**REVIEW ARTICLE** 



## An overview on the nomenclatural and phylogenetic problems of native Asian brine shrimps of the genus Artemia Leach, 1819 (Crustacea, Anostraca)

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#### Abstract

The genus *Artemia* Leach, 1819 is a cosmopolitan halophilic crustacean, consisting of bisexual species and obligate parthenogenetic populations. Asia is rich in *Artemia* biodiversity. More than 530 *Artemia* sites have been recorded from this area and more than 20 species/subspecies/variety names have been used for them. There exist various problems in the nomenclature, identification, and phylogenetic status of *Artemia* native to Asia, which are discussed in this paper.

#### Keywords

Artemia, phylogeny, nomenclature, taxonomy, Asia

The brine shrimp Artemia Leach, 1819 is a halophilic zooplankton, distributed in saline habitats worldwide, with the exception of Antarctica (Van Stappen 2002). The genus consists of several bisexual species and a large number of polyphyletic obligate parthenogenetic populations including di-, tri-, tetra-, and pentaploid (Asem et al. 2016). Asia is rich in Artemia biodiversity, where more than 530 Artemia sites have been recorded (count based on Walter 1888; Sars 1901; Bond 1934; Van Stappen 2002; John et al. 2004; Mura and Nagorskaya 2005; Abatzopoulos et al. 2006; Shadrin and Anufriieva 2012; Salman et al. 2012; Vikas et al. 2012; Zheng and Sun 2013; Naganawa and Mura 2017). Many of the sites are now inhabited by the invasive species Artemia franciscana Kellogg, 1906 (in some cases, co-existing with other bisexual species or parthenogenetic populations), whose identification were mostly confirmed by molecular analyses (e.g., Van Stappen et al. 2007; Vikas et al. 2012; Eimanifar et al. 2014). For Artemia native to Asia, more than 20 binomial/trinomial names have been used by different authors (see below). Several problems emerged in the past related to the nomenclature, identification or the phylogenetic status of Artemia species. Some of these issues have found a solution and scientific consensus, whereas others still persist.

As far as we are aware, 14 binomens (including the one questionably reported as a species of Branchinecta Verrill, 1869) and nine trinomens, as well as unidentified species/subspecies/varieties, have appeared in the form of scientific names (combined with a genus name and typeset in italics) in literature relating to native Asian brine shrimps. As shown in Table 1, almost all of the names have some kind of nomenclatural problem. Among the 13 binomens proposed for Artemia, eleven fulfil the availability requirements of International Code of Zoological Nomenclature (ICZN, 4<sup>th</sup> edition) and are considered to be available species names [Artemia salina (Linnaeus, 1758); Artemia asiatica Walter, 1887; Artemia urmiana Günther, 1899; Artemia parthenogenetica Bowen & Sterling, 1978; Artemia sinica Cai, 1989; Artemia barkolica Qian and Wang in Qian et al. 1992; Artemia urumuqinica Qian and Wang in Qian et al. 1992; Artemia ebinurica Qian and Wang in Qian et al. 1992; Artemia tibetiana Abatzopoulos, Zhang & Sorgeloos, 1998; Artemia frameshifta Naganawa & Mura, 2017; Artemia murae Naganawa in Naganawa and Mura 2017], and the other two are unavailable [Artemia kazakhastan Vikas et al., 2012; Artemia china Vikas et al., 2012]. Branchinecta orientalis Sars, 1901 sensu Chiang, 1983 is supposed to be a misuse for brine shrimp (vide post). Three trinomens (Artemia sinica sinica Cai, 1989; Artemia sinica tibetiana Abatzopoulos, Zhang & Sorgeloos, 1998; Artemia salina arietina Fischer, 1851) are available subspecies names, with all their subspecific names first proposed as (available) specific names. The other six trinomens are unavailable names.

Many Asian *Artemia* populations were reported as *A. salina* in earlier publications but most of these records were later revealed to be parthenogenetic *Artemia*, *A. tibeti-ana*, *A. sinica* or unidentified bisexual populations (Mura and Nagorskaya 2005; Salman et al. 2012; Shadrin and Anufriieva 2012; Zheng and Sun 2013; Eimanifar et al. 2014; Litvinenko et al. 2016). Even so, the inadequate use of the name still appeared

in very recent papers, e.g., Alas et al. (2017) identified the Salt Lake (= Tuz Lake, Turkey) population as *A. salina* although they have been aware that Başbuğ (1999) already documented the population reproducing parthenogenetically.

Padhye and Lazo-Wasem (2018) indicated that the Sambhar Lake (Rajasthan, India) population was a valid report of *A. salina*, whereas the several hundred specimens from this lake at the Indian Museum seem to be all females (Belk and Esparza 1995). Confirmed distribution of this species in Asian countries is restricted to Cyprus, an island in the eastern Mediterranean (Baxevanis et al. 2006).

Bond (1934) reported *A. salina* from Tso Kar, Ladakh, Jammu & Kashmir. Padhye and Lazo-Wasem (2018) studied Bond's specimens deposited in the Yale Peabody Natural History Museum, and referred them as an *Artemia* sp. that is morphologically close to *A. sinica*. However, this study made a mistake in citing literature and erroneously stated that *A. tibetiana* does not have a basal spine on the male gonopod, even though it is well developed in this species (Mura and Brecciaroli 2004; Zheng and Sun 2008). Given that Tso Kar is closer to the sites of *A. tibetiana* (than those of *A. sinica*), and that they live in similar high altitude habitats, this population awaits a (molecular) comparison with *A. tibetiana*, as well as with other bisexual phylogenetic lineages from adjacent areas such as the "Kazakhstan" and "Kyêbxang Co" population (vide post).

Two varieties of *A. salina* were reported from Asia. Gurney (1921) identified the Amara (Iraq) population as *Artemia salina* var. *arietina* Fischer 1851. Since the Amara population is parthenogenetic (Salman et al. 2012), the population is by no means assignable to *A. salina*. Walter (1888) identified his specimens from Molla-kary (Turkmenistan) as "*Artemia salina* L. var." and thought it to be a variety between *A. salina* and *Artemia milhausenii* (Fischer, 1834). Because Walter's specimens contained only females, the Molla-kary population may also be parthenogenetic and is not assignable to any bisexual species.

Artemia urmiana was originally described as a bisexual species based on specimens from Urmia Lake, Iran (Günther 1899), but Barigozzi and Baratelli (1989) documented that all samples collected from Urmia Lake in 1987 were parthenogenetic and contained di-, tetra-, and pentaploid individuals. Azari Takami (1989) reported that bisexual and parthenogenetic populations coexisted in the Lake. Agh et al. (2007) concluded that parthenogenetic samples had likely been collected from lagoons neighbouring Urmia Lake or its coastal areas, whereas bisexual A. urmiana dominated in the main body of the lake. However, a later study documented the existence of parthenogenetic populations in both the lagoons and the main body of Urmia Lake, with significant morphometric differentiation (Asem et al. 2009). In addition to Iran, several populations in Altai (Russia) may belong to this species (Shadrin and Anufriieva 2012), Turkey and Turkmenistan (Eimanifar et al. 2014). The record of this species from Basrah (Iraq) should be A. franciscana (see Salman et al. 2012). Outside Asia, Abatzopoulos et al. (2009) identified A. urmiana from Lake Koyashskoe (Crimea). Another study based on sequence variation of the mitochondrial COI marker suggested the occurrence of A. urmiana in Bulgaria, China, Greece, Crimea, Turkey, and Turkmenistan (Eimanifar et al. 2015). However, these populations need to be further

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**Table 1.** Names that have ever been used for native brine shrimps *Artemia* of Asia, their availability, information of type specimens and nomenclatural problems

Taxon names	Availability	Type specimens	Type locality	Comments and/or references	
A. salina (Linnaeus, 1758)	Available	Not mentioned in original description	Salt works at Lymington, England	Linnaeus 1758	
A. asiatica Walter, 1887	Available	Syntypes. Number of specimens and deposit place not mentioned in original description	Murgab, Tajikistan	This species is known only from type locality (Walter 1887, 1888). It was considered to be a <i>nomen dubium</i> (Belk and Brtek 1995)	
A. urmiana Günther, 1899	Available	Syntypes. Number of specimens and deposit place not mentioned in original description	Urmia Lake, Iran	Günther 1899	
A. parthenogenetica Bowen & Sterling, 1978	Available	Syntypes, containing cysts from five localities. Deposit place not mentioned in original description	Madras and Kutch, India; Port Hedland, Australia; Sète, France; Yamaguchi-ken, Japan	Though the term "parthenogenetica" was used by earlier authors, Belk and Brtek (1995) clarified its authorship should be Bowen and Sterling (1978). Type specimens should include all specimens that Bowen and Sterling (1978) studied, and type localities include all 5 sites collecting the specimens (ICZN Articles 73.2.3, 76.1). Rogers (2013) listed this name as a nomen dubium.	
<i>A. sinica</i> Cai, 1989	Available	Syntypes, probably containing cysts and laboratory cultured adults, which are believed to have been lost (Yaneng Cai, pers. comm.)	Yun Cheng Salt Lake, Shanxi, China	Artemia sinica was referred to Cai (1989b) in some publications (e.g., Abatzopoulos et al. 1998; Hou et al. 2006; Van Stappen et al. 2009; Zheng and Sun 2013). However, Cai (1989a; an abridged version of Cai 1989b) has nomenclatural priority because it was published earlier (January 1989) than Cai (1989b; spring and fall 1989 / mailed 17 July 1990). Cai (1989a) described only the morphology of adults, but according to Cai (1989b), the type series of this species might contain cysts and laboratory cultured adults (ICZN Article 72.4)	
<i>A. barkolica</i> Qian & Wang in Qian et al. 1992	Available	"Holotypes" 5♀♀, 5♂♂; paratypes 56♀♀, 4♂♂. Deposited at Xinjiang August First Agricultural College (now Xinjiang Agricultural University), Xinjiang, China	Barkol Lake, Xinjiang, China	Multiple specimens were designated as 'holotype' in the original description (Qian et al. 1992). All type specimens may be regarded as syntypes, with original authors' "holotypes" having the priority in designating as a lectotype when necessary	
<i>A. urumuqinica</i> Qian & Wang in Qian et al. 1992	Available	"Holotypes" 5♀♀; paratypes 60♀♀. Deposited at Xinjiang Agricultural University	Urumqi Caiwuo Pu Yan Hu (= Dabancheng Salt Lake / Dabancheng Dong Salt Lake), Xinjiang, China	Ibid	
<i>A. ebinurica</i> Qian & Wang in Qian et al. 1992	Available	"Holotypes" 6♀♀, 2♂♂; paratypes 60♀♀. Deposited at Xinjiang Agricultural University	Ebinur (=Aibi Lake used in many publications), Xinjiang, China	Ibid	
<i>A. tibetiana</i> Abatzopoulos, Zhang & Sorgeloos, 1998	Available	Syntypes, probably consisting of cysts (two batches collected in different time), nauplii and adults. Deposit place not mentioned in original description	Lagkor Co, Tibet, China	Abatzopoulos et al. (1998) studied adults, nauplii and cysts, all should be components of type series (ICZN Article 72.4). Later studies showed Lagkor Co population was a mixture of bisexual and parthenogenetic Artemia (Van Stappen et al. 2003; Maccari et al. 2013). So the type series may contain specimens of more than one species, given that samples studied by Abatzopoulos et al. (1998) were not contaminated after collection and parthenogens were not introduced to the lake after harvesting of type samples	
<i>A. kazakhastan</i> Vikas et al. 2012	Unavailable	N/A	N/A	This name appeared in Vikas et al. (2012: 135, 138) in the form of binomen, which seemed to refer to "Artemia sp. Kazakhstan" mentioned in the same paper. It is obvious that the authors did not intend to establish any new taxa, therefore is unavailable (ICZN Article 16)	

Taxon names	Availability	Type specimens	Type locality	Comments and/or references	
A. china Vikas et al. 2012	Unavailable	N/A	N/A	Same as the last name, but this name seemed to refer to " <i>Artemia</i> sp. China" (Kyêbxang Co population)	
<i>A. frameshifta</i> Naganawa & Mura, 2017	Available	Holotype ♀; Deposited at Kyoto University Museum	Bajan-Onjul, Tov aimag, Mongolia	Naganawa and Mura 2017	
<i>A. murae</i> Naganawa in Naganawa & Mura, 2017	Available	Holotype ♂; allotype ♀. Deposited at Kyoto University Museum	Tonkhil nuur (Tonkhil Lake), Gobi-Altai, Mongolia	In addition to the type specimens, Naganawa and Mura (2017) observed 232 other specimens including 124 ♂♂ and 108 ♀♀	
<i>? Branchinecta orientalis</i> Sars, 1901 sensu Chiang, 1983	N/A	N/A	N/A	Sars (1901) described this anostracan based on specimens from Lake Chuntu-nor, Dornod, Mongolia. Chiang's (1983) record from Kyébxang Co, Tibet, China might be <i>Artemia</i> (see text)	
A. urmiana parthenogenetica Barigozzi, 1980 non A. parthenogenetica Bowen & Sterling, 1978	Unavailable	N/A	N/A	Barigozzi (1980) used this trinomen as an example to discuss the nomenclature of parthenogenetic <i>Artemia.</i> It is unavailable because of no description or diagnosis (ICZN Article 13)	
A. parthenogenetica urmiana Barigozzi, 1980 non A. urmiana Günther, 1899	Unavailable	N/A	N/A	Ibid	
A. sinica sinica Cai, 1989	See A. sinica	See A. sinica	See A. sinica	Zhou et al. (2003b)	
A. sinica tibetiana Abatzopoulos, Zhang & Sorgeloos, 1998	See A. tibetiana	See A. tibetiana	See A. tibetiana	Zhou et al. (2003b)	
A. sinica jingyuhuensis Yin, Zhang & You, 2013	Unavailable	N/A	N/A	Referring to the bisexual population from Jingyu Lake, Xinjiang, China, this name appeared first in the MSc degree thesis of Zhou (2001), and then in Yin et al. (2013). The former was an unpublished work, the later did not describe it as a new taxon and designate name-bearing type(s), thus the name is unavailable (ICZN Articles 13 and 16)	
<i>A. sinica xiaochaidanensis</i> Yin, Zhang & You, 2013	Unavailable	N/A	N/A	Same as the last name, but referring to the bisexual population from Xiao Qaidam Lake, Qinghai, China	
<i>A. sinica gahaiensis</i> Yin, Zhang & You, 2013	Unavailable	N/A	N/A	Yin et al. (2013) used this name for the parthenogenetic <i>Artemia</i> population from Ga F Qinghai, China. It is unavailable because thes authors did not describe it as a new taxon and designate name-bearing type(s) (ICZN Articles and 16)	
<i>A. sinica aibihuensis</i> Yin, Zhang & You, 2013	Unavailable	N/A	N/A	Same as the last name, but referring to the parthenogenetic <i>Artemia</i> population from Ebinur, Xinjiang, China	
<i>A. salina</i> var. <i>arietina</i> Fischer, 1851	Available	Syntypes, including several specimens. Deposit place not mentioned in original description	Odessa, Ukraine	Fischer (1851) described Artenia arietina, which might be bisexual because both sexes were mentioned. The name is now thought to be a nomen dubium (Belk and Brtek 1995). Gurney (1921) reported the Amara (Iraq) population as A. salina var. arietima	
Artemia sp.	N/A	N/A	N/A	Many Asian <i>Artemia</i> populations were reported as <i>Artemia</i> sp.	
Artemia s. subsp. (=A. sinica subsp.)	N/A	N/A	N/A	Yin et al. (2011) reported the <i>Artemia</i> from Jingyu Lake (Xinjiang, China) and Xiao Qaidam Lake (Oinghai, China) as A. s. subsp.	
A. salina var.	N/A	N/A	N/A	Walter (1888) reported Molla-kary population (Turkmenistan) as "Artemia salina L. var." and considered it a variety between A. salina and Artemia milhausenii (Fischer, 1834) ["in die Reihe der von Artemia salina L. (Milne Edw.) zur Artemia milhausenii Fisch."]. The name A. milhausenii was established for Artemia from Crimmea (Fischer, 1834) and is now considered a nomen dubium or nomen nodum (Belk and Brtek 1995; Rogers 2013)	

explored with special emphases on the status of reproductive mode (bisexual or parthenogenetic) to confirm the coexistence of *A. urmiana* and parthenogenetic populations in these localities or/and existence of shared COI haplotype(s) between *A. urmiana* and parthenogenetic gene pools.

For two decades, Tibetan bisexual populations have been considered as belonging to a single species, A. tibetiana, originally described as a bisexual species from Lagkor Co, Tibet, China (Abatzopoulos et al. 1998). However, as that in Urmia Lake, a parthenogenetic population was also documented from this lake (Van Stappen et al. 2003; Maccari et al. 2013; see Table 1). Wang et al. (2008) documented four Tibetan bisexual populations clustering in two different clades using the mitochondrial COI marker, with one clade only hosting the type locality population (Lagkor Co) and a second distinct clade hosting the others (Kyêbxang Co (=Qixiang Lake or Qi Xiang Cuo), Nima, and Yangnapeng Co). Two other studies have shown that the Tibetan populations clustered in two different groups in a phylogenetic tree based on the COI marker, while all of them represented a single clade based on the nuclear marker ITS1 (Maccari et al. 2013; Eimanifar et al. 2014). Thus, the taxonomic status of these populations awaits to be clarified by future investigations. Chiang (1983) reported Branchinecta orientalis from Kyêbxang Co, whereas later studies showed that the anostracan in this lake was Artemia (e.g., Zhou et al. 2003a, 2003b; Hou et al. 2006; Yu and Xin 2006; Wang et al. 2008). We suppose that Chiang (1983) might have confused his specimens (no Tb-76-2012) for two reasons: 1) Chiang (1983: 451) reported the altitude of the lake as 4740 m, while it was listed as 4660 m in the chapter "General Account" of the same book (Chiang et al. 1983: 29); and 2) during the time (1976) he collected specimens, the salinity of this lake was as high as 63.27 g/L (Zheng et al. 2002), salinity that is not suitable for *B. orientalis*.

The validity of *Artemia sinica*, described based on specimens from Yuncheng Salt Lake, China (Cai 1989a) has rarely been questioned, and nearly 30 bisexual populations from China (see review of Zheng and Sun 2013), and several populations from Russia (Shadrin and Anufriieva 2012; Litvinenko et al. 2016), and Mongolia (Gajardo and Beardmore 2012; Eimanifar et al. 2014) have been identified as this species so far. The molecular clock divergence analysis indicated that *A. sinica* had already diverged in the late Miocene (19.99 Mya), whereas *A. urmiana*, *A. tibetiana*, and "Eurasian Haplotype Complex" (EHC refer to group of parthenogenetic *Artemia* lineages) shared a common ancestor in the late Pliocene (5.41 Mya) (Eimanifar et al. 2015).

Another controversial topic in taxonomy of *Artemia* relates to a batch of bisexual *Artemia* cysts from Kazakhstan (KAZ; ARC no. 1039), supplied by Catvis Co. (s-Hertogenbosch, The Netherlands) in 1988. However, no information about the exact origin(s) of the sample (Pilla and Beardmore 1994; Ben Cattel, pers. comm. 2017) was provided. An earlier study documented morphological differentiations between this bisexual sample and other Asian species (Pilla and Beardmore 1994). In molecular analyses, this population was located in a separate phylogenetic clade in the mitochondrial COI tree (Maccari et al. 2013), but was clustered together with *A. urmiana* and *A. tibetiana* using ITS1 nuclear marker (Vikas et al. 2012). Under these circumstances,

the systematic position and geographical origin of this sample remains a source of debate. So far only one site, the salt lake Margen-sor (district of Atbassar), was reported to be inhabited by bisexual *Artemia* in Kazakhstan (Sars 1901: reported as *A. salina*). Future sampling in Margen-sor may help to elucidate if KAZ and the Margen-sor population belong to the same species/lineage.

Qian et al. (1992) described three species based on specimens from three different salt lakes in Xinjiang, China, namely *Artemia barkolica* Qian & Wang, 1992, *Artemia urumuqinica* Qian & Wang, 1992 and *Artemia ebinurica* Qian & Wang, 1992 (Table 1). Except for 12 papers in the special Chinese-language issue "Studies on *Artemia* of Barkol Lake" (Journal of August 1<sup>st</sup> Agriculture College, 1994, Vol. 17 no. 2) and Qian et al. (1993), which used the names *A. barkolica* and *A. urumuqinica*, respectively, these three species have not been recognised by other researchers. As commented by Zheng and Sun (2013), the very biased sex ratios in the original description, as well as the results of many other studies, have indicated that all three populations were parthenogenetic. Since all these populations consist of strains of different ploidies (for reference review see Zheng and Sun 2013), and diploid/triploids and tetraploid/pentaploids are assumed to have originated separately (Maniatsi et al. 2011; Asem et al. 2016); each of the three nominal species may represent more than one phylogenetic clade (or species) or they may be synonyms.

Recently, in Mongolia, two new species have been described: *A. frameshifta* and *A. murae* (see Naganawa and Mura 2017). These species have been described using primary morphological characters and a single COI sequence, whereas morphometric differentiation and population genetic analysis have not been studied. Although both species were said to reproduce bisexually in the original descriptions, males have not been observed in *A. frameshifta*. Considering the 'sex ratio' (125 males and 109 females were observed), phylogenetic position (sister to *A. sinica*) and genetic distance (p-distance between *A. murae* and *A. sinica* is 4.8%) (Naganawa and Mura 2017), *A. murae* may represent a lineage close to *A. sinica*. Moreover, no sequences of parthenogenetic *Artemia* were included in the phylogenetic analysis of Naganawa and Mura (2017). Therefore, the taxonomic status of these species also needs to be re-confirmed by future multidisciplinary studies on their biology and phylogeny.

*Artemia asiatica* was described according to only female specimens from Murgab, Tadjikistan (Walter 1887). It may be a parthenogenetic population, and the name is now treated as a nomen dubium (Belk and Brtek 1995).

Theoretically, the ability of interfertility and producing offspring able to reproduce is a common criterion to confirm subspecies status (Mayr 1969). In nature however, subspecies populations mostly have an allopatric distribution. Due to geographical isolation, proof of natural interbreeding is practically impossible except in rare cases. The results of cross-breeding tests with different Asian bisexual *Artemia* were inconsistent among different studies. Pilla and Beardmore (1994) documented complete interfertility among *Artemia* sp. (the KAZ sample), *A. urmiana*, and *A. sinica*. Zhou et al. (2003b) showed interfertility between *A. sinica* and *A. tibetiana*, and considered them as subspecies (*Artemia sinica sinica sinica and Artemia sinica tibetiana*). In other studies, however, an isolating barrier was found between *A. urmiana* and *A. sinica* (see Zheng and Sun 2008), and between *A. sinica* and *A. tibetiana* (see Abatzopoulos et al. 2002b; Zheng and Sun 2008). Due to the possible effect of laboratory rearing conditions (ionic composition of the medium, salinity and temperature) on the reproductive potential of *Artemia* (Abatzopoulos et al. 2003; Velasco et al. 2016, 2018), it seems that a fertility test is not suitable as a single biological tool to determine *Artemia* population/ species ranks. Although reproductive isolation in captivity/laboratory circumstances might refer to a lack of gene flow in nature, laboratory cross-fertility even for F1 and later generations cannot evidence that such cross-fertility would occur under natural conditions if there is no hybrid zone (Helbig et al. 2002).

Two other bisexual populations, the Jingyu Lake (Xinjiang, China) population and the Xiao Qaidam Lake (Qinghai, China) population, were considered to represent different subspecies of *A. sinica*. The subspecies names *Artemia sinica jingyuhuensis* Yin, Zhang & You, 2013 and *Artemia sinica xiaochaidanensis* Yin, Zhang & You, 2013 were proposed for them, respectively, though they are not available (Table 1). Zheng and Sun (2008) documented some morphological differences between Jingyu Lake and Lagkor Co populations though they identified the former population as *A. tibetiana*. In phylogenetic analyses the Jingyu Lake population located in a clade containing *A. tibetiana* (Wang et al. 2008; Yin et al. 2011, 2013), while the Xiao Qaidam Lake population is clustered together with *A. sinica* (Yin et al. 2011, 2013).

In addition, there are also a number of allegedly bisexual populations, generally based on visual observation and personal communication, in Asian saline habitats, especially in Siberia and China, which have not been identified to any nominal species (Van Stappen et al. 2009; Zheng and Sun 2013). The phylogenetic status of these populations still needs to be determined.

The nomenclatural and taxonomic status of Artemia parthenogenetica have been discussed by previous authors (Barigozzi 1980; Belk and Brtek 1995; Baxevanis et al. 2006; see also Table 1). Since Bowen and Sterling (1978), this name has been used in numerous publications (sometimes followed by a population site), whereas some authors preferred to refer parthenogenetic Artemia as "populations" (Abatzopoulos et al. 2002a; Baxevanis et al. 2006; Asem et al. 2010). Recent molecular analyses have shown that parthenogenetic Artemia is a polyphyletic group, with diploids/triploids being evolutionally close to A. urmiana while tetraploids/pentaploids sharing a common ancestor with A. sinica (Asem et al. 2016). In another molecular analysis conducted by Eimanifar et al. (2015), the parthenogenetic Artemia has been named as "Eurasian Haplotype Complex" comprising a group of putative parthenogenetic Artemia lineages which were genetically close to two bisexual species, A. urmiana and A. tibetiana. The molecular divergence analysis has indicated that a recent population expansion in A. urmiana and "Eurasian Haplotype Complex" occurred in the Pleistocene (1.72 Mya) and Holocene (0.84 Mya), respectively. Males of parthenogenetic Artemia (produced only by diploids; Saleem Chang et al. 2017) are fertile when mating with bisexual A. sinica, A. tibetiana, A. urmiana and KAZ (Cai 1993; Liu et al. 2007; Maccari et al.

2014), but not fertile when mating with the female of the other bisexual species (Mac-Donald and Browne 1987). Parthenogenetic females mating with males produce only parthenogenetic offspring (Barigozzi 1974). These results suggest that parthenogenetic *Artemia* may originate from bisexual species native to Asia; the gene pool of bisexual *Artemia* may be affected by diploid parthenogenetic *Artemia* but not vice versa; gene exchange can hardly happen among parthenogenetic individuals of different ploidies. In other words, parthenogenetic individuals cannot readily be considered as belonging to a single species as commented by Abatzopoulos et al. (2002a), and can be assigned to taxa different from bisexuals. There are already some available names referring to parthenogenetic *Artemia*, e.g., *Artemia bivalens* Artom, 1912 (Artom 1912 proposed this name for a tetraploid parthenogenetic population from Capo d' Istria; also as *Artemia salina bivalens*), and the aforementioned *A. asiatica*, *A. barkolica*, *A. urumuqinica*, *A. ebinurica*, and *A. frameshifta* (?). These names could be candidates to be assigned to parthenogenetic populations, but this requires the type specimens to be re-examined, or/and topotypes to be studied using multidisciplinary methods.

Artemia is a taxon of difficult classification because of the lack of discernible characters. Some morphological characters are documented to be useful in delimitate species. For instance, the conical frontal knob on male antenna and the absence of basal spine on gonopod can segregate A. salina from the other bisexual species (e.g., Mura and Brecciaroli 2004; Zheng and Sun 2008), the "orthostichous spines" on gonopod are present only in species from China (A. sinica, A. tibetiana) (Zheng and Sun 2008). Molecular analyses based on genetic markers, like mitochondrial COI and 16SrRNA, and nuclear ITS1 sequences, have successfully resolved the phylogenetic relationships of different populations and are useful to assign a population to a known species/clade (e.g., Baxevanis et al. 2006; Wang et al. 2008; Yin et al. 2011, 2013; Eimanifar et al. 2014, 2015; Asem et al. 2016). However, these methods cannot resolve all taxonomic problems existing in Asian Artemia, particularly they cannot tell the taxonomic rank (species/subspecies) of a certain morphological group or phylogenetic clade. As discussed earlier, the test of reproductive isolation, though theoretically supposed to be a gold criterion for diagnosing species, cannot resolve these problems, either. Therefore, based on present knowledge it is hard to tell an exact number of valid species of Artemia living in Asia.

As far as we know, there are three ongoing comprehensive projects related to *Ar*temia genomics, including the *Artemia* genome project, in which the full nuclear genome of inbred *A. franciscana* from Great Salt Lake was assembled and annotated (Ghent University, Belgium), also a study involving the creation of whole-genome SNP-based second-generation genetic maps (Ocean University of China, China), and a study devoted to complete the mitochondrial genomes of all described bisexual species (including both bisexual clades from Tibet) and parthenogenetic *Artemia* with different ploidy degrees (Hainan Tropical Ocean University, China). We believe that the final results of these studies will further contribute to deciphering the phylogenetic relationships in the genus *Artemia*.

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RESEARCH ARTICLE



# Vietnamellidae (Insecta, Ephemeroptera) of Thailand

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#### Abstract

The genus *Vietnamella* Tshernova, 1972 is investigated in detail for the first time in Thailand. As a consequence, four species are recognized, namely *Vietnamella maculosa* **sp. nov**., *Vietnamella thani* Tshernova, 1972, *Vietnamella* sp. B and *Vietnamella* sp. C. Herein, larvae and eggs of *V. maculosa* **sp. nov.** are described and reported from Chiang Rai Province. The larva of *Vietnamella* sp. B from Tak Province is also described, but not named due to insufficient material, and the imaginal stages and eggs of *V. thani* Tshernova, 1972 are described and presented for the first time. Our morphological evidence is supported with COI data. The phylogeny showed that four different lineages of the genus *Vietnamella* occur in Thailand, one of them, viz., *Vietnamella* sp. C, only known from a couple of COI sequences retrieved from the Barcode of Life Data System (BOLD). Diagnoses for all known Oriental species are also presented.

#### **Keywords**

COI, Ephemerelloidea, mayfly, phylogeny, Vietnamella

## Introduction

The monogeneric family Vietnamellidae was originally established by Tshernova (1972) [type species: *Vietnamella thani* Tshernova, 1972] based on larval specimens. The status of the family and the taxonomic history of the genus *Vietnamella* Tsher-

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nova, 1972 are reviewed by Jacobus et al. (2005) and Hu et al. (2017) respectively. Nowadays, three species have been described from the Oriental region. They are *V. ornata* (Tshernova, 1972), *V. sinensis* (Hsu, 1936) [=*V. dabieshanensis* You & Su, 1987, *V. qingyuanensis* Zhou & Su, 1995, *V. guadunensis* Zhou & Su, 1995], and *V. thani* (Hu et al. 2017). The larval and imaginal stages of *V. sinensis* have been described, whereas *V. thani* is only known from the larval stage and *V. ornata* (Tshernova, 1972) only from the sub-imaginal stage. Recently, *Vietnamella* sp. A was described from India, based on its larvae which present the unique character of outer projections on the head with serrations (Selvakumar et al. 2018). However, the authors were unable to confirm species identification using current morphological and molecular data because the larval morphology of *V. ornata* has never been described, and the distribution of *Vietnamella* sp. A is close to the type locality of *V. ornata* (Selvakumar et al. 2018).

The genus *Vietnamella* is endemic in the Oriental region and is distributed in China, Thailand, India and Vietnam (Tshernova 1972; Jacobus et al. 2005; Hu et al. 2017; Selvakumar et al. 2018). In Thailand, *Vietnamella* has never been reported at the species level. Here, we review the species of *Vietnamella* in Thailand and describe a new species in the genus and the imaginal stages of *V. thani* based on reared specimens. A distribution map and mitochondrial COI sequence data are also provided.

#### Materials and methods

#### **Ethics statement**

The present study was approved by the ethics committee of Kasetsart University (approval no. ACKU61-SCI-029) for rearing and collecting the mayfly specimens.

#### Morphological observations

The vietnamellid larvae were collected from fast-flowing areas of streams in northern and western Thailand. The imagoes were reared from mature larvae in the laboratory. Measurements (mm) and photographs were taken using a Nikon SMZ800 stereoscopic microscope and a Canon EOS 6D camera with MP-E 65 mm macro lens. For scanning electron microscopy (SEM), eggs were dried, coated with gold and observed with a FEI Quanta 450 SEM instrument. Final plates were prepared with Adobe Photoshop CC 2017. The specimens are deposited in the collection of the Zoological Museum at Kasetsart University in Bangkok, Thailand (**ZMKU**) and at the Museum of Zoology in Lausanne, Switzerland (**MZL**). The distribution map was generated via the Simple Mapper website using GPS coordinates (http://www. simplemappr.net).

#### Molecular analysis

The collected specimens were fixed in absolute ethanol and preserved under refrigeration for description and DNA extraction. Collection details of the specimens of the three species used for the DNA experiment are shown in Table 1. Part of the specimens was extracted by using non-destructive methods. Total DNA was extracted using a genomic DNA purification kit (NucleoSpin, Macherey-Nagel, Germany) following the manufacturer's protocol. A fragment of the mitochondrial cytochrome oxidase I (COI) was amplified (658 bp) using the primers LCO1490 (5'-GGT CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'), designed by Folmer et al. (1994). Polymerase chain reaction (PCR) conditions were as follows: a 25 µl final total volume containing 12 µl of PCR Master Mix solution, 1.5  $\mu$ l (10  $\mu$ M) of each primer, 5  $\mu$ l of DNA and 5  $\mu$ l of sterile water. PCR was performed as follows: 5 minutes at 94 °C, then 30 seconds at 94 °C, 30 seconds at 48 °C and 60 seconds at 72 °C (40 cycles), and a final elongation step at 72 °C for 10 minutes (Gattolliat et al. 2015). Purification and sequencing were conducted by Macrogen, Inc. (South Korea). Sequence alignment and editing were performed using ClustalW. The phylogenetic tree was analysed by Bayesian inference using MrBayes. The evolution model obtained was General Time Reversible Model and Gamma distributed with invariant sites (GTR+G+I). Nucleotide sequences obtained in this study have been deposited in GenBank database. Other analysed mayfly sequences were obtained from the Barcode of Life Data System (BOLD): Vietnamella sp. C (THMAY031-09.COI-5P, THMAY148-12.COI-5P and THMAY149-12. COI-5P); and GenBank: Vietnamella sp. 1 (KM207084.1; KM244655.1) and V. dabieshanensis (HM067837.1). Other Ephemerelloidea COI sequences from GenBank including Dudgeodes palnius (LC057264.1), Teloganella indica (LC057266.1), Teloganopsis deficiens (HQ958649.1) were added to the analysis. Potamanthellus edmundsi (MN186576) was used as an outgroup.

#### Taxonomy

Family Vietnamellidae Allen, 1984 Genus *Vietnamella* Tshernova, 1972

Vietnamella maculosa sp. nov.

http://zoobank.org/E75B83EC-A077-4533-83EC-224D5A26E1DA Figs 1A, 2A–G, 3A–L, 4A, D, G, J, 5A, B, 6A–C

**Material examined.** *Holotype*: 1 male larva Thailand, Chiang Rai Province, Mueang Chiang Rai, Pong Phra Bat waterfall, 20°00'41.0"N, 99°48'15.0"E, 470 m, 6.V.2019, D. Chainthong leg. [ZMKU]. *Paratypes*: 2 larvae same data as holotype; 1 larva on

Species	Code	Collection locality	Collector	Date	GenBank Accession
_					Number
V. maculosa sp. nov.	VmCR01	Chiang Rai	D. Chainthong	6-5-2019	MN510862
	VmCR02				MN510863
<i>Vietnamella</i> sp. B	VbTK01	Tak	A. Watcharangkool	12-1-2016	MN204621
V. thani	VtKN01	Kanchanaburi	B. Boonsoong	20-2-2016	MN204618
V. thani	VtKN02	Kanchanaburi	B. Boonsoong	20-2-2016	MN204619
V. thani	VtKN03	Kanchanaburi	B. Boonsoong	21-2-2016	MN204620
V. thani	VtPK01	Prachuap Khiri Khan	D. Chainthong	19-4-2019	MN318306

Table 1. Collection details of the sequenced specimens.

slide [ZMKU] and another in ethanol [MZL GBIFCH00673059]. Both paratypes were used for DNA extraction.

**Description.** Mature larva (in alcohol, Fig. 1A). Body length 11.25 mm without cerci; cerci 4.8 mm; body brown with dark brown markings on thorax and femora.

*Head.* Brown with a pair of occipital tubercles, a single sub-occipital tubercle medially; two projections below eyes; inner pairs of projections small, spine-like and sharp (Fig. 2A); outer pair large, triangular, cone shaped without any serrated spines (Fig. 4A, D). Labrum, similar to other vietnamellid mayflies, anterior half of dorsal surface and margins with relatively long setae, ventral surface with short setae (Fig. 2B). Labium, glossae width greater than length, glossae and paraglossae with dense setae on surface, setae on dorsal surface and margins longer; labial palpi three segmented, basal segment broader and longer than the second, apical segment very small; palpi with tiny setae (Fig. 2C). Left mandible, slender, a little concave at sub-median area; molar block-like shape with a tuff of short setae below inner molar margin (Fig. 2D). Right mandible, slender, slightly concave at sub-median area; molar block-like shape with a tuff of short setae; length ratio from basal to apical = 1.3:1.2:1 (Fig. 2F). Hypopharynx, lingua square and superlinguae nearly round, with setae on surface (Fig. 2G)

**Thorax.** Pronotum with small moderately sharp anterolateral projections, and slightly pointed protuberances below the anterolateral projection (Fig. 3A). Forefemur, strongly expanded with serrations or teeth projections on ventral margin (Fig. 3B); transverse ridge serrated with small rounded setae (Fig. 5A, B) and long thin setae near inner dorsal margin. Midfemur without any projection, expanded, dorsal margin convex apically and with a row of hair-like setae (Fig. 3C). Hindfemur without any projection, expanded, longer than midfemur, dorsal margin with a row of hair-like setae (Fig. 3D). All claws similar, strongly hooked with a single small denticle basally (Fig. 3E).

**Abdomen.** Tergites I–X with a pair of median ridges or tubercles; posterolateral angles of tergites II–X extended into sharp projection; tergite VII with a pair of tubercles (Fig. 4G) and tergite X with well-developed a pair of tubercles (Fig. 4J); lateral margins of tergite with dense setae. Gill on segments I-VII: gill I finger-like with setae (Fig. 3F); gills on segments II–VI similar in structure, with dorsal and ventral lamellae, the latter further divided into two clusters, each with several smaller lobes



**Figure 1.** *Vietnamella* spp. **A** *Vietnamella maculosa* sp. nov., habitus in dorsal view **B** *Vietnamella* sp. B, habitus in dorsal view. Scale bars: 1 mm.

(Fig. 3G–K); gill on segment VII small, with two lamellae but ventral lamella divided into three lobes (Fig. 3L). Caudal filaments with dense lateral setae on inner and outer margins of middle part.



**Figure 2.** *Vietnamella maculosa* sp. nov. **A** ventral view of head with outer projection and small inner projection (arrow) **B** labrum **C** labium **D** left mandible **E** right mandible **F** maxilla **G** hypopharynx. Scale bars: 1 mm (**A**); 0.1 mm (**B**-**G**).

*Eggs.* (dissected from mature larva). Length 200  $\mu$ m, width 144  $\mu$ m; oval shape, chorionic surface with small protuberances, half of egg covered with helmet-shaped polar cap (Fig. 6A); rod shaped KCT (Knob Terminated Coiled Thread) around egg body (Fig. 6B); 2 or 3 tagenoform-type micropyles at centre (Fig. 6C).

**Diagnosis.** The larva of *Vietnamella maculosa* sp. nov. is most similar to that of *V. thani* and *V. sinensis* in the lack of serrations on the outer projection on the head, but it can be separated from *V. thani* based on the following characteristics: i) the pattern of serration on the ventral margin of the forefemur, ii) the setae on the transverse ridge of the forefemur, iii) a well-developed pair of median ridge projections of tergite X. It can be separated from *V. sinensis* by the proportion of the maxillary palp on the second segment, which is slightly longer than on the third segment (1.2:1), whereas in *V. sinensis* it is clearly longer (1.6:1).

**Remarks.** The larvae of *Vietnamella maculosa* sp. nov. have a unique colour pattern, with brown and dark brown banding spread over the body and femurs. However, this colour can change in intensity depending on the life stage and environment. Other species, like *V. thani*, have variation in body colour but do not have this pattern.



**Figure 3.** *Vietnamella maculosa* sp. nov. **A** thorax **B** forefemur **C** middle femur **D** hind femur **E** foreleg claw **F** first gills on segment I (arrow) **G** gill II **H** gill III I gill IV J gill V **K** gill V **L** gill VII. Scale bars: 1 mm (**A–D, F**); 0.2 mm (**G–L**); 0.1 mm (**E**).

**Etymology.** The name *maculosa* (Latin for having spot or macula), refers to the brown and dark brown banding of the species.

**Distribution.** Chiang Rai Province **Adult.** Unknown

*Vietnamella* sp. B Figs 1B, 4B, E, H, K

**Material examined.** Thailand; 1 larva (immature) on slide, Tak Province, Mae Ra-Merng, 17°31'18.7248"N, 98°3'36.8064"E, 26.X.2015, A. Watcharangkool leg. (ZMKU).

**Description.** Larva (in alcohol, Fig. 1B). Body length 6 mm without cerci; body reddish with dense setae lateral and on margin of head.

*Head.* Reddish with a sharp pair of occipital tubercles and a single sub-occipital tubercle medially; two projections below eyes: inner pairs of projections small, spine-like and sharp; outer pair large, triangular, cone shaped with five unequal serrated spines (Fig. 4B, E), basal spine the largest. Labrum, similar to *V. thani* and *V. maculosa* sp. nov. Labium, glossae width greater than length, glossae and paraglossae with dense setae on surface, labial palpi three segmented, basal segment broader and longer than the second, apical segment small; palpi with tiny setae. Left mandible and right mandible slender, mostly similar to the other *Vietnamella* species. Maxillae slender, maxillary palpi three segmented with tiny setae, length ratio from basal to apical = 1.3:1:1. Hypopharynx, lingua and superlinguae nearly round with setae on surface.

**Thorax.** Pronotum with small sharp anterolateral projections, slight protuberances below the anterolateral projections. Forefemur strongly expanded with serrations or teeth projections on ventral margin; transverse ridge serrated with small setae. Midfemur without any projection. Hindfemur without any projection, expanded, longer than midfemur; dorsal margin with a row of hair-like setae. All claws similar, with one small denticle basally.

**Abdomen.** Tergite I–VI and VIII–X with pair of median ridges or tubercles progressively; tergite VII with a single tubercle (Fig. 4H); posterolateral angles of terga II–IX extended into sharp projection; tergite X with a pair of moderately-developed tubercles (Fig. 4K); lateral margins of tergite with dense setae. Gills on segments III–VI similar in structure with dorsal and ventral lamella, the latter further divided into two clusters, each with several smaller lobes; gill VII small, with two lamellae but ventral lamella divided into three lobes.

**Diagnosis.** The larva of *Vietnamella* sp. B can be separated from those of other species based on the following characteristics: i) outer pairs of projections on the head are large and stout, triangular, and cone shaped, with five unequally serrated spines (one large spine + four small spines), and ii) the abdominal tergites II–IX have a pair of projections or tubercles at the posterolateral margin except for tergite VII which has only a single projection (Fig. 4H).

**Remarks.** The larval description given herein agrees with larvae of other species belonging to the genus *Vietnamella*, including the presence of a pair of projections on the head, the expanded femur and the forefemur with serrations on the outer dorsal margin. The larva described here has serrated spines on the outer projection of the head that differ from the other valid species of *Vietnamella*. The outer serrated projection is similar to that of *Vietnamella* sp. A from India (Selvakuma et al. 2018), but it differs in the number and character of the outer projection spines (four equal serrated spines in *Vietnamella* sp. B, five unequal serrated spines in *Vietnamella* sp. B. Although *Vietnamella* sp. B was not a mature larva, the phylogenetic analysis showed it belonged to a clearly different clade and had a high genetic distance compared to the other species (Fig. 12; Table 2). Formal description of this species is pending more material.

**Distribution.** Tak Province. **Adult and egg.** Unknown.



**Figure 4.** *Vietnamella* spp. *Vietnamella maculosa* sp. nov. (**A**, **D**, **G**, **J**): **A** outer projection on head (arrow) **D** inner and outer projections on head **G**, **J** tubercle on tergite VII and X; *Vietnamella* sp. B (**B**, **E**, **H**, **K**): **B** outer projection on head (arrow) **E** inner and outer projections with serration on head **H**, **K** tubercles on tergite VII and X; *Vietnamella thani* (**C**, **F**, **I**, **L**): **C** outer projection on head (arrow) **F** inner and outer projections on head **I**, **L** tubercles on tergite VII and X. Scale bars: 1 mm (**A**–**C**); 0.5 mm (**G**); 0.1 mm (**D**–**F**, **H**, **I**); 0.05 mm (**J**–**L**).

Taxa	K2P genetic distances					
-	1	2	3	4	5	6
1. Vietnamella maculosa sp. nov.						
2. Vietnamella thani	0.253					
3. Vietnamella sp. B	0.286	0.267				
4. Vietnamella sp. C	0.276	0.160	0.286			
5. Vietnamella sp. 1	0.254	0.224	0.278	0.242		
6. Vietnamella dabieshanensis	0.258	0.184	0.309	0.185	0.217	
7. Potamanthellus edmundsi	0.267	0.206	0.229	0.248	0.289	0.242

Table 2. Pairwise genetic distances (COI) between species of Vietnamella using the Kimura 2-parameter.

#### Vietnamella thani Tshernova, 1972

Figs 4C, F, I, L, 5C, 7A–O, 8A–O, 9A–N, 10B, C, 11A–C

*Vietnamella thani* Tshernova, 1972: 604–614, fig. 4 (orig.); Hu et al. 2017: 381–390, fig. 7 (distribution).

**Material examined.** Thailand; Kanchanaburi Province, Thong Pha Phum, Huai Pak Kok, 14°35'01.4"N, 98°34'54.0"E, 161 m, 15.X.2015, 1 larva; 20.II.2016, 1 larva; 21.II.2016, 3 larvae, 1 female imago; 31.I.2019, 9 larvae, 1 male subimago (reared), 1 male imago (reared). Huai Khayeng, Ban Prachum Mai, 14°39'34.0"N, 98°32'02.0"E, 233 m, 20.II.2016, 4 larvae, [ZMKU]; 13.XII.2014, 1 larva, all B. Boonsoong leg; 15.X.2015, 3 larvae, B. Boonsoong & M. Sartori leg. [MZL]. Prachuap Khiri Khan Province, Kui Buri, Huai Samrong, 12°03'55.0"N, 99°37'38.0"E, 103 m, 11 larvae, D. Chainthong leg, [ZMKU].

**Diagnosis.** The larva of *Vietnamella thani* can be distinguished from those of other *Vietnamella* based on the following characteristics: i) outer pairs of projections on the head are long, triangular, and cone shaped without serrated spines; ii) the first and second segments of the maxillary palpi have an equal length ratio; iii) the forefemur has a serrated transverse ridge with spatulate setae (Fig. 5C); and iv) the abdominal tergite X either lacks or has a poorly-developed pair of tubercles (Fig. 4L).

Description of imagoes. Male imago (in alcohol, Figs 7, 10C). Head. Eyes rounded with ventral part brown-yellowish and dorsal part yellowish (Fig. 7A, B). **Thorax.** Forelegs (8.07 mm), length ratio of femur and tibia = 1:1.45; length ratio of four tarsal segments is 3:2:1.5:1 (Fig. 7C). Midlegs (4.76 mm), length ratio of femur and tibia = 1.2:1; length ratio of four tarsal segments is 1:1:1:3 (Fig. 7D). Hindlegs (4.97 mm), length ratio of femur and tibia = 1.4:1; length ratio of four tarsal segments is 1:1:1:4 (Fig. 7E). Mesonotum with a notable median longitudinal suture, two medioparapsidal sutures (Fig. 7F). Mesosternum with a square basisternum, broad furcasternum (Fig. 7G). Forewings, numerous crossviens. MA forked middle of wing, MP forked basally, three intercalaries between MP<sub>1</sub> and MP<sub>2</sub>; CuA and CuP adjacent at base; cubital field with three bifurcate veins arising from CuA (Fig. 7H). Hind wings rounded, leading margin slightly concave, with clear crossveins; seven crossveins with one bifurcate between Sc and RA; three crossveins between MA and MP (Fig. 7I). *Abdomen.* Genitalia with three-segmented forceps (1.2 mm), first segment = 0.6 mm, second segment = 0.45 mm, apical segment = 0.15, small and nearly rounded; penes (0.76 mm) totally fused with a shallow median cleft; subgenital plate slightly convex (Fig. 7J–L). Abdominal segment IX with lateral projection, white stripe on sternites VII-IX (Fig. 7M-O).

**Male subimago.** (in alcohol, Fig. 8). *Head.* Eyes rounded; dorsal part dark grey; ventral part brown (Fig. 8A, B). *Thorax.* Forelegs (6.38 mm), length ratio of femur and tibia = 1:1.1; length ratio of four tarsal segments is 1.6:1:1:1.3 (Fig. 8C). Midlegs (5.41 mm), length ratio of femur and tibia = 1.4:1; length ratio of four tarsal segments is 1.3:1:1.1:3.4 (Fig. 8D). Hindlegs (5.58 mm), length ratio of femur and tibia = 1.6:1;



**Figure 5.** Forefemur and setae **A** forefemur of *Vietnamella maculosa* sp. nov. with serration of transverse ridge (arrow) **B** setae on transverse ridge of *Vietnamella maculosa* sp. nov. **C** setae on transverse ridge of *V. thani*. Scale bars: 1 mm (**A**); 0.01 mm (**B**, **C**).



**Figure 6.** SEM of egg structure of *Vietnamella maculosa* sp. nov. **A** overview **B** detail of Knob Terminated Coiled Thread (KCT) **C** micropyle and chorionic surface. Scale bars: 50 μm (**A**); 20 μm (**B**); 10 μm (**C**).

length ratio of four tarsal segments is 1.4:1:1.2:3.4 (Fig. 8E). Mesonotum, brown with a notable median longitudinal suture (Fig. 8F). Mesosternum, pale red with a square basisternum, broad furcasternum (Fig. 8G). Forewing and hindwing are similar to imago but more opaque and having more visible crossveins (Fig. 8H, I). *Abdomen.* Genitalia



Figure 7. Male imago of *Vietnamella thani* A dorsal view of head B lateral view of head C foreleg D middle leg E hindleg F, G dorsal and ventral view of thorax H forewing I hindwing J ventral view of genitalia
K dorsal view of genitalia L lateral view of genitalia M dorsal view of abdomen N lateral view of abdomen
O ventral view of abdomen. Scale bars: 1 mm.

similar to those of imago but forceps and penes shorter and broader; forceps with total length = 1.0 mm, first segment = 0.53 mm, second segment= 0.40 mm and third segment = 0.07 mm; penes length = 0.7 mm (Fig. 8J–L). Abdomen brown and pale red dorsally, segment IX with notable lateral projections (Fig. 8M–O).



**Figure 8.** Male subimago of *Vietnamella thani* **A** dorsal view of head **B** lateral view of head **C** foreleg **D** middle leg **E** hindleg **F**, **G** dorsal and ventral view of thorax **H** forewing **I** hindwing **J** ventral view of genitalia **K** dorsal view of genitalia **L** lateral view of genitalia **M** dorsal view of abdomen **N** lateral view of abdomen. Scale bars: 1 mm.

**Female imago.** (in alcohol, Fig. 9). *Head.* Eyes rounded, dark brown without dorsal eyes (Fig. 9A, B). *Thorax.* Forelegs (7.2 mm), length ratio of femur and tibia = 1.4:1; length ratio of four tarsal segments is 1:1:1:3.7 (Fig. 9C). Midlegs (6.4 mm), length ratio of femur and tibia = 1.2:1; length ratio of four tarsal segments is 1:1:1.2:2.8 (Fig. 9D). Hindlegs (7.2 mm), length ratio of femur and tibia = 1:1, length ratio of



**Figure 9.** Female imago of *Vietnamella thani* **A** dorsal view of head **B** lateral view of head **C** foreleg **D** middle leg **E** hindleg **F**, **G** dorsal and ventral view of thorax **H** forewing **I** hindwing **J**, **K** ventral and dorsal view of genitalia **L** dorsal view of abdomen **M** lateral view of abdomen. Scale bars: 1 mm.

four tarsal segments is 1:1.2:1.1:2.6 (Fig. 9E). Mesonotum brown with a notable median longitudinal suture (Fig. 9F). Mesoternum pale red with rectangle basisternum, broad furcasternum (Fig. 9G). Forewing, 14 crossveins in stigmatic area; MA forked middle of wing; MP forked basally, 3 intercalaries between MP1 and MP2; CuA and CuP adjacent at base (Fig. 9H). Hindwing rounded, leading margin slightly concave, with clear crossveins, 7 crossveins between MA and MP (Fig. 9I). *Abdomen.* Tergites brown, sternites pale red, sternites VIII-IX brown (Fig. 9K–N). Subanal plate brown with shallow median cleft (Fig. 9J). Subgenital plate weakly developed, pale, with shallow median emargination (Fig. 9N)



Figure 10. *Vietnamella thani* A habitat B habitus of larva C habitus of male imago (incomplete molting).

*Eggs.* (dissected from female imago). Length 175  $\mu$ m, width 125  $\mu$ m; oval shape, chorionic surface with small protuberances, half of egg covered with helmet-shaped polar cap (Fig. 11A); many KCT around egg body (Fig. 11B); 1 or 2 tagenoform-type of micropyles at centre (Fig. 11C).

Distribution. Kanchanaburi and Prachuap Khiri Khan Provinces, Thailand.

**Remarks.** The larvae of *Vietnamella thani* are widely distributed in Thailand. They inhabit fast-flowing streams (Fig. 10A) but have never been reported in southern Thailand. *Vietnamella thani* have notable outer pairs of projections on head without serrated spines that differ from *Vietnamella* sp. B. The larvae show colour variations and can be greenish, yellow or brown. The imaginal stages of *V. thani* have similar characters to those of *V. sinensis* but lack the longitudinal vein on the stigmatic area of the forewing and they have less crossveins between Sc and RA on the hindwings (Table 4). The egg structure is covered by a membrane, which leads to unclear sculpturing of the surface, especially in the posterior part of the egg. Thus, the egg from this study showed little difference from the egg structure of *V. sinensis*.

#### Molecular analysis

The phylogenetic tree of vietnamellid mayflies and the other families of Ephemerelloidea was constructed from 658 bp of COI sequences by Bayesian interference. The results show that Vietnamellidae is clearly separated from the others. Six clades can be



**Figure 11.** SEM of egg structure of *Vietnamella thani* **A** overview **B** detail of Knob Terminated Coiled Thread (KCT) and chorionic surface **C** micropyle. Scale bars: 50 μm.

Characters	V. thani	V. sinensis	Vietnamella	Vietnamella sp. A	Vietnamella sp. B
			<i>maculosa</i> sp. nov.		
Maxillary palp segment	1.3:1.3:1	1:1.6:1	1.3:1.2:1	1:0.9:0.7	1.3:1:1.1
ratio					
Outer pair of	Without	Without	Without serration	With serration	With serration
projections on head	serration	serration			
Median ridge projection	Pair: I-IX	Pair: I-X	Pair: I-X	Pair: II–IX	Pair: II–VI,
of abdominal terga					VIII–X; single: VII
Posterolateral projection	Less developed	Moderately	Well developed	Moderately	Moderately
on tergite X	_	developed <sup>a</sup>	_	developed b	developed
Distribution	Vietnam,	China	Thailand	India	Thailand
	Thailand, China				

Table 3. Comparison of larval characteristics of known Vietnamella species.

<sup>a</sup> Hu et al. (2017). Definition based on fig. 1A, p. 383.

<sup>b</sup> Selvakumar et al. (2018). Definition based on fig. 1, p. 995.

Table 4. Comparison of adult characteristics of known Vietnamella species.

Characters	V. thani (imago)	V. sinensis (imago)
Stigmatic area of forewing	Not divided by longitudinal vein	Divided by longitudinal vein
Penes	Slender, shallow median cleft	Slender, shallow median cleft
Subgenital plate	Convex	Slightly convex
Hindwing	8 or 9 crossveins between Sc and RA	12 crossveins between Sc and RA

recognized within Vietnamellidae with high posterior probability value support for the morphospecies: *Vietnamella maculosa* sp. nov., *Vietnamella* sp. B, *Vietnamella* sp. C, *Vietnamella* sp. 1, *V. dabieshanensis* and *V. thani* (Fig. 12) Moreover, K2P genetic distance was analyzed to confirm species delimitation. The intraspecific genetic distances vary between 0–6.7 % whereas interspecific distances are very high, ranging from 16–31% (Table 2). The phylogenetic tree and K2P value result confirm that there are four different species of *Vietnamella* in Thailand.

## Discussion

Comparison of the larvae of *Vietnamella*, including *V. thani*, *V. sinensis*, *Vietnamella* sp. A, *Vietnamella* sp. B and *Vietnamella maculosa* sp. nov., is presented in Table 3. *Vietnamella ornata*, which was previously reported from Yunnan, China is not included because its larvae are still unknown (Tshernova 1972). The comparisons showed that a major character, the serration of the outer projection on the head, can distinguish *Vietnamella* into two groups (serration and non-serration groups). The non-serration group consists of *V. thani* and *Vietnamella maculosa* sp. nov., which is most similar to *V. sinensis*. They have a second segment of the maxillary palp that is longer than the other segments in *V. sinensis* but is of medium or nearly equal length in *V. thani* and *Vietnamella maculosa* sp. nov. The serration group includes *Vietnamella* sp. A (India) and *Vietnamella* sp. B (Thailand).



**Figure 12.** Bayesian interference of Ephemerelloidea. The COI phylogenetic construction of *Vietnamella* and sister groups with the percentages indicating branch probability support. *Potamanthellus edmundsi* was used as the outgroup.

Egg structure of *Vietnamella* species has a similar pattern of a polar cap covering half of the egg chorion; however, we found little difference between the three species known at that stage: *V. maculosa* sp. nov., *V. thani* and *V. sinensis*. In addition, *V. maculosa* sp. nov. has a rod-shaped KCT that is different from that in *V. thani* and *V. sinensis* which have oval-shaped KCT. The chorionic surface of *V. maculosa* sp. nov. and *V. thani* have a protuberance which is smaller than in *V. sinensis* (Hu et al. 2017, fig. 6). From our results, it appears that the egg structure can be useful for species identification at least in the three *Vietnamella* species investigated. Considering other ootaxonomic investigations, egg structure of some mayflies can be used for identification at the species level (Sivaramakrishnan and Venkataraman 1987; Ubero-Pascal and Puig 2007).

Our phylogeny indicated the existence of six different species of *Vietnamella* in the Oriental region, with four of them found in Thailand. Only one species, *V. thani*, can be found in western Thailand. Surprisingly, three species distributed in northern Thailand are revealed here by molecular analysis. Herein, only *V. maculosa* can be described as new species. However, we suppose that *Vietnamella* sp. B and *Vietnamella* sp. C are putative new species which could be formally described when more material becomes available. Although our molecular phylogenetic result clearly showed species delimitation in Thailand, there are still ambiguities in other areas where COI sequence or morphological data are incomplete. Thus, we infer that the sequences of *Vietnamella* sp. 1 (KM207084.1; KM244655.1) may belong to *V. ornata* because their specimens were collected near the type locality (Fig. 13) (Tang et al. 2014). *Vietnamella* sp. A was recently reported from India and the authors suspect it can represent the unknown larvae of *V. ornata* (Selvakumar et al. 2018). In addition, *V. dabieshanensis* (HM067837.1) is



**Figure 13.** Distribution map of *Vietnamella* in the Oriental region. Each different shape represents a different species. Our specimens are only distributed in Thailand and including the data of *Vietnamella* sp. C from the BOLD system. Outside of Thailand distribution of *Vietnamella* is based on previous records including *V. ornata* (Tshernova, 1972), *V. sinensis* (Hu et al. 2017), *Vietnamella* sp. 1 (Tang et al. 2014), *Vietnamella* sp. A (Selvakumar et al. 2018) and *V. thani* (Hu et al. 2017 and unpublished data).

now considered as a junior synonym of *V. sinensis* (Hu et al. 2017) and this sequence likely refers to this species.

Although ambiguous classifications of Vietnamellidae still remain, our results allow us to conclude that at least four valid species (*V. thani*, *V. ornata*, *V. sinensis* and *V. maculosa* sp. nov.) exist, as supported by our morphological and molecular analyses. The findings of this study also extend the species diversity, imaginal description and phylogeny for future considerations of the Vietnamellidae.

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RESEARCH ARTICLE



# Taxonomic studies of the ground beetle subgenus Falcinebria Ledoux & Roux, 2005 (Coleoptera, Carabidae, Nebria) from Honshû, Japan

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#### Abstract

*Nebria (Falcinebria) reflexa* Bates (Carabidae), a Japanese endemic flightless ground beetle, is geographically polytypic and was thought to be composed of four subspecies (including nominotypical subspecies). Populations from Honshû recognized as three subspecies were taxonomically revised based primarily on the shape of the endophallus, a membranous inner sac everted from the aedeagus. Three known taxa, *Nebria reflexa, Nebria niohozana* Bates, and *Nebria uenoi* Nakane, are redefined based on endophallus morphology, and the latter two are described as distinct species rather than subspecies of *N. reflexa.* Seven new species are described: *N. sagittata* **sp. nov.**, *N. iidesana* **sp. nov.**, *N. furcata* **sp. nov.**, *N. pisciformis* **sp. nov.**, *N. kuragadakensis* **sp. nov.**, *N. dichotoma* **sp. nov.**, and *N. chugokuensis* **sp. nov.** Comparative morphology, primarily of the endophallus, indicated that *N. furcata*, *N. pisciformis*, *N. kuragadakensis*, and *N. uenoi* form basal lineages, and the remaining six species form a clade in which *N. niohozana* and *N. dichotoma* are sister taxa. Species phylogeny and known distributions suggest that the initial diversification of these species occurred in the western Chûbu and eastern Kinki regions of Japan.

#### Keywords

Biogeography, cryptic species, endophallus, ground beetle, Japan, male genitalia, phylogeny, taxonomy

## Introduction

*Nebria (Falcinebria) reflexa* Bates (Carabidae) is a ground beetle first described from Mount Iwaki, Honshû, Japan (Bates 1883). The species occurs within mountainous areas of Honshû, Shikoku, and Kyûshû in the Japanese Archipelago (Uéno 1985) and is geographically polytypic among these areas due to poor dispersal ability caused by atrophied hind wings. Two subspecies on Honshû and one from Kyûshû have been distinguished from the nominotypical subspecies. Among the four known subspecies, *N. r. hikosana* Habu (known from Kyûshû) is readily distinguished based on external morphology (Uéno 1985). Differences among the remaining three, *N. r. reflexa, N. r. niohozana* Bates, and *N. r. uenoi* Nakane, are less clear (Mori 2018), and the subspecies identification of populations outside of type localities has been determined largely without evidence (Yoshitake et al. 2011; Yoshimatsu et al. 2018).

In some species groups of *Nebria*, the endophallus (a membranous inner sac everted from the aedeagus) is known to be diversified and therefore useful for species-level taxonomy (e.g., Dudko and Shilenkov 2001; Ledoux and Roux 2005; Sa-sakawa 2016). This genital character had not been examined in *N. reflexa*, but the endophallus is in fact diversified and thus taxonomically useful. This study aims to revise unclear subspecies-level taxonomy for populations of *N. reflexa* on Honshû. To this end, the three subspecies known from Honshû are redefined based on endophallus morphology and, based on these results, taxonomic revisions to other Honshû populations are presented.

#### Materials and methods

A total of 120 specimens from various localities in Honshû were examined (Fig. 1). Two specimens, lectotypes of *N. r. reflexa* ( $\eth$ ) and *N. r. niohozana* ( $\circlearrowright$ ), which are deposited at the Muséum national d'Histoire naturelle (MNHN), were investigated through high-quality photos that are available for external macromorphological and morphometric studies (Figs 2, 3). In the remaining 118 specimens ( $72 \circlearrowright$ ,  $46 \circlearrowright$ ) examined directly, specimens from the exact type localities of *N. r. reflexa* and *N. r. uenoi* were included, as well as specimens from three sites in the surrounding area (12-14 km) of the type locality of *N. r. niohozana* (Mount Tanigawa, Doai, Mount Naeba). For comparison, specimens from Kyûshû (type materials of *N. r. hikosana:* holotype male and paratype  $1 \circlearrowright 1 \circlearrowright 1 \circlearrowright$ , deposited at the National Agriculture and Food Research Organization [NARO], Tsukuba, Ibaraki, Japan) were also examined. Examined specimens are preserved in the collections of NARO and in the author's (KS) collection deposited in the Laboratory of Zoology, Department of Science Education, Faculty of Education, Chiba University, Chiba, Japan.

Body length was measured as the distance from the mandible apices to the end of the elytra. As an index of body shape, the ratio of pronotal posterior margin width (PPW) to elytral length (EL) was determined; PPW was measured as the length between hind



**Figure 1.** Distribution of 10 species previously classified as *Nebria reflexa* in Honshû. Only records with unambiguous identities (i.e., collection sites of type materials and records based on specimens identified by the endophallus) are presented. **1** *N. reflexa* Bates, **2** *N. sagittata* sp. nov., **3** *N. iidesana* sp. nov., **4** *N. niohozana* Bates, **5** *N. furcata* sp. nov., **6** *N. pisciformis* sp. nov., **7** *N. kuragadakensis* sp. nov., **8** *N. dichotoma* sp. nov., **9** *N. uenoi* Nakane, **10** *N. chugokuensis* sp. nov. **\*** indicates the locality where the sympatric occurrence of *N. iidesana* and *N. niohozana* was confirmed. Red letters denote the type localities of each species. The blue letter denotes the locality described as the collection site of cotypes of *N. niohozana* but was not designated as the type locality in the lectotype designation.



Figures 2, 3. Habitus dorsal views (a) and attached labels (b) of lectotypes of *Nebria reflexa* (2) and *N. niohozana* (3).

angle apices, and EL was measured from the scutellum base to the elytral apex with the elytral lateral margins maintained horizontally. In general, an individual with a smaller PPW/EL has a more slender habitus and more posteriorly constricted pronotum.

To compare genital structures among males, the endophallus was everted by injecting toothpaste at the basal end of the aedeagus. All Honshû specimens except for the lectotypes were dissected. In some older specimens, the gonopore and some surface lobes could not be fully everted, as toothpaste injection can cause the endophallus to burst. To prevent damaging specimens, the endophallus of such specimens was observed with the gonopore protrusion and the lobes not fully everted. Comparison of fully everted gonopore protrusions among species indicated that morphological differences in the protrusion are smaller than those of other lobes. Character observations of incompletely everted lobes on the endophallus surface were complemented by observations of these same lobes everted in specimens collected at, or close to, the same collection site. Terminology of endophallus structures followed Sasakawa (2016), but the new terminology of "dorsomedian lobe" is defined herein as the lobe between the dorsobasal and dorsoapical lobes. To observe female genitalia, muscles around the genitalia were dissolved using 5% potassium hydroxide, and organs were cleaned and observed in pure water.

To confirm species identities based on genital morphology, discriminant analysis based on external morphometrics was performed. In addition to PPW and EL, the following two external body parts were also measured in the lectotypes of *N. reflexa* and *N. niohozana* and 72 male specimens of the 10 species described here: PL, pronotum length along median line; and PAW, pronotal anterior margin width. The linear discriminant analysis was first performed for data set excluding the lectotypes, with "species" as a response variable and the four morphometric measurements (EL, PL, PAW, and PPW) as the explanatory variables. The species identities of the two lectotypes were then determined using the obtained function. All statistical analyses were performed in the statistical package R v.3.4.3 (R Development Core Team 2017). The raw data of the analysis are available in Supplementary material 1, Table S1.

#### Taxonomy

Specimens were classified into 10 species based primarily on the shape of the endophallus. Species identities were further determined by comparing the distribution of each species and the type localities of known taxa (Fig. 1). These results agree with those of the discriminant analysis. In the discriminant function analysis, the lectotypes of *N. reflexa* and *N. niohozana* were classified to *N. reflexa* and *N. niohozana*, respectively, although the former was situated in an intermediate position among *N. reflexa*, *N. iidesana*, and *N. sagittata* on the scatter plot of the first two canonical variates (Fig. 4).

The 10 species described here are similar to one another (Figs 5–16) and share the following adult morphology:

**External characters:** Body flat. Dorsal surface of body reddish black, shiny, not opaque; mouthpart appendages, antennae, legs, pronotal and elytral margins yellowish to reddish brown. Hind wings completely reduced.

Head widest at mid-eye level; eyes large and convex; frontal impression shallow; frons smooth; mentum tooth shallowly bifid. Antennae long, with apices reaching the apical 1/3-1/2 in both sexes.



Figure 4. Scatter plot of the first two canonical variates obtained from the discriminant analysis. 1 *N. re-flexa*, 2 *N. sagittata* sp. nov., 3 *N. iidesana* sp. nov., 4 *N. niohozana*, 5 *N. furcata* sp. nov., 6 *N. pisciformis* sp. nov., 7 *N. kuragadakensis* sp. nov., 8 *N. dichotoma* sp. nov., 9 *N. uenoi*, 10 *N. chugokuensis* sp. nov., **r** lectotype male of *N. reflexa*, **n** lectotype male of *N. niohozana*.

Pronotum cordate, widest in front of the middle, convex; anterior margin as wide as or slightly wider than posterior margin; lateral margins arcuate in the apical 2/3, straight in the basal 1/3, but more or less sinuate in front of hind angle; anterior angles pronounced, with corners widely rounded; hind angles square to somewhat acute, with sharp corners; lateral margins reflexed throughout; laterobasal impressions large and deep on the posterior part, with the anterior part shallow and reaching the apical half of the pronotum; laterobasal impressions connected by a transverse impression; median line distinct on the central part, but less distinct near the anterior and posterior margins; surface of central part smooth; surface near the posterior margin punctate; surface near lateral and anterior margins punctate in most individuals, but sparser than that of the posterior margin; two marginal setae on each side, anterior setae at widest pronotal point and posterior setae in front of hind angle.

Elytra oblong, widest slightly behind the middle; shoulders and apices rounded, not denticulate; intervals barely convex; scutellar stria present, not connected to stria 1; interval 1 with one setigerous puncture adjoining stria 1 at the level of posterior end of scutellum; interval 3 with five or seven setigerous punctures adjoining or near stria 3.

Ventral surface of the body with punctations on thorax and some sterna, with setae on mentum, gula, and other sterna; meso- and metathorax and sterna 2 and 3 punctate in all individuals, prothorax punctate in some individuals; mentum with two pairs of setae, the anterior pair at the base of mentum tooth and the posterior pair behind the



Figures 5–16. Habitus dorsal view of *Nebria* spp. 5 *N. reflexa*, a male from the type locality 6 *N. sagit-tata* sp. nov., holotype male 7 *N. iidesana* sp. nov., holotype male 8–10 *N. niohozana* males from Mount Chôkai (8), Doai, a locality close to the type locality designated for the lectotype (9), and Mount Hakusan (10); 11 *N. furcata* sp. nov., holotype male 12 *N. pisciformis* sp. nov., holotype male 13 *N. kuragadakensis* sp. nov., holotype male 14 *N. dichotoma* sp. nov., holotype male 15 *N. uenoi*, a male from the type locality 16 *N. chugokuensis* sp. nov., holotype male.

anterior pair; gula with 13–14 setae along the anterior margin; sterna 4–7 with one to three (usually two) setae on each ventrolateral side.

**Male genital characters:** Aedeagus stout and strongly arcuate; apex short and widely rounded. Endophallus with gonopore narrowly protruding; five lobes present on the surface in all species, two on laterobasal surface (laterobasal lobes), two on later-

oapical surface (lateroapical lobes), and one on dorsoapical surface (dorsoapical lobe); two additional lobes present in some species, one on the dorsal surface at a position more anterior than the level of the gonopore protrusion (dorsobasal lobe), and the other on the dorsal surface at a position between the level of the gonopore protrusion and dorsoapical lobe (dorsomedian lobe). Both right and left parameres spatulate, with the former larger than the latter.

**Female genital characters:** Gonocoxite 2 semi-triangular and weakly bent posterolaterally. Membranous parts without pigmentation; openings of spermatheca and common oviduct adjacent; innermost part of vagina elongate, forming the bursa copulatrix; spermatheca not uniform in shape between the basal and apical halves; the basal half straight tubular and glued to the wall of the bursa copulatrix; the apical half about half the thickness of the basal half and zigzag tubular.

#### Nebria (Falcinebria) reflexa Bates, 1883

Figs 2, 5, 17 Japanese name: Iwaki-hime-marukubi-gomimushi

- *Nebria reflexa*: Bates (1883): 218 (original description), type locality: "Iwakisan" (originally stated), "Iwaki Japan", as stated in lectotype designation by Ledoux and Roux (1992: 45); Nakane (1963b): 19 (part); Uéno (1985): 56 (part).
- *Nebria reflexa reflexa*: Ledoux and Roux (1992): 45 (lectotype ()) designation).

Nebria (Orientonebria) reflexa reflexa: Farkač and Janata (2003): 94.

Nebria (Falcinebria) reflexa reflexa: Ledoux and Roux (2005): 829, plate-fig. 625; Yoshitake et al. (2011): 32; Huber (2017): 50.

**Notes.** This species is known only from the type locality of Mount Iwaki and has the smallest body size among all species previously regarded as *N. reflexa*. *Nebria reflexa* is distinguished from *N. sagittata* sp. nov. and *N. niohozana*, which are relatively closely distributed and thus may be sympatric, by a T-shaped apex of the dorsoapical lobe. The morphological features of the lectotype male (Fig. 2) are compatible with specimens examined here as *N. reflexa* with respect to all macromorphological features, body length (8.88 mm), and PPW/EL (0.343).

**Description.** Body length: 3, 8.92-9.06 mm (mean  $\pm$  SD:  $9.00 \pm 0.08 \text{ mm}$ , n = 3); 9, 9.19 mm (n = 1). PPW/EL: 3, 0.339-0.356 (mean  $\pm$  SD:  $0.346 \pm 0.009$ , n = 3); 9, 0.312 (n = 1). Ventral surface of aedeagal apex not concave. Dorsomedian lobe largely swollen, directed right-laterally. Dorsoapical lobe with the basal part protruding right-dorsolaterally; the protrusion similar in size to the right laterobasal lobe; the apical portion directed right-ventrolaterally, bifurcated in a T shape. Right laterobasal lobe small, with the width from a ventral view narrower than the width of the gonopore protrusion from a lateral view. Left laterobasal lobe small, with the width from a ventral view narrower than the midth of the gonopore protrusion from a lateral view. Right laterobasal lobe small, with the width from a ventral view narrower than the width from a ventral view. Right laterobasal lobe small, with the width from a ventral view. Left laterobasal lobe small, with the width from a ventral view. Right laterobasal lobe small, with the width from a ventral view. Right laterobasal lobe large, with the apex slightly protruding and bent anteriorly. Left



Figures 17–21. Right lateral (a), ventral (b), dorsal (c), and posterodorsal (d) views of the endophallus and the left dorsolateral view of the dorsoapical lobe (e) of *Nebria* spp. 17 *N. reflexa* male from the type locality 18 *N. sagittata* sp. nov., holotype male 19 *N. sagittata* sp. nov., a paratype male from "Asahi-mura"
20 *N. iidesana* sp. nov., holotype male 21 *N. iidesana* sp. nov., paratype male from Mount Kitamata. Abbreviations: da, dorsoapical lobe; dm, dorsomedian lobe; go, gonopore; gp, gonopore protrusion; la, left lateroapical lobe; lb, left laterobasal lobe; ra, right lateroapical lobe; rb, right laterobasal lobe. Asterisk indicates that the gonopore protrusion or lobes are not fully everted.

lateroapical lobe bifurcated at the base, with one apex large and directed ventrally and the other similar in size to right laterobasal lobe and directed anteriorly. Ventrobasal surface almost flat, without swelling.

**Materials examined.** 3∂1♀ (NARO), Yunosawa, Mount Iwaki, Aomori Prefecture, Japan, 18.viii.1959, S. Ueno leg.

#### Nebria (Falcinebria) sagittata sp. nov.

http://zoobank.org/264650A4-C1B0-4347-AB83-5FE19B651D75 Figs 6, 18, 19 Japanese name: Asahi-hime-marukubi-gomimushi

Nebria reflexa: Uéno (1985): 56 (part); Nakane (1963b): 19 (part).

**Notes.** This species is known from the Asahi Mountains and the adjacent Mount Gassan. It is likely sympatric with *N. niohozana* and is distinguished from that species by a smaller body size and a larger PPW/EL. Among known related species, *N. sagittata* sp. nov. is most similar to *N. iidesana* sp. nov., in both external and endophallic structures, but it is distinguished by a strong bend in the apical portion of the right lateroapical lobe.

**Description.** Body length: 3, 9.22-9.87 mm (mean  $\pm$  SD:  $9.54 \pm 0.26$  mm, n = 9); 9, 9.77-10.92 mm (mean  $\pm$  SD:  $10.07 \pm 0.43$  mm, n = 6). PPW/EL: 3, 0.317-0.345 (mean  $\pm$  SD:  $0.331 \pm 0.009$ , n = 9); 9, 0.307-0.333 (mean  $\pm$  SD:  $0.320 \pm 0.009$ , n = 6). Ventral surface of aedeagal apex not concave. Dorsobasal lobe absent. Dorsomedian lobe absent. Dorsoapical lobe with the basal part protruding anterodorsally; the protrusion longer than the right laterobasal lobe; the apical portion directed dorsally, bifurcated at the apex; the left apex more than twice the size of the right apex. Right laterobasal lobe small, with the width from the ventral view narrower than the width of the gonopore protrusion from the lateral view. Left laterobasal lobe small, with the width of the gonopore protrusion from the lateral view narrower dapically; apical half strongly bent in an anterior direction. Left lateroapical lobe large, narrowed apically; apical half strongly bent in an anterior direction. Left lateroapical lobe large, narrowed apically; Neutrobasal surface almost flat, without swelling.

**Type materials.** Holotype: 3 (KS), Riv. Higuresawa, ca 625 m, Nishikawa-machi, Yamagata Prefecture, Japan (38.320729N, 139.942376E), 9–12.ix.2004, K. Sasakawa leg. Paratypes: 6369 (KS), same data as the holotype; 13 (NARO), "Tachiyazawa-Vill." [Tachiyazawa, Shônai-machi], Yamagata Prefecture, Japan, 2.viii.1960, Y. Watanabe leg.; 13 (NARO), "Asahi-mura" [a part of Murakami-shi], Niigata Prefecture, Japan, K. Baba leg.

**Etymology.** The specific name derives from the Latin word *sagittata* (arrow-shaped) and refers to the dorsal view of dorsoapical lobe.

*Nebria* (*Falcinebria*) *iidesana* sp. nov. http://zoobank.org/1E60020F-32F9-4252-B8D4-CC73E99A1EF1 Figs 7, 20, 21 Japanese name: Iide-hime-marukubi-gomimushi

Nebria reflexa: Uéno (1985): 56 (part); Nakane (1963b): 19 (part). Nebria (Paranebria) reflexa niohozana: Habu and Baba (1972): 3 (part). **Notes.** This species is known from the Iide Mountains. Sympatry with *N. niohozana* is considered confirmed based on an observation from the NARO collection, where paper cards affixed to a paratype male of this species and male specimen of *N. niohozana* were held together with the same pin. In external and endophallus characters, *N. sagittata* sp. nov. is a similar species. *Nebria iidesana* is distinguished from *N. niohozana* by a smaller body size and larger PPW/EL, and from *N. sagittata* sp. nov. by a right lateroapical lobe that is not narrowed apically.

**Description.** Body length:  $3, 9.16-9.87 \text{ mm} (\text{mean} \pm \text{SD}: 9.53 \pm 0.29 \text{ mm}, n = 5); <math>9, 10.25-10.64 \text{ mm} (\text{mean} \pm \text{SD}: 10.39 \pm 0.17 \text{ mm}, n = 6)$ . PPW/EL: 3, 0.319-0.350 (mean  $\pm$  SD:  $0.334 \pm 0.013, n = 5$ ); 9, 0.321-0.338 (mean  $\pm$  SD:  $0.330 \pm 0.006, n = 6$ ). Ventral surface of aedeagal apex not concave. Dorsobasal lobe absent. Dorsomedian lobe absent. Dorsoapical lobe with the basal part protruding anterodorsally; the protrusion slightly smaller than the right laterobasal lobe; the apical portion directed dorsally, bifurcated at the apex; the right and left apices almost similar in size. Right laterobasal lobe small, with the width from a ventral view narrower than the width of the gonopore protrusion from a lateral view. Left laterobasal lobe small, with the width from a ventral lobe small, with the width of the gonopore protrusion from a lateral view. Right laterobasal lobe small, with the width of the gonopore protrusion from a lateral view. Right laterobasal lobe small, with the width of the gonopore protrusion from a lateral view. Right laterobasal lobe large, almost semispherical, slightly concave at the top. Left lateroapical lobe large, narrowed apically. Ventrobasal surface almost flat, without swelling.

**Type materials.** Holotype:  $\Diamond$  (NARO), Mount Takizawamine, alt. 1300 m, Kurokawa, Niigata Prefecture, Japan, 27.vii.1957, K. Baba leg. Paratypes (NARO):  $3\Diamond 6 \heartsuit$ , Kurokawa, Niigata Prefecture, Japan, K. Baba leg. (1 $\Diamond$ , Wataba, 25.vii.1957; 1 $\heartsuit$ , Wataba, 23.vii.1957; 1 $\heartsuit$ , Wataba, 24.vii.1957; 1 $\heartsuit$ , Wataba, 25.vii.1957; 1 $\heartsuit$ , Tamogi, alt. 600 m, 25.vii.1957; 1 $\Diamond$ , 18.ix.1959; 1 $\Diamond 2 \heartsuit$ , 23.vi.1964); 1 $\Diamond$ , Mount Kitamata, the Iide Mountains, Niigata Prefecture, Japan, 15.viii.1964, K. Baba leg.

**Etymology.** The specific name refers to the Iide Mountains, where the type materials were collected.

#### Nebria (Falcinebria) niohozana Bates, 1883

Figs 8–10, 22–26

Japanese name: Chûbu-hime-marukubi-gomimushi

- *Nebria reflexa* var. *Niohozana*: Bates (1883): 218 (original description), type locality: "Niohozan" (originally stated), changed to "Mikuni-toge Japan" through lectotype designation by Ledoux and Roux (1992: 42).
- Nebria (Paranebria) reflexa niohozana: Habu and Baba (1972): 3 (part).

Nebria reflexa: Uéno (1985): 56 (part).

*Nebria reflexa niohozana*: Ledoux and Roux (1992): 42, fig. 7b (lectotype (♂) designation); Nakane (1963b): 19, plate 10 fig. 1a (part).

Nebria (Orientonebria) reflexa niohozana: Farkač and Janata (2003): 94.

*Nebria (Falcinebria) reflexa niohozana*: Ledoux and Roux (2005): 831, plate-fig. 626; Yoshitake et al. (2011): 32 (part); Huber (2017): 50; Yoshimatsu et al. (2018): 37 (part).



**Figures 22–26.** Right lateral (**a**), ventral (**b**), dorsal (**c**), and anterodorsal (**d**) views of the endophallus of *Nebria niohozana* males from Mount Chôkai (**22**), Yumoto, a locality close to the collection site of cotypes in the original description (**23**), Doai, a locality close to the type locality designated in the lectotype designation (**24**), Renge-Onsen (**25**), and Mount Hakusan (**26**). Abbreviations are described in the caption of Figures 17–21.

**Notes.** This species was originally described as a variety of *N. reflexa* and was later treated as a subspecies. Although specimens described by Bates (1883) are from "Niohozan" (meaning Mount Nyohô of Nikkô in Tochigi Prefecture), the male specimen designated as the lectotype by Ledoux and Roux (1992) is from "Mikuni-toge" (the Mikuni Pass on the border between Niigata and Nagano prefectures), which is far from "Niohozan" (Fig. 1). Thus, the designated type locality differs between the original description and the lectotype designation. Comparative male genital morphology and macroscale distributions show that populations from "Niohozan" and "Mikuni-toge"

should be regarded as the same taxon. Here, the taxon *N. r. niohozana* is upgraded from a subspecies of the species *N. reflexa* to a distinct species, based on marked differences in endophallus morphology and confirmed and putative sympatry with other related species. The confirmed distribution of *N. niohozana* is wide, ranging from Mount Chôkai in the north to Mount Hakusan in the west. The species may be collected with other, related species in areas near Mount Gassan, and Hakusan and the Asahi and Iide Mountains. *Nebria niohozana* can be distinguished from similar species by a larger body size, smaller PPW/EL, and endophallus shape (in particular, Y-shaped lateroapical lobes). The morphological features of the male lectotype (Fig. 3) well match those of the specimens treated here as *N. niohozana* with respect to all macromorphological features, body length (10.18 mm), and PPW/EL (0.296).

**Description.** Body length: 3, 9.45-11.30 mm (mean  $\pm$  SD:  $10.49 \pm 0.39 \text{ mm}$ , n = 37;  $\bigcirc$ , 11.04–12.19 mm (mean  $\pm$  SD: 11.44  $\pm$  0.36 mm, n = 16). PPW/EL:  $\bigcirc$ , 0.281-0.334 (mean  $\pm$  SD:  $0.303 \pm 0.013$ , n = 37);  $\bigcirc$ , 0.273-0.317 (mean  $\pm$  SD:  $0.296 \pm 0.014$ , *n* = 16). Ventral surface of aedeagal apex not concave. Dorsobasal lobe absent. Dorsomedian lobe small or rudimentary, or sometimes absent. Dorsoapical lobe cylindrical, with the apex in an almost ventral direction; slightly curved anteriorly from a lateral view and left-laterally from a posterior view. Right laterobasal lobe small, with the width from a ventral view narrower than the width of the gonopore protrusion from a lateral view. Left laterobasal lobe small, with the width from a ventral view narrower than the width of the gonopore protrusion from a lateral view. Right lateroapical lobe bifurcated at the middle in a Y shape, with one apex directed posterolaterally and the other directed anterolaterally; the posterolateral apex smaller than the anterolateral apex; the anterolateral apex larger than right laterobasal lobe. Left lateroapical lobe bifurcated at the middle in a Y shape, with one apex directed posterolaterally and the other directed anterolaterally; the posterolateral apex similar in size to right laterobasal lobe. Ventrobasal surface with a pair of swelling adjoining each laterobasal lobe; swellings unconnected; ventrobasal swelling small, shaped as a gently sloped mountain from a lateral view.

**Materials examined.** 1 $\circ$  (NARO), Fushiogamidake, Mount Chôkai, Yuza-machi, Yamagata Prefecture, Japan, 23.vii.1970, K. Shirahata leg.; 1 $\oarphi$  (NARO), Mount Chôkai, alt. 1700 m, Yuza-machi, Yamagata Prefecture, 20.viii.1957, K. Shirahata leg.; 2 $\circ$  (NARO), Mount Gassan, Yamagata Prefecture, Japan, 20.viii.1959, K. Shirahata leg.; 2 $\circ$  (NARO), Mount Gando, Yamagata Prefecture, Japan, 6.vi.1954, S. Kimata leg.; 1 $\circa$  (KS), Ichimaiishi-sawa, alt. 1375 m, Mount Zaô, Shichikashuku-machi, Miyagi Prefecture, Japan (38.117581N, 140.425892E), 11.ix.2004, K. Sasakawa leg.; 1 $\circa$  (NARO), Ôtori-ike, alt. 1000 m, the Asahi Mountains, Yamagata Prefecture, Japan, 23.vii.1959, K. Baba leg.; 1 $\circa$  (NARO), Mount Kitamata, the Iide Mountains, Niigata Prefecture, Japan, 15.viii.1964, K. Baba leg.; 1 $\circa$  (NARO), Mount Monnai, alt. 1800 m, the Iide Mountains, Niigata Prefecture, 28.vii.1957, K. Baba leg.; 1 $\circa$  (NARO), Mount Futastumine, the Iide Mountains, Niigata Prefecture, Japan, K. Baba leg. (1 $\circa$ , alt. 1600 m, 27.viii.1957; 1 $\circa$ , 14.vii.1960); 6 $\circaa$  (KS), Kuratani-sawa, alt. 480 m, Ôaza-Iine, Okugawa, Nishiaizu-machi, Fukushima Prefecture, Japan, 22–24.

ix.2016, H. Itô leg.; 1d (NARO), Mount Ôtaki, Aizumisato-machi, Fukushima Prefecture, 15.vii.1950, Y. Kurosawa leg.;  $1\sqrt[3]{22}$  (KS), Makukawa-Onsen, Tsuchuyuonsen-machi, Fukushima-shi, Fukushima Prefecture, Japan, 23-24.v.2003, K. Sasakawa leg.; 1 (NARO), "Egawamura" [a part of Shimogou-machi], Fukushima Prefecture, Japan, 2.vi.1951, Y. Kurosawa leg.; 2Å, Mount Ôjôgo, the Asahi Mountains, Niigata Prefecture, 21.vii.1959, K. Baba leg.; 1d (NARO), Sandogoya-Onsen, Nasushiobarashi, Tochigi Prefecture, Japan, 22.viii.1963, S. Ueno leg.; 2d (NARO), Yumoto, Nikkô-shi, Tochigi Prefecture, Japan, K. Tanaka leg. (13, 21.vi.1963; 13, 22.vi.1963); 2Å, Oze (NARO), on the border between Hinoemata-mura, Fukushima Prefecture and Katashina-mura, Gunma Prefecture, 24.vii.1954, A. Habu leg.;  $1^{\circ}_{\circ}1^{\circ}_{\circ}$  (NARO), Shimizu Pass, alt. 1450 m, on the border between Uonuma-shi, Niigata Prefecture and Minakami-machi, Gunma Prefecture, 1.x.1969, K. Baba leg.; 1 (NARO), Doai, Minakami-machi, Gunma Prefecture, 2.x.1942, T. Takei leg.; 13 (NARO), Mount Tanigawa, Minakami-machi, Gunma Prefecture, 24.x.1955, K. Baba leg.; 432 (KS), Mount Naeba, Sakae-mura, Nagano Prefercture, Japan ( $432^{\circ}$ , 30.vi.2003, W. Toki leg.; 19, 3.viii.2003, J. Ogawa leg); 29 (NARO), Mount Korenge, Itoigawa-shi, Niigata Prefecture, Japan, 25.vii.1961, K. Baba leg (12, no data for altitude; 12, alt. 2500 m).; 1♂ (NARO), Renge-Onsen, Mount Shirouma, Niigata Prefecture, Japan, 24.vii.1961, K. Baba leg.; 3Å, Mount Hakusan (NARO), 1300-2000 m, Gifu Prefecture, Japan, 20.vi.1972, K. Tanaka & H. Ohira leg.; 1 (NARO), Keimatsudaira, Mount Hakusan, Ishikawa Prefecture, Japan, 1.viii.1961, A. Uchimura leg.

#### Nebria (Falcinebria) furcata sp. nov.

http://zoobank.org/34A51377-A57D-4266-91F0-0AE9D158D797 Figs 11, 27, 28 Japanese name: Inoue-hime-marukubi-gomimushi

Nebria reflexa: Uéno (1985): 56 (part); Nakane (1963b): 19 (part).

**Notes.** This species in known from two localities near Mount Hakusan where *N. ni-ohozana* and *N. pisciformis* sp. nov. co-occur. This species is distinguished from *N. niohozana* by a smaller body size and larger PPW/EL (values for male *N. niohozana* from Mount Hakusan: body length, 10.24–10.90 mm; PPW/EL, 0.289–0.321 (n = 4); values for females are not shown due to unavailability of specimens), and from *N. pisciformis*, by larger PPW/EL and a trifurcate apical margin of the dorsoapical lobe.

**Description.** Body length: 3, 9.27-9.65 mm (mean  $\pm$  SD:  $9.48 \pm 0.19 \text{ mm}$ , n = 3); 9, 10.21-10.60 mm (mean  $\pm$  SD:  $10.46 \pm 0.13 \text{ mm}$ , n = 4). PPW/EL: 3, 0.323-0.341 (mean  $\pm$  SD:  $0.334 \pm 0.009$ , n = 3); 9, 0.311-0.320 (mean  $\pm$  SD:  $0.316 \pm 0.004$ , n = 4). Ventral surface of aedeagal apex not concave. Dorsobasal lobe present. Dorsomedian lobe absent. Dorsoapical lobe with the basal part protruding dorsally; the protrusion as long as and 1.5 times as wide as the right laterobasal lobe; the apical portion directed ventrally, twice as wide as the subapical constriction; the apical mar-

gin divided into four projections. Right laterobasal lobe small, with the width from the ventral view narrower than the width of the gonopore protrusion from the lateral view. Left laterobasal lobe small, with the width from the ventral view narrower than the width of the gonopore protrusion from the lateral view. Right lateroapical lobe small, strongly bent at the middle in an anterior direction. Left lateroapical lobe moderate in size, more or less bifurcate. Ventrobasal surface almost flat, without swellings.

**Type materials.** Holotype: 3 (KS), Arashiguchi, Kamiuchinami, Ôno-shi, Fukui Prefecture, Japan, 26.v.2019, S. Inoue leg. Paratypes:  $1^{\circ}$  (KS), same data as the holotype;  $2^{\circ}_{3^{\circ}}$  (NARO), Amô Pass–Shoyashiki, Hida-shi, Gifu Prefecture, Japan, 19.vi.1972, K. Tanaka & H. Ohira leg.

**Etymology.** The specific name derives from the Latin word *furcata* (forked) and refers to the apical margin of the dorsoapical lobe.

#### Nebria (Falcinebria) pisciformis sp. nov.

http://zoobank.org/DBFF170F-058B-45D2-BB86-6FC3F261FEF1 Figs 12, 29 Japanese name: Hakusan-hime-marukubi-gomimushi

Nebria reflexa: Uéno (1985): 56 (part); Nakane (1963b): 19 (part).

**Notes.** This species is known only from the type locality, Öshirakawa-dani, which is situated on the eastern foot of Mount Hakusan. It is distinguished from *N. niohozana*, which is known from high altitude areas of Mount Hakusan, by a smaller body size, and from *N. furcata* sp. nov. by a lower PPW/EL and the shape of the dorsoapical lobe.

**Description.** Body length: 3, 9.51–9.54 mm (n = 2). PPW/EL: 3, 0.309–0.320 (n = 2). Ventral surface of aedeagal apex not concave. Dorsobasal lobe present. Dorsomedian lobe absent. Dorsoapical lobe with the basal part protruding anterodorsally; the protrusion as long as and 1.5 times as wide as right laterobasal lobe; the apical portion directed ventrally, less than twice the width of subapical constriction; the apical margin simple, not furcate. Right laterobasal lobe small, with the width from the ventral view narrower than the width of the gonopore protrusion from the lateral view. Left laterobasal lobe small, with the width from the ventral view narrower than the middle in an anterior direction. Left lateroapical lobe small, strongly bent at the middle in size and weakly bent at the middle in an anterior direction; the anterior part weakly swollen. Ventrobasal surface almost flat, without swelling.

**Type materials.** Holotype:  $\Im$  (NARO),  $\hat{O}$ shirakawa-dani, 800–1250 m, Shirakawa-mura, Gifu Prefecture, Japan, 19.vi.1972, K. Tanaka & H. Ohira leg.;  $1\Im$ , same data as the holotype.

**Etymology.** The specific name derives from a combination of the Latin words *piscis* (fish) and *-formis* (-shaped) and refers to the dorsal view of the dorsoapical lobe.



Figures 27-30. Right lateral (a), ventral (b), left lateral (c), and anterior (d) views of the endophallus and dorsal (e), posterodorsal (f), and left dorsolateral (g) views of the dorsoapical lobe of *Nebria* spp. 27 *N. furcata* sp. nov., holotype male 28 *N. furcata* sp. nov., paratype males from Amô Pass-Shoyashiki 29 *N. pisciformis* sp. nov., holotype male 30 *N. kuragadakensis* sp. nov., holotype male. db, dorsobasal lobe; other abbreviations are given in Figures 17–21. In *N. furcata* (27, 28), a<sub>1.4</sub> denotes apices of the dorsoapical lobe, and 28d' is presented to show individual variation in the focal endophallus structures.

# Nebria (Falcinebria) kuragadakensis sp. nov.

http://zoobank.org/C0496598-8A20-49F9-88FE-1CE7F6DCE9A4 Figs 13, 30 Japanese name: Kuragadake-hime-marukubi-gomimushi

Nebria reflexa: Uéno (1985): 56 (part); Nakane (1963b): 19 (part).

**Notes.** This species is known only from the type locality, Mount Kuragatake, a low mountain located northwest of Mount Hakusan. It is distinguished from *N. nioho-zana*, which is also distributed on Mount Hakusan, by a larger PPW/EL.

**Description.** Body length: 3, 9.59 mm (n = 1); 9, 10.28 mm (n = 1). PPW/EL: 3, 0.342 (n = 1); 9, 0.334 (n = 1). Ventral surface of aedeagal apex not concave. Dorsobasal lobe present. Dorsomedian lobe absent. Dorsoapical lobe with the basal part weakly swollen; the left dorsolateral surface behind the subapical constriction weakly swollen; the apical portion directed ventrally, less than twice the width of the subapical constriction; the apical margin very slightly bifurcated. Right laterobasal lobe small, with the width from the ventral view narrower than the width of the gonopore protrusion from the lateral view. Left laterobasal lobe small, with the width of the gonopore protrusion from the lateral view. Left lateroapical lobe small, weakly bent at the middle in an anterolateral direction. Left lateroapical lobe small, weakly bent as the middle in an anterolateral direction; the anterior part largely swollen, directed left-dorsoposteriorly. Ventrobasal surface almost flat, without swelling.

**Type materials.** Holotype:  $\Im$  (NARO), Mount Kuragadake, Kanazawa-shi, Ishikawa Prefecture, Japan, 20.v.1962, S. Takaba leg.; Paratype: 1 $\Im$  (NARO), same data as the holotype.

**Etymology.** The specific name refers to Mount Kuragadake, the type locality of this new species.

#### Nebria (Falcinebria) dichotoma sp. nov.

http://zoobank.org/A6290AFF-8569-4594-BFDC-BEEF9C11B1DF Figs 14, 31, 32 Japanese name: Dando-hime-marukubi-gomimushi

Nebria reflexa: Uéno (1985): 56 (part); Nakane (1963b): 19 (part).

**Notes.** This species is known only from the type locality, Mount Takanosu. Among known related species, it is similar to *N. chugokuensis* sp. nov. in having a relatively large-sized body and larger PPW/EL but is distinguished by a lack of concavity on the ventral surface of the aedeagal apex.

**Description.** Body length: 3, 9.80-10.34 mm (mean  $\pm$  SD:  $10.14 \pm 0.22 \text{ mm}$ , n = 5); 9, 10.49-11.12 mm (mean  $\pm$  SD:  $10.79 \pm 0.30 \text{ mm}$ , n = 6). PPW/EL: 3, 0.314-0.335 (mean  $\pm$  SD:  $0.322 \pm 0.010$ , n = 5); 9, 0.302-0.324 (mean  $\pm$  SD:  $0.312 \pm 0.008$ , n = 6). Ventral surface of aedeagal apex not concave. Dorsobasal lobe absent. Dorsomedian lobe small, directed right-laterally. Dorsoapical lobe with two small protrusions at the basal part; the apical portion directed ventrally, bifurcated in a Y-shape. Right laterobasal lobe large, with the width from a ventral view wider than the width of the gonopore protrusion from a lateral view. Left laterobasal lobe large, with the width from a ventral view directed posterolaterally and the other directed anterolaterally; the posterolateral apex very small; the anterolateral apex smaller than right laterobasal lobe. Left lateroapical lobe bifurcated at the middle in a Y shape, with one apex directed posterolateral apex; the anterolateral apex smaller than the anterolateral apex; the anterolateral apex smaller than the approximation approximation



**Figures 31–35.** Right lateral (**a**), ventral (**b**), dorsal (**c**), and posterodorsal (**d**) views of the endophallus and left laterodorsal view of the dorsoapical lobe (**e**) of *Nebria* spp. **31** *N. dichotoma* sp. nov., holotype male **32** *N. dichotoma* sp. nov., paratype male from the type locality **33** *N. uenoi*, male from the type locality **34** *N. chugokuensis* sp. nov., holotype male **35** *N. chugokuensis*, paratype male from Sandan-kyô. ac, concavity of aedeagal apex; remaining abbreviations are provided in Figures 17–21.

eral apex smaller than the right laterobasal lobe. Ventrobasal surface with a pair of swelling adjoining each laterobasal lobe; swellings conjoined; ventrobasal swelling absent.

**Type materials.** Holotype: 3 (NARO), Mount Takanosu (= Mount Dando), alt. 1000 m, Shitara-machi, Aichi Prefecture, Japan, 8.vi.1971, K. Tanaka & H. Ohira leg. Paratypes (NARO): 234, same data as the holotype; 232, same locality and collector (132, 24.vi.1972; 13, 4.viii.1972).

**Etymology.** The specific name derives from the Greek word *dichotoma* (divided into two) and refers to the apical portion of the dorsoapical lobe.

#### Nebria (Falcinebria) uenoi Nakane, 1963

Figs 15, 33 Japanese name: Kinki-hime-marukubi-gomimushi

*Nebria reflexa uenoi*: Nakane (1963a): 218 (original description), type locality: "Kasuga, Nara, Honshu"; Nakane (1963b): 19, plate 10 fig. 1b.

Nebria reflexa: Uéno (1985): 56 (part).

Nebria (Orientonebria) reflexa uenoi: Farkač and Janata (2003): 94.

Nebria (Falcinebria) reflexa uenoi: Ledoux and Roux (2005): 831, plate-fig. 627; Yoshitake et al. (2011): 32; Huber (2017): 50; Yoshimatsu et al. (2018): 37.

Nebria (Falcinebria) reflexa ssp.: Yoshitake et al. (2011): 33.

**Notes.** This species is known only from the type locality, Mount Kasuga. Although similar in body size and PPW/EL to *N. furcata*, *N. shirokawa*, and *N. kuagadakensis*, it is distinguished from these species by the shape of the endophallus.

**Description.** Body length: 3, 9.92-10.00 mm (n = 2); <math>9, 9.94-10.56 mm (mean  $\pm$  SD: mean  $\pm$  SD: 10.34  $\pm$  0.34 mm, n = 3). PPW/EL: 3, 0.332-0.345 (n = 2); 9, 0.314-0.330 (mean  $\pm$  SD: 0.324  $\pm$  0.009, n = 3). Ventral surface of aedeagal apex not concave. Dorsobasal lobe present. Dorsomedian lobe small. Dorsoapical lobe with basal part protruding posterodorsally; protrusion smaller than the right laterobasal lobe; the apical portion directed ventrally. Right laterobasal lobe large, with the width from the ventral view wider than the width of the gonopore protrusion from the lateral view. Left laterobasal lobe large, with the width from the ventral view mider than the lateral view. Right lateroapical lobe small, simply swollen. Left lateroapical lobe bifurcated at the base, with one apex directed posterolaterally and the other directed anterolaterally; the posterolateral apex and slightly smaller than the right laterobasal lobe. Ventrobasal surface with a pair of swelling adjoining each laterobasal lobe; swellings unconnected; ventrobasal swelling absent.

**Materials examined.** 2∂3♀ (NARO), Mount Kasuga, Nara Prefecture, Japan (1♀, 17.v.1952, H. Ishida leg.; 2∂, 27.v.1950, S. Ueno leg.; 2♀, 29.v.1950, S. Ueno leg.)

# *Nebria (Falcinebria) chugokuensis* sp. nov. http://zoobank.org/70FDF936-491C-4637-B851-35202CE729EA Figs 16, 34, 35 Japanese name: Chûgoku-hime-marukubi-gomimushi

Nebria reflexa: Uéno (1985): 56 (part); Nakane (1963b): 19 (part). Nebria (Falcinebria) reflexa uenoi: Yoshiake et al. (2011): 33 (part). **Notes.** This species is widely distributed in the Chûgoku Mountains. It is readily distinguished from other species previously considered *N. reflexa* distributed in Honshû by the concavity of the ventral surface of the aedeagal apex. It is distinguished from *N. hikosana*, described from nearby northern Kyûshû, by two pairs of setae on the ventral side of the sterna, versus one pair in *N. hikosana*.

**Description.** Body length: 3, 9.97-10.32 mm (mean  $\pm$  SD:  $10.12 \pm 0.13 \text{ mm}$ , n = 5); 9, 10.69-11.75 mm (mean  $\pm$  SD:  $11.22 \pm 0.53 \text{ mm}$ , n = 3). PPW/EL: 3, 0.312-0.324 (mean  $\pm$  SD:  $0.320 \pm 0.005$ , n = 5); 9, 0.305-0.317 (mean  $\pm$  SD:  $0.313 \pm 0.007$ , n = 3). Ventral surface of aedeagal apex deeply concave. Dorsobasal lobe absent. Dorsomedian lobe absent. Dorsoapical lobe with the basal part protruding dorsally; the protrusion as long as and narrower than the right laterobasal lobe; apical portion directed anteroventrally. Right laterobasal lobe large, with the width from a ventral view wider than the width of the gonopore protrusion from a lateral view. Left laterobasal lobe large, with the width of the gonopore protrusion from a lateral view. Right lateroapical lobe bifurcated at the middle, with one apex directed posterolaterally and the other directed anterolaterally; the anterolateral apex further bifurcated. Left lateroapical lobe bifurcated at the middle, with one apex directed posterolaterally and the other directed anterolaterally; the anterolateral apex further bifurcated. Ventrobasal surface without swellings adjoining the laterobasal lobes; ventrobasal swelling large, semispherical from a lateral view.

**Etymology.** The specific name refers to the Chûgoku Mountains, where this new species is distributed.

## Discussion

The previously recognized *N. reflexa* is composed of several "cryptic species" that are distinguished by the shape of the endophallus, an organ of the male genitalia (Fig. 36). Although these species are broadly similar in external appearance, some are distinguishable by morphometric values (Fig. 4). Sympatric occurrence was confirmed for one species pair (*N. iidesana* and *N. niohozana*), providing definitive evidence that these two species are not subspecies of a single species (i.e., geographical races) but rather are reproductively isolated and distinct. Further cryptic species previously considered as *N. reflexa* (e.g., Kasahara 1985; Satake and Kasahara 1985; The Japan Coleopterological Society 2007; Morita and Hirai 2010; Yoshitake et al. 2011; Hiramatsu and Usio 2018; Mori 2018; Yoshimatsu et al. 2018; Nakahama et al. 2019) could not be identified here, in some instances due to the unavailability of male specimens (Yoshitake et al. 2011;



**Figure 36.** Phylogenetic relationships of the 10 *Nebria* species inferred from two unambiguous synapomorphies. Right lateral views of the endophallus of all species are also shown to demonstrate the morphological diversity.

Yoshimatsu et al. 2018). In addition, populations on Shikoku (Yoshida et al. 2007; Yoshitomi et al. 2012) and those on Kyûshû from localities outside the type locality of *N. r. hikosana* (e.g., Nishida 2005) remain unexamined. Future examination of specimens from these populations is encouraged.

Although this work was not comprehensive of all collection sites, as described above, comparative morphology, mainly of the endophallus, and comparison of distribution patterns provided insight into the differentiation process of these species in the Japanese Archipelago. Among the endophallus characters observed here, the absence of a dorsobasal lobe in some species is noteworthy. In most *Nebria* species for which the endophallus has been examined (e.g., Dubko and Matalin 2002; Ledoux and Roux 2005; Sasakawa 2016; Sasakawa and Kubota 2006) and in a species of *Leistus* (Morita 2012), the sister taxon of *Nebria*, the dorsobasal lobe is well developed, suggesting that the absence of a dorsobasal lobe is an apomorphic character condition in *Nebria*. Thus, the six species

lacking a dorsobasal lobe, namely N. reflexa, N. sagittata, N. iidesana, N. niohozana, N. dichotoma, and N. chugokuensis, are considered to form a clade among the species examined. Within this clade, N. niohozana is likely the most derived, as it has the largest body size and widest distribution range, both of which are typical characteristics of derived species and/or lineages in other groups of Carabidae (e.g., Ishikawa 1979; Sota et al. 2005; Fujisawa et al. 2019). Nebria niohozana is likely sister to N. dichotoma, as they share Y-shaped lateroapical lobes. This lobe shape is found only in these two species and is considered to be apomorphic within the genus (Dubko and Matalin 2002; Ledoux and Roux 2005; Sasakawa and Kubota 2006; Sasakawa 2016). Phylogenetic relationships of the 10 species presented here are inferred as shown in Figure 36. Importantly, four species of the basal lineages (N. furcata, N. pisciformis, N. kuragadakensis, and N. uenoi) and N. dichotoma, which is the putative sister taxon of the derived species N. niohozana, are all distributed in the western Chûbu and eastern Kinki regions. This pattern indicates that the initial differentiation of these species likely occurred in these regions. This finding is similar to those in other flightless groups of Carabidae, e.g., the Carabus subgenus Ohomopterus (Carabini), the Nebria subgenus Sadonebria (Nebrini), Apatrobus (Patrobini), and Jujiroa (Platynini), in which the most ancestral species are distributed in the western Chûbu and/or eastern Kinki region and derived species occur in other regions (Sasakawa 2006; Sasakawa and Kubota 2006; Dejima and Sota 2017; Fujisawa et al. 2019). The western Chûbu and eastern Kinki regions are therefore likely to have been the main areas in Japan where the initial differentiation of various groups of Carabidae occurred.

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# Supplementary material I

## Table S1. Raw data for discriminant analysis

Authors: Kôji Sasakawa

Data type: statistical data

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RESEARCH ARTICLE



# Fourteen new species of the spider genus Psiloderces Simon, 1892 from Southeast Asia (Araneae, Psilodercidae)

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## Abstract

The genus *Psiloderces* Simon, 1892 is the second largest genus of Psilodercidae, a family restricted to Asia, occurring from East India to the Philippines. Fourteen new species of *Psiloderces* from Indonesia, the Philippines, and Vietnam are described: *P. heise* **sp. nov.** ( $\mathcal{J} Q$ ), *P. gawanaensis* **sp. nov.** ( $\mathcal{J} Q$ ), *P. bontocensis* **sp. nov.** ( $\mathcal{J} Q$ ), *P. cattienensis* **sp. nov.** ( $\mathcal{J} Q$ ), *P. pingguo* **sp. nov.** ( $\mathcal{J} Q$ ), *P. palopoensis* **sp. nov.** ( $\mathcal{J} Q$ ), *P. penajamensis* **sp. nov.** ( $\mathcal{J} Q$ ), *P. pingguo* **sp. nov.** ( $\mathcal{J} Q$ ), *P. bolang* **sp. nov.** ( $\mathcal{J} Q$ ), *P. mangen prov.* ( $\mathcal{J} Q$ ), *P. bolang* **sp. nov.** ( $\mathcal{J} Q$ ), *P. mangou* **sp. nov.** ( $\mathcal{J} Q$ ), *P. bolang* **sp. nov.** ( $\mathcal{J} Q$ ), *P. mangen* **sp. nov.** ( $\mathcal{J} Q$ ), *P. bolang* **sp. nov.** ( $\mathcal{J} Q$ ), *P. mangen* **sp. nov.** ( $\mathcal{J} Q$ ), *P. bolang* **sp. nov.** ( $\mathcal{J} Q$ ), *P. mangen* **sp. nov.** ( $\mathcal{J} Q$ ), *P. bolang* **sp. nov.** ( $\mathcal{J} Q$ ), *P. mangen* **sp. nov.** ( $\mathcal{J} Q$ ), *P. bolang* **sp. nov.** ( $\mathcal{J} Q$ ), *P. mangen* **sp. nov.** ( $\mathcal{J} Q$ ), *P. bolang* **sp. nov.** ( $\mathcal{J} Q$ ), *P. mangen* **sp. nov.** ( $\mathcal{J} Q$ ), *P. bolang* **sp. nov.** ( $\mathcal{J} Q$ ), *P. mangen* **sp. nov.** ( $\mathcal{J} Q$ ), and *P. malinoensis* **sp. nov.** ( $\mathcal{J} Q$ ). Types are deposited in the Institute of Zoology, Chinese Academy of Sciences (IZCAS) in Beijing.

#### Keywords

Borneo, Indonesia, Kalimantan, Philippines, Sulawesi, Vietnam

# Introduction

The spider family Psilodercidae Machado, 1951 was treated as a subfamily of Ochyroceratidae Fage, 1912 until Wunderlich (2004) elevated it to the family level. Currently, it is known by 11 genera and 151 species distributed in Southern Asia from India to the Philippines (Li and Quan 2017; WSC 2019). The number of species in Psilodercidae has increased nearly threefold during the 21<sup>th</sup> century (Platnick 2000). More than half of psilodercid genera have been described only recently: *Flexicrurum* Tong & Li, 2007, *Luzonacera* Li & Li, 2017, *Priscaleclercera* Wunderlich, 2017, *Qiongocera* Li & Li, 2017, *Sinoderces* Li & Li, 2017, and *Thaiderces* Li & Li, 2017.

At present, 24 species of *Psiloderces* Simon, 1892 are known from China, Indonesia, Malaysia, the Philippines, Sri Lanka, and Thailand (WSC 2019). About three quarters of the known species are found in Indonesia and Thailand. Up to now, the type species *Psiloderces egeria* Simon, 1892 and another four species, i.e., *P. elasticus* Brignoli, 1975, *P. kalimantan* Deeleman-Reinhold, 1995, *P. penaeorum* Deeleman-Reinhold, 1995, and *P. tesselatus* Deeleman-Reinhold, 1995 are known only from female specimens, and *P. dicellocerus* Li, Li & Jäger, 2014, *P. fredstonei* Deeleman-Reinhold, 1995, *p. nasicornis* Baert, 1988 are known only from male specimens. Deeleman-Reinhold (1995) provisionally classified *Psiloderces* species into nine groups based on a combination of characteristics of the vulva and palp.

During the examination of a spider collection from Southeast Asia, we found fourteen new species of *Psiloderces* from Kalimantan and Sulawesi (Indonesia), the Philippines, and Vietnam. The goals of this paper are to provide detailed descriptions of the new species with images of their copulatory organs and chelicerae, as well as discuss their placement in the aforementioned species groups (Deeleman-Reinhold 1995).

#### Materials and methods

Types are deposited in the Institute of Zoology, Chinese Academy of Sciences (IZCAS) in Beijing. All specimens were observed and preserved in 95% ethanol. The specimens were measured and examined using a Leica M205 C stereomicroscope, and further morphological details were observed with an Olympus BX41 compound microscope. The left palp of the male was detached for further examination (except for *Psiloderces penajamensis*, the right palp was detached). Carapace length was measured excluding the clypeus. The internal genitalia and male palpal bulb were dissected and immersed in lactic acid. An Olympus C7070 wide zoom digital camera (7.1 megapixels) mounted on an Olympus SZX12 stereomicroscope was used to take photos in different focal planes. The photos were then transferred to Helicon Focus 6.7.1 image stacking software to improve depth of field before further revision with Adobe Photoshop CC 2014. Leg measurements are shown as total length (femur, patella, tibia, metatarsus, and tarsus). Leg segments were measured from their retrolateral side. All measurements are given in millimetres (mm). All terminology follows Li et al. (2014).

#### Taxonomy

#### Family Psilodercidae Machado, 1951

#### Genus Psiloderces Simon, 1892

*Psiloderces* Simon, 1892: 40. *Psiloderces*: Deeleman-Reinhold, 1995: 7. Type species. Psiloderces egeria Simon, 1892 from the Philippines.

Emended diagnosis. Psiloderces resembles Thaiderces by having a shallow, dark brown fovea, a cheliceral promargin with a lamina bearing 3 triangular extensions, the retromargin with 2 small teeth, and the anterior part of the thoracic region is distinctly elevated, but it can be differentiated by the following characters: 1) the presence of a cymbial protrusion (vs. absent in Thaiderces); 2) the presence of an inconspicuous clypeal protrusion in *P. enigmatus* Deeleman-Reinhold, 1995, *P. pulcher* Deeleman-Reinhold, 1995, *P. incomptus*, *P. nasicornis*, *P. gawanaensis* sp. nov., *P. cuyapoensis* sp. nov., *P. xichang* sp. nov., *P. cattienensis* sp. nov., *P. pingguo* sp. nov., and *P. penajamensis* sp. nov. (vs. absent in Thaiderces); and 3) the presence or absence of a laminar apophysis or a bulge. If present, then the embolus and laminar apophysis are not separated basally, or the bulge is separated basally from the embolus (vs. absence of bulge and such combination of embolus and laminar apophysis in Thaiderces).

**Remarks.** The type species of the genus is known from a female, and the female genitalia is insufficient for genus identification. However, the somatic morphology together with the morphology of male of the species and DNA barcoding data all confirm that these species belong to the genus *Psiloderces*.

**Species groups.** Nine *Psiloderces* species groups were established by Deeleman-Reinhold (1995). They are:

- *althepoides*-group: endogyne with a pair of sessile spermathecae; bulb pyriform, with tapering tip, extremely long legs. Species included: *P. althepoides* Deeleman-Reinhold, 1995 (♂♀).
- *egeria*-group: endogyne with a pair of sausage-like spermathecae; bulb syringiform. Species included: *P. palopoensis* sp. nov. (♂♀) and *P. egeria* (♀) (type species).
- *enigmatus*-group: endogyne with a pair of sessile or sausage-like spermathecae; embolus arises distally from bulb; male with clypeus projection. Species included: *P. gawanaensis* sp. nov. ( $\mathcal{J} Q$ ), *P. cuyapoensis* sp. nov. ( $\mathcal{J} Q$ ), *P. xichang* sp. nov. ( $\mathcal{J} Q$ ), *P. penajamensis* sp. nov. ( $\mathcal{J} Q$ ), *P. enigmatus* ( $\mathcal{J} Q$ ), *P. pulcher* ( $\mathcal{J} Q$ ), and *P. tesselatus* ( $\mathcal{Q}$ ).
- *howarthi*-group: endogyne with membranous projection, guiding ridges, and a pair of sausage-like, sessile spermathecae; bulb syringiform. Species included: *P. howarthi* Deeleman-Reinhold, 1995 (♂♀).
- *leucopygius*-group: endogyne with 2 pairs of pedunculated spermathecae; palp with simple syringiform bulb. Species included: *P. pingguo* sp. nov. ( $\mathscr{J} Q$ ), *P. elasticus* (Q), *P. dicellocerus* ( $\mathscr{J}$ ), *P. incomptus* ( $\mathscr{J}$ ), *P. leucopygius* Deeleman-Reinhold, 1995 ( $\mathscr{J} Q$ ), *P. vallicola* Deeleman-Reinhold, 1995 ( $\mathscr{J} Q$ ), *P. limosa* ( $\mathscr{J}$ ), *P. coronatus* Deeleman-Reinhold, 1995 ( $\mathscr{J} Q$ ), and *P. penaeorum* (Q).
- *ligula*-group: endogyne with sausage-like spermathecae; male with coiled embolus with spatula-shaped tip. Species included: *P. ligula* Baert, 1988 (♂♀).
- *longipalpis*-group: endogyne with a pair of sessile spermathecae; bulb constricted medially, embolus arises distally; male without clypeus protrusion. Species included: *P. grohotensis* sp. nov. (♂♀), *P. bangkiraiensis* sp. nov. (♂♀), *P. bolang* sp. nov. (♂♀),

*P. wangou* sp. nov.  $(\mathcal{J} \mathcal{Q})$ , *P. malinoensis* sp. nov.  $(\mathcal{J} \mathcal{Q})$ , *P. longipalpis* Baert 1988  $(\mathcal{J} \mathcal{Q})$ , *P. nasicornis*  $(\mathcal{J})$ , *P. torajanus* Deeleman-Reinhold, 1995  $(\mathcal{J} \mathcal{Q})$ , *P. leclerci* Deeleman-Reinhold, 1995  $(\mathcal{J} \mathcal{Q})$ , and *P. kalimantan*  $(\mathcal{Q})$ .

- *mulcatus*-group: endogyne with a pair of stalked or pedunculated spermathecae; bulb with indentation medially, embolus arises distally. Species included: *P. heise* sp. nov.  $(\stackrel{\frown}{\bigcirc} \bigcirc)$  and *P. bontocensis* sp. nov.  $(\bigcirc)$ .
- *septentrionalis*-group: endogyne with 2 pairs of sessile sausage-like spermathecae; bulb simple syringiform. Species included: *P. cattienensis* sp. nov. ( $\Diamond \heartsuit$ ), *P. septentrionalis* Deeleman-Reinhold, 1995 ( $\Diamond \heartsuit$ ), *P. suthepensis* Deeleman-Reinhold, 1995 ( $\Diamond \heartsuit$ ), *P. albostictus* Deeleman-Reinhold, 1995 ( $\Diamond \heartsuit$ ), and *P. fredstonei* ( $\Diamond$ ).

**Distribution.** The genus is known from China, Vietnam to the Philippines, and south to Indonesia (Kalimantan and Sulawesi).

#### Psiloderces heise Li & Chang, sp. nov.

http://zoobank.org/301F1310-5C27-488B-A335-2C26D5CFCC9B Figs 1, 2, 28C, 30

**Types.** *Holotype*: ∂ (IZCAS), Philippines, Luzon Island, Tarlac Province, Tarlac City, near Monasterio de Tarlac, 15°26.8998'N, 120°25.6710'E, 123 m, 20.V.2015, F. Ballarin & Y. Li. *Paratype*: 1♀ (IZCAS), same data as holotype.

**Etymology.** The species name is a noun in apposition derived from the Chinese pinyin "hēisè" (black) and refers to the unique black color of the embolus.

**Diagnosis.** Males of *P. heise* sp. nov. can be distinguished from all other species of the genus by the structure of the bulb with a distinct prolateral indentation (Fig. 2C, D), a cymbial protrusion with a right-angled attachment (Fig. 2C), and the thick and dark embolus (vs. the absence of an indentation, a right-angled attachment, and different colored embolus in congeners); females can be differentiated from congeners by a pair of horizontally stalked spermathecae that width almost equally wide with globose distal part (Fig. 1A).

**Description. Male** (Holotype). Total length 1.46; carapace 0.56 long, 0.63 wide; abdomen 0.90 long, 0.70 wide. Carapace round and brown, with 3 longitudinal dark brown bands, median band half length of carapace, lateral bands eight times wider than median band (Fig. 1C). Chelicerae pale brown (Fig. 28C). Clypeus slanting, brown. Endites brown. Labium dark brown. Sternum dark brown, delimiting light brown band medially. Abdomen elongated, dorsum with purplish stripes posteriorly, antero-ventrally brown with pair of circular patches followed by semi-circular patch, posterior half with indistinct brown and light brown patterns. Legs uniformly brown; measurements: I 3.86 (1.00, 0.20, 1.09, 1.02, 0.55), II 4.38 (1.25, 0.20, 1.20, 1.10, 0.63), III 3.75 (1.00, 0.25, 1.00, 1.00, 0.50), IV 5.31 (1.41, 0.20, 1.60, 1.40, 0.70). Palp (Fig. 2A–D): femur slender, 3 times longer than patella; patella not swollen; tibia 2 times shorter than femur but almost as wide as cymbium; cymbium pale, 2 times



**Figure 1.** *Psiloderces heise* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** male habitus, dorsal view **D** female habitus, dorsal view **E** female habitus, ventral view. Abbreviation: SP = spermatheca.



**Figure 2.** *Psiloderces heise* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: BT = bristle, CP = cymbial protrusion, EM = embolus, LA = laminar apophysis.

wider than femur but almost as long as femur, cymbial protrusion with right-angled stick-out attachment and distinct bristles basally and anteriorly; bulb light yellow, pyriform, with slight indentation promarginally, laminar apophysis and embolus arise distally; laminar apophysis not separated from embolus basally, shorter than embolus; embolus thicker and darker than laminar apophysis, bent apically (Fig. 2B).

**Female** (Paratype). General features and coloration similar to those of male (Fig. 1D, E). Measurements: total length 1.40; carapace 0.50 long, 0.47 wide; abdomen 0.90 long, 0.63 wide. Leg measurements: I 4.16 (1.10, 0.16, 1.25, 1.02, 0.63), II 3.45 (0.90, 0.20, 0.94, 0.86, 0.55), III 3.72 (1.00, 0.13, 1.10, 1.02, 0.47), IV 2.81 (0.70, 0.16, 0.78, 0.70, 0.47). Endogyne (Fig. 1A): transverse stalked spermathecae bearing globose distal parts, heads almost as wide as stalks, stalks 10 times longer than heads.

**Distribution.** Known only from the type locality (Fig. 30).

#### Psiloderces gawanaensis Li & Chang, sp. nov.

http://zoobank.org/15281939-437A-4A72-B5E3-ABF4BA47B4A7 Figs 3, 4, 28F, 30

**Types.** *Holotype*: ∂ (IZCAS), Philippines, Luzon Island, Mountain Province, Bontoc Town, road to Gawana Town, 17°3.6396'N, 121°3.0402'E, 1674 m, 26.V.2015, F. Ballarin & Y. Li. *Paratype*: 1♀ (IZCAS), same data as holotype.

Etymology. The species name is an adjective referring to the type locality.

**Diagnosis.** Males of *P. gawanaensis* sp. nov. can be distinguished from congeners by the human heart-shaped bulb (inverted pyriform) (Fig. 4B) bearing a threadlike embolus, the presence of 4 distinct bristles on the cymbial protrusion, and the clypeal projection bearing a few setae (Fig. 4C, D) (vs. absence or less than 4 bristles on cymbial protrusion); females can be differentiated from congeners by the widely spaced, horseshoe-shaped spermathecae (Fig. 3A).

**Description.** Male (Holotype). Total length 1.54; carapace 0.60 long, 0.63 wide; abdomen 0.94 long, 0.56 wide. Carapace wider than long, brown, with 3 longitudinal dark brown bands, median band half length of carapace, median band two times wider than lateral band (Fig. 3D). Chelicerae pale brown (Fig. 28F). Clypeus dark brown, with long and slightly bent medial projection, bearing few setae apically (Fig. 3C, D). Endites dark brown. Labium dark brown delimiting pair of light brown circular spots. Sternum dark brown, delimiting light brown band medially. Abdomen elongated, dorsum with brown stripes, antero-ventrally brown with elliptical patch, posterior part with indistinct brown pattern. Legs uniformly brown; measurements: I 5.21 (1.33, 0.25, 1.63, 1.20, 0.80), II 4.03 (1.09, 0.20, 1.25, 0.94, 0.55), III 3.49 (0.94, 0.20, 0.94, 0.86, 0.55), IV 4.88 (1.25, 0.25, 1.38, 1.25, 0.75). Palp (Fig. 4A–D): femur slender, 4 times longer than patella; patella not swollen; tibia 3 times shorter than femur; cymbium purplish distally, 2 times shorter than wide, and 2 times wider than femur, protrusion with threadlike embolus arising distally (Fig. 4B).



**Figure 3.** *Psiloderces gawanaensis* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** male habitus, lateral view **D** male habitus, dorsal view **E** female habitus, ventral view. Abbreviations: PR = clypeal protrusion, SP = spermatheca.



**Figure 4.** *Psiloderces gawanaensis* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: BT = bristle, CP = cymbial protrusion, EM = embolus.

**Female** (Paratype). General features and coloration similar to those of male (Fig. 3E, F). Measurements: total length 1.64; carapace 0.70 long, 0.60 wide; abdomen 0.94 long, 1.20 wide. Leg measurements: I 5.23 (1.25, 0.25, 1.60, 1.25, 0.88), II 4.30 (1.10, 0.20, 1.30, 1.00, 0.70), III missing, IV 5.24 (1.30, 0.23, 1.60, 1.33, 0.78). Endogyne (Fig. 3A): encircled spermathecae resemble a horseshoe, curving downwards (Fig. 3A).

**Distribution.** Known only from the type locality (Fig. 30).

#### Psiloderces bontocensis Li & Chang, sp. nov.

http://zoobank.org/F99F7890-8D40-449E-900D-E3B3FD7FB52E Figs 5, 28E, 30

**Types.** *Holotype*:  $\bigcirc$  (IZCAS), Philippines, Luzon Island, Mountain Province, Bontoc Town, road to Banawe Town, 16°59.6630'N, 121°1.1120'E, 1470 m, 25.V.2015, F. Ballarin & Y. Li.

**Etymology.** The species name is an adjective referring to the type locality.

**Diagnosis.** The female of *P. bontocensis* sp. nov. can be distinguished from others by a pair of stalked spermathecae with bulbous distal parts, spermathcae almost 8 times wider than stalk, resembling balloons (vs. balloon-shaped spermathecae absent in congeners) (Fig. 5A).

**Description. Female** (Holotype). Total length 2.00; carapace 0.60 long, 0.70 wide; abdomen 1.40 long, 0.81 wide. Carapace almost round, wider than long, brown, with 3 longitudinal dark brown bands, median band extends only half length of carapace, lateral bands almost as wide as the middle band (Fig. 5C). Chelicerae brown (Fig. 28E). Clypeus slanting, dark brown. Endites dark brown, light brown basally. Labium dark brown, delimiting pair of light brown circular spots. Sternum dark brown, delimiting light brown patch medially. Abdomen elongated, dorsum with mixture of dark and pale brown forming indistinct pattern (Fig. 5C), antero-ventrally dark brown with semi-circular brown patch medially, posterior part with light brown dots laterally (Fig. 5D). Legs uniformly brown; measurements: I 5.20 (1.25, 0.25, 1.60, 1.30, 0.80) II 4.32(1.09, 0.20, 1.25, 1.09, 0.69), III 3.48 (0.88, 0.20, 0.90, 0.90, 0.60), IV 5.15(1.25, 0.20, 1.60, 1.30, 0.80). Endogyne (Fig. 5A): stalked spermathecae with bulbous distal part, stalks two times longer than bulbous part, bulbous part 8 times wider than stalks (Fig. 5A).

Male. Unknown.

Distribution. Known only from the type locality (Fig. 30).

#### Psiloderces cuyapoensis Li & Chang, sp. nov.

http://zoobank.org/7E6FFA31-1180-4D0D-9F08-AF683147B829 Figs 6, 7, 28D, 30

**Types.** *Holotype*: ♂ (IZCAS), Philippines, Luzon Island, Nueva Ecija Province, Cuyapo City, 15°47.8086'N, 120°39.3294'E, 60 m, 22.V.2015, F. Ballarin & Y. Li. *Paratype*: 1♀ (IZCAS), same data as holotype.



**Figure 5**. *Psiloderces bontocensis* sp. nov., female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** female habitus, dorsal view **D** female habitus, ventral view. Abbreviation: SP = spermatheca.



**Figure 6.** *Psiloderces cuyapoensis* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** male habitus, lateral view **D** male habitus, dorsal view **E** female habitus, dorsal view **F** female habitus, ventral view. Abbreviations: PR = clypeal protrusion, SP = spermatheca.


**Figure 7.** *Psiloderces cuyapoensis* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: BT = bristle, CP = cymbial protrusion, EM = embolus.

Etymology. The species name is an adjective referring to the type locality.

**Diagnosis.** Males of *P. cuyapoensis* sp. nov. resemble those of *P. xichang* sp. nov. but can be distinguished by the rounded embolus (vs. wavy embolus), 3 distinct bristles on the cymbial protrusion (vs. 2 distinct bristles on cymbial protrusion), length of palp ca 6 times the length of bulb (vs. extremely slender and long palp, ca 10 times the length of bulb (Fig. 9C, D), and coloration of male and female are pale brown (vs. coloration of male and female are darker brown); females can be distinguished by tubular spermathecae (vs. earlobe-shaped spermathecae).

**Description. Male** (Holotype). Total length 1.60; carapace 0.60 long, 0.67 wide; abdomen 1.00 long, 0.78 wide. Carapace round and brown, with 3 longitudinal dark brown bands, median band half carapace length, median band almost as wide as the lateral bands (Fig. 6D). Chelicerae pale brown (Fig. 28D). Clypeus dark brown, with long, slightly bent medial projection (Fig. 6C, D). Endites light brown. Labium dark brown delimiting pair of light brown circular dots. Sternum dark brown, delimiting light brown band medially. Abdomen elongated, dorsum with brown stripes, anteroventrally pale brown with elliptical patch, posterior with purplish patterns. Legs uniformly brown; measurements: I–III missing, IV 5.21 (1.33, 0.25, 1.50, 1.38, 0.75). Palp (Fig. 7A–D): femur slender, 4 times longer than patella; patella not swollen; tibia 1.50 times shorter than femur; cymbium 2 times shorter and almost as wide as femur, protrusion with 3 distinct bristles basally and anteriorly; bulb pale brown, bulging pyriform with embolus arising distally; embolus rounded and blunt (Fig. 7B).

**Female** (Paratype). General features and coloration similar to those of male (Fig. 6E, F). Measurements: total length 1.64; carapace 0.47 long, 0.63 wide; abdomen 1.17 long, 0.78 wide. Leg measurements: I–IV missing. Endogyne (Fig. 6A): tubular spermathecae with indistinct concave ducts posteriorly (Fig. 6A).

Distribution. Known only from the type locality (Fig. 30).

#### Psiloderces xichang Li & Chang, sp. nov.

http://zoobank.org/A219F896-1005-440F-B783-6E8A08B3252E Figs 8, 9, 28G, 30

**Types.** *Holotype*: ∂ (IZCAS), Philippines, Luzon Island, Rizal Province, Quezon City, Rodriguez area, Montalban Village, Pamitinan Cave, 14°43.7916'N, 121°11.3580'E, 102 m, 5.VI.2015, F. Ballarin & Y. Li. *Paratype*: 1♀ (IZCAS), same data as holotype.

**Etymology.** The species name is a noun in apposition derived from the Chinese pinyin "xìcháng" (slender and long) and refers to the extremely slender palp structures.

**Diagnosis.** Diagnostic features of males and females are discussed in the diagnosis of *P. cuyapoensis* sp. nov.

**Description. Male** (Holotype). Total length 2.00; carapace 0.70 long, 0.90 wide; abdomen 1.30 long, 1.00 wide. Carapace round and brown, with 2 longitudinal dark brown bands laterally, medially with dark brown line (Fig. 8D). Chelicerae brown (Fig. 28G). Clypeus dark brown, with long, slightly bent medial projection, bearing few setae



**Figure 8.** *Psiloderces xichang* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** male habitus, lateral view **D** male habitus, dorsal view **E** female habitus, dorsal view **F** female habitus, ventral view. Abbreviations: PR = clypeal protrusion, SP = spermatheca.



**Figure 9.** *Psiloderces xichang* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: BT = bristle, CP = cymbial protrusion, EM = embolus.

(Fig. 8C, D). Endites dark brown, light brown basally. Labium dark brown delimiting pair of light brown circular dots. Sternum dark brown, delimiting light brown band medially. Abdomen elongated, dorsum with complex brown patterns, antero-ventrally dark brown with pair of circular patches followed by semi-circular patch, posterior with indistinct dark brown and light brown patterns. Legs uniformly brown; measurements: I 10.30 (2.80, 0.25, 3.20, 2.80, 1.25), II 8.91 (2.40, 0.31, 2.60, 2.60, 1.00), III 6.23(1.72, 0.25, 1.75, 1.63, 0.88), IV 9.34 (2.25, 1.00, 2.66, 2.34, 1.09). Palp equally wide throughout its length (Fig. 9A–D): femur extremely slender, 6 times longer than patella; patella not swollen; tibia 2.50 times shorter than femur; cymbium 2 times shorter than femur, protrusion with 2 distinct bristles basally and anteriorly; bulb pale brown, bulging pyriform with embolus arising distally; embolus irregular, resembles a petal (Fig. 9A).

**Female** (Paratype). General features and coloration similar to those of male (Fig. 8E, F). Measurements: total length 2.16; carapace 0.75 long, 0.86 wide; abdomen 1.41 long, 1.02 wide. Leg measurements: I 9.66 (2.60, 0.31, 3.00, 2.66, 1.09), II 7.91 (2.13, 0.31, 2.34, 2.19, 0.94), III 6.63 (1.63, 0.25, 1.50, 1.50, 1.75), IV 10.96 (2.80, 0.31, 3.60, 3.00, 1.25) Endogyne (Fig. 8A): widely spaced, earlobe-like spermathecae, ratio of spermathecae interdistances and spermathecae width 1:4 (Fig. 8A).

Distribution. Known only from the type locality (Fig. 30).

## Psiloderces cattienensis Li & Chang, sp. nov.

http://zoobank.org/EA2D9AD0-68C6-406A-89AF-E33968EC8712 Figs 10, 11, 28H, 30

**Types.** *Holotype*: ∂ (IZCAS), Vietnam, Dong Nai Province, Cat Tien National Park, 11°27.3620'N, 107°26.4980'E, 168 m, 4.IX.2015, Q. Zhao, Y. Li & Z. Chen. *Paratype*: 1♀ (IZCAS), same data as holotype.

Etymology. The species name is an adjective referring to the type locality.

**Diagnosis.** Males of *P. cattienensis* sp. nov. resemble those of *P. pingguo* sp. nov. but can be distinguished by the relatively long embolus, which is equal to the length of the tegulum (vs. embolus two times shorter than the tegulum), the bulb is rather angular (vs. bulging); females can be distinguished by the merged tubular spermathecae (vs. stalked spermathecae globose distally).

**Description.** Male (Holotype). Total length 1.32; carapace 0.44 long, 0.47 wide; abdomen 0.88 long, 0.50 wide. Carapace round and brown, with trident brown stripes medially and dark brown patches laterally (Fig. 10D). Chelicerae brown, cheliceral promargin with lamina bearing 2 triangular extensions (Fig. 10C, D). Endites dark brown. Labium dark brown delimiting pair of indistinct light brown circular dots. Sternum dark brown, delimiting light brown band medially. Abdomen elongated, dorsum with complex dark brown patterns, antero-ventrally dark brown with elliptical patch, posterior with 4 pairs of light brown vertical lines laterally and medially. Legs uniformly brown; measurements: I missing, II 3.45 (0.94, 0.13, 0.88, 0.94, 0.56), III 2.94 (0.81, 0.13, 0.81, 0.75, 0.44), IV missing. Palp (Fig. 11A–D): femur slender, 4 times longer than patella; patella



**Figure 10.** *Psiloderces cattienensis* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** male habitus, lateral view **D** male habitus, dorsal view **E** female habitus, ventral view. Abbreviations: PR = clypeal protrusion, SP = spermatheca.



**Figure 11.** *Psiloderces cattienensis* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: CP = cymbial protrusion, EM = embolus.

not swollen; tibia 2 times shorter than femur; cymbium almost as long and wide as tibia, cymbial protrusion darkens distally; bulb pale brown, widely turbinate with embolus arising distally; embolus slightly bent at tip, almost as long as entire bulb (Fig. 11B).

**Female** (Paratype). General features and coloration similar to those of male (Fig. 10E, F). Measurements: total length 1.28; carapace 0.47 long, 0.55 wide; abdomen 0.81 long, 0.47 wide. Leg measurements: I 4.49 (1.13, 0.16, 1.41, 1.09, 0.70), II 3.53 (0.90, 0.20, 1.02, 0.86, 0.55), III 2.94 (0.75, 0.16, 0.81, 0.75, 0.47), IV 4.11 (1.00, 0.13, 1.33, 1.02, 0.63). Endogyne (Fig. 10A): spermathecae with lobes, cylindrical anteriorly and oval posteriorly, anterior lobes extended anteriorly and posterior lobes extended laterally.

**Distribution.** Known only from the type locality (Fig. 30).

#### Psiloderces pingguo Li & Chang, sp. nov.

http://zoobank.org/4D0C497A-B952-41BF-861B-A92F7113EE7F Figs 12, 13, 28I, 30

**Types.** *Holotype*: ∂ (IZCAS), Vietnam, Ninh Thuan Province, Nui Chua National Park, 11°43.9830'N, 107°11.1300'E, 102 m, 31.VIII.2015, Q. Zhao, Y. Li & Z. Chen *Paratype*: 1♀ (IZCAS), same data as holotype.

**Etymology.** The species name is a noun in apposition derived from the Chinese pinyin "píngguð" (apple) and refers to the structure of the bulb which resembles an apple.

**Diagnosis.** Diagnostic features of the males and females are discussed in the diagnosis of *P. cattienensis* sp. nov.

**Description.** Male (Holotype). Total length 1.25; carapace 0.47 long, 0.47 wide; abdomen 0.78 long, 0.47 wide. Carapace round and brown, with trident brown stripes medially and dark brown patches laterally (Fig. 12D). Chelicerae brown, cheliceral promargin with lamina bearing 2 triangular extensions (Fig. 28I). Clypeus dark brown, with bifurcate medial projection (Fig. 12C, D). Endites brown, light brown basally. Labium dark brown, delimiting pair of light brown circular dots. Sternum dark brown. Abdomen elongated, dorsum with complex brown patterns, antero-ventrally dark brown with pair of kidney-shaped patches followed by ovate patch, posterior with light brown vertical lines laterally. Legs uniformly brown; measurements: I–IV missing. Palp (Fig. 13A–D): femur slender, 3 times longer than patella; patella not swollen; tibia 1.5 times shorter than femur; cymbium almost as long and wide as tibia, protrusion darkens distally; bulb pale yellow, wide and cuneate with embolus arising medially; embolus slightly bent, half length of tegulum (Fig. 13B).

**Female** (Paratype). General features and coloration similar to those of male (Fig. 12E, F). Measurements: total length 1.56; carapace 0.47 long, 0.55 wide; abdomen 1.09 long, 0.86 wide. Leg measurements: I–IV missing. Endogyne (Fig. 12A): spermathecae stalked, with globose distal part, globose part almost as wide as stalk, stalks 4 times longer than globose part, spermathecae anteriorly elliptical with a pair of tubular ducts (Fig. 12A).

**Distribution.** Known only from the type locality (Fig. 30).



**Figure 12.** *Psiloderces pingguo* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** male habitus, lateral view **D** male habitus, dorsal view **E** female habitus, ventral view. Abbreviations: PR = clypeal protrusion, SP = spermatheca.



**Figure 13.** *Psiloderces pingguo* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: CP = cymbial protrusion, EM = embolus.

http://zoobank.org/97B946FD-697D-4C94-AFEF-7DE3CA26DB4F Figs 14, 15, 29C, 30

**Types.** *Holotype*:  $\delta$  (IZCAS), Indonesia, Sulawesi, mountain in Palopo, 2°59.9210'S, 120°08.5650'E, 465 m, 2.IX.2017, H. Liu & Z. Chen. *Paratype*: 1 $\bigcirc$  (IZCAS), same data as holotype.

Etymology. The species name is an adjective referring to the type locality.

**Diagnosis.** Males of *P. palopoensis* sp. nov. can be distinguished from all other species of the genus by the swollen ovate shape of the bulb bearing a short embolus distally (Fig. 15B), 5 times shorter than the length of the tegulum (vs. absence of the extreme length difference of the embolus and tegulum in congeners), and relatively pale color of the male and female; female can be distinguished by widely separated, short, digitiform spermathecae (Fig. 14A).

**Description.** Male (Holotype). Total length 1.41; carapace 0.55 long, 0.63 wide; abdomen 0.86 long, 0.50 wide. Carapace round and pale brown, with trident brown stripes medially and brown patches laterally (Fig. 14C). Chelicerae pale brown (Fig. 29C). Clypeus slanting, purplish. Endites purplish, light brown basally. Labium purplish, delimiting pair of light brown circular dots. Sternum purplish. Abdomen elongated, dorsum with indistinct dark brown pattern posteriorly, antero-ventrally brown with semi-circular patch, posterior with dark brown pattern delimiting light brown dotted vertical lines laterally and V-shaped medially. Legs uniformly brown; measurements: I 5.72 (0.55, 0.16, 1.88, 2.13, 1.00), II 5.78 (1.60, 0.20, 1.60, 1.63, 0.75), III missing, IV 8.33 (2.19, 0.25, 2.60, 2.20, 1.09). Palp (Fig. 15A–D): femur slender, 5 times longer than patella; patella not swollen; tibia 2 times shorter than femur; cymbium almost as wide and long as tibia, with protrusion; bulb pale brown, bulging ovate with embolus arising distally; embolus thinly, sheet-liked, 5 times shorter than entire length of tegulum (Fig. 15B).

**Female** (Paratype). General features and coloration similar to those of male (Fig. 14D, E). Measurements: total length 1.49; carapace 0.55 long, 0.60 wide; abdomen 0.94 long, 0.55 wide. Leg measurements: I 8.27 (2.19, 0.20, 2.50, 2.25, 1.13), II 6.28 (1.75, 0.25, 1.65, 1.75, 0.88), III 4.30 (1.30, 0.20, 1.40, 1.20, 0.20), IV 6.91 (1.88, 0.23, 2.00, 1.80, 1.00). Endogyne (Fig. 14A): widely spaced, digitiform spermathecae, ratio of length of spermatheca and the interdistance of digitiform spermathecae –1:10 (Fig. 14A).

**Distribution.** Known only from the type locality (Fig. 30).

## *Psiloderces penajamensis* Li & Chang, sp. nov. http://zoobank.org/82D11755-9750-46AD-A399-281473327D80 Figs 16, 17, 29A, 30

**Types.** *Holotype*: ∂ (IZCAS), Indonesia, East Kalimantan, Penajam, Camp of International Timber Corporation of Indonesia, 1°5.2915'S, 116°41.0938'E, 64 m, 16.VIII.2014, H. Zhao & Z. Yao.



**Figure 14.** *Psiloderces palopoensis* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** male habitus, dorsal view **D** female habitus, dorsal view **E** female habitus, ventral view. Abbreviation: SP = spermatheca.



**Figure 15.** *Psiloderces palopoensis* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: CP = cymbial protrusion, EM = embolus.



**Figure 16.** *Psiloderces penajamensis* sp. nov., male holotype. **A** Male habitus, lateral view **B** male habitus, dorsal view **C** male habitus, ventral view. Abbreviation: PR = clypeal projection.

**Etymology.** The species name is an adjective referring to the type locality.

**Diagnosis.** The male of *P. penajamensis* sp. nov. can be recognized by the structure of laminar apophysis of the bulb that arises distally, bearing the embolus and bulge (Fig. 17B) (vs. absence of laminar apophysis in congeners), a relatively short clypeal protrusion in which the length does not exceed the anterior tip of the carapace (vs. a clypeal projection that exceeds the anterior tip of carapace).

**Description.** Male (Holotype). Total length 1.20; carapace 0.50 long, 0.60 wide; abdomen 0.70 long, 0.50 wide. Carapace round and brown, with 3 longitudinal dark brown bands, median band and lateral bands nearly the same width (Fig. 16B). Chelicerae brown (Fig. 29A). Clypeus dark brown, bearing few setae, with short medial projection, length does not exceed the anterior edge of carapace, (Fig. 16A, B). Endites brown. Labium dark brown. Sternum dark brown. Abdomen elongated, dorsum with dark brown patches concentrated posteriorly (Fig. 16B), antero-ventrally dark brown with complex patterns (Fig. 16C). Legs uniformly brown; measurements: I–III missing, IV 6.30 (1.56, 0.16, 1.90, 1.80, 0.88). Palp (Fig. 17A–D): femur slender, 3 times longer than patella; patella not swollen; tibia 1.5 times shorter than femur; cymbium almost as long and wide as tibia, protrusion darkens distally; bulb pale yellow, pyriform with bulge, laminar apophysis and embolus arise distally; laminar apophysis sheet-like, almost as long as width of tegulum; laminar apophysis adjacent to embolus, embolus dark short, and pointed; blunt bulge adjacent to embolus (Fig. 17B).

Female. Unknown.

Distribution. Known only from the type locality (Fig. 30).



**Figure 17.** *Psiloderces penajamensis* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: CP = cymbial protrusion, BG = bulge, EM = embolus, LA = laminar apophysis.

#### Psiloderces grobotensis Li & Chang, sp. nov.

http://zoobank.org/15F30792-C43A-4D79-AC45-18836A3B24E5 Figs 18, 19, 29D, 30

**Types.** *Holotype*: ∂ (IZCAS), Indonesia, East Kalimantan, Tanah Grohot, 1°48.6260'S, 115°51.1250'E, 62 m, 20.VIII.2017, H. Liu & Z. Chen. *Paratype*: 1♀ (IZCAS), same data as holotype.

**Etymology.** The species name is an adjective referring to the type locality.

**Diagnosis.** Males of *P. grohotensis* sp. nov. can be distinguished from all other species of the genus by the structure of the bulb which has 2 bulges, and a laminar apophysis connected to the embolus (Fig. 19B) (vs. one bulge or absent); the female can be distinguished by transversal, tubular spermathecae resembling caterpillars (Fig. 18A) (vs. the absence of transversal, tubular spermathecae).

**Description. Male** (Holotype). Total length 1.30; carapace 0.50 long, 0.60 wide; abdomen 0.80 long, 0.50 wide. Carapace round and pale brown, with trident dark brown stripes medially and dark brown bands laterally (Fig. 18C). Chelicerae pale brown (Fig. 29D). Clypeus slanting, dark brown. Endites dark brown, light brown basally. Labium dark brown, delimiting pair of light brown circular dots. Sternum dark brown. Abdomen elongated, dorsum with indistinct brownish stripes, antero-ventrally with pair of circular dark brown patches followed by elliptical light brown patch, posterior dark brown, delimiting light brown dotted vertical lines laterally and V-shaped medially. Legs uniformly brown; measurements: I 7.47 (2.00, 0.20, 2.25, 2.00, 1.02), II 5.85 (1.60, 0.20, 1.75, 1.50, 0.80), III 4.10 (1.09, 0.16, 1.20, 1.10, 0.55), IV 6.00 (1.60, 0.20, 1.80, 1.60, 0.80). Palp (Fig. 19A–D): femur slender, 4 times longer than patella; patella not swollen; tibia 2.5 times shorter than femur; cymbium 1.5 times shorter than femur, protrusion darkens distally; bulb pale brown, diamond-shaped with embolus and laminar apophysis located distally; embolus thin, connected basally with laminar apophysis; 2 acute bulges directed ventrally (Fig. 19B)

**Female** (Paratype). General features and coloration similar to those of male (Fig. 18D, E). Measurements: total length 1.49; carapace 0.47 long, 0.55 wide; abdomen 1.02 long, 0.78 wide. Leg measurements: I 3.96 (1.30, 0.16, 1.50, 1.30, 0.70), II 4.91 (1.25, 0.16, 1.50, 1.30, 0.70), III 3.89 (1.00, 0.13, 1.13, 1.00, 0.63), IV 5.41 (1.38, 0.13, 1.70, 1.40, 0.80). Endogyne (Fig. 18A): spermathecae tubular, elongate mesally, median tips pointed, lateral ends rounded, receptacles separated by about 3 diameters (Fig. 18A).

Distribution. Known only from the type locality (Fig. 30).

#### Psiloderces bangkiraiensis Li & Chang, sp. nov.

http://zoobank.org/A55D77D3-F71B-46B8-ADD8-21259DCDF0EE Figs 20, 21, 28A, 30

**Types.** *Holotype*: ♂ (IZCAS), Indonesia, East Kalimantan, Kutai Kaetanegara, Bukit Bangkirai, 1°1.2247'S, 116°51.9580'E, 92 m, 18.VIII.2014, Y. Li *Paratype*: 1♀ (IZ-CAS), same data as holotype.



**Figure 18.** *Psiloderces grohotensis* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view, **B** female epigastric area, ventral view **C** male habitus, dorsal view **D** female habitus, dorsal view **E** female habitus, ventral view. Abbreviation: SP = spermatheca.



**Figure 19.** *Psiloderces grohotensis* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: CP = cymbial protrusion, BG = bulges, EM = embolus, LA = laminar apophysis.



**Figure 20.** *Psiloderces bangkiraiensis* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** male habitus, dorsal view **D** female habitus, dorsal view **E** female habitus, ventral view. Abbreviation: SP = spermatheca.



**Figure 21.** *Psiloderces bangkiraiensis* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: CP = cymbial protrusion, BG = bulge, EM = embolus, LA = laminar apophysis.

Etymology. The species name is an adjective referring to the type locality.

**Diagnosis.** Males of *P. bangkiraiensis* sp. nov. can be distinguished from all other species of the genus by the relatively slender and elongated bulb bearing a distinct pointed bulge posteriorly (vs. bulb not elongated and slender), the angular tip of the cymbial protrusion (vs. tip of cymbial protrusion rounded), the tibia of the male palp swollen anteriorly (vs. tibia not swollen); females can be distinguished by the curled spermathecae (Fig. 20A).

Description. Male (Holotype). Total length 1.49; carapace 0.47 long, 0.60 wide; abdomen 1.02 long, 0.50 wide. Carapace round and brown, with trident dark brown stripes medially and dark brown bands laterally (Fig. 20C). Chelicerae brown (Fig. 28A). Clypeus slanting, dark brown. Endites dark brown, light brown basally. Labium dark brown, delimiting pair of light brown circular dots. Sternum dark brown. Abdomen elongated, dorsum with indistinct brownish stripes posteriorly, with pair of circular dark brown patches antero-ventrally followed by elliptical light brown patch, posterior dark brown pattern delimiting light brown dotted vertical lines laterally. Legs uniformly brown; measurements: I 7.88 (1.88, 0.16, 2.50, 2.34, 1.00), II 5.79 (1.63, 0.16, 1.75, 1.50, 0.75), III 4.41 (1.20, 0.16, 1.25, 1.17, 0.63), IV 6.71 (1.88, 0.20, 2.00, 1.75, 0.88). Palp (Fig. 21A–D): femur slender, 4 times longer than patella; patella not swollen; tibia swollen anteriorly, 1.5 times shorter and 2 times wider than femur; cymbium 2.5 times shorter and 2 times wider than femur, protrusion darkens distally, tip angled; bulb light brown, lanceolate with laminar apophysis and embolus arising distally; laminar apophysis attached to embolus anteriorly and basally; embolus directed apically away from tegulum, thin and slender, bulge directed toward the base of bulb (Fig. 21B).

**Female** (Paratype). General features and coloration similar to those of male (Fig. 20D, E). Measurements: total length 1.38; carapace 0.44 long, 0.55 wide; abdomen 0.94 long, 0.86 wide. Leg measurements: I missing, II missing, III 3.62 (1.00, 0.13, 1.00, 0.94, 0.55), IV 5.51 (1.40, 0.13, 1.72, 1.48, 0.78). Endogyne (Fig. 20A): curled spermathecae connected with bent ducts, equally wide as long (Fig. 20A).

Distribution. Known only from the type locality (Fig. 30).

#### Psiloderces bolang Li & Chang, sp. nov.

http://zoobank.org/A62B1BD7-D14F-4DBF-869F-31938299D18A Figs 22, 23, 29E, 30

**Types.** *Holotype*: ∂ (IZCAS), Indonesia, Sulawesi, mountain Palopo, 2°57.6000'S, 120°6.0'E, 509 m, 3.IX.2017, H. Liu & Z. Chen. *Paratype*: 1♀ (IZCAS), same data as holotype.

**Etymology.** The species name is a noun in apposition derived from the Chinese pinyin "bolang" (wave) and refers to the unique undulated base of the bulb which resembles a wave pattern.

**Diagnosis.** Males of *P. bolang* sp. nov. resemble those of *P. torajanus* by the trilobate base of the bulb and indented ventrally but can be distinguished by the undi-



**Figure 22.** *Psiloderces bolang* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** male habitus, dorsal view **D** female habitus, dorsal view **E** female habitus, ventral view. Abbreviation: SP = spermatheca.



**Figure 23.** *Psiloderces bolang* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view. **D** palp, retrolateral view. Abbreviations: CP = cymbial protrusion, EM = embolus.

vided bulb (vs. bulb distinctly divided into two parts (proximal and distal); figs 8, 9 in Deeleman-Reinhold 1995), the bulb with a crooked periphery and rounded tip (vs. bulb with smooth periphery and pointed tip), the length of cymbial protrusion is at least half the width of the bulb (vs. length of cymbial protrusion narrower than the width of the bulb); females of both species resemble looped spermathecae but can be distinguished by rippled spermathecae (vs. smooth elliptical spermathecae).

**Description.** Male (Holotype). Total length 1.49; carapace 0.47 long, 0.60 wide; abdomen 1.02 long, 0.65 wide. Carapace round and brown, with 3 longitudinal dark brown bands, median band half length the carapace, lateral bands 3 times wider than median band (Fig. 22C). Chelicerae brown, promargin with lamina bearing 2 triangular extensions (Fig. 29E). Clypeus dark brown. Endites dark brown. Labium dark brown delimiting pair of indistinct light brown circular dots. Sternum dark brown, delimiting light brown patch medially. Abdomen elongated, dorsum with dark brown stripes, anteroventrally with elliptical patch, posterior part with pair of light brown longitudinal lines laterally. Legs uniformly brown; measurements: I 7.73 (2.00, 0.20, 2.40, 2.13, 1.00), II 5.50 (1.30, 0.20, 1.70, 1.50, 0.80), III 5.34 (1.09, 0.16, 1.71, 1.75, 0.63), IV missing. Palp (Fig. 23A–D): femur slender, 3 times longer than patella; patella not swollen; tibia 2 times shorter than femur; cymbium almost as long and wide as tibia, protrusion darkens distally; bulb pale brown, undulate basally and at the margins, pyriform with embolus located anteriorly, with indentation ventrally; embolus laminar and gradually tapering (Fig. 23B).

**Female** (Paratype). General features and coloration similar to those of male (Fig. 22D, E). Measurements: total length 1.30; carapace 0.50 long, 0.60 wide; abdomen 0.80 long, 0.55 wide. Leg measurements: I 4.52 (1.00, 0.16, 1.71, 1.02, 0.63), II missing, III 3.12 (0.80, 0.13, 0.88, 0.81, 0.50), IV 4.60 (1.20, 0.20, 1.40, 1.10, 0.70). Endogyne (Fig. 22A): spermathecae looped transverse anteriorly, rippled posteriorly (Fig. 22A).

Distribution. Known only from the type locality (Fig. 30).

#### Psiloderces wangou Li & Chang, sp. nov.

http://zoobank.org/696D6C23-F5FC-496D-95BB-30BA6F6EBAEC Figs 24, 25, 28B, 30

**Types.** *Holotype*: ∂ (IZCAS), Indonesia, South Sulawesi, Maros, Cenrana Village, East of Maros Water Park, 5°3.2573'S, 119°44.3747'E, 229 m, 24.VII.2014, Y. Li. *Paratype*: 1♀ (IZCAS), same data as holotype.

**Etymology.** The species name is a noun in apposition derived from the Chinese pinyin "wāngōu" (hook) and refers to the hook-shaped embolus.

**Diagnosis.** Males of *P. wangou* sp. nov. resemble those of *P. malinoensis* sp. nov. but can be distinguished by the relatively long, protruding, bent embolus (vs. relatively short and embedded embolus in *P. malinoensis* sp. nov.); females can be distinguished by the enclosed spermathecae (vs. ribbon-like spermathecae with 3 branches).

**Description. Male** (Holotype). Total length 1.30; carapace 0.50 long, 0.56 wide; abdomen 0.80 long, 0.50 wide. Carapace round and brown, with 3 longitudinal dark brown bands, median band half length of carapace, lateral bands 2 times wider than



**Figure 24.** *Psiloderces wangou* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** male habitus, dorsal view **D** female habitus, dorsal view **E** female habitus, ventral view. Abbreviation: SP = spermatheca.



**Figure 25.** *Psiloderces wangou* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: CP = cymbial protrusion, EM = embolus.

median band (Fig. 24C). Chelicerae brown, promargin with lamina bearing 2 triangular extensions (Fig. 28B). Clypeus dark brown. Endites dark brown, light brown basally. Labium dark brown with pair of light brown circular dots. Sternum dark brown, with light brown median stripe. Abdomen elongated, dorsum with dark brown stripes concentrated posteriorly, antero-ventrally dark brown with elliptical patch, posterior with pair of lateral light brown longitudinal lines. Legs uniformly brown; measurements: I 8.13 (2.19, 0.16, 2.50, 2.19, 1.09), II missing, III 5.04 (1.25, 0.20, 1.25, 1.71, 0.63), IV 6.55 (1.75, 0.20, 2.00, 1.70, 0.90). Palp (Fig. 25A–D): femur slender, 3 times longer than patella; patella not swollen; tibia 2 times shorter than femur; cymbium 1.5 times shorter than femur, protrusion darkens distally; bulb pale brown, pyriform, with embolus arising apically; embolus protruded and slightly bent at tip, resembling a hook, almost 4 times thinner than width of tegulum, length of embolus 2.5 times shorter than tegulum (Fig. 25B).

**Female** (Paratype). General features and coloration similar to those of male (Fig. 24D, E). Measurements: total length 1.50; carapace 0.50 long, 0.63 wide; abdomen 1.00 long, 0.75 wide. Leg measurements: I 6.33 (1.63, 0.20, 2.00, 1.60, 0.90), II missing, III missing, IV 4.90 (1.50, 0.20, 1.70, 0.94, 0.56). Endogyne (Fig. 24A): enclosed spermathecae formed by a horizontal posterior part, and pair of anterior part slanted at 45°, resembling a clothes hanger (Fig. 24A).

Distribution. Known only from the type locality (Fig. 30).

## Psiloderces malinoensis Li & Chang, sp. nov.

http://zoobank.org/51DB8482-8B37-4B6D-937C-23358E00E8F5 Figs 26, 27, 29B, 30

**Types.** *Holotype*:  $\mathcal{J}$  (IZCAS), Indonesia, Sulawesi, Makassar, mountain around Malino, 5°16.2000'S, 119°50.4000'E, 881 m, 7.IX.2017, H. Liu & Z. Chen. *Paratype*: 1 (IZCAS), same data as holotype.

**Etymology.** The species name is an adjective referring to the type locality. **Diagnosis.** See diagnosis of *P. wangou* sp. nov.

**Description.** Male (Holotype). Total length 1.40; carapace 0.50 long, 0.60 wide; abdomen 0.90 long, 0.63 wide. Carapace round and brown, with trident dark brown stripes medially and dark brown bands laterally (Fig. 26C). Chelicerae brown (Fig. 29B). Clypeus slanting, dark brown medially, light brown laterally. Endites dark brown, light brown basally. Labium dark brown with pair of light brown circular dots. Sternum dark brown. Abdomen elongated, dorsum with dark brown patches followed by semi-circular patch, posterior with light brown dotted vertical lines laterally. Legs uniformly brown; measurements: I 6.64 (1.88, 0.16, 2.00, 1.70, 0.90), II missing, III 4.33 (1.20, 0.16, 1.25, 1.09, 0.63), IV 5.79 (1.50, 0.20, 1.75, 1.48, 0.86). Palp (Fig. 27A–D): femur slender, 4 times longer than patella; patella not swollen; tibia 2.5 times shorter than femur; cymbium 1.5 times shorter than femur, protrusion darkens



**Figure 26.** *Psiloderces malinoensis* sp. nov., male holotype and female paratype. **A** Endogyne, dorsal view **B** female epigastric area, ventral view **C** male habitus, dorsal view **D** female habitus, dorsal view **E** female habitus, ventral view. Abbreviation: SP = spermatheca.



**Figure 27.** *Psiloderces malinoensis* sp. nov., male holotype. **A** Palp, ventral view **B** bulb, ventral view **C** palp, prolateral view **D** palp, retrolateral view. Abbreviations: CP = cymbial protrusion, EM = embolus.



Figure 28. Cheliceral retromargin, posterior view. A *Psiloderces bangkiraiensis* sp. nov. B *P. wangou* sp. nov.
C *P. heise* sp. nov. D *P. cuyapoensis* sp. nov. E *P. bontocensis* sp. nov. F *P. gawanaensis* sp. nov. G *P. xichang* sp. nov.
H *P. cattienensis* sp. nov. I *P. pingguo* sp. nov. Abbreviations: CL = cheliceral laminar, RT = retromargin teeth.



**Figure 29.** Cheliceral retromargin, posterior view. **A** *Psiloderces penajamensis* sp. nov. **B** *P. malinoensis* sp. nov. **C** *P. palopoensis* sp. nov. **D** *P. grohotensis* sp. nov. **E** *P. bolang* sp. nov. Abbreviations: CL = cheliceral laminar, RT = retromargin teeth.

distally; bulb pale brown, pyriform, with embedded embolus located distally, 4 times shorter than tegulum; tip of embolus pointed (Fig. 27B).

**Female** (Paratype). General features and coloration similar to those of male (Fig. 26D, E). Measurements: total length 1.47; carapace 0.47 long, 0.63 wide; abdomen 1.00 long, 0.63 wide. Leg measurements: I 5.63 (1.50, 0.20, 1.75, 1.38, 0.80), II 4.61 (1.25, 0.16, 1.41, 1.09, 0.70), III missing, IV missing. Endogyne (Fig. 26A): ribbon-like spermathecae with 3 branches, lateral pairs longest and directed anteriorly, median and second pairs similar in length, directed laterally (Fig. 26A).

Distribution. Known only from the type locality (Fig. 30).



Figure 30. Distribution of new *Psiloderces* species in Southeast Asia. 1 *P. heise* sp. nov. 2 *P. gawanaensis* sp. nov. 3 *P. bontocensis* sp. nov. 4 *P. cuyapoensis* sp. nov. 5 *P. xichang* sp. nov. 6 *P. cattienensis* sp. nov. 7 *P. ping-guo* sp. nov. 8 *P. palopoensis* sp. nov. 9 *P. penajamensis* sp. nov. 10 *P. grohotensis* sp. nov. 11 *P. bangkiraiensis* sp. nov. 12 *P. bolang* sp. nov. 13 *P. wangou* sp. nov. 14 *P. malinoensis* sp. nov.

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RESEARCH ARTICLE



# A new species of *Enteromius* (Actinopterygii, Cyprinidae, Smiliogastrinae) from the Awash River, Ethiopia, and the re-establishment of *E. akakianus*

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## Abstract

In the present study, populations of small-sized smiliogastrin barbs with a thickened and serrated last simple dorsal-fin ray distributed in the Main Ethiopian Rift were analysed. An integrated approach combining genetic markers and a variety of morphological methods based on a wide set of characters, including osteology and sensory canals, proved to be very productive for taxonomy in this group of fishes. The results showed that Ethiopian Enteromius species with a serrated dorsal-fin ray are distant from the true E. paludinosus (with E. longicauda as a synonym) and the so-called E. paludinosus complex involves several supposedly valid species with two distinct species occurring in the Main Ethiopian Rift area. A new species, Enteromius yardiensis sp. nov., is described from the Afar Depression in the north-eastern part of the Northern Main Ethiopian Rift. Enteromius akakianus is resurrected as a valid species including populations from the Central Main Ethiopian Rift (basins of lakes Langano, Ziway, and Awasa). No genetic data were available for *E. akakianus* from its type locality. *Enteromius yardiensis* sp. nov. is clearly distant from E. akakianus from the Central Main Ethiopian Rift by CO1 and cytb barcodes: pairwise distances between the new species and the Ethiopian congeners were 5.4 % to 11.0 %. Morphologically, the new species most clearly differs from all examined Ethiopian congeners by three specialisations which are unique in the group: the absence of the anterior barbel, the absence of the medial branch of the supraorbital sensory canal, and few, 1-3, commonly two, scale rows between the lateral line and the anus.

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#### Keywords

East Africa, Main Ethiopian Rift, morphology, CO1 and cytb sequences, zoogeography

## Introduction

Small-sized smiliogastrin barbs are typical representatives of the fish fauna in sub-Saharan Africa (Lévêque and Daget 1984; Skelton 2001; Stiassny et al. 2007) with several hundred species distributed in almost all drainages of the continent (Lévêque and Daget 1984). Despite their widespread occurrence, phylogenetic relationships within the group and taxonomy of most species have not been fully resolved yet (Yang et al. 2015; Ren and Mayden 2016; Hayes and Armbruster 2017). In previous taxonomic studies, small-sized African smiliogastrin barbs were commonly referred to as a polyphyletic assemblage named Barbus sensu lato (e.g., Greenwood 1962; Berrebi et al. 1996; Golubtsov and Berendzen 2005; Mina et al. 2017). Recent molecular phylogenetic studies of barbeled cypriniforms supported a different opinion (e.g., Karaman 1971; Roberts 2010) that the assemblage of Palearctic true barbs - Barbus Daudin, 1805, typified by the species Cyprinus barbus Linnaeus, 1758 and close genera – do not occur in sub-Saharan Africa (Tsigenopoulos and Berrebi 2000; Wang et al. 2013; Yang et al. 2015; Ren and Mayden 2016). Though the phylogenetic resolution was considered by some authors as rather limited (Schmidt and Bart 2015; Stiassny and Sakharova 2016), all available genetic data clearly indicate that diploid African taxa belong to a phylogenetically distinct clade, the tribe Smiliogastrini or the subfamily Smiliogastrinae of the family Cyprinidae (in case the subfamily Cyprininae is given the family rank) and are not closely related to Barbus sensu stricto (Yang et al. 2015; Ren and Mayden 2016).

Accordingly, the oldest available name for this group, *Enteromius* Cope, 1867, was resurrected at the generic level (Yang et al. 2015). The decision was criticised (Schmidt and Bart 2015; Stiassny and Sakharova 2016; Stiassny et al. 2016; Schmidt et al. 2017) but has been accepted (Schmidt et al. 2018; Mamonekene et al. 2018; Mipounga et al. 2019) based on a summarising review by Hayes and Armbruster (2017) as the first step for taxonomic delimitation of small-sized African smiliogastrin barbs. The phylogenetic trees in Yang et al. (2015) and Ren and Mayden (2016) adopted by Hayes and Armbruster (2017) indicate heterogeneity of the genus, which contains at least two putative distinct genera, Clade Enteromius I and Clade Enteromius II of Hayes and Armbruster (2017). Which one of the two represents the genus Enteromius is not clear because the placement of the type species of Enteromius (E. potamogalis Cope, 1867) within Enteromius sensu lato is still uncertain (Ren and Mayden 2016; Hayes and Armbruster 2017). If the opinion of Roberts (2010) that *E. potamogalis* is closely related to Central and West African taxa is proved to be correct, then the clade *Enteromius* I represents the true Enteromius. This issue is beyond the goal of our study and we do not discuss a valid taxonomic name (or the absence of it) for the clade Enteromius II of Hayes and Armbruster (2017). We use the genus name *Enteromius* as a convenient taxonomic compromise at the present level of knowledge on phylogenetic interrelationships in the group.
Based on morphology, a supposedly non-monophyletic but readily diagnosable group of Enteromius occurs in Ethiopia - comparatively small-sized smiliogastrin barbs with a thickened, segmented only at the tip, and serrated last unbranched ray in the dorsal fin. It includes taxa of the species level originally described under five available names as follows: E. paludinosus (Peters, 1852), E. kerstenii (Peters, 1868), E. pleurogramma (Boulenger, 1902), E. amphigramma (Boulenger, 1903) (in Boulenger 1903a) and E. akakianus (Boulenger, 1911). They were considered valid species or synonymised in various ways by different authors (Greenwood 1962; Golubtsov and Krysanov 1993; Admassu and Dadebo 1997; Golubtsov et al. 2002; Golubtsov and Krysanov 2003; Golubtsov and Berendzen 2005; De Graaf et al. 2007; Vijverberg et al. 2012; Mina et al. 2017) and commonly divided into two phenotypic groups. One contains *E. paludinosus*-like taxa characterised by the absence of an orange or yellow blotch on the operculum and over 30 total lateral-line scales (E. pleurogramma, E. akakianus and E. amphigramma) and the other, the E. kerstenii complex, with species possessing an orange or yellow blotch on the operculum and fewer than 30 total lateral-line scales (Greenwood 1962; Golubtsov and Krysanov 1993; Golubtsov et al. 2002; Golubtsov and Berendzen 2005; Mina et al. 2017).

Morphological observations indicated that most Ethiopian populations of the first group are similar to *E. paludinosus* (Golubtsov and Berendzen 2005). Initially described from the Lower Zambezi River (Peters 1852), *E. paludinosus* was thought to be widely distributed, from South Africa in the south to Ethiopia in the north (Lévêque and Daget 1984; Seegers 1996; Skelton 2001; Marshall 2011). In contrast, genetic studies (De Graaf et al. 2007; Mwita 2013; Schmidt et al. 2017) highlighted distinct differences between groups of populations of *E. cf. paludinosus* in East Africa. Based on data of Schmidt et al. (2017), Mina et al. (2017) recently re-assigned all Ethiopian populations, commonly identified as *E. paludinosus*, to "*E. pleurogramma* complex" but did not provide any data that could support this conclusion.

Twelve nominal species are synonymised (Seegers 1996; Seegers et al. 2003; Hayes and Armbruster 2017) with *E. paludinosus* originally described from Quellimane, Mozambique (Zambezi River delta). They are as follows (drainage of their type localities in parentheses), all described originally as *Barbus*:

akakianus (Akaki River, Awash (endorheic), Ethiopia),

*amphigramma* (Nairobi River, Athi, Kenya); in the original description the location is given as "Nairobi River, Kilimanjaro",

helleri Hubbs, 1918 (Athi River, Athi, Kenya),

ivongoensis Fowler, 1934 (Ivongo River, Ivongo, South Africa),

*longicauda* Boulenger, 1905 (Zambezi River, Zambezi, Mozambique); replacement name for *B. gibbosus* Peters, 1852, *longicauda* is a noun in apposition, not be changed to agree in gender with the masculine generic name according to Art. 34.2.1. of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999),

*macropristis* Boulenger, 1904 (Lake Victoria [Victoria Nyanza], Nile, Kenya), *macropristis meruensis* Lönnberg, 1907 (River Ngare na nyuki, Nile, Tanzania), taitensis Günther, 1894 (unknown drainage, Taita, Kenya),

thikensis Boulenger, 1905 (Thika River, Tana, Kenya),

*tsotsorogensis* Fowler, 1935 (Tsotsoroga Pan, northeastern edge of the Mababe Flats (possibly endorheic), Okawango, Botswana),

*vinciguerraii* Pfeffer, 1896 (Wembere River, Lake Kitangiri basin (endorheic), Tanzania), *welwitschii* Günther, 1868 (unknown drainage, Huilla and Benguella provinces, Angola).

During recent field trips, samples of small smiliogastrin barbs with a thickened and serrated last unbranched ray in the dorsal fin were collected in central Ethiopia. Preliminary observations showed strong phenotypic variations and suggested an undescribed species of *Enteromius* in the Lower Awash River. In the present paper, we only discuss *E. paludinosus*-like fishes with a serrated dorsal-fin ray. Herein, we present genetic and morphological analyses of Ethiopian samples from the Main Ethiopian Rift endorheic drainages and compare them with type series of *E. akakianus, E. longicauda, E. paludinosus* and *E. pleurogramma* in order to evaluate their taxonomic status and identity.

## Materials and methods

## Sampling and preservation

During recent field trips (2017–2019), the fish fauna of the Awash River was investigated from the source region in the Chilimo forest to the lakes of the Afar Depression (Englmaier 2018). Sampled localities are given in Fig. 1 including those where no *Enteromius* were found. *Enteromius* were collected (Fig. 1: sample sites 1–10) in endorheic drainages of the Main Ethiopian Rift (MER; same as the Ethiopian Rift Valley of Paugy (2010)) and the Lower Awash River (the Afar Depression). The abbreviation CMER refers to the Central Main Ethiopian Rift (definition and abbreviation follow Bonini et al. (2005)). Length of sampled aquatic segments was between 5 and 20 m (maximum water depth 1.5 m). Collections were made from the main river channel, side arms and shoreline habitats using beach seines (mesh size 1.5 mm) and frame nets (mesh size 1.5 mm). Fish specimens were first euthanised with etheric clove oil (*Eugenia caryophyllata*) diluted in water, and later fixed in 6 % pH neutral formalin or 96 % ethanol. Formalin-preserved specimens were later transferred to ethanol.

Museum samples included specimens deposited in the collections of the Natural History Museum Vienna (**NMW**; Fig. 1: sample sites 1–10); the British Museum of Natural History (**BMNH**; Fig. 1: sample sites 11–15); Museum für Naturkunde, Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Berlin, Germany (**ZMB**); and the fish collection of the National Fisheries and Aquatic Life Research Centre, Sebeta, Ethiopia (**NFALRC**) (see comparative material in Table 1). Data for the type series of the new species are in the species description part. In case of uncertain identification, the term "cf." is used to indicate that a taxon is considered as close or to be compared with another one (Lucas 1986).



**Figure 1.** Map of Ethiopia, presenting sampling sites and examined material. Sampling sites: **1–13** Main Ethiopian Rift (**1–5** Lower Awash R., distribution of *Enteromius yardiensis* sp. nov.; **6–12** lakes and rivers in Central Main Ethiopian Rift; **13** Akaki R., type locality of *E. akakianus*); **14–15** Blue Nile drainage, type locality of *E. pleurogramma*. In blue, known populations of small-sized *E. paludinosus*-like smiliogastrin barbs in Ethiopia. Locations in southern Africa included in CO1 and cytb analyses in red, green, and purple. Distribution data for Ethiopian populations from Golubtsov and Berendzen (2005).

# Genetic analyses

Total genomic DNA was extracted from ethanol preserved tissue (fin clips) using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, USA). Two mitochondrial regions, cytochrome c oxidase subunit 1 (CO1) and cytochrome b (cytb), were used for species differentiation. Partial CO1 (approximately 660 bp) was amplified with primers Fish-Co1-F and Fish-Co1-R (Baldwin et al. 2009). Volumetric composition of the PCR mix and thermal setting during 35 cycles of PCR followed Meulenbroek et al. (2018). The partial cytb gene (approximately 650 bp) was amplified

Taxon name	Museum number	-	Tynee	SI mm	Information
		: -		000	
Barbus akaktanus	68.02.1.8061 HNIMB	_	holotype	66.0	Akaki Kiver, Hawash [Awash] system (site 1.5), Ethiopia, coll. F. Zaphiro.
Barbus akakianus	BMNH 1908.1.20.82–283	5	non-types	67.8-88.5	Akaki River, Hawash [Awash] system (site 13), Ethiopia, coll. P. Zaphiro.
Barbus akakianus	BMNH 1908.1.20.84	-	non-type	88.1	Dry skeleton, Akaki River, Hawash [Awash] system (site 13), Ethiopia, coll. P. Zaphiro.
Barbus amphigramma	BMNH 1902.11.8.24-26	3	syntypes	31.1 - 34.9	Nairobi River, Kilimanjaro, Tanzania.
Barbus longicauda	ZMB 4735	-	lectotype	66.3	Tette [Tete], Revugo, Mozambique
Barbus longicauda	ZMB 32377	5	paralectotypes	61.9-67.8	Tette [Tete], Revugo, Mozambique
Barbus macropristis	BMNH 1904.5.19.22-23	7	syntypes	98.3-111.6	Lake Victoria, coll. W. Doggett
Barbus macropristis	ZMB 16580		syntype	62.1	Meru Nied.: Floden Ngare na nyuki, mount Meru, Tanzania
meruensis					
Barbus paludinosus	ZMB 4732		lectotype	71.3	Quellimane, Mozambique
Barbus paludinosus	ZMB 32375	3	paralectotypes	67.3-70.4	Quellimane, Mozambique
Barbus paludinosus	ZMB 4733	4	paralectotypes	42.1-54.7	Quellimane, Mozambique
Barbus paludinosus	ZMB 4734	2	paralectotypes	35.9-48.3	Quellimane, Mozambique
Barbus paludinosus	ZMB 4738	2	paralectotypes	43.5-46.6	Quellimane, Mozambique
Barbus paludinosus	NMW 54476	7	paralectotypes	50.7-65.5	Quellimane, Mozambique
Barbus paludinosus	BMNH 1861.3.10.6–7	3	paralectotypes	50.0-53.0	Quellimane, Mozambique, don. Peters
Barbus pleurogramma	BMNH 1902.12.13.356		syntype	28.5	Unfras River, Lake Tsana [Tana] (site 14), Ethiopia, coll. E. Degen.
Barbus pleurogramma	BMNH 1902.12.13.353-355	3	syntypes	30.3-35.4	Bahardar, Lake Tsana [Tana] (site 15), Ethiopia, coll. E. Degen
Barbus vinciguerraii	ZMB 14496	10	syntypes	26.3-39.5	Wembere brook, Njagaua, Tanzania
Enteromius sp. CMER	BMNH 1903.11.16.10–12	2	non-types	48.1-61.2	Suksuk [Bulbula] R., tributary to Lake Abijata [Abiyata] (site 12), Lake Ziway basin, Ethiopia, coll. C. Erlanger et al.
Enteromius sp. CMER	BMNH 1985.7.16.101–105	5	non-types	38.9-55.7	Lake Awasa [Awassa] (site 11), Rift Valley, Ethiopia, don. A. Harrison.
Enteromius sp. CMER	NMW 99236	5	non-types	33.8-38.20	Western shore of Lake Ziway (site 7; 8°1'44"N, 38°44'32"E), Ethiopia, 22.05.2018, coll. G.K. Englmaier and G.
					Tesfaye (and two specimens, both vouchers for CO1 and cytb; MN747020, MN747030, 45.2 mm SL;
					and MN747021, MN747031, 37.7 mm SL)
Enteromius sp. CMER	NMW 99237	7	non-types	38.9-41.0	South-western shore of Lake Ziway (site 8; 7~567,"N, 38°43'41"E). Ethiopia, 22.05.2018, coll. G.K. Englmaier and G. Tesfave (and one specimen, C&S, 34.8 mm SL)
Enteromius sp. CMER	NMW 99238	4	non-types	42.2-45.4	Western shore of Lake Ziway (site 7; 8°1'44"N, 38°44'32"E), Ethiopia, 22.05.2018, coll. G.K. Englmaier and G.
					Tesfaye (and one specimen C&S, 39.4 mm SL)
Enteromius sp. CMER	NMW 99239	4	non-types	50.4-70.4	Labo River, a tributary of the Meki River (site 6; 8°14'18"N, 38°28'58"E). Lake Ziway basin, Ethiopia, 13.09.2008. coll. F. Wicker and K. Borkenhaven (and one specimen. C&S. 53.4 mm SU)
Enteromius sp. CMER	NMW 99260	9	non-types	33.2-46.4	Western shore of Lake Ziway (site 7; 8°1'44"N, 38°44'32"E), Ethiopia, 22.05.2018, coll. G.K. Englmaier and G.
					Tesfaye
Enteromius sp. CMER	NMW 99261	33	non-types	31.0–35.3	South-western shore of Lake Ziway (site 8; 7°567"N, 38°43'41"E), Ethiopia, 22.05.2018, coll. G.K. Englmaier
					and G. Icstaye
Enteromius sp. CMER	NMW 99643	7	non-types	42.4-48.7	Shoreline of Lake Ziway (site 8), Ethiopia, 2018, coll. G. Testaye.
Enteromius sp. CMER	NMW 99644	2	non-types	43.1–63.1	Lake Langano (site 9), Ethiopia, coll. G. Tesfaye.

Table 1. Comparative material. CMER referring to Central Main Ethiopian Rift as defined in text.

with primers L15267 and H15891 (Briolay et al. 1998). The temperature profile was set at 95 °C for 2 min (initial denaturation), followed by 32 cycles of 95 °C for 40 s, 55 °C for 40 s, 72 °C for 1:20 min, and a final extension at 72 °C for 10 min. Gel electrophoresis was used to verify the size of the amplified DNA fragments. PCR products were then sent to Eurofins Genomics (Ebersberg, Germany) for sequencing (Sanger sequencing). Data for specimens used in the genetic analyses and GenBank accession numbers of sequences are in Suppl. material 1, Table S1. We used two different sets of specimens. The first contained data for CO1 (611 bp; 40 specimens) and the second for cytb (520 bp; 66 specimens). Only four specimens were used for both analyses.

CO1 and partial cytb were edited in MEGA7 (Kumar et al. 2016) and aligned with ClustalW. Maximum Likelihood analysis (ML) (1000 bootstrap replications), using RAxML-HPC2 (Stamatakis 2014), and Bayesian Inference analysis (BI), using MrBayes v. 3.2.6 (Ronquist et al. 2012), were performed via the CIPRES Science Gateway (Miller et al. 2011). Both analyses were run under the most general model (GTR+G+I), following the approach of Sayyadzadeh et al. (2015). Two independent runs were performed during BI and Markov chains were run for 5 million generations. Trees were sampled every 100 generations. Tracer v. 1.7.1 (Rambaut et al. 2018) was used to discard the first 25 % as burn-in. The variation between groups (p-distances) was calculated in MEGA7 (Kumar et al. 2016). Asian smiliogastrins were included as outgroups (Yang et al. 2015, Schmidt et al. 2017). FigTree v. 1.4.4 (Rambaut 2018) and CorelDRAW Graphics Suite X7 were used for visualisation.

#### Morphological analyses

Data for all examined specimens (total 146) are presented below in the text of the new species description. Comparative material included type specimens (either syntypes or lectotypes and paratypes) of eight nominal species involved in taxonomy of the Ethiopian *Enteromius*.

Therefore, we examined a wide set of morphological characters (Suppl. material 1, Table S2), including shape and number of the axial skeleton elements and infraorbital bones as suggested by Mamonekene et al. (2018).

In total, 31 measurements were made point to point using a digital calliper to the nearest 0.1 mm. The fin insertion is the posterior-most point where the last fin ray connects with the body. Most measurements follow Hubbs and Lagler (1958) and Holčík et al. (1989). Standard length (SL) is measured from the anteriormost point of the head to the posterior margin of the hypurals at midline. Head length (HL) excludes the skin fold on the operculum. Body depth was measured at pelvic-fin origin and maximum caudal-peduncle depth at the anal-fin insertion. Additional measurements of the cranium, jaws and operculum were made point to point from the anteriormost extremity to the lower-most extremity (depths), and between the lateralmost extremities (widths). Length of the cranial roof was measured from the anterior margin of the base

of the supraoccipital crest. Length of the pelvic splint was also measured (between posterior and anterior extremities). Three measurement of the last unbranched dorsal-fin ray (lengths of lower non-serrated part, serrated part and unsegmented part) were taken from radiographs. Total length of the last unbranched dorsal-fin ray was taken in relatively few specimens because the uppermost segmented part of the ray is often broken.

For morphometric analyses, we used 57 characters, including proportional measurements as specified in Tables 2–3 and Suppl. material 1, Table S3.

Definitions of the used meristic characters, 28, are given in Table 4 and in Suppl. material 1, Table S2. The posterior two branched rays in the dorsal and anal fins were counted as two. As scales are often lost while sampling and preservation, we calculated total number of scales in the lateral series (bearing the lateral-line canal or scale pockets in case of scale loss) including scales at the caudal-fin base and the number of lateral-line scales to the posterior margin of the hypurals at midline. Vertebral counts and terminology follow Naseka (1996) and were taken from radiographs. Terminology of barbels and coding for barbel length follow Lévêque et al. (1987). The sample from Lake Awasa lacks data on five scale counts (Suppl. material 1, Table S3).

Infraorbital bones (io1-io5) and the cephalic sensory canals were examined from alizarin Red S stained (C&S) specimens. The cephalic sensory canal terminology is based on Reno (1969) following Skelton (1980: fig. 3.48) in its application to barbin cyprinids.

Multivariate data analyses included *forward stepwise* discriminant function analysis (DFA), principal component analysis (PCA), cluster analysis (CA) (using the *complete linkage method* with *Euclidean distance*), and multidimensional scaling (MDS). The statistical analyses were performed using Microsoft Excel, Statistica 6.0 (Statistic for Windows. StatSoft) and PAST v. 3.16 (Hammer 1998–2012) software.

In some specimens, due to damage or poor preservation condition, individual measurements could not be obtained; to remain important specimens in the analyses, group means were used to substitute missing data. These cases are highlighted in Suppl. material 1, Table S3.

#### Results

#### Genetic analyses

An analysis of 611 bp of the mitochondrial CO1 (Fig. 2) included Ethiopian samples (Lower Awash and Lake Ziway) and *Enteromius (E. paludinosus, E. cf. paludinosus* in Fig. 2) from multiple drainage systems in southern Africa. Two specimens from the Lower Zambezi River in Mozambique (LT629216 and LT629217, Tete, type locality of *E. longicauda*) represent a locality geographically closest to the type locality of *E. paludinosus* (Quellimane). The two sister lineages from Ethiopia clustered clearly outside this group (Bayesian posterior probability, BPP 1.00; bootstrap value, bs 96) and the pairwise distance between them (the Ziway and the Lower Awash) is 5.4–6.0



**Figure 2.** Maximum Likelihood (ML) analysis, 611 bp of CO1. Posterior probabilities from BI analysis and bootstrap (bs) values for ML (1000 bootstrap replications) above and below slash. Values below 0.70/50 considered as collapsed. Colours corresponding to those in Fig. 1. In bold, samples LT629216 and LT629217 representing locality geographically closest to type locality of *Enteromius paludinosus*.

%. Pairwise distance between Ethiopian and southern African lineages ranged from 10.5 % to 16.3 %. The sample from the Lower Awash is diverged from *E. paludinosus* (Lower Zambezi River) by a mean p-distance of 12.0 %.

The analysis of 520 bp of the partial cytb included voucher specimens of *E. paludinosus*-like fishes from South Africa, Tanzania, Kenya and Ethiopia (Fig. 3). Con-



**Figure 3.** Maximum Likelihood (ML) analysis, 520 bp of partial cytb. Posterior probabilities from BI analysis and bootstrap (bs) values for ML (1000 bootstrap replications) above and below slash. Values below 0.70/50 considered as collapsed. Colours corresponding to those in Fig. 1.

siderable genetic divergence was observed within the group of Ethiopian small-sized smiliogastrin barbs with a thickened and serrated dorsal-fin ray. All Ethiopian lineages from the White Nile system, the Blue Nile system and the MER belong to a weakly-supported clade (bs 74) but are clearly separated from specimens in East and South Africa (BPP 1.00, bs 100). Pairwise distance between these clades ranged from 12.4 % to 17.8 %. The ML analysis supports a monophyletic Ethiopian clade (Fig. 3) but lacks nodal support from the BI analysis, which revealed an unresolved trichotomy (Suppl. material 2, Fig. S1).

The Lower Awash River sample is a distinct lineage (BPP 1.00, bs 100). Pairwise distance (Suppl. material 1, Table S4) between the newly described population from the Lower Awash River and Ethiopian congeners ranged from 8.4 % to 11.0 %, with the lowest divergence to specimens from the CMER and the highest difference to *E. pleurogramma* from Lake Tana. Specimens from Lake Ziway and Lake Awasa do not form differentiated lineages. Pairwise distance within this group ranged from 0.0 % to 1.0 %. The highest divergence (1.0 %) is between Lake Awasa (AY740710) and Lake Ziway (MN747031). The two lakes share the same haplotype (AY740708, AY740713, AY740714). The CMER clade is well differentiated from *E. pleurogramma* (9.4 %–10.2 %) and is the closest sister-clade to the White Nile *Enteromius* sp. (8.0 %–9.0 %).

## Morphological analyses

The results of CA, MDS, PCA, and DFA (based on individual data per specimen) are given in Figs 4–5. For the analyses, 40 morphometric (proportional measurements), 16 meristic, and two coded characters were used. For routine statistics see Suppl. material 1, Tables S5–S10.

Based on the distribution (Fig. 1), the data for CO1 and cytb presented above as well as on primary morphological data that demonstrate a high level of similarity (Tables 3–5, Suppl. material 1, Table S3), we combined all samples from the CMER (Lake Ziway and its basin, Lake Langano and Lake Awasa) into one sample (Tables 3–5).

Both CA and MDS based on means per group (Fig. 4) clustered *E. paludinosus* and *E. longicauda* (both from the Zambezi drainage) together (distance 0.597) while they are distinct from all Ethiopian congeners (0.725–1.984) though *E. pleurogramma* is closest to *E. paludinosus* in CA. Among the Ethiopian samples, the Lower Awash sample (see description of the new species) is closest to *E. pleurogramma* (0.724) and the specimens of *E. akakianus* are closest to the CMER group (the Meki River, Lake Ziway, Lake Langano and Lake Awasa).

As a further step, PCA and DFA were performed based on data per individual (Fig. 5). Based on the PCA, the most influential variables are length of posterior barbel (both % of horizontal eye diameter and % of HL) and length of anterior barbel (both % of horizontal eye diameter and % of HL). Statistics of factor coordinates of the variables can be seen in Suppl. material 1, Table S6. The syntypes of *E. pleurogramma* are separated but lay comparatively close to the Central Main Ethiopian Rift samples includ-



**Figure 4. A** CA and **B** MDS results for six samples based on means for 40 morphometric, 15 meristic and two coded qualitative characters (as in Tables 2–5). CMER referring to Central Main Ethiopian Rift as defined in the text.

ing *E. akakianus*. All Ethiopian samples are well separated from *E. paludinosus* and *E. longicauda*, which are morphologically very close (Fig. 5A, Suppl. material 1, Table S6).

The DFA (Fig. 5B) demonstrated a similar pattern of morphological affinities. Predicted classifications for *E. paludinosus, E. longicauda, E. pleurogramma, E. akakianus* and the samples from the Lower Awash and CMER were 100 % correct (Suppl. material 1, Table S8). DFA statistics: Wilks' Lambda 0.00001, approx. F (170,357) = 19.832, p < 0.0000. Variables that contribute most for discrimination of the samples (Partial Lambda < 0.6) were the length of the lower non-serrated section of the last unbranched dorsal-fin ray (0.344), number of serrae on the last unbranched dorsal-fin ray (0.407), minimal caudal peduncle depth (two proportional measurements: % SL 0.531 and % caudal peduncle length 0.576), and caudal peduncle length (0.554).



**Figure 5. A** PCA and **B** DFA results for six samples based on individual data. CMER referring to Central Main Ethiopian Rift as defined in the text.

The Lower Awash sample (described below as a new species) is morphologically the most distant from *E. longicauda*, *E. akakianus* and E. p*aludinosus* (Squared Mahalanobis Distance equals 463.68, 373.59, and 275.90, respectively). The CMER samples are closer to *E. pleurogramma* (81.76) and *E. akakianus* (126.43) and the most distant from *E. longicauda* (388.89) being well separated also from the Lower Awash sample (206.79).

To summarise, in all statistical analyses 1) the Lower Awash sample is distinct from all Ethiopian congeners and the type series of *E. paludinosus* and *E. longicauda*; 2) the holotype of *E. akakianus* and two non-type specimens from the Akaki River are closest to (or imbedded into) the CMER group (Lake Langano, the Meki River, Lake Ziway and Lake Awasa); and 3) *E. paludinosus* and *E. longicauda* are morphologically closest taxa.

These results combined with the CO1 and cytb data provide a solid support to consider the Lower Awash River population of *Enteromius* as a distinct species described below.

## Enteromius yardiensis sp. nov.

http://zoobank.org/444F1EDC-BA2C-4922-B8DE-0396EB6A343C Figs 6–8, 9A, 10A

**Material examined. Holotype** (Fig. 6A). BMNH 2018.10.10.1, holotype, 40.2 mm SL (voucher specimen for CO1: MN747019), side channel of the Awash River at Kada Bada, north of the bridge on the way to Herto Bouri (site 2; 10°13'53"N, 40°34'43"E; 565 m a.s.l.), Afar Region, Ethiopia, 28.01.2018, coll. G.K. Englmaier, G. Tesfaye, P. Meulenbroek and H. Waidbacher.

Paratypes. BMNH 2018.10.10.2-4, 3, 35.6-38.8 mm SL, same date and locality as holotype. BMNH 2018.10.10.5-24, 20, 17.9-26.2 mm SL, same date and locality as holotype. NMW 99232, 1, 34.1 mm SL, same locality as holotype, 21.05.2018, coll. G.K. Englmaier and G. Tesfaye. NMW 99233, 18, 23.1-41.0 mm SL, same date and locality as NMW 99232. NMW 99234, 16, 24.9-31.2 mm SL, four voucher specimens for CO1 (MN747015, 29.5 mm SL; MN747016, 27.2 mm SL; MN747017, 24.3 mm SL; and MN747018, 22.8 mm SL), two vouchers for cytb (MN747022, 29.5 mm SL; and MN747023, 27.2 mm SL) and three C&S specimens in a separate jar (23.4–29.0 mm SL), all same date and locality as holotype. NMW 99235, 21, 17.4-24.1 mm SL, same date and locality as holotype. NMW 99259, 13, 24.4-33.4 mm SL, three C&S specimens in a separate jar (25.6-37.6 mm SL), same date and locality as NMW 99232. NMW 99487, 25, 20.4-42.2 mm SL, Awash River at Aditu [Adayitu], downstream of the bridge (site 3; 11°7'46"N, 40°45'52"E; 465 m a.s.l.), Afar Region, Ethiopia, 12.03.2019, coll. G.K. Englmaier, G. Tesfaye, P. Meulenbroek and H. Waidbacher. NMW 99488, 13, 20.9-32.6 mm SL, two voucher specimens for cytb (MN747024, 32.9 mm SL; and MN747025, 28.4 mm SL), same date and locality as NMW 99487. NMW 99493, 17, 26.2-33.8 mm SL, Awash River at Dubti (site 4; 11°41'50"N, 41°7'22"E; 375 m a.s.l.), Afar Region, Ethiopia, 13.03.2019, coll. G.K. Englmaier, G. Tesfaye, P. Meulenbroek and H. Waidbacher. NMW 99494, 8, 17.2–33.9 mm SL, two voucher specimens for cytb (MN747026, 41.0 mm SL; and MN747027, 42.8 mm SL) same date and locality as NMW 99493. NMW 99500, 4, 8.0-22.2 mm SL, western shore of Lake Gamari (site 5; 11°30'54"N, 41°38'57"E; 342 m a.s.l.), Afar



**Figure 6.** General appearance of *Enteromius yardiensis* sp. nov. **A** holotype, BMNH 2019.10.10.1, side channel of Awash R. at Kada Bada (site 2), 40.2 mm SL, The Trustees of the Natural History Museum, London **B** longest paratype, NMW 99642, Lake Gamari (site 5), 52.8 mm SL.

Region, Ethiopia, 14.03.2019, coll. G.K. Englmaier, G. Tesfaye, P. Meulenbroek and H. Waidbacher. NMW 99501, 5, 8.9–23.2 mm SL, two voucher specimens for cytb (MN747028, 36.5 mm SL; and MN747029, 43.1 mm SL), same date and locality as NMW 99500. NMW 99639, 1, 28.7 mm SL, eastern shore of Lake Yardi (site 1; 10°14'41"N, 40°32'44"E; 565 m a.s.l.), Afar Region, Ethiopia, 21.05.2018, coll. G.K. Englmaier and G. Tesfaye. NMW 99640, 5, 30.3–42.9 mm SL, same date and locality as NMW 99487. NMW 99641, 8, 31.9–46.7 mm SL, same date and locality as NMW 99493. NMW 99642, 2, 25.6–52.9 mm SL, same date and locality as NMW 996500.

**Diagnosis.** *Enteromius yardiensis* sp. nov. belongs to a phenotypic group characterised by small size and the last unbranched dorsal-fin ray thickened and serrated. The new species is readily distinguished from its Ethiopian congeners by three unique specialisations: the absence of the anterior barbel, the absence of the medial branch of the supraorbital cephalic canal and few, 1–3, commonly two, scale rows between lateral line and anus. It further differs by posterior barbel usually shorter than half eye diameter; eye large, its diameter 24–34 % HL; snout short and pointed; lateral line complete and strongly curved; scales in the lateral series 32–35; few transversal scale rows between lateral line and pelvic-fin base (1–3); scale rows between dorsal- and pelvic-fin origins 7–10; often four unbranched dorsal-fin rays; few branched pectoral-fin rays, commonly 12 or 13; 17 or 18 abdominal vertebrae; 10–12 predorsal abdominal vertebrae; and 6–9 vertebrae between first pterygiophores of dorsal and anal fins.

**Description.** Morphometric and meristic characters of the holotype are in Tables 2, 4–5. The general appearance of *E. yardiensis* is shown in Figs 6, 7, dorsal fin in Fig. 8, sensory canals in Fig. 9A, axial skeleton in Fig. 10A and relative measurements of the holotype and paratypes are given in Table 2 and Suppl. material 1, Table S3. Variations in 14 meristic characters and numerically coded types of posterior barbel length are provided in Table 4, and data on the number of serrae on last unbranched dorsal-fin ray in Table 5.

Longest examined specimen 52.8 mm SL (NMW 99642, Fig. 6B). Body markedly compressed. Dorsal head profile slightly concave, its transition to back smooth, slight nuchal hump only present in few larger individuals (more than 35 mm SL). Head commonly longer than body depth at pelvic-fin origin.

In most specimens, predorsal back outline steeply rising to dorsal-fin origin. Postdorsal back outline slightly convex or straight to end of caudal peduncle. Head depth at nape not exceeding HL. Snout pointed and relatively short, its length not exceeding eye horizontal diameter. Mouth terminal, tip of mouth cleft on about level of middle of eye, mouth cleft straight. Posterior barbel short (coded length 2), shorter than half eye diameter. Anterior barbel absent in all specimens (17.9–52.8 mm SL) but foramen for its nerve present in maxillary (examined in six C&S specimens). Eye large, its horizontal diameter greater than snout length but shorter than lower jaw length. Eye diameter negatively correlated with SL (R = -0.72 Spearman's rank correlation, N = 39). Interorbital width is commonly less than eye horizontal diameter.

Dorsal fin with three or four unbranched and eight branched rays. Last unbranched ray moderately thickened and densely serrated. Serration extending over more than 76 % of ray non-segmented part (range 62-86 %) and lower non-serrated part relatively short and not strongly thickened (Table 2, Fig. 8). Number of serrae ranging from 10 (< 25 mm SL) to 27 (> 50 mm SL) and positively correlated with size (R = 0.96, Spearman's rank correlation). In case of four unbranched rays (in 32 % of examined specimens, Table 4), first ray often small and only visible in radiographs or in C&S individuals (Fig. 8). Predorsal body long and dorsal-fin origin placed behind origin of pelvic fin. Dorsal-fin depth slightly shorter than HL. Anal fin with three unbranched and six branched rays, reaching to about middle of caudal peduncle. Pelvic fin with one unbranched and commonly seven branched rays (Table 3), commonly reaching behind anus and rarely to anal-fin origin. Pelvic splint variable in length but relatively short (19-32% of pelvic-fin length). Pectoral fin with one unbranched and commonly 12 or 13 branched rays (Table 4), commonly not reaching pelvic-fin origin. Pectoral, pelvic and anal fins of about similar length (Table 2). Caudal fin forked with 2+17 principal rays (commonly eight in upper lobe and seven in lower lobe). Upper procurrent rays 7 (11), 8 (17) or 9 (1), lower procurrent rays 6 (2), 7 (25) or 8 (2).

Lateral line complete (in all specimens with well-preserved scales) and clearly downwardly curved on the body going along midline on posterior half of caudal peduncle. Total lateral series with 32–35, commonly 33, scales (Table 4). Circumpeduncular scale rows 12–14 (mode 13 (6), N = 11). Back, belly and chest fully scaled. Short axillary scale present at pelvic-fin base.

**Table 2.** Morphometrics of *Enteromius yardiensis* sp. nov.; holotype, BMNH 2018.10.10.1 (in separate column); paratypes, BMNH 2018.10.10.2–4, NMW 99232, NMW 99233, NMW 99639, NMW 99640, NMW 99641, NMW 99642.

Measurements	BMNH 2018.10.10.2-4,		holot	vpe and p	aratypes	
	holotype	n	Min	Max	Mean	S.D.
SL, mm	52.80	69	17.9	52.80	30.5	7.1
Body depth at pelvic-fin origin (% SL)	28.4	39	23.5	28.4	26.1	1.4
Minimum caudal-peduncle depth (% SL)	12.2	39	10.4	12.9	11.8	0.5
Minimum caudal-peduncle depth (% caudal-peduncle length)	54.0	39	45.3	59.1	52.7	3.1
Maximum caudal-peduncle depth (% SL)	14.8	39	11.8	15.2	14.0	0.8
Maximum caudal-peduncle depth (% caudal-peduncle length)	65.7	39	50.1	68.7	62.0	4.0
Predorsal length (% SL)	55.5	39	53.0	58.1	55.7	1.4
Prepelvic length (% SL)	48.3	39	47.6	51.1	49.3	0.9
Preanal length (% SL)	67.4	39	67.4	71.6	69.8	0.9
Pectoral – pelvic distance (% SL)	22.6	39	19.7	23.3	21.2	1.0
Pelvic – anal distance (% SL)	21.3	39	18.8	23.2	21.3	1.0
Caudal-peduncle length (% SL)	22.6	39	21.0	24.5	22.5	1.0
Dorsal-fin depth (% SL)	27.6	39	22.3	31.3	28.0	1.6
Dorsal-fin depth (% HL)	100.4	39	76.9	113.2	99.8	7.0
Length of last unbranched dorsal-fin ray excluding the segmented	24.1	39	18.3	24.2	21.6	1.6
part (% SL)						
Length of last unbranched dorsal-fin ray including the segmented	0.0	16	23.4	26.7	25.3	1.1
part (% SL)						
Anal-fin depth (% SL)	18.4	39	16.7	20.5	19.1	0.8
Pectoral-fin length (% SL)	20.2	39	16.4	20.8	19.3	0.9
Pectoral-fin length (% pectoral – pelvic distance)	89.4	39	81.7	101.7	90.9	5.1
Pelvic-fin length (% SL)	19.9	39	17.7	21.0	19.3	0.9
Pelvic-fin length (% pelvic – anal distance)	93.2	39	81.8	101.6	90.6	5.1
Pelvic-splint length (% pelvic-fin length)	21.4	39	13.2	31.8	21.6	3.2
Head length (% SL)	27.4	39	25.5	31.5	28.1	1.5
Head length (% body depth at pelvic-fin origin)	96.5	39	94.5	131.5	108.1	7.4
Head depth at nape (% SL)	18.8	39	17.7	21.8	19.5	1.0
Head depth at nape (% HL)	68.6	39	62.9	75.9	69.4	3.1
Head depth at posterior eye margin (% SL)	16.2	39	15.8	20.5	17.7	1.1
Head depth at posterior eye margin (% HL)	59.1	39	57.9	72.6	63.1	3.4
Head width (% SL)	14.8	39	12.6	17.4	14.9	1.3
Head width (% HL)	54.1	39	46.2	65.3	53.0	3.4
Snout length (% SL)	7.3	39	6.1	7.7	6.7	0.4
Snout length (% HL)	26.6	39	20.8	26.7	24.0	1.5
Eye horizontal diameter (% SL)	6.4	39	6.4	10.1	8.1	0.9
Eye horizontal diameter (% HL)	23.5	39	23.5	33.6	28.8	2.2
Eye horizontal diameter (% interorbital width with skin fold)	63.3	39	63.3	93.1	78.1	6.6
Interorbital width with skin fold (% SL)	10.2	39	9.3	11.8	10.4	0.6
Interorbital width with skin fold (% HL)	37.1	39	33.2	40.4	36.9	1.5
Interorbital width between frontal margins (% SL)	6.6	39	4.9	8.7	6.2	0.9
Interorbital width between frontal margins (% HL)	24.2	39	17.1	29.4	22.2	3.8
Lower-jaw length (% SL)	9.6	39	8.1	10.1	9.2	0.4
Lower-jaw length (% HL)	35.0	39	29.6	36.1	32.9	1.5
Lower-jaw length (% interorbital width with skin fold)	94.3	39	79.4	97.2	89.2	4.8
Lower-jaw length (% operculum depth)	85.9	39	71.2	91.2	80.5	4.9
Lower-jaw length (% maximum cranium width)	81.6	39	66.5	81.6	74.6	3.5
Operculum depth (% SL)	11.2	39	10.1	13.4	11.5	0.8
Operculum depth (% HL)	40.8	39	37.9	44.5	40.9	1.5
Cranial-roof length (% SL)	17.6	39	15.9	21.6	18.4	1.4
Cranial-roof length (% HL)	64.2	39	55.7	71.6	65.6	3.4
Maximum cranium width (% cranial-roof length)	66.7	39	59.8	78.8	67.4	4.3
Anterior barbel length (% HL)	0.0	39	0.0	0.0	0.0	0.0

Measurements	BMNH 2018.10.10.2-4,		holot	ype and p	aratypes	
	holotype	n	Min	Max	Mean	S.D.
Anterior barbel length (% eye horizontal diameter)	0.0	39	0.0	0.0	0.0	0.0
Posterior barbel length (% HL)	14.0	39	4.2	17.8	11.5	3.0
Posterior barbel length (% eye horizontal diameter)	59.5	39	13.9	65.0	40.4	12.3
Length of unsegmented part from x-ray (% dorsal-fin depth)	86.9	62	76.3	95.1	88.3	4.9
Length of lower non-serrated part from x-ray (% dorsal-fin	11.9	62	11.9	32.9	20.5	4.9
depth)						
Length of upper serrated part from x-ray (% dorsal-fin depth)	75.0	62	49.8	77.5	67.7	5.7
length of lower non-serrated part from x-ray (% length of	13.7	62	13.7	38.0	23.2	5.2
unsegmented part)						
length of upper serrated part from x-ray (% length of	86.3	62	62.0	86.3	76.7	5.3
unsegmented part)						

Five infraorbital bones (io) but io4 often fragmented into two. Bones io3 to io5 wide, covering most surface of cheek in front of preoperculum; io5 as wide as deep or wider as deep (in larger specimens, Fig. 9A).

Supraorbital canal complete lacking medial branch of supraorbital canal in all specimens (Fig. 9A). Infraorbital and supratemporal canals complete. Preoperculomandibular canal complete between lower jaw and preoperculum and not communicating with postocular commissure of infraorbital canal; preopercular section of this canal terminating at upper margin of preoperculum or continuing into suprapreopercular segment fused to antedorsal process of operculum terminating by free pore over its upper margin or somewhat below.

Total vertebrae few, 33 (most commonly, Fig. 10A) or 34; other vertebral counts given in Table 4. Supraneurals 5–6, first three or four square-shaped and two or three in front of dorsal fin deeper and elongated.

Gill rakers in outer row of first gill arch 10 (5), 11 (4) or 12 (1), with eight or nine on lower limb and two on upper limb. Pharyngeal teeth thin and slightly hooked, not serrated, 2.3.5–5.3.2.

In four examined specimens (23.4–29.1 mm SL), length of digestive tract (not stretched) about 82–107 % of SL. Intestine folded in simple loop before reaching anus.

Mature females were observed during mid of dry season at sizes less than 40 mm SL (36.7–39.4 mm SL, N = 4). Early stage of maturation (the developing phase of the reproductive cycle according to Brown-Peterson et al. (2011)) in females was found between 28.9–29.5 mm SL (N = 2).

**Colouration.** In life (Fig. 7), overall silvery, with greenish brown back. Greenish iridescence especially at nape and upper eye. Fins pale, base of pectoral, pelvic and anal fins sometimes faintly pinkish, caudal-fin base brownish. No stripe or blotches. Most ethanol-preserved specimens (Fig. 6A) overall silvery with much shine at midline, ventral and opercular regions. Other body parts brownish orange or silvery grey. Fins pale, base of caudal fin and, often, anterior part of dorsal-fin base ash-grey. Formalin (initial fixation) and later transferred to 75 % ethanol (Fig. 6B) specimens creamy to yellowish, formalin-deposited whitish. Back greyish brown, posterior head brownish black. Narrow black mid-lateral stripe usually of increasing intensity at caudal peduncle but not reaching caudal-fin base. Ventral body (especially bases of pectoral and anal fins)

ank spaces indicating missing	
i, and E. longicauda; bl	1.
. CMER, E. paludinosu	er specimen as in Table
ramma, Enteromius sp.	in text. Information p
us akakianus, E. pleuro,	niopian Rift as defined
etric data of <i>Enteromi</i>	g to Central Main Eth
Table 3. Morphom	data. CMER referrin

Measurements	E. akakianus,	E. akakianus	E. akakianus		E. ple	urogran	nma		En	teromiu	ssp. CN	IER		E	. palud	inosus			E.	longica	nda	
	holotype			u u	1 I	Max M	fean S	.D.	n M	in Ma	x Meau	n S.L	. n	Min	Max	Mean	S.D.	a	Min	Max	Mean	S.D.
SL, mm	66.0	88.5	67.8	4 2	8.5	35.4 3	1.7	3.0	47 31	.4 70.	4 45.8	.6	3 2(	35.5	70.5	51.1	11.3	3	61.1	67.0	64.6	3.1
Body depth at pelvic-fin origin (% SL)	30.2	28.4	26.0	4	3.1	26.4 2	4.4	1.4	43 22	3 28.	8 25.5	1.0	2 5(	25.1	30.8	27.8	1.8	ŝ	26.3	28.1	27.2	6.0
Minimum caudal-peduncle depth (% SL)	14.0	12.0	12.6	4 1	. <u>6.0</u>	2.9 1	2.0 (	0.8	42 8.	.4 13.	6 12.1	1.0	2(	12.6	14.7	13.5	0.5	ŝ	11.6	12.1	11.9	0.3
Minimum caudal-peduncle depth (% caudal-peduncle length)	66.0	56.8	57.8	4	9.9	5 8.65	2.0	5.6	42 35	6.6 65.	1 54.4	5	5 5(	48.0	62.2	55.2	3.7	$\omega$	41.6	45.2	43.8	1.9
Maximum caudal- peduncle depth (% SL)	14.7	15.1	12.9	4 1	3.2 j	14.0 1	3.6 (	0.3	42 10	.2 16.	6 14.0	1.	2 5	14.9	16.6	15.8	0.7					
Maximum caudal- peduncle depth (% caudal- peduncle length)	69.1	71.7	59.3	4	9.9	53.8	.6.6	3.2	42 43	6.0 74.	4 62.7	0.0	2	62.7	70.1	66.0	3.3					
Predorsal length (% SL)	53.5	54.5	54.5	4 5	1.7	i4.3 5	3.1		41 49	.3 56.	0 52.6	1.5	5 20	50.9	56.3	53.0	1.5	3	49.4	54.2	51.7	2.4
Prepelvic length (% SL)	51.9	49.7	49.8	4 5	0.6	3.0 5	1.9	1.0	42 46	.2 52.	7 50.2	÷	<u>1 2(</u>	45.1	51.2	47.8	1.8	3	45.0	46.5	45.9	0.8
Preanal length (% SL)	72.6	70.9	72.6	4 7	3.5 7	74.4 7	4.1	0.4	42 68	.1 74.	5 71.5	- -	<u></u> 12(	65.6	74.7	71.0	2.0	3	9.99	68.3	67.4	0.9
Pectoral – pelvic distance (% SL)	25.4	21.5	21.6	4 2	0.3	23.9 2	22.4	1.5	43 20	0.0 25.	7 22.4		3 5	20.6	22.9	21.4	0.9					
Pelvic – anal distance (% SL)	23.0	24.2	24.1	4 2	1.7	23.7 2	1.0	0.9	42 20	0.1 27.	8 22.5	2	5	24.6	26.4	25.5	0.7					
Caudal-peduncle length (% SL)	21.3	21.1	21.7	4 2	1.6	23.7 2	3.0	1.0	42 20	.5 25.	1 22.4		0 2(	22.2	26.8	24.5	1.4	3	25.6	29.1	27.2	1.8
Dorsal-fin depth (% SL)	28.7	23.4	25.2	4 2	1.0	2 22	4.6	2.8	40 19	.9 29.	8 25.0	2.7	2 15	23.2	30.5	26.9	1.8	3	21.9	25.5	23.3	1.9
Dorsal-fin depth (% HL)	108.5	86.2	95.3	4 8	4.4 1	00.5 9	0.5	7.1	40 71	.7 108	.2 89.8	7	1,5	94.3	119.3	103.4	5.6	3	91.2	106.5	96.4	8.8
Length of last unbranched dorsal-fin ray excluding the	21.8	19.7	18.5	3 1	7.1	1 8.9 1	7.8	0.9	33 13	3.5 24.	1 19.1	5.	3 17	18.8	24.5	21.6	1.7	3	18.8	22.0	19.9	1.8
segmented part (% SL)																						
Length of last unbranched dorsal-fin ray including the			<u> </u>						23 18	3.0 26.	7 22.6	5	_									
segmented part (% 5L)	0 11		110		5	-	0	0	¢1 C7		-	-		160	000	7 11	-	,	15 4	16.2	15.0	1
Anal-fin depth (% SL)	1/.0	10.1	10.2	4 	7.0	8.0 1	0.1	0.0	42 15	0.0 19.	0 10./	-		10.0	20.0	1/.0	1.0	0 0	17.6	10.0	2.CI	0.4
Pectoral-fin length (% SL) Pectoral-fin lenorh (%	78.9	10.4	893	4 4	0.0	1 0.2	1./.1	2.0	43 L3 43 68	0.20.	1 80.9	<u>و</u> ۱	7 v 7 v	80.4	21./ 93.4	C.71 88 1	۶.0 د 1	c	1/.9	18.8	18.4	0.4
pectoral – pelvic distance)		1					2	ì	3	2		5					:					

Measurements	E. akakianus,	E. akakianus	E. akakianus		E. pl	eurogru	ımma		F	nteron	tius sp.	CMEF	~		E. p.	aludino	sus			E. lo	ngican	ła	
	holotype			u	Min	Max	Mean	S.D.	u u	Min 1	Max N	fean	S.D.	u u	1 In	Aax N	fean S	Ū.	Z u	fin N	ax M	ean S	ġ.
Pelvic-fin length (% SL)	18.4	16.7	19.7	4	13.5	15.8	14.8	1.0	43 ]	5.0	19.5	7.1	0.8	20 1	6.2 2	2.9	9.3	1.5	3 15	8.0 1	3.2 1	8.1	0.1
Pelvic-fin length (% pelvic – anal distance)	7.97	0.69	82.0	4	59.1	68.8	64.7	4.3	42 5	53.9	35.7 7	'5.2	6.2	5	3.1	6.7 (	59.3	5.4					
Pelvic-splint length (% pelvic-fin length)	24.0	15.0	18.8	4	23.2	35.5	29.6	5.5	43 ]	2.8	34.9 2	3.4	4.1	5	2 6.2	6.3	21.3	3.2					
Head length (% SL)	26.5	27.2	26.5	4	24.9	28.6	27.2	1.6	47 2	5.9	31.1 2	8.0	1.3	20 2	4.0 2	7.5	26.0	6.0	3 2	3.9 2	4.4 2	4.2	0.3
Head length (% body	87.6	95.7	101.7	4 1	07.7	116.6	111.6	4.2	43 9	3.7 1	22.7 1	09.2	7.0	20 8	1.5 1	09.5	94.1	7.5	<i>6</i> 0	5.5 9	8 6.2	8.7	3.8
deptn at pelvic-nn origin)				+	1				+					+	+			1	+		+	+	
Head depth at nape (% SL)	18.4	17.9	17.4	4	17.7	19.9	19.0	6.0	46 ]	17.2	21.4	9.5	6.0	2	8.2	1.03	6.8	0.8					
Head depth at nape (% HL)	69.5	65.9	65.8	4	9.05	71.3	70.0	1.0	46 (	53.7	0.77	5.6	2.9	5	9.2	4.5	71.2	2.0					
Head depth at posterior eye margin (% SL)	17.1	15.9	16.5	4	15.4	18.9	17.4	1.4	42 ]	5.3	0.61	7.6	1.0	20 1	5.2	7.6	16.2	0.8	3 1	4.1 1	5.0 1	4.5	0.5
Head depth at posterior	64.6	58.5	62.2	4	52.1	66.1	63.7	1.9	42	58.5	59.5 (	3.1	2.6	20	8.0	5.4 (	52.4	2.0	3	7.6 6	2.6 6	0.0	2.5
eye margin (% HL)																							
Head width (% SL)	13.8	12.4	13.1	4	11.0	13.2	12.1	1.0	42	2.2	16.8 ]	4.3	1.2	20 1	2.0	4.9	3.5	0.9	3 11	2.7 1	3.4 1	3.1	9.4
Head width (% HL)	51.9	45.6	49.6	4	£1.3	46.1	44.4	2.2	42 4	í4.5 (	50.0	1.4	4.0	20 4	5.6 5	57.5	61.9	3.4	3 5	2.6 5	5.5 5	4.3	1.5
Snout length (% SL)	6.9	6.8	6.9	4	6.1	6.9	6.7	0.4	42	6.0	8.1	7.1	0.5	20	5.1	8.0	6.9	0.5	3 6	5.1 6	.8	5.5	0.3
Snout length (% HL)	26.0	25.1	25.9	4	23.7	25.0	24.5	0.6	42 2	22.1	28.7 2	5.4	1.6	20 2	4.2 3	0.4	9.93	1.7	3 2	5.5 2	7.6 2	6.8	1.1
Eye horizontal diameter	6.7	6.1	6.7	4	7.6	8.0	7.7	0.2	42	5.4	9.0	7.1	0.8	20	5.3	7.8	6.9	0.4	3 6	6.3 (	9	5.5	0.1
(% SL)														-					_		_		
Eye horizontal diameter (% HL)	25.2	22.4	25.4	4	26.5	30.4	28.5	1.7	42	6.7	32.7 2	5.5	2.7	20	3.9 3	0.4	26.6	1.6	3 2	6.0 2	7.6 2	6.8	0.8
Eye horizontal diameter	76.4	70.9	76.7	4	31.2	95.0	91.3	6.7	42	50.5 1	08.5 7	4.2	11.3	20 6	3.1 8	3.5	72.3	5.2	3 6	5.4 7	0.2 6	7.5	2.4
(% interorbital width with skin fold)																							
Interorbital width with	8.7	8.6	8.8	4	8.0	9.3	8.5	0.6	42	7.7	12.0	5.7	0.9	20	8.8	0.4	9.6	0.5	3	.4	L.	9.0	0.2
skin fold (% SL)																							
Interorbital width with	33.0	31.5	33.2	4	29.5	32.7	31.3	1.4	42 2	28.7	<u>14.0</u>	4.7	2.9	20 3	3.9 4	0.1	36.9	1.5	3.35	9.3 4	0.0 3	9.7	9.4
skin fold (% HL)																							
Interorbital width between	6.4	6.6	6.3	4	6.3	7.5	6.8	0.6	46	3.4	7.1	5.6	0.0	20	5.3	8.1	7.3	0.4	3	6.9	5	5.2	0.3
frontal margins (% SL)																			_				
Interorbital width between	24.3	24.3	23.8	4	22.9	26.2	24.8	1.4	46 ]	2.0	25.3	0.1	3.2	20	3.9	1.2	28.1	1.7	3	4.3 2	7.0 2	5.8	1.4
frontal margins (% HL)				+					-					+	-				-	+	-	-	
Lower-jaw length (% SL)	9.1	8.8	8.2	4	9.0	9.8	9.4	0.4	47	7.8	9.9	9.1	0.4	Ś	0.0	9.6	9.2	0.2	_	_	_	_	
Lower-jaw length (% HL)	34.4	32.5	31.1	4	33.0	36.1	34.7	1.4	47 2	8.8	36.1 3	32.7	1.8	5	3.9 3	<u>6.0</u>	34.7	1.0					

Measurements	E. akakianus,	E. akakianus	E. akakianus		E. pl	00100Th	mma		En	iteromi	us sp.	CMER	F		E. Da	Indino	SHS			E. 10	ngican	da	
	holotype			q	Min	Max	Mean	S.D.	u N	1in N	fax M	ean S	Ū.	N N	lin V	1ax N	fean S	S.D.	Z Z	lin N	lax N	Iean 3	S.D.
Lower-jaw length (% interorbital width with skin fold)	104.2	103.2	93.8	4	05.3	114.7	111.1	4.1	42 6	9.6 12	21.6 9	5.4 1	11.2	5	6 6.6	7.4 5	02.7	2.8					
Lower-jaw length (% operculum depth)	84.1	87.1	78.0	4	80.0	92.2	87.9	5.5	47 7	0.1 9	3.8 8	0.7	5.4	5 8	2.1 8	6.5 8	33.8	1.7					
Lower-jaw length (% maximum cranium width)	73.8	78.5	71.9	4	76.6	80.4	78.5	1.7	46 6	5.0 8	6.4 7	5.0	5.0	5 7.	2.4 8	0.3 7	76.7	3.8					
Operculum depth (% SL)	10.8	10.2	10.6	4	9.8	11.4	10.8	0.7	47 5	.9 1.	3.2 1	1.4	0.7	5 1(	0.7 1	1.5 1	11.0	0.3					
Operculum depth (% HL)	40.9	37.3	39.9	4	38.4	41.2	39.6	1.2	47 30	6.3 4.	3.9 4	0.6	1.6	5 4(	0.7 4	2.8 4	11.4	0.8					
Cranial-roof length (% SL)	15.8	16.6	16.2	4	18.2	19.8	19.2	0.8	46 1	6.4 2	1.0 1	7.8	1.0	5 15	5.5 1	6.5 1	16.2	0.4					
Cranial-roof length (% HL)	59.7	61.1	61.2	4	68.0	73.3	70.5	2.5	46 5	6.5 6	9.9.6	3.6	3.1	5	8.9 6	2.5 6	6.05	1.4					
Maximum cranium width	78.0	67.8	70.7	4	61.2	64.1	62.7	1.2	46 5	8.5 7	8.2 6	8.7	4.3	5 7.	2.5 7	7.8 7	74.3	2.2					
(%) cranial-root length)																							
Anterior barbel length (% HL)	21.9	16.0	17.5	4	8.4	10.0	9.2	0.7	46 4	i.6 2	4.0 1	. 1.9	4.6	20 4	1	1.6	7.3	2.1	3 1(	1 0.0	3.6	6.11	1.5
Anterior barbel length (% eye horizontal diameter)	86.6	71.4	68.9	4	31.3	33.8	32.4	1.2	42 1	6.6 1(	9.7 4	9.5 2	22.4	20 1	5.6 4	5.4 2	27.6	6.7	3 4(	0.7 4	9.2	<del>14</del> .2	4.4
Posterior barbel length	33.8	27.6	29.3	4	14.2	19.7	17.2	2.5	46 1	5.4 3	8.7 2	4.4	5.3	20 1-	4.2 2	5.7 1	2.61	2.7	3 18	8.1 2	3.7	21.0	2.8
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Posterior barbel length (% eye horizontal diameter)	134.0	123.6	1.611	4	46.7	74.1	61.0	12.1	42 5	4.1 17	7.3 9	3.0	29.2	20 2	2.2 7	) 6./	8.00	6.5	<i>2</i>	8.0	6.6	/8.4	8.1
length of unsegmented part from x-ray (% dorsal-	88.7	92.9	92.5	$\tilde{\omega}$	80.1	6.06	86.8	5.8	35 7.	3.1 9	6.1 8	5.1	6.7	17 8:	3.3 9	6.5 5	91.4	4.5	<u>6</u> 29	2.4 9	3.4	33.0	0.5
fin depth)									_	_	_	_		_	_	_	_				_		
length of lower non- serrated part from x-ray (%	15.0	11.5	17.2	$\tilde{c}$	24.2	29.8	26.5	2.9	35 1	0.4 2	5.4 1	7.4	3.3	17 3.	2.8 4	3.1 5	37.4	2.9	<i>3</i> 3	2.6 3	8:2	35.2	2.8
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length of upper serrated	/2./	61.5	5.0	ŝ	04.5	1.00	00.7	5.4	ŝ	8 8.0	1.2 0	/./	6./	4	0 1.0	. /.1	0.4.0	0.0	ς 	^ 	7./	8./0	7.4
part from x-ray (% dorsal- fin depth)																							
length of lower non-	16.9	12.3	18.6	Э	27.1	32.8	30.6	3.1	35 1.	2.7 3	1.0 2,	0.6	4.5	17 3.	5.9 4	6.6 4	£1.0	3.4	3.3	5.3 4	0.0	37.8	2.9
serrated part from x-ray																							
(% length of unsegmented part)																							
length of upper serrated	83.1	87.7	81.4	Э	67.2	72.9	69.4	3.1	35 6	9.0 8	7.3 7	9.4	4.5	17 5	3.4 6	4.1 5	59.0	3.4	3 59	9.1 6	4.7 (	52.2	2.9
part from x-ray (% length of unsegmented part)																							

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Character states	E. yardiensis	E. akakianus	E. pleurogramma	Enteromius sp. CMER	E. paludinosus	E. longicauda
Number of unbranched dorsal-	$3(47), 4^{*}(22)$ [3.3±0.5]	$3^{*}(2), 4(1) [3.3\pm0.6]$	$3(1), 4(3) [3.8\pm0.5]$	$2(1), 3(38), 4(7) [3.1\pm0.4]$	$2(3), 3^*(14), 4(3)$	$3^{*}(2), 4(1) [3.3\pm0.6]$
fin rays					$[3.0\pm0.6]$	
Number of branched pelvic-fin	7*(35), 8(4) [7.1±0.3]	7*(1), 8(2) [7.7±0.6]	$8(4) [8.0\pm0.0]$	6(1), 7(3), 8(38), 9(5)	$7(1), 8^*(18), 9(1)$	$7(1), 8^{*}(2) [7.7\pm0.6]$
rays				$[8.0\pm0.5]$	$[8.0\pm0.3]$	
Number of branched pectoral-fin	$12^{*}(11), 13(23), 14(5)$	$16^{*}(3) [16.0\pm0.0]$	13(1), 14(1), 15(2)	12(1), 14(5), 15(25),	$13(2), 14^{*}(9), 15(9)$	15*(2), 16(1) [15.3±0.6]
rays	$[12.8\pm0.6]$		$[14.3\pm1.0]$	$16(16) [15.2\pm0.8]$	$[14.4\pm0.7]$	
Total number of vertebrae	$33^{*}(53), 34(16)$	$35(1), 36(1), 37^{*}(1)$	$35(4)$ [ $35.0\pm0.0$ ]	34(2), 35(29), 36(13),	34*(17), 35(3) [34.2±0.4]	$35(2), 36^{*}(1) [35.3\pm0.6]$
	$[33.2\pm0.4]$	$[36.0\pm1.0]$		37(2) [35.3±0.6]		
Number of abdominal vertebrae	$17(18), 18^{*}(50) [17.7\pm0.4]$	20(2), 21*(1) [20.3±0.6]	19(1), 20(3) [19.8±0.5]	19(14), 20(27) [19.7±0.5]	18*(18), 19(2) [18.1±0.3]	$19^{*}(3) [19.0\pm0.0]$
Number of caudal vertebrae	15*(35), 16(32), 17(1)	$15(1), 16^{*}(2) [15.7\pm0.6]$	15(3), 16(1) [15.3±0.5]	14(1), 15(25), 16(15),	$15(1), 16^{*}(17), 17(2)$	16(2), 17*(1) [16.3±0.6]
	$[15.5\pm0.5]$			$17(1) [15.4\pm0.6]$	$[16.1\pm0.4]$	
Number of predorsal abdominal	$10(8), 11^*(60), 12(1)$	$10^{*}(2), 11(1) [10.3\pm0.6]$	11(3), 12(1) [11.3±0.5]	9(1), 10(20), 11(23), 12(1)	$9(1), 10^{*}(19) [10.0\pm0.2]$	$10^{*}(3) [10.0\pm0.0]$
vertebrae	$[10.9\pm0.3]$			$[10.5\pm0.6]$		
Number of preanal caudal	$0^{*}(39), 1(19), 2(1)$	$0^{*}(2), 1(1) [0.3\pm0.6]$	$0(3), 1(1) [0.3\pm0.5]$	0(24), 1(21), 2(1)	$0^{*}(19), 1(1) [0.1\pm0.2]$	$0^{*}(3) [0.0\pm0.0]$
vertebrae	$[0.4\pm0.5]$			$[0.5\pm0.5]$		
Number of vertebrae between	$6(5), 7^*(52), 8(11), 9(1)$	$10(2), 11^{*}(1) [10.3\pm0.6]$	$8(1), 9(3) [8.8\pm0.5]$	8(1), 9(11), 10(29), 11(2),	8*(15), 9(5) [8.3±0.4]	$8(1), 9^{*}(2) [8.7\pm0.6]$
first pterygiophores of dorsal and	$[7.1 \pm 0.5]$			12(1) [9.8±0.7]		
anal nns				1211		
lotal number of lateral-series scales	$32(4), 33(23), 34^*(10),$ $35(2) [33.3\pm0.7]$	$36(2), 37^{*}(1) [36.3\pm0.6]$	34(1), 35(2), 36(1) [ $35.0\pm0.8$ ]	$34(6), 35(17), 36(16), 37(3) [35.4\pm0.8]$	$33^{*}(5), 34(7), 35(5), 36(3)$ [ $34.3\pm1.0$ ]	$35(1), 36^{*}(1), 37(1)$ [ $36.0\pm1.0$ ]
Number of lateral-series scales to	31(7), 32(19), 33*(9).	35*(2). 36(1) [35.3+0.6]	32(1). 34(3) [33.5+1.0]	32(1), 33(9), 34(11).	32*(8). 33(5). 34(7)	34*(2). 35(1) [34.3+0.6]
posterior margin of hypurals	34(4) [32.3±0.9]			35(17), 36(4) [34.3±1.0]	[33.0±0.9]	
Number of scale rows between	6(28), 7*(11) [6.3±0.5]	$6^{*}(3) [6.0\pm0.0]$	6(3), 7(1) [6.3±0.5]	$5(1), 6(35), 7(6) [6.1\pm0.4]$	6*(7), 7(13) [6.7±0.5]	$7^{*}(3)$ [7.0±0.0]
lateral line – dorsal-fin origin						
Number of scale rows between	$1(1), 2^*(30), 3(8)$	$3(2), 4^*(1) [3.3\pm0.6]$	$4(4) [4.0\pm0.0]$	$3(4), 4(37) 5(1) [3.9\pm0.3]$	$3(3), 4^{*}(17) [3.9\pm0.4]$	$3(1), 4^{*}(2) [3.7\pm0.6]$
lateral line – pelvic fin origin	$[2.2\pm0.5]$					
Number of scale rows between	$1(1), 2^*(33), 3(5)$	$4(2), 5^{*}(1) [4.3\pm0.6]$	$4(2), 5(2) [4.5\pm0.6]$	$3(1), 4(35), 5(6) [4.1\pm0.4]$	$4^{*}(16), 5(4) [4.2\pm0.4]$	$4^{*}(3)$ [4.0±0.0]
lateral line – anus	$[2.1\pm0.4]$					
Anterior barbel coded length;	absent	$2^{*}(3)$	1(4)	1(36), 2(10)	$1^{*}(20)$	$1^{*}(3)$
note that the character is not						
applicable for E. yardiensis sp. nov.						
with anterior barbel absent in all						
specimens						
Posterior barbel coded length	1(7), 2*(32)	3(2), 4*(1)	2(3), 3(1)	2(9), 3(35), 4(2)	2*(17), 3(3)	$2^{*}(3)$

and E. longicauda. Values with \* indicating counts found in holotypes and lectotypes. CMER referring to Central Main Ethiopian Rift as defined in text. Values are Table 5. Number of serrae on last unbranched dorsal-fin ray in Enteromius yardiensis sp. nov., E. akakianus, E. pleurogramma, Enteromius sp. CMER, E. paludinosus, minimum - maximum and mean in parentheses. Information per specimen as in Table 1.

	< 25 mm SL	25-30 mm SL	30-35 mm SL	35-40 mm SL	40-45 mm SL	45-50 mm SL	50-55 mm SL	55-60 mm SL	60-65 mm SL	65-70 mm SL	> 70 mm SL
<i>E. yardiensis</i> $n = 39$	8-11 (10)	9-15 (13)	15-19 (17)	17-21 (20)	20*-24 (22)	20	27				
E. akakianus n = 3										24-26* (25)	35
E. pleurogramma $n = 3$		10	12	13							
Enteromius sp. CMER n = 32			10-12 (11)	12-13 (13)	14-18 (16)	14-18 (16)	15-20 (17)	15-20 (17)	15-19 (17)		22
E. paludinosus n = 17				11	15-19 (18)	19-20 (20)	20-23 (21)			20-23 (21)	27*
E. longicauda $n = 3$									20	20-23* (22)	
			~								



Figure 7. *Enteromius yardiensis* sp. nov. alive, paratype, NMW 99640, Awash R. at Aditu (site 3), 35.4 mm SL. Photograph by W. Graf.



**Figure 8.** Dorsal fin in *Enteromius yardiensis* sp. nov., paratype, NMW 99259, side channel of Awash R. at Kada Bada (site 2), 37.6 mm SL, with four unbranched rays. Arrow 1 showing first unbranched dorsal-fin ray, arrow 2 showing relative positions of tip of penultimate (3<sup>rd</sup>) unbranched dorsal-fin ray and lowermost limit of serrated part of last (4<sup>th</sup>) unbranched dorsal-fin ray.

often yellowish. Fins pale, anterior part of dorsal-fin base brownish. Sparse melanophores on rays of dorsal, caudal and anal fins.

**Distribution and habitat.** The new species was found so far only in the Lower Awash River and interconnected lakes (Fig. 1). The altitude ranges from 565 m a.s.l. (10°14'41"N, 40°32'44"E) to 342 m a.s.l. (11°30'54"N, 41°38'57"E). This wetland area is a part of the extensive Afar lowland which is a geological depression caused by



**Figure 9.** Alizarin-stained specimens showing cephalic sensory canals and infraorbitals in **A** *Enteromius yardiensis* sp. nov. (same specimen as in Fig. 8) **B** *Enteromius* sp. CMER, NMW 99237, Lake Ziway (site 8), 34.8 mm SL. Arrows showing part of frontal with no canal in **A** and medial branch of supraorbital canal (CSO) in **B**.

the Afar Triple Junction, connected in the south to the north-eastern segment of the Main Ethiopian Rift (Beyene and Abdelsalam 2005).

Specimens were abundant in shallow shoreline habitats of the main channel (low flow velocity), deep (max. 1.5 m) stretches of side channels, stagnant water bodies of the adjacent floodplain, and the shoreline of lakes (Fig. 11). A preference for structured habitats with aquatic plants, woody debris, dense river bank vegetation and fine substrate (sand and finer fractions) was noted. A pronounced difference between wet and dry season is characteristic for the area. The water was usually turbid (suspended solids). Water temperature ranged from 26.1 °C to 31.9 °C.

Gut contents examined from type locality (N = 15) contained unidentifiable fine organic detritus and remains of planktonic crustaceans (exuviae of Phyllopoda (Cladocera), Copepoda, Rotatoria) but also nematodes, remains of terrestrial arthropods (beetles), diatoms, larger plant material (e.g., leaf parts), seeds, and wood debris, and some non-organic material (sand grains).



**Figure 10.** Axial skeletons of *Enteromius.* **A** *E. yardiensis* sp. nov. (same specimen as in Fig. 6B), 11 showing last predorsal abdominal vertebra and 18 last abdominal vertebra, total vertebrae 33:18+15 **B** *E. akakianus*, holotype, BMNH 1908.1.20.85, Akaki R. (site 13), 66.0 mm SL, 10 showing last predorsal abdominal vertebra and 21 last abdominal vertebra, total vertebrae 37:21+16, The Trustees of the Natural History Museum, London **C** *E. paludinosus*, paralectotype, BMNH 1861.3.10.6–7, Quellimane Mozambique, 50.0 mm SL, 10 showing last predorsal abdominal vertebrae 34:19+15, The Trustees of the Natural History Museum, London. Arrows showing relative positions of tip of penultimate unbranched dorsal-fin ray and lowermost limit of serrated part of last unbranched dorsal-fin ray.

The lowland fish fauna of the Awash River is dominated by cyprinids (*E. yardiensis* sp. nov., *Garra makiensis* (Boulenger, 1903) (in Boulenger 1903b), *G. dembeensis* (Rüppell, 1835), *Labeobarbus intermedius* (Rüppell, 1835) and introduced *Cyprinus carpio* 



Figure 11. Habitat of *Enteromius yardiensis* sp. nov. **A** Side channel of Awash R. at Kada Bada (site 2; 10°13'53"N, 40°34'43"E; 562 m a.s.l.), type locality of *E. yardiensis* sp. nov. **B** Lake Yardi (site 1; 10°15'10"N, 40°32'9"E; 562 m a.s.l.).

Linnaeus, 1758 but also includes *Micropanchax antinorii* (Vinciguerra, 1883), *Oreo-chromis niloticus* (Linnaeus, 1758), *Coptodon* cf. *zillii* and *Clarias gariepinus* (Burchell, 1822) (Golubtsov et al. 2002; Englmaier 2018).

**Etymology.** The species name *yardiensis* refers to Lake Yardi, where the new species is abundant.

**Comparative remarks.** Our data confirm the assumption that *E. yardiensis* sp. nov. belongs to the group of *E. paludinosus*-like smiliogastrin barbs. Based on data from Greenwood (1962) and Golubtsov and Berendzen (2005) (the latter for *E. kerstenii* from Lake Chamo-Abaya basin), the new species is different from the *E. kerstenii* complex by the absence of an orange or yellow blotch on the operculum (vs. presence) and 32–35 total lateral-series scales (vs. 23–27 in the lateral line that equals to lateral series in case lateral line is complete).

*Enteromius yardiensis* sp. nov. clearly differs from all examined species (Tables 2–5) and still unidentified forms (or undescribed species) in the group of *E. paludinosus*-like fishes by the absence of the anterior barbel, the absence of the medial branch of the supraorbital cephalic canal and few, 1–3, commonly two, scale rows between lateral line and anus.

**Comparison of** *E. yardiensis* **sp. nov. with Ethiopian congeners.** Besides the characters mentioned above, *E. yardiensis* **sp. nov.** is readily distinguished from the *E. pleurogramma* syntypes (Lake Tana basin, Upper Blue Nile) by a set of characters: commonly seven branched pelvic-fin rays (vs. eight); 33–34 total vertebrae (vs. 35); 17–18 abdominal vertebrae (vs. 19–20); 6–9, commonly seven, vertebrae between first ptery-giophores of the dorsal and anal fins (vs. 8–9, commonly nine); 32–35, commonly 33, total lateral-series scales (vs. 34–36); and 1–3, commonly two, scale rows between the lateral line and the pelvic-fin origin (vs. four) (Table 4).

Literature data confirm the distinctiveness of the new species and *E. pleurogramma* from Lake Tana which is characterised by 7–9, commonly eight, branched pelvic-fin rays; 34–36, commonly 35, total vertebrae; 32–37, commonly 35, total lateral-line scales; and 4–6 scale rows between the lateral line and the pelvic-fin origin (Dejen et al. 2002; Golubtsov and Berendzen 2005).

*Enteromius yardiensis* sp. nov. can be further distinguished from *E. akakianus* (including the holotype of the latter species, Fig. 12A) by 12–14, commonly 13, branched pectoral-fin rays (vs. 16); 33–34 total vertebrae (vs. 35–37, Fig. 10B); 17–18 abdominal vertebrae (vs. 20–21); 6–9, commonly seven, vertebrae between first pterygio-phores of the dorsal and anal fins (vs. 10–11); 32–35, commonly 33, total lateral-series scales (36–37); and posterior barbel coded length 1–2 (vs. 3–4) (Table 4).

*Enteromius akakianus* (Akaki River, Upper Awash drainage) is similar to the CMER combined sample (Fig. 12B, C) by most characters (Tables 3–5).

*Enteromius yardiensis* sp. nov. differs from the CMER *Enteromius* by 12–14, commonly 13, branched pectoral-fin rays (vs. 12–16, commonly 15); 33–34 total vertebrae (vs. 34–37); 17–18 abdominal vertebrae (vs. 19–20, commonly 20); 6–9, commonly seven, vertebrae between first pterygiophores of the dorsal and anal fins (vs. 8–12, commonly 10); 32–35, commonly 33, total lateral-series scales (vs. 34–37, commonly 35); posterior barbel coded length 1–2 (vs. 2–4, commonly 3) (Table 4). *Enteromius yardiensis sp. nov.* is further distinguished from the CMER samples by a wider than deep io5 (vs. deeper than wide, Fig. 9B).



Figure 12. General appearance of **A** *Enteromius akakianus* (same specimen as in Fig. 10B) The Trustees of the Natural History Museum, London **B** *Enteromius* sp. CMER, NMW 99239, Labo R., Meki R. drainage (site 6), 70.4 mm SL **C** *Enteromius* sp. CMER, NMW 99238, Lake Ziway (site 7), 45.4 mm SL.



**Figure 13.** General appearance of *Enteromius paludinosus* (same specimen as Fig. 10C) The Trustees of the Natural History Museum, London.

We did not examine specimens from the Didessa River (tributary of the Blue Nile), the White Nile and the Omo River and refer to published data (Golubtsov and Berendzen 2005) for a comparison. These populations are identified as *E. paludinosus* (Golubtsov and Berendzen 2005) or as a member of the *E. pleurogramma* complex (Mina et al. 2017). *Enteromius yardiensis* sp. nov. can be readily distinguished by the absence of the anterior barbel (vs. always present); 12–14 branched pectoral-fin rays (vs. 13–17); 33–34, commonly 33, total vertebrae (vs. 33–36, commonly 34–35); predorsal length 53–58 % SL (vs. 46–55 % SL). Data on cytb located Didessa *Enteromius* close to *E. pleurogramma* but as a still distant (p-distance 6.6–6.8 %) and well supported clade (Fig. 3, Suppl. material 1, Table S4).

Comparison of E. yardiensis sp. nov. with East African congeners outside Ethiopia. All morphological analyses of the type series of *E. paludinosus* and *E. longicauda* (both are from the Lower Zambezi) revealed their closest morphological affinities. This brings additional support to Greenwood's (1962) and Seegers' (1996) synonymisation of the two species, with priority to the name paludinosus. Enteromius yardiensis sp. nov. differs clearly from *E. paludinosus* samples (which combine the type specimens of E. paludinosus and E. longicauda) (Fig. 13) by the absence of the anterior barbel (vs. presence) and a set of counts (Table 4), including fewer, 1-3, mean 2.2, transversal scale rows between the lateral line and the pelvic-fin base (vs. 3-4) and between the lateral line and the anus, 1–3, mean 2.1 (vs. 4–5); fewer total vertebrae, 33–34, mean 33.2 (vs. 34–36, mean 34.3); and a higher number of predorsal abdominal vertebrae, 10–12, mean 10.9 (vs. 9–10, mean 10.0). The ranges of number of lateral-series scales largely overlap (32-35, mean 33.3 vs. 33-37, mean 34.5), but the highest count, 36 and 37, recorded in *E. paludinosus* were not found in the new species. A character distinguishing the two species is also the structure of the last unbranched dorsal-fin ray: in *E. yardiensis* sp. nov., the lower (non-serrated) part of the ray is short (less than onethird of the entire unsegmented part of the ray) (Fig. 10A) vs. a markedly longer (much longer than one-third) lower non-serrated part of the ray in *E. paludinosus* (Fig. 10C). Respectively, in *E. yardiensis* sp. nov., the upper serrated part is commonly longer than 75 % of the entire unsegmented part of the ray (vs. 59 % and less in *E. paludinosus*).

Enteromius amphigramma (Nairobi River, Kenya [Nairobi River, Kilimanjaro]), E. loveridgii (Amala River, Kenya), E. macropristis (Lake Victoria), E. macropristis meruensis (Mount Meru, Tanzania) and E. vinciguerraii (Wembere River, Tanzania) are currently synonymised with E. paludinosus. The type series of these nominal species examined in the present study showed that they are different from E. yardiensis sp. nov., first of all, by the presence of the anterior barbel, which is well-developed in all species including small-sized E. vinciguerraii.

*Enteromius yardiensis* sp. nov. shares with *E. macropristis meruensis* and *E. vinciguerraii* such characters as a high number of predorsal abdominal vertebrae (10–12) and a lower number of vertebrae between the first pterygiophores of the dorsal and anal fins (6–9). However, the new species is well distinguished from the two by fewer vertebrae: 33–34 total and 17–18 abdominal (vs. 35–36 and 19, respectively) and the absence of a small distinct dark spot at the end of the caudal peduncle (vs. presence).

Within the group of small-sized African smiliogastrin barbs with a thickened and serrated last unbranched dorsal-fin ray outside Ethiopia, a very short or vestigial anterior barbel was reported in *E. apleurogramma* (Boulenger, 1911) from Lake Victoria, *E. amboseli* (Banister, 1980) from the Middle Athi River in Kenya (Boulenger 1911, Banister 1980, Schmidt et al. 2018), and specimens identified as *E. paludinosus* from Satansplatz in South Africa (nowadays Namibia, Orange River drainage, Atlantic basin) (Greenwood 1962: 162). Neither *E. apleurogramma* nor *E. amboseli* has been reported from Ethiopian drainage systems. *Enteromius yardiensis* sp. nov. is readily distinguished from both species by a complete lateral line (vs. incomplete) and a higher number of lateral-series scales (32–35 vs. 20–25).

## Discussion

Combined morphological and mitochondrial data obtained in this study clearly show a distinctiveness of the Lower Awash *E. yardiensis* sp. nov. from *Enteromius* species distributed in the CMER region. This latter form, as shown above, was supported as a distinct unit on the species level.

The two most distinguishing characters, the absence of the anterior barbel and the absence of the medial branch of the supraorbital sensory canal, are both apparently specialisations (derived states) representing reductions of structures commonly present in the studied group of species.

The conclusion that the absence of the anterior barbel in all examined specimens of different size (8.0–52.8 mm) is a secondary reduction in the new species, is supported by the presence of a respective foramen in the maxillary for the maxillary branch of the trigeminal nerve innervating the anterior barbel (in species with the anterior barbel present). The presence of the anterior (rostral) barbel and the maxillary foramen for the nerve are assumed apomorphies of the subfamily Cyprininae (now at the family level) of the family Cyprinidae (Howes 1981). The secondary reduction of the anterior barbel might be related to the small size of E. yardiensis sp. nov. Greenwood (1962) recorded the reduction of the anterior barbel in *E. paludinosus* from Satansplatz. However, small-sized E. vinciguerraii and, apparently, small-sized E. paludinosus-like fishes (maximum SL does not exceed 40 mm) possess a well-developed anterior barbel. In E. vinciguerraii, the anterior barbel is present in smallest examined specimens (SL less than 30 mm). Barnard (1943: 172) analysed a series of small E. paludinosus-like fishes from the Fish River at Aiais, South West Africa, Orange River drainage (nowadays Namibia) and found that the anterior barbel was already developed in fishes 29-30 mm long (probably TL).

The medial branch, even a very short segment, of the supraorbital canal was not found in the examined material of the new species. In all other examined species, it was present though variably long – the longest state is the terminal pore of the branch located at the frontal parietal border and the shortest is the branch reduced to a tiny canaliculum. Among the examined set of species, the cephalic sensory canal pattern (disjunctions between the canals and the lack of particular canal segments, for example, on the operculum) is very diverse. It is much more variable than described by Skelton (1980), for South African redfin barbs, who distinguished two stable patterns: type A with 1) the preopercular-mandibular and infraorbital canal connected with the operculum and 2) the medial branch of the supraorbital canal present (serrated-rayed redfins); and type B with 1) the preopercular-mandibular and infraorbital canal disconnected and 2) the medial branch of the supraorbital canal absent (flexible-rayed redfins) (Skelton 1980: fig. 3.48). The cephalic canal pattern found in *E. yardiensis* sp. nov. belongs to Type B though the species is characterised by a thickened and serrated ray in the dorsal fin.

As E. yardiensis sp. nov. is not conspecific with E. akakianus, the important issue was to identify the CMER specimens. No clear morphological difference was found between the holotype and topotypical specimens of E. akakianus and the CMER Enteromius, so, we preliminary identify the latter as E. akakianus. However, there is no genetic data available at present to check this hypothesis. We did not manage to collect Enteromius in the Upper Awash River and its tributaries downstream to the Koka Reservoir (Fig. 1, unnumbered localities: Chilimo Forest, Gare Arera, Awash Belo, Awash Kunture, Sulula, Lafessa) (see also Englmaier 2018: fig. 4a). Furthermore, small-sized smiliogastrin barbs with a serrated last unbranched dorsal-fin ray were not found in the entire Upper Awash by other authors either (Getahun and Stiassny 1998, Golubtsov et al. 2002). An upstream migration of *Enteromius* to the source region of the Awash River is supposedly blocked by the chain of cascades at Awash Kunture (Fig. 14) that was established at least with the onset of the rifting process at approximately 6-5 Ma (Bonini et al. 2005). It was shown that the upstream dispersal of the fish assemblages in the Awash River is considerably influenced by these cascades (Englmaier 2018).

So far, the only known locality in the Upper Awash is the type locality of *E. akakianus*, the Akaki River. It is a tributary to the Awash River downstream of the Awash Kunture rapids. This might explain why *Enteromius* could penetrate into the Akaki River from the CMER (Fig. 14). The Akaki River was sampled by GE and GT in March 2019, but no *Enteromius* were found. The river is heavily polluted by domestic and industrial sewage from Addis Ababa (see also Golubtsov et al. 2002).

In geological scales, the recent Awash River course is relatively young (Sagri et al. 2008) and the ancient Upper Awash system (upstream of the Koka Reservoir) was situated within the northern part of the CMER (Sagri et al. 2008; Benvenuti and Carnicelli 2015). Paleo-hydrological data indicate that the Upper paleo-Awash emptied into the lake basin of the CMER at least until the beginning of the Holocene (Sagri et al. 2008; Benvenuti and Carnicelli 2015). Connections between the recent Upper Awash drainage and the lakes in the CMER via rivers (now dry valleys Fesesa, Koye, Sulula Hafa and Cheleleka, Fig. 14) are well documented. Sediment records date the lacustrine (Megalake and Makrolake) phases in the Pleistocene at 100,000–22,000 years BP and in the Holocene at 10,000–5,000 years BP (Laury and Albritton 1975; Street 1979; Le Turdu et al. 1999; Sagri et al. 2008; Benvenuti and Carnicelli 2015).



**Figure 14.** Dispersal pathways (**A–G**) and tectonic setting in Central Main Ethiopian Rift. (**A**) pathway into Akaki R. (**B**) Fesesa and Koye paleovalleys (**C**) Sulula Hafa, and Cheleleka palaeovalleys (**D**) interconnection between CMER lakes (**E**) pathway into L. Awasa (**F**) connection to Omo drainage (**G**) connection to Southern Main Ethiopian Rift. Based on Bonini et al. (2005); Sagri et al. (2008: figs 6, 12); Maslin et al. (2014); and Benvenuti and Carnicelli (2015: fig. 17.13).

These data allow us to hypothesise that the lower part of the Upper paleo-Awash system including the Akaki River was in contact with the CMER at least until the beginning of the Holocene providing pathways for fish dispersal (Fig. 14). Fish taxa shared by the Upper Awash and the CMER include *Garra makienis*, *G. quadrimaculatal aethipica* complex, *G. dembeensis*, *Labeobarbus intermedius*, *Oreochromis niloticus*, *Clarias gariepinus*, *Micropanchax antinorii* and an *E. paludinosus*-like smiliogastrin barb

(Golubtsov et al. 2002; Stiassny and Getahun 2007; Vijverberg et al. 2012; Englmaier 2018). However, *L. ethiopicus* (Zolezzi, 1939) endemic to the Lake Ziway basin and *L. beso* (Rüppell, 1835) distributed in the Awash downstream to Nur Sada and in the Blue Nile may indicate a more complex scenario of vicariant and dispersal events.

Another important aspect is the pattern of geographic distribution of *E. yardiensis* sp. nov. and substantial level of morphological and genetic divergence from *E. akaki-anus* in the concept accepted above with little evidence of any gene flow.

In general, distribution of *Enteromius* in endorheic basins of central Ethiopia is congruent with its geological and zoogeographical delineations. The MER is a geologically heterogeneous system that was traditionally differentiated into three main segments: (1) the Southern (SMER), (2) the Central (CMER), and (3) the Northern MER (NMER) (Bonini et al. 2005 and references therein). The NMER extends from the Afar Depression south-west to the Yerer-Tullu Wellel major transversal fault following the middle course of the Awash River valley. The CMER encompasses most of the Lakes Region from the Koka Reservoir south to the Lake Awasa area separated from the SMER by the Goba-Bonga major transversal fault (Fig. 14). The SMER is not clearly separated from the Kenyan Rifts extending in the south into a system of basins and ranges referred to as the broadly rifted zone of Southern Ethiopia connected with both the Kenyan Rift and the Albertine (Western Branch) Rift (Bonini et al. 2005; Corti 2009; Mairal et al. 2017).

*Enteromius paludinosus*-like fishes are absent from the SMER. This area is inhabited by an *E. kerstenii*-like species (Lakes Chamo-Abaya) and at least two species without a serrated last unbranched dorsal-fin ray (Lakes Chamo-Abaya, Lake Chew Bahir, Lake Turkana) (Seegers et al. 2003; Golubtsov et al. 2002; Golubtsov and Habteselassie 2010). Zoogeographically, the SMER belongs to the Nilo-Sudan Province of Roberts (1975) and Paugy (2010) which is characterised by a higher number of species including typical nilotic elements when compared to the fish fauna of the CMER and NMER. These two latter areas are part of the Ethiopian Rift Valley Province (Paugy 2010; fig. 5).

CMER and NMER both have their individual, geographically isolated, species of *E. paludinosus*-like smiliogastrin barbs. *Enteromius yardiensis* sp. nov. was found only in the wetland area at Gewanae - site 1 (Lake Yardi) and site 2 (Kada Bada) and further downstream (Fig. 1) (approximately 400 km between the CMER and Gewanae). It was absent from five sampled localities in the CMER and the NMER (Wonji, Korkada, Nur Sada, Yimre, and Worer) downstream to Gewanae (Englmaier 2018: fig. 4b).

An isolation of the Lower paleo-Awash from the CMER occurred between the latest Pleistocene (100,000 years BP) and early Holocene (5,000 years BP), as indicated by paleo-hydrological data (Sagri et al. 2008; Benvenuti and Carnicelli 2015). The recent course of the Awash was established in the mid-Holocene and followed an opening of the Afar Depression accompanied by volcanic activities (Sagri et al. 2008). The Upper paleo-Awash system was subsequently disconnected from the CMER lakes (Sagri et al. 2008) and followed the rift to the north-east (connection to the Lower paleo-Awash drainage). River networks in earlier geological stages of the CMER are still unknown, but the course of the paleo-Awash drainage must have been subject to frequent changes (Gallotti et al. 2010). Tectonic and volcanic activities, geomorphological changes (erosion, downcutting) and a changing paleoclimate (wet and dry periods) are well documented for the MER until the Miocene (Kalb et al. 1982; Kalb 1995; Benvenuti et al. 2002; Abebe et al. 2007; Gallotti et al. 2010; Abbate et al. 2015; Benvenuti and Carnicelli 2015). This has not only affected distribution patterns of terrestrial animals (e.g., Bibi et al. 2017) and vegetation cover (e.g., Bonnefille et al. 2004; WoldeGabriel et al. 2009; Bibi et al. 2017) but presumably also the evolution of the river networks.

The presence of fish in the Lower paleo-Awash is known from excavations in the area of Gewanae which date back to the Miocene (Murray and Stewart 1999; Wold-eGabriel et al. 2009; Stewart and Murray 2017). In late Miocene deposits, the earliest fossil evidence of a cyprinid with a serrated dorsal-fin ray in the paleo-Awash corridor was discovered (Stewart and Murray 2017). This is well in accordance with the formation of the MER at approximately 5 Ma BP (Bonini et al. 2005). However, it is uncertain if this fossil record represents an ancestor of small-sized African smiliogastrin barbs (Stewart and Murray 2017).

To summarise, the results of the present study provide solid support for some conclusions. First, Ethiopian *Enteromius* species with a serrated dorsal-fin ray are distant from true *E. paludinosus* (with *E. longicauda* as a synonym) and the so-called *E. paludinosus* complex involves several distinct species in accordance with molecular data of Schmidt et al. (2017). Second, two distinct species occur in the Main Ethiopian Rift area – a new species, *E. yardiensis* sp. nov., endemic to the Afar Depression in the north-eastern part of the NMER, and *E. akakianus*, endemic to the CMER lake region and the lower reaches of the Upper Awash River. An integrated approach combining genetic markers and a variety of morphological methods based on a wide set of characters, including osteology and sensory canals, proved to be very productive for taxonomy in this group of fishes.

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# Supplementary material I

# Supplementary tables S1–S10

Authors: Gernot K. Englmaier, Genanaw Tesfaye, Nina G. Bogutskaya Data type: species data

Explanation note: Table S1. Voucher specimens included in present study, with Gen-Bank accession numbers and localities. Table S2. List of character states (morphometric characters, coded qualitative characters, and meristic characters). Table S3. Primary data (morphometric and meristic) for all examined specimens. Values highlighted in blue mark cases where means used to substitute missing data; characters in red not used for statistical analyses (because of incomplete data); blank spaces

indicating missing data. **Table S4.** Pairwise genetic distances (p-distances) for CO1 and cytb. **A** comparison of 611 bp of CO1 between Ethiopian populations and *Enteromius paludinosus* from Lower Zambezi River (LT629216, LT629217, locality closest to type locality of *E. paludinosus*) **B** comparison of 520 bp of partial cytb between Ethiopian populations; (minimum-maximum and mean in parentheses). For localities see Fig. 1 and Table 1. **Table S5.** MDS: Distances in final configuration, for six samples based on means for 40 morphometric, 15 meristic and two coded qualitative characters (as in Tables 2–5). **Table S6.** PCA: Factor coordinates only containing two first factors (82.6 % of variance) for 40 morphometric, 15 meristic and two coded qualitative characters (as in Tables 2–5). Most contributing characters highlighted. **Table S7.** DFA statistics (*forward stepwise*, minimal tolerance). **Table S8.** DFA: Classification matrix. **Table S10.** DFA: Squared Mahalanobis distances (distances between group centroids). **Table S10.** DFA: Standardised coefficients. Most contributing characters highlighted.

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- Link: https://doi.org/10.3897/zookeys.902.39606.suppl1

# Supplementary material 2

# Figure S1. Bayesian Inferences (BI) analysis, 520 bp of partial cytb (same data set as in Fig. 3)

Authors: Gernot K. Englmaier, Genanaw Tesfaye, Nina G. Bogutskaya

Data type: phylogenetic data

- Explanation note: Posterior probabilities from BI analysis and bootstrap (bs) values for ML (1000 bootstrap replications) above and below slash. Values below 0.70/50 considered as collapsed. Colours corresponding to those in Fig. 1.
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Link: https://doi.org/10.3897/zookeys.902.39606.suppl2



# First report on the gall wasp Ophelimus near migdanorum (Hymenoptera, Eulophidae) and its parasitoid Closterocerus chamaeleon (Hymenoptera, Eulophidae) in Eucalyptus globulus in Bogotá, Colombia

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# Abstract

*Ophelimus* near *migdanorum*, a foliage gall wasp-inducer of *Eucalyptus*, is recorded for the first time in Colombia, infesting both mature ornamental trees of *Eucalyptus globulus* (Labill.) and monoculture plantations in the periurban forest in the city of Bogotá. The parasitoid *Closterocerus chamaeleon* was also emerged from the galled foliage. The spread of this pest and its parasitoid in other *Eucalyptus* species planted in Colombia has not been evaluated.

# Keywords

Biological control, introduced pest, natural enemy

# Introduction

*Eucalyptus* (Myrtaceae) is the most planted tree genus for commercial purposes in Colombia. *Eucalyptus globulus* (Labill.) was introduced in Colombia at the beginning of the 20<sup>th</sup> century, and during the decades of 20's and 30's was used in reforestation programs to protect soils around the rivers San Cristóbal, San Francisco, and Arzobispo, around the city of Bogotá (Bustos and Venegas 1975). Currently, *E. globulus* is one of the predominant species in the oriental periurban areas of the city, where occupies approximately 2300 has (Secretaria Medio Ambiente – JBB 2007). *Eucalyptus globulus* in Colombia, up to the present time, records two exotic sap-sucker pest species (Hemiptera: Aphalaridae) attacking the foliage: *Ctenarytaina eucalypti* Maskell (Pinzón et al. 2002) and *Ctenarytaina spatulata* Taylor (Rendón-Mera et al. 2017). To date, no gall-forming insects had been reported in *Eucalyptus* trees planted in Colombia.

# Materials and methods

In order to characterize the damage and associated insects, samples of mature leaves of *E. globulus*, fallen on the ground, were collected during July 2019 in a city park of Bogotá under a number of trees older than 30 years. Also, apical sections 20 cm long, containing young leaves, were collected from mature branches of about ten years old-trees, in a periurban area of the city. Affected trees are growing at 2650 m of altitude and temperatures ranging from 8 to 18 degree Celsius.

The collected samples were maintained in transparent airtight containers, in the Forest Health Laboratory of the Universidad Distrital "Francisco José de Caldas", at environment temperature ranging from 8 to 18 °C, until the wasps' emergence.

Emerged wasps were preserved in 95 % ethanol, and samples of the wings and the antennae were prepared for microscopic analysis using PVA mounting media (Bioquip, Rancho Dominguez, California, USA). Voucher specimens are maintained in the Forest Entomological Collection hosted in the Forest Health Lab. under the code CE-FUDFJC-LS2019100. Photographs were taken using a 506 Axiocam camera mounted in a Carl Zeiss Discovery 8 Stereo-microscope and stacked using Zen software (version1.04). Size and density of galls in the leaf blade were estimated using the measure option available in the Zen software. A total of 100 fully developed galls were measured and ten leaves were used to estimate average number of galls per square centimeter.

Taxonomic identification of the wasps was performed following characters described in Protasov et al. (2007a, b), the key presented by Borowiec et al. (2019), and the description of Molina-Mercader et al. (2019a, b).

# Results

Affected leaves display numerous tiny, slightly ellipsoid, galls (44 galls per cm<sup>2;</sup> average area of a single fully developed gall: 0.06 mm<sup>2</sup>) (Fig. 1). The galls are perceptible to the touch on both the upper and the underside of the leaves and are randomly distributed mostly in the leaf blade but sometimes near the central nerve or in the petiole. Most of the galls are individual, but frequently form clusters of three or four. Likely, the formation of the galls induces premature fall of the foliage under the affected trees. The accumulation of the leaves under the infested trees facilitated the detection, since the height of the trees prevents the direct observation of the foliage. Both a gall-forming species of *Ophelimus* (Eulophidae: Opheliminae) and its parasitoid *Closterocerus chamaeleon* (Girault) (Eulophidae: Entedoninae) were obtained from the collected samples.



**Figure 1.** Different stages of gall development on *E. globulus* foliage. **A** Initial stage **B** fully developed galls **C** fully developed galls in the petiole. Scale bar: 1 mm.



Figure 2. Ophelimus sp. Body in lateral view. Scale bar: 1 mm.

Body length of the *Ophelimus* reported here (Fig. 2) range from 1 to 1.1 mm, body color dark brown with metallic shine, and both eyes and ocelli are dark. Legs are dark brown except for the apical region of the femur and tibia and the three first tarsomeres of the meso and metathoracic legs, which are pale. Fore wings have one or two setae on the submarginal vein. Antennal scape is a dark color with flagellomeres and clava being pale brown.

Ophelimus reported here does not fit precisely the morphological characters or types of galls reported for O. maskelli (Ashmead), Ophelimus eucalypti (Gahan) or O. mediterraneus Borowiec & Burks. According with the keys compiled by Borowiec et al. (2019), O. eucalypti have more submarginal setae on the forewings and is larger than Ophelimus sp. On the other hand, although O. maskelli induces galls that are visible on both sides of the leaves, they are larger and, also, the wasps have only one submarginal seta on the fore wing. Similarly, O. mediterraneus differs in having 2–4 submarginal seta on the fore wings, and although induces small galls, they are only visible on the upper side of the leaves.

Conversely, length of the wasp of *Ophelimus migdanorum* (Molina-Mercader et al. 2019b), general color pattern of the body, number of submarginal seta on the fore



**Figure 3.** *Closterocerus chamaeleon* emerged from mature *E. globulus* leaves infested by *Ophelimus* sp. **A** Dorsal view **B** lateral view. Scale bars: 1 mm.

wings, as well as size of the galls (small), developing in both sides of the leaves and the petiole, resemble the case reported here. *Ophelimus migdanorum* also attacks *E. globulus*, and considering that Chile is relatively close to Colombia, it may likely be the same species but further detailed comparisons are needed.

A second wasp, found in the same samples of *E. globulus* foliage, was identified as *Closterocerus chamaeleon* (Fig. 3), which, in addition to the keys, was identified using characters included by Molina- Mercader et al. (2019a), such as eye color and body shape, that were useful to fully separate the gall wasp identity from that of the parasitoid.

# Discussion

The genus Ophelimus Haliday, native of Australia, comprises approximately 51 species (Noyes 2019) of gall-inducing wasps. To date, four *Ophelimus* species are known to occur outside Australia attacking eucalypts. Ophelimus eucalypti was detected in New Zealand attacking E. globulus (Withers et al. 2000); O. maskelli, is widely distributed in North America and the Mediterranean basin, attacking E. camaldulensis and E. tereticornis (Burks et al. 2015a, b; Protasov et al. 2007b); O. mediterraneus occurring in France, Italy and Portugal attacking E. globulus, E. cinerea, E. gunni and E. parvula (Borowiec et al. 2019) and O. migdanorum attacking E. globulus in Chile (Molina-Mercader et al. 2019). The Ophelimus reported here, corresponds to a third of the fortuitous introductions of *Ophelimus* species and its parasitoid in South America, being the previous registers in Argentina (O. maskelli, attacking E. camaldulensis, Aquino et al. 2015) and Chile (O. migdanorum, attacking E. globulus, Molina-Mercader et al. 2019a). Most likely O. nr migdanorum infestation found in Bogotá, along with its parasitoid, correspond to an accidental introduction occurring several years ago but that had gone unnoticed. The size of the galls is small, the wasps are microscopic, and this may have contributed to the fact that the case has gone unnoticed.

*Closterocerus*, on the other hand, is also an Australian genus that comprises 74 species (Noyes 2019), of which *Closterocerus chamaeleon* have been described as an ectoparasite with a narrow range of host species among eulophids forming galls on eucalypts (Protasov et al. 2007a). This parasitoid was effectively introduced from Australia to Israel and Italy, to content *O. maskelli* attacks (Protasov et al. 2007a). However, it was naturally dispersed and reduced *O. maskelli* populations in neighboring countries such as Tunisia and Portugal (Branco et al. 2014), and from there likely to North and South America (Aquino et al. 2015, Molina et al. 2019). In the areas of Bogotá where *O.* nr *migdanorum* is being reported, the parasitoid *C. chamaeleon* seems to be well established, as also indicated for *O. maskelli* in California (Burks et al. 2015a). Perhaps, the high effectiveness of *C. chamaeleon* has prevented the intensity of the attacks of *Ophelimus* sp. in urban trees of *E. globulus* in Bogota and surrounding areas, since heavily defoliated areas have not been observed.

Several species of *Eucalyptus*, other than *E. globulus*, are planted in Colombia for industrial purposes; therefore, a further study aimed to detect and estimate the distribution and prevalence of *Ophelimus* sp. and parasitism success of *C. chamaeleon* on those species is valuable.

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