodon furcidens* was the older of the two names and thus had priority, so the name *I. xantusi* is no longer considered valid. Some ichthyologists and aquarists consider populations from the upper Ameca River basin to be a separate

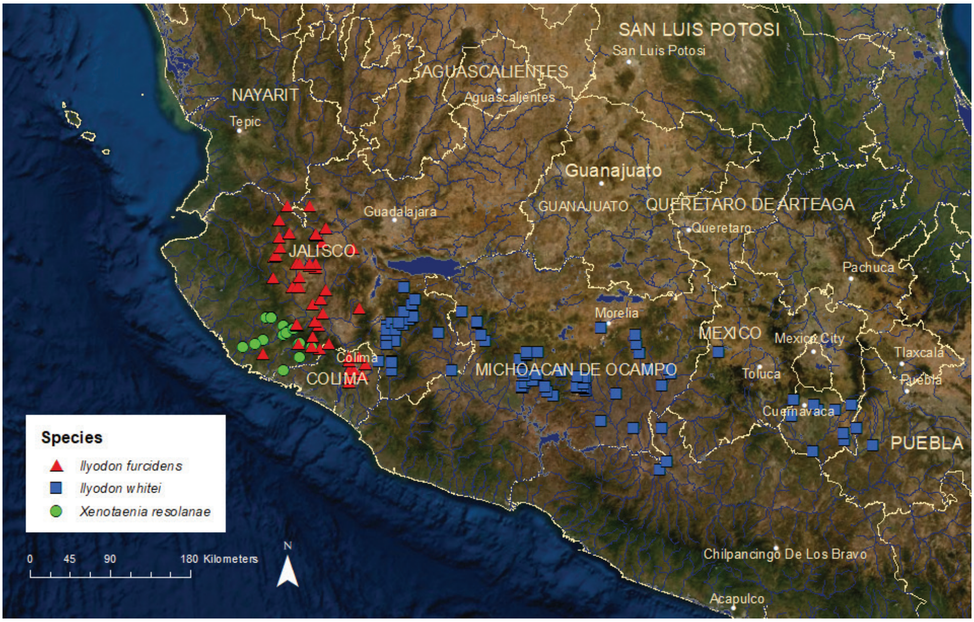

Figure 13. Distribution of *Ilyodon furcidens*, *I. whitei*, and *Xenotaenia resolanae*.

species, *I. "amecae"* (Doadrio and Domínguez-Domínguez 2004). However, genetic and morphological differences between Ameca and Armería populations are small and more recent analyses do not consider the Ameca populations worthy of even separate ESU status (Beltrán-López et al. 2017). An introduced population of what appears to be *I. furcidens* was discovered in 2019 in the Citala Reservoir in the Lake Sayula basin (Köck, unpublished data).

Ilyodon furcidens is often the most common species at the localities where it occurs. It can reach a relatively large size (150 mm) and is sometimes consumed as food. However, numbers appear to be decreasing since 2000. Populations have declined or disappeared from several streams in the Ameca River basin because of shrinking water levels and invasions of non-native species. Within the Armería River basin, the expansion of the non-native *M. salmoides*, a top predator, and *Poeciliopsis gracilis* (Poeciliidae), a likely competitor and fry predator, has apparently resulted in the near elimination of *I. furcidens* from long stretches of the Ayuquila River, Jalisco (Lyons, unpublished data).

Ilyodon whitei: Vulnerable/Declining/5 ESUs (Figure 13) – As we define it, this species is found in the Coahuayana and Balsas river basins, where it occurs in ca. 60 sites over a wide range. Specimens from the Chacambero River, a tributary to the Balsas River near the town of Ciudad Altamirano in the state of Guerrero, were described as a separate species, *I. lennoni* (Meyer and Förster 1983), but based on limited and inconsistent morphological and genetic differences, we and most other ichthyologists (e.g., Doadrio and Domínguez-Domínguez 2004; Domínguez-Domínguez et al. 2005b; Miller et al. 2005; Beltrán-López et al. 2017) do not consider this species to be valid. Similarly, populations in Tacámbaro River drainage in the upper Balsas River basin were described as a separate species, *I. cortesae* (Paulo-Maya and Trujillo-Jiménez 2000), but again morphological and genetic differences between this and other populations are small and inconsistent, and we also do not consider this species to be valid.

Many populations of *I. whitei* have declined or disappeared during the last 25 years, largely because of predation by or competition from non-native fish species (e.g., Contreras-MacBeath et al. 1998) and water pollution. Non-native species now dominate many areas of the Balsas River basin. Of the five ESUs we recognize, Ilywh1, found in the upper Coahuayana River basin in Jalisco is vulnerable. It has declined from many areas but remains abundant where it occurs. Similarly, Ilywh2, distinguished from Ilywh1 based on morphology, from the lower Coahuayana basin in Jalisco, is also vulnerable. It too has declined but is still numerous in several areas. Ilywh3, in the central and western portion of the Balsas River basin and including the nominal *I. cortesae* and *I. lennoni*, is the most common and widespread ESU, occurring at ca. 30 sites, but still qualifies as vulnerable. Ilywh4 is classified as vulnerable and is found at ca. 12 sites in the Amacuzac River drainage in the eastern Balsas River basin in the state of Morelos. Ilywh5 is classified as vulnerable and has been reported from approximately ten sites in the Atoyac River drainage of the far eastern Balsas River basin in the state of Puebla.

***Neoophorus*:** This genus currently consists of one species, *N. regalis*, which some ichthyologists place in the genus *Allotoca* (Webb et al. 2004; Miller et al. 2005). We concur with Doadrio and Domínguez-Domínguez (2004) that genetic information supports recognition of *Neoophorus* as a distinct genus, but additional genetic and morphological studies to confirm this view are warranted.

Neoophorus regalis: Critically Endangered/Declining/1 ESU (Neore1) (Figure 8) – At present, this species survives in only one small unnamed stream near the town of Los Reyes, Michoacán, in the upper Balsas River basin. Historically, the species was widespread and moderately common in streams and wetlands in the Valley of Los Reyes (Miller et al. 2005), but distribution and abundance have declined steadily during the last 25 years as wetlands have been drained and streams have been channelized and diverted for agriculture. Introductions of non-native *Xiphophorus helleri* (Poeciliidae) and *Oreochromis aureus* (Cichlidae) may also have contributed to losses. As of 2000, *N. regalis* was known from four locations (Domínguez-Domínguez et al. 2005b), but 2008 and 2011 surveys found the species at only one of these locations, where it was uncommon.

***Neotoca*:** This genus consists of one species, *N. bilineata*, which many ichthyologists place in the genus *Skiffia* (Webb et al. 2004; Domínguez-Domínguez et al. 2005b; Miller et al. 2005). We concur with Doadrio and Domínguez-Domínguez (2004) that genetic information supports recognition of *Neotoca* as a distinct genus, but as in the case for *Neoophorus*, additional genetic and morphological studies to confirm this view are warranted.

Neotoca bilineata: Endangered/Declining/2 ESUs (Figure 14) – Historically, this species was reported from three distinct areas: Lake Chapala and adjacent portions of its outlet, the upper Santiago River; the Lerma River and its tributaries the Laja River, Turbio River, and Lake Yuriria in the middle Lerma River basin; and the endorheic Lake Cuitzeo/Grande de Morelia River basin near the city of Morelia (Miller et al. 2005), but it has declined dramatically throughout its range. We recognize two ESUs based on genetic analyses (Ornelas-García et al. 2012). Neobi1, from Lake Chapala and vicinity, appears to be extinct with no captive populations. Most records are from the early 1900s, and no individuals have been collected there for at least 70 years (Lyons et al. 1998). Neobi2 is critically endangered and occupies the rest of the species' range. Only a remnant population remains in the middle Lerma River near the city of Salamanca and in two small springs tributary to the Turbio River near Penjamo, and the species has been lost from the Laja River drainage and Lake Yuriria (Soto-Galera et al. 1998; Mercado-Silva et al. 2006; Mercado and Piller unpublished data). Populations persist in the Lake Cuitzeo/Grande de Morelia River basin in Lake Cuitzeo, Cointzio Reservoir, Querendaro River, and Borbollon Springs, but none of these are particularly large and numbers fluctuate greatly within and among years. Pollution and habitat modifications had eliminated the species from nearly all of Lake Cuitzeo proper and from most of the Grande de Morelia River drainage before 2000 (Soto-Galera et al. 1999). Population declines have continued since then, and the long-term survival of this species in the wild is uncertain (De la Vega-Salazar et al. 2003a; Domínguez-Domínguez et al. 2005b).

***Skiffia*:** As we define it, this genus has three species, all limited to central and west-central Mexico where they are found mainly in springs and spring-fed lakes and streams (Miller et al. 2005).

Skiffia francesae: Extinct in the Wild/No records since 2008/2 ESUs (Figure 14) – This species had long been thought to be endemic to the Teuchitlán Springs in the upper Ameca River basin and was already believed extinct in the wild when it was first formally described as a species in 1978 (Kingston 1978; Miller et al. 1989). Fortunately, captive populations exist in North America and Europe, although many of these are inbred (Domínguez-Domínguez et al. 2005b). In 2007, Omar Domínguez-Domínguez discovered a new population of *Skiffia* that suggested that *S. francesae* might still persist in wild. These fish were found at the El Molino spring pond near the town of Cuyucapán in the endorheic Lake Sayula basin, which is located ca. 80 km south of the Teuchitlán Springs. Some individuals from El Molino appeared identical to *S. francesae*, whereas others had pigmentation that was more reminiscent of *S. multipunctata.* Genetically, the El Molino fish were more similar to *S. francesae* than to *S. multipunctata*. Consequently, we have chosen to treat the two populations as separate ESUs of *S. francesae*, Skifr1 for Teuchitlán and Skifr2 for El Molino. Unfortunately, the El Molino population was eliminated when the pond dried completed during a drought in 2010. Thus, both ESUs are extinct in the wild. Moderate numbers of cap-

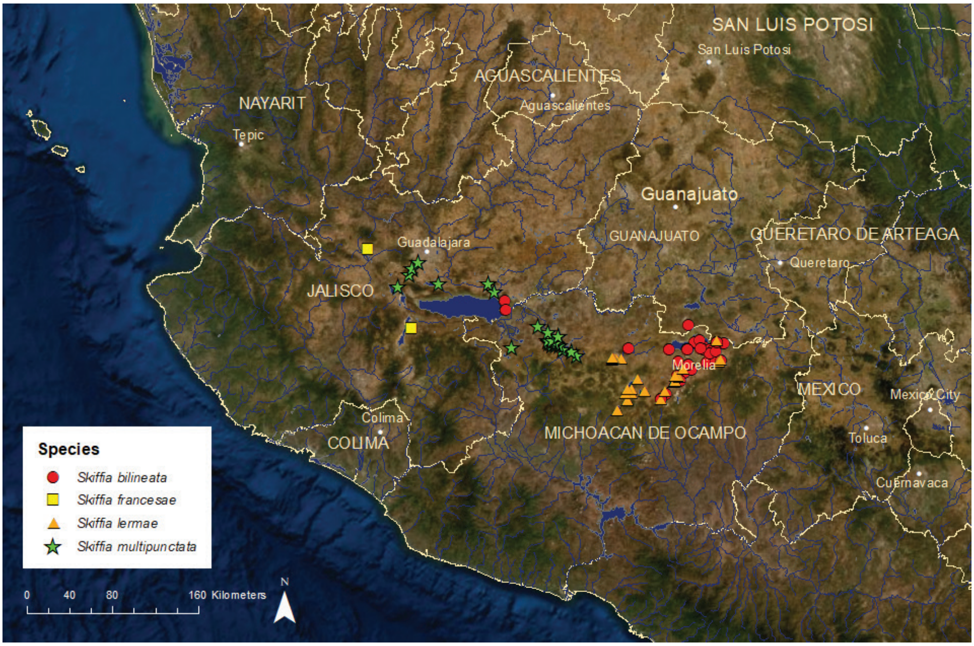

Figure 14. Distribution of *Neotoca bilineata* and the three species of *Skiffia*.

tive populations of Skifr1 exist in Mexico, the United States, and Europe, but captive populations of Skifr2 are scarce.

Skiffia lermae: Endangered/Declining/4 ESUs (Figure 14) – The historical range of this species encompassed many sites in central Mexico including Lake Zacapu, Lake Yuriria, and the Laja River in the middle Lerma River basin, and the endorheic Lake Pátzcuaro, Lake Zirahuén, and Lake Cuitzeo/Grande de Morelia River basins. Distribution and abundance of *S. lermae* have declined steadily during the last 50 years, due to water pollution, habitat degradation, and non-native species, with continued losses through the 2000s. The species has disappeared from nearly all of the Laja River drainage, Lake Yuriria, Lake Cuitzeo, and the entire Lake Zirahuén basin, and has become uncommon and limited to Lake Zacapu and a few small springs in the Lake Pátzcuaro and Grande de Morelia River basins (Lyons et al. 1998; Soto-Galera et al. 1998, 1999; De la Vega-Salazar 2003a; Domínguez-Domínguez et al. 2005b, 2008a; Mercado-Silva et al. 2006). Four ESUs are recognized, all in trouble. Skile1 occupies Lake Zacapu in the middle Lerma River basin where it is endangered. Skile2 has been reported from Lake Yuriria and the endorheic Lake Cuitzeo/Grande de Morelia River basin and is endangered. Populations from Lake Yuriria and Lake Cuitzeo are gone, but this ESU persists in the La Mintzita Springs, tributary to the Grande de Morelia River. Skile3 is known from the endorheic Lake Zirahuén and Lake Pátzcuaro basins and is also endangered. This ESU has been eliminated from Lake Zirahuén and Lake Pátzcuaro but persists in the Molino de Chapultepec Springs in the Lake Pátzcuaro basin. Skile4 is known from the Laja River drainage and is critically endangered. Historically this ESU was common throughout the drainage but now it is restricted to the Charco del Ingenio Reserve on the De Las Colonias Reservoir in the city of San Miquel de Allende, Guanajuato.

Skiffia multipunctata: Endangered/Declining/1 ESU (Figure 14) – This species was found historically in Lake Chapala, the upper part of the Santiago River basin near the city of Guadalajara, including Lake Cajititlán, and the lower Lerma River basin, particularly the Duero River drainage (Domínguez-Domínguez et al. 2005b). Pollution, habitat modifications, and introductions of non-native species have eliminated *S. multipunctata* from Lake Chapala, the Santiago River basin, and parts of the lower Lerma River basin (Lyons et al. 1998; Soto-Galera et al. 1998). The only area where the species remains is the Duero River drainage, but the species has disappeared from the lower portion of the drainage because of stream channelization and water diversions for agriculture. Only six or seven populations remain, with the largest found in the spring-fed La Luz and Orandino lakes. Populations in both lakes are threatened by habitat modifications for recreation and introductions of non-native fishes. Information on the larval ecology of *S. multipunctata* in captivity is provided by Escalera-Vázquez et al. (2004) and Kelley et al. (2005).

***Xenoophorus*:** As presently defined, this genus has only one species, *X. captivus*, which is known from three hydrologically and geographically isolated areas on the Atlantic slope in the state of San Luis Potosí that are located to the northeast of the main body of the overall goodeid range (Miller et al. 2005). Populations from each area are somewhat distinctive morphologically and genetically, and they were formerly considered three different species until synonymized as one by Fitzsimons (1979). We consider them three separate ESUs based on their genetic and morphological characteristics.

Xenoophorus captivus: Endangered/Declining/3 ESUs (Figure 10) – The distribution and abundance of this species has shrunk considerably since the 1970's and 1980's owing to groundwater pumping and spring diversions that have lowered water levels and degraded water quality. Of the three ESUs, Xenca1 is critically endangered. Two or three small and somewhat interconnected populations are known from the upper portion of the Santa María del Río drainage in the upper portion of the Pánuco River basin in southern portion of the state of San Luis Potosí. Xenca2 is extinct in the wild. Historically it was found in the endorheic Illescas spring system near the border of the states of Zacatecas and San Luis Potosí, with the last confirmed collection from 1994 (Artigas-Azas 1995). A few captive populations exist in Mexico, the United States, and Europe. Xenca3 is endangered. Populations were known from three small springs, Venados, Moctezuma, and Agua de Enmedio, located in a small endorheic basin in the northern part of San Luis Potosí. These three populations still exist but are small.

***Xenotaenia*:** This genus has a single species, *X. resolanae*, limited to streams and small rivers in the Marabasco and Purificación river basins on the Pacific slope of west-central Mexico. The Marabasco and Purificación populations differ morphologically (Lyons 1996), and we consider them separate ESUs. The species is most closely related to *Allodontichthys* and *Ilyodon* (Doadrio and Domínguez-Domínguez 2004; Webb et al. 2004; Miller et al. 2005).

Xenotaenia resolanae: Vulnerable/Stable/2 ESUs (Figure 13) – This species is known historically from a total of ca. 20 locations. Xenre1, which occupies the upper Marabasco River basin, is vulnerable, and occurs at approximately ten locations. The populations, while small, seem to be stable (Lyons and Navarro-Pérez 1990; Lyons 1996; Domínguez-Domínguez et al. 2005b; Lyons unpublished data). Xenre2, which occupies ca. ten locations in the upper Purificación River basin, is also vulnerable. Water pollution from sugar mill discharges, human sewage, and animal wastes had eliminated or reduced several populations by the 1980s, but since then the remaining populations seem to have stabilized.

***Xenotoca*:** Based on the morphological analyses of Fitzsimons (1972) and the genetic and morphological analyses of Domínguez-Domínguez et al. (2016), this genus is currently considered to have five species, the long-established *X. eiseni*, *X. melanosoma*, and *X. variata*, and the recently described *X. doadrioi* and *X. lyonsi* (Domínguez-Domínguez et al. 2016). However, Webb (1998), in his Ph.D. dissertation, provided genetic and morphological evidence that *X. eiseni* and *X. melanosoma* are not the closest relatives of *X. variata.* This conclusion was supported by further genetic analyses by Doadrio and Domínguez (2004), Webb et al. (2004), and Domínguez-Domínguez et al. (2016). Webb (1998) proposed that *X. variata* remain in *Xenotoca*, and the remaining species be placed in a new and separate genus "*Xenotichthys*". Consequently, in some publications, the two species were referred to as "*Xenotoca*" *eiseni* and "*Xenotoca*" *melanosoma* to indicate the likelihood that their genus would eventually change (e.g., Miller et al. 2005). To date, the proposal by Webb (1998) to apply "*Xenotichthys*" as the genus for *X. eiseni* and *X. melanosoma* has not yet been formally published.

Xenotoca doadrioi: Endangered/Declining/1 ESU (Xendo1) (Figure 15) – This species was shown to be genetically distinctive by Piller et al. (2015) and was recently separated from *X. eiseni* (Domínguez-Domínguez et al. 2016). It is found in the San Marcos drainage of the upper Ameca River Basin and the adjacent endorheic Lake Magdalena basin. Since 2000, it has disappeared from many locations due to water diversions and groundwater pumping that have eliminated many springs and small streams, and probably also from competition with or predation by the non-native *Pseudoxiphophorus bimaculatus* (Poeciliidae), which appears to have displaced *X. doadrioi* in some places. Only a handful of populations of *X. doadrioi* persist.

Xenotoca eiseni: Endangered/Declining/2 ESUs (Figure 15) – This species was recently split into three: *Xenotoca doadrioi*, *X. eiseni*, and *X. lyonsi* (Domínguez-Domínguez et al. 2016). As currently defined, *X. eiseni* is known from the upper Santiago River basin near the city of Tepic, and the upper portions of direct Pacific Ocean drainages near the city of Compostela, all in the state of Nayarit. We recognize two ESUs based on genetic differences. Xenei1 is endangered and is found in the Santiago River basin near the city of Tepic. Many populations have disappeared owing to lack of water, habitat destruction, and introductions of non-native species, and only a

Figure 15. Distribution of *Xenotoca doadrioi*, *X. eiseni*, and *X. lyonsi*.

handful of small populations remain. Xenei2 is also endangered and known from the direct drainages to the Pacific Ocean near the city of Compostela. It too has declined dramatically for the same reasons as Xenei1 and survives in small numbers in just a few springs and streams.

Xenotoca lyonsi: Endangered/Declining/1 ESU (Xenly1) (Figure 15) – This species was recently separated from *X. eiseni* (Domínguez-Domínguez et al. 2016). It is known from the upper Coahuayana River basin and is endangered. Historically it was widespread, but lack of water and water pollution have eliminated most populations.

Xenotoca melanosoma: Vulnerable/Declining/4 ESUs (Figure 16) – This species is found in the Santiago, Ameca, Armería, and Coahuayana river basins and the endorheic Magdalena, Atotonilco, San Marcos, Zacoalco, Sayula, and Zapotlán lake basins in Jalisco (Miller et al. 2005). We recognize four ESUs based on genetics and zoogeography (Domínguez-Domínguez et al. 2010; Mar-Silva 2018). Xenme1 is by far the most numerous and widely distributed and is classified as vulnerable. It is found in the Ameca, Magdalena, Atotonilco, San Marcos, Zacoalco, and Sayula basins at a total of ca. 15 locations. It has declined since 2000 owing to water pollution, habitat degradation, and non-native species. It has disappeared from the San Marcos and Zacoalco basin and persists at only 1–3 locations in the Magdalena, Atotonilco, and Sayula basins. The best remaining populations are in the Ameca basin. Xenme2 is critically endangered and is known from Lake Zapotlán where it is rare and in decline from habitat modifications and non-native species. Xenme3 is endangered and currently known from two locations on the upper Tamazula River where it is threatened by water

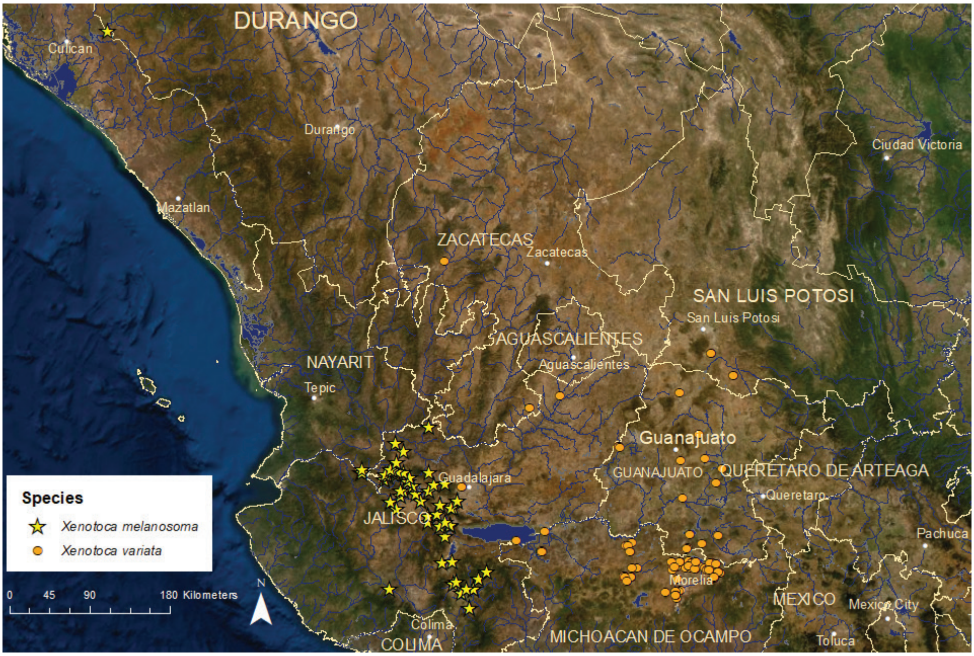

Figure 16. Distribution of *Xenotoca melanosoma* and *X. variata*.

diversions and introduced species. Xenme4 is also endangered and is limited to a short reach of the Ayuquila River downstream of the city of El Grullo in the Armería River basin, where chronic water quality degradation limits the population.

Xenotoca variata: Least Concern/Declining/5 ESUs (Figure 16) – This species is broadly distributed in central Mexico throughout the Lerma and upper Santiago river basins on the Pacific slope, the endorheic Lake Cuitzeo/Grande de Morelia River basin in central Mexico, and a small area of the upper Pánuco River basin on the Atlantic slope (Miller et al. 2005). It reaches a relatively large total length (~100 mm) and is caught and eaten in Zacapu Lake, Michoacán. It is highly tolerant of pollution and habitat modifications and, along with *Goodea atripinnis*, still persists in areas where other goodeid species have been eliminated. *Xenotoca variata* is currently found at many locations throughout its historic range. Nonetheless, the species has declined in recent years, disappearing from heavily polluted areas of the Santiago and Lerma basins and from reservoir and lake habitats where the non-native *Micropterus salmoides* (Centrarchidae) has become established (Lyons et al. 1998; Soto-Galera et al. 1998, 1999; Domínguez-Domínguez et al. 2005b; Mercado-Silva et al. 2006). We recognize five ESUs based on genetic analyses (Domínguez-Domínguez et al. 2010). Xenva1 is classified as least concern and broadly distributed in the Santiago, Lerma, and Pánuco basins. Many populations are still present, but others have disappeared from the Lerma River and its major tributaries due to water pollution. Xenva2 is vulnerable and known from a single location, Lake Los Negritos (also known as La Alberca). The population there remains moderately large but appears to have declined because of non-native species. Xenva3 is vulnerable and found in the Angulo River drainage in the middle Lerma River basin. It has declined in abundance in the river but remains relatively common in the headwaters at Lake Zacapu. Xenva4 is vulnerable and known from the endorheic Aquanaval River basin. It has declined because of overuse of water and habitat loss. Xenva5 is least concern and found in Lake Cuitzeo and the Grande de Morelia River basin. It has declined in the lake proper owing to habitat loss and poor water quality and has been eliminated from the Grande de Morelia River near the city of Morelia, but still remains common at several locations. This last ESU is particularly distinctive genetically and may eventually be described as a separate species.

***Zoogoneticus*:** This genus is found over a large portion of central and west-central Mexico. Until the late 1990's, *Zoogoneticus* was thought to have only one species, the wide-ranging *Z. quitzeoensis.* Then in 1998, *Z. tequila* was described (Webb and Miller 1998) and in 2008, *Z. quitzeoensis* was split into two species, *Z. quitzeoensis* and *Z. purhepechus* (Domínguez-Domínguez et al. 2008b).

Zoogoneticus purhepechus: Vulnerable/Declining/3 ESUs (Figure 17) – This species was recently separated from *Z. quitzeoensis* based on genetic and morphological differences (Domínguez-Domínguez et al. 2007, 2008b). The historical range of this species, as currently defined, encompassed the lower Lerma, upper Santiago (including Lake Chapala), upper Ameca, and upper Armería river basins on the Pacific slope, and the endorheic Lake Magdalena, Atotonilco, San Marcos, and Sayula basins in west-central Mexico (Miller et al. 2005; Domínguez-Domínguez et al. 2008b). Lake drying, water pollution, and introductions of non-native species have eliminated *Z. purhepechus* from many areas. We recognize three ESUs based on genetics and zoogeography. Zoopu1 is vulnerable and occurs in the upper Santiago and lower Lerma River basins including Lake Chapala. It has become scarce in Lake Chapala due to non-native species and has disappeared from much of the Santiago and Lerma basins owing to water pollution and habitat destruction. The best remaining populations occur in four springs that drain to the Duero River, a tributary of the Lerma River (Lyons et al. 1998; Soto-Galera et al. 1998; Moncayo-Estrada et al. 2015). Zoopu2 is endangered and is known from the Upper Ameca River basin and the endorheic Lago Magdalena basin. Once moderately common, it is now rare at one location in the Magdalena basin and occurs at only one or two locations in the Ameca basin, declining because of water pollution, habitat loss, and non-native species. Zoopu3 is critically endangered and known historically from the Armería River and Atotonilco and San Marcos lake basins. Habitat destruction, water diversions, water pollution, and non-native species have eliminated this ESU from the Armería and Atotonilco basins, and it persists in very small numbers at one site in the San Marcos basin (Lyons et al. 1998; Domínguez-Domínguez et al. 2008b; Domínguez unpublished data).

Zoogoneticus quitzeoensis: Endangered/Declining/2 ESUs (Figure 17) – As currently defined, this species was known historically from the Angulo, Turbio, and Laja river drainages and Lake Yuriria in the middle Lerma River basin, and from throughout the endorheic Lake Cuitzeo/Grande de Morelia basin in central Mexico (Domínguez-Domínguez et al. 2007, 2008b). Since 2000, *Z. quitzeoensis* has disappeared from

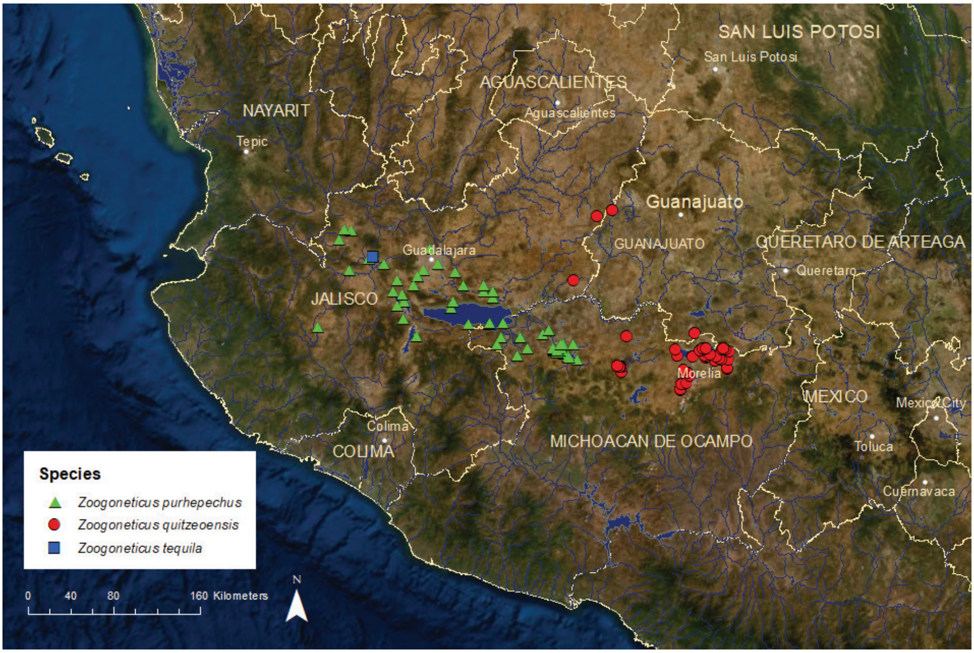

Figure 17. Distribution of the three species of *Zoogoneticus*.

many areas owing to a combination of water pollution, habitat loss from water diversions, and introduction of non-native species (Lyons et al. 1998; Soto-Galera et al. 1997, 1998; De la Vega-Salazar et al. 2003a, De la Vega-Salazar and Macías-Garcia 2005; Domínguez-Domínguez et al. 2005b, 2008a; Mercado-Silva et al. 2006). We recognize two ESUs based on genetic analyses (Domínguez-Domínguez et al. 2008b). Zooqu1 is critically endangered and was known historically from the Laja and Turbio river drainages. Populations in the Laja are now gone, and nearly eliminated from the Turbio. One or two populations may still persist in springs draining to the Turbio River. Zooqu2 is endangered and known historically from Lake Yuriria, the endorheic Lake Cuitzeo/Grande de Morelia basin, and the Angulo River drainage. The populations in Lake Yuriria, Lake Cuitzeo, and the Grande de Morelia River have been eliminated, and the species persists at only a few locations. The best remaining populations are in Lake Zacapu at the headwaters of the Angulo River drainage, and La Mintzita Springs, which drains to the Grande de Morelia River near Morelia.

Zoogoneticus tequila: Extinct in the Wild/Last Record 2008/1 ESU (Figure 17) – This species, endemic to the Teuchitlán Springs in the upper Ameca River basin in the state of Jalisco, was thought to be already extinct in the wild when it was formally described in 1998 (Webb and Miller 1998; Miller et al. 2005). However, in 2000, a tiny remnant wild population was discovered in a small and isolated area of the Teuchitlán Springs (De la Vega-Salazar 2003b; Domínguez-Domínguez et al. 2005b, 2008a). This population was so small (< 100 individuals) that it was already inbred (Bailey et al. 2007). The last collection from there was in 2008. A major drought in

2010 completely dried the habitat of *Z. tequila*, and when the drought ended and water levels increased, the area was invaded by the non-native *Pseudoxiphophorus bimaculatus* (Poeciliidae), a species that has been associated with the decline of several goodeid species. Many efforts to find *Z. tequila* were undertaken after 2010 without success and eventually the species was declared truly extinct in the wild. Fortunately, captive populations are relatively common in Mexico, the United States, and Europe. In 2014, faculty, students, and staff at the Universidad Michoacana de San Nícolas de Hidalgo, in Morelia, state of Michoacán, began a project to re-introduce captive stocks of *Z. tequila* into the Teuchitlán Springs. They exposed the captive fish to semi-wild conditions in outdoor ponds for several generations and then in 2016 added pond fish to a different semi-isolated area of the springs from which nearly all non-native species had been removed. Thus far the stocked fish are surviving and reproducing. However, continued monitoring and removal of non-native species will probably be required to ensure that *Z. tequila* persists in the Teuchitlán Springs.

Discussion

The Mexican goodeids are at a crossroads. Once the most diverse, widespread, and numerous fishes in central Mexico (Miller et al. 2005), they now have been reduced to only a shadow of their former distribution and abundance. If they are to survive the 21st century, or even the next few decades, major conservation initiatives must be undertaken. We believe three steps are essential. First, the best remaining habitats for each species and ESU should be protected. For those goodeids that are able to live in springs or the standing water of small spring-fed lakes, habitat protection may be a practical goal. Many of the springs and spring-fed lakes containing the most viable remaining goodeid populations are already formally or informally protected as municipal or even national parks or as sources of water for drinking and irrigation. These designations do not fully protect them from habitat modifications for recreation, agriculture, or water extraction, or reductions in the water table caused by regional groundwater pumping (e.g., Scott and Shah 2004) or climate-change-induced severe droughts (e.g., Mulholland et al. 1997). The local and national governmental entities charged with managing them sometimes work at cross purposes, but at least these springs and lakes have groups likely to advocate for their conservation. A more insidious problem is non-native species. In many springs and lakes of central Mexico, non-native fish species such as *Cyprinus carpio* (Cyprinidae), *Ictalurus punctatus* (Ictaluridae), *Oncorhynchus mykiss* (Salmonidae), *Micropterus salmoides* (Centrarchidae), or *Oreochromis aureus* (Cichlidae) have been stocked to provide a food source (Lyons et al. 1998; Moncayo-Estrada et al. 2012; Gesundheit and Macías-García 2018). Smaller-bodied non-native fish species such as poeciliids often arrive as contaminants from these stockings or as escapees or direct releases of aquarium fish (Contreras-MacBeath et al. 1998; Lyons et al. 1998; Moncayo-Estrada et al. 2012, 2015; Gesundheit and Macías-García 2018). The interactions of goodeids with non-native species are generally poorly documented or understood (Ramírez-Carrillo and Macías-García 2015; Ramírez-García et al. 2018), but in almost all cases the establishment of one or more non-natives is associated with declines in abundance of the goodeids (e.g., Soto-Galera et al. 1998, 1999; Gesundheit and Macías-García 2018). Introductions of non-native species have been and will likely continue, and once established, non-native species are difficult to eradicate, and long-term goodeid preservation may be challenging even in undegraded and well-protected springs.

For those goodeids requiring flowing waters (e.g., *Allodontichthys*, *Ilyodon*, and *Xenotaenia*) a much larger area of land may need to be managed to encompass the watershed of the stream or river occupied by the goodeids. Most parks or protected areas will be insufficient by themselves, but in concert with less restrictive land management schemes, such as biosphere reserves (Batisse 1997), it may be possible to provide at least partial protection from major water diversions, industrial or municipal pollution, and habitat destruction. For example, the Sierra de Manantlán Biosphere Reserve in the state of Jalisco helps implement land and water management practices that conserve populations of *Allodontichthys zonistius*, *Ilyodon furcidens*, and *Xenotaenia resolanae* (Lyons and Navarro-Pérez 1990). However, some stream and river-dwelling goodeid species and ESUs have essentially no protected or sustainably managed land in their watersheds, and their future survival is uncertain.

Second, where practical, degraded habitats should be rehabilitated. The key is choosing habitats where some recovery of goodeid populations is realistic through re-colonization or re-introduction once habitat quality is improved. Many habitats in central Mexico have been completely obliterated or are so modified that goodeid restoration is infeasible (Lyons et al. 1998; Soto-Galera et al. 1998, 1999; Mercado-Silva et al. 2006; Moncayo-Estrada et al. 2015). However, where only a single type of habitat or water quality/quantity degradation limits the goodeid population, recovery may be possible. For example, a 50-km segment of the Ayuquila River in the Upper Armería Basin, had been rendered fishless from severe water pollution from a sugar mill (Lyons et al. 1998). Fortunately, goodeids and other species persisted further downstream and in tributaries. Diversion of the sugar mill wastes into irrigation canals for several kilometers allowed for partial breakdown of the wastes, and water quality in the river improved. *Ilyodon furcidens*, a relatively tolerant goodeid, and two tolerant native poeciliids were able to re-colonize most of the 50-km segment, avoiding only the 10-km stretch immediately below the discharge of the sugar mill wastes into the river from the irrigation canals. As a second example, in the Teuchitlán Springs, habitat and water quality and quantity remained adequate for goodeid survival, but abundant non-native species had eliminated two species. Manual reduction of non-native fish populations to low levels allowed for the successful re-introduction of *Zoogoneticus tequila*.

Third, and finally, captive populations of rare goodeid species and ESUs should be established. Relying on only protecting and restoring wild populations is too risky. Many goodeid species and populations face such daunting environmental challenges that they will likely disappear soon from the wild even with the best-possible on-the-ground conservation efforts. Fortunately, most goodeids can be maintained and bred relatively easily in captivity. Currently, two Mexican universities, the Universidad Michoacana de San Nícolas de Hidalgo (UMSNH) in Morelia, Michoacán, and the Universidad Autónoma de Nuevo León (UANL) in Monterrey, Nuevo León, maintain goodeids. The UMSNH has nearly all of the extant goodeid species and the UANL has a selection of some of the rarest species. Several zoos and public aquariums in the United States and Europe also maintain one or more goodeid species. But collectively, the total holdings of all public institutions in Mexico, the United States, and Europe do not cover all of the goodeid ESUs, and many species are represented by a single population at a single institution, vulnerable to accidental or catastrophic loss. Funding and technical support for goodeid conservation at many institutions is also limited and uncertain for the future.

We believe that a key component of goodeid captive maintenance is participation by aquarium hobbyists (Maceda-Veiga et al. 2014). A relatively small but passionate group of hobbyists already maintains goodeids, and we are working to increase their numbers and holdings. We have developed a framework and network to facilitate goodeid conservation by aquarists through the Goodeid Working Group (GWG: http://www.goodeidworkinggroup.com/). This voluntary organization of scientists, conservationists, and hobbyists provides a mechanism for exchanges of information, promotion of good conservation principles in captive maintenance, and fund-raising to support goodeid conservation in Mexico. Communication is facilitated through a website, regular email and Facebook updates, and annual face-to-face meetings in Mexico, the United States, and Europe that allow hobbyists and goodeid scientists and conservationists to interact. Hobbyists often have important data to share on the husbandry and behavior of goodeid species. The captive populations that hobbyists maintain can also be a source for re-introductions in the wild. Indeed, the *Zoogoneticus tequila* that were recently re-introduced into the Teuchitlán Springs were ultimately derived from a captive population maintained by an English aquarist, the late Ivan Dibble. He kept the species for many years and returned live specimens to the UMSNH in the late 1990's.

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