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A new flatworm species of *Temnocephala* (Rhabdocoela, Temnocephalidae) ectosymbiont on the freshwater crab *Valdivia serrata* (Decapoda, Trichodactylidae) from Amazonas, Colombia

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Abstract

A new species of temnocephalan is described from the branchial chambers of *Valdivia serrata* in Colombia as *Temnocephala ivandarioi* **sp. nov.** The most distinctive characters of the new species are in the cirrus and the epidermal 'excretory' syncytial plates. In the present study, the terminology to describe the cirrus of species of *Temnocephala* is updated. Comparison between the shape of the cirrus of the temnocephalans associated with trichodactylid crabs is also provided.

Keywords

Crustacea, Reserva Natural Tanimboca, taxonomy, *Temnocephala ivandarioi* sp. nov.

Introduction

The genus *Temnocephala* Blanchard, 1849 includes 37 species of symbiotic freshwater rhabdocoels from the Neotropics, which are associated with a large variety of hosts including chelonians (3), mollusks (6), insects (7) and crustaceans (21) (Garcés et

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al. 2013, Martínez-Aquino et al. 2014, Arias-Pineda et al. 2015, Seixas et al. 2015a, Ponce de León and Volonterio 2018, Seixas et al. 2018). At present, only two species have been described from Colombia, *T. colombiensis* Garcés, Puerta, Tabares, Lenis & Velásquez, 2013, ectosymbiont of the gastropod *Pomacea* sp., and *T. icononcensis* Arias-Pineda, Damborenea & Castro, 2015, hosted by the pseudothelphusid crabs *Hypolobocera bouvieri* (Rathbun 1898), *Phallangothelphusa dispar* (Zimmer 1912) and *Strengeriana cajaensis* Campos & Rodríguez, 1993. In this paper, we describe and illustrate a new species of *Temnocephala* associated with the trichodactylid crab *Valdivia serrata* from the Colombian Amazonas.

Materials and methods

Eleven specimens of *Valdivia serrata* White, 1847 (crab) were manually collected from the Reserva Natural Tanimboca, Leticia, Amazonas (4°07'39.8"S, 69°57'13.0"W), Colombia. The specimens were transported alive to the Programa de Estudio y Control de Enfermedades Tropicales (PECET) laboratory and identified using a decapod key (Campos 2014). Specimens of *Temnocephala* were removed from the branchial chambers of host crabs under a stereomicroscope, rinsed in saline solution, and preserved in cold alcohol-formalin-acetic acid (AFA) and 70% ethanol for morphological identification by light microscopy; or fixed in 2.5% glutaraldehyde for observation by Scanning Electron Microscopy (SEM). The temnocephalans were identified by focusing on the morphology of the reproductive complex, the shape of the epidermal excretory syncytial plates (DLSPs) and the deposit areas of the eggs in the host. Terminology to describe the cirrus of the temnocephalans was updated from Sewell et al. (2007), Seixas et al. (2011), Garcés et al. (2013) and Ponce de León et al. (2015). Morphology of the male and female reproductive system was studied by light microscopy in specimens mounted as permanent slides in Canada balsam, stained in Meyer's paracarmine and Borax carmine. Samples were also observed by SEM to determine the shape and position of the egg filament, the fracture plane of the eggshell, the shape of the DLSPs and the relative position of the excretory pore. Measurements were in micrometres (µm) unless otherwise indicated; ranges were determined followed by the arithmetic mean, the standard deviation and the number of specimens measured for a given character (mean, standard deviation, n). Photomicrographs of the temnocephalans were taken with a Nikon Alphaphot YS-2 microscope. Drawings were made using a drawing tube Nikon 1.25X. Line drawings and photographic images were prepared using Inkscape 0.92. The SEM preparations were examined with a Hitachi S-4800 SEM at the Servicio de Microscopía, SCSIE, Universitat de València, Spain. Type specimens were deposited in the Colección Colombiana de Helmintos (CCH.116), Universidad de Antioquia, Medellín, Colombia.

Taxonomy

Phylum Platyhelminthes Minot, 1876
Order Rhabdocoela Ehrenberg, 1831
Suborder Dalytyphloplanida Willems, Wallberg, Jondelius, Littlewood, Backeljau, Schockaert & Artois, 2006
Infraorder Neotyphloplanida Willems, Wallberg, Jondelius, Littlewood, Backeljau, Schockaert & Artois, 2006
Parvorder Limnotyphloplanida Van Steenkiste, Tessens, Willems, Backeljau, Jondelius & Artois, 2013
Section Temnocephalida Blanchard, 1849
Superfamily Temnocephaloidea Baer, 1953
Family Temnocephalidae Monticelli, 1899

Genus *Temnocephala* Blanchard, 1849

***Temnocephala ivandarioi* sp. nov.**
http://zoobank.org/E7284E9B-D311-46C3-B5CF-26CA544B15EF
Figs 1A–C, 2A–C

Type host. *Valdivia serrata* White, 1847 (Fig. 1D–F).

Site of infection. Branchial chambers.

Prevalence. 36% of the eleven hosts were infected.

Type locality. Kilómetro 11, Reserva Natural Tanimboca, Leticia, Amazonas (4°07'39.8"S, 69°57'13.0"W), Colombia.

Type specimens. Holotype: CCH.116 (159); Paratypes: CCH.116 (160).

Examined material. 10 whole mounted specimens; 5 stained in Meyer's paracarmine; 5 stained in Borax carmine; 6 dissected cirrus; 2 samples observed by SEM, 5 unhatched eggs observed by SEM.

Description. ***External characteristics.*** Body (without tentacles) 1.36–2.26 mm (1.75 ± 0.25) long by 1.18–1.56 mm (1.36 ± 0.11) wide; adhesive disk ventral, subterminal 280–520 (370 ± 82) long by 320–520 (400 ± 40) wide (Figs 1A–C, 2A); eyespots with red pigmentation (observations made on live specimens; Fig. 3E). DLSPs small, elliptical-shaped (Fig. 3A, B), 167 long by 141 wide (N = 2); excretory pore "subcentral" in the DSLP, displaced towards the internal limit (Fig. 3A); length ratio of DLSPs:total body length, without tentacles, 1.0:10.7.

Alimentary system. Mouth surrounded by a large muscular sphincter 200–280 (220 ± 26) long by 210–310 (248 ± 32) wide; pharynx 330–620 (417 ± 56) long by 450–620 (511 ± 52) wide; intestine saccular, without septations (Fig. 2A).

Glands. Rhabditogenic glands forming bunches in the lateral fields of the body extending from the pharynx to the middle level of the adhesive disk. Haswell cells in

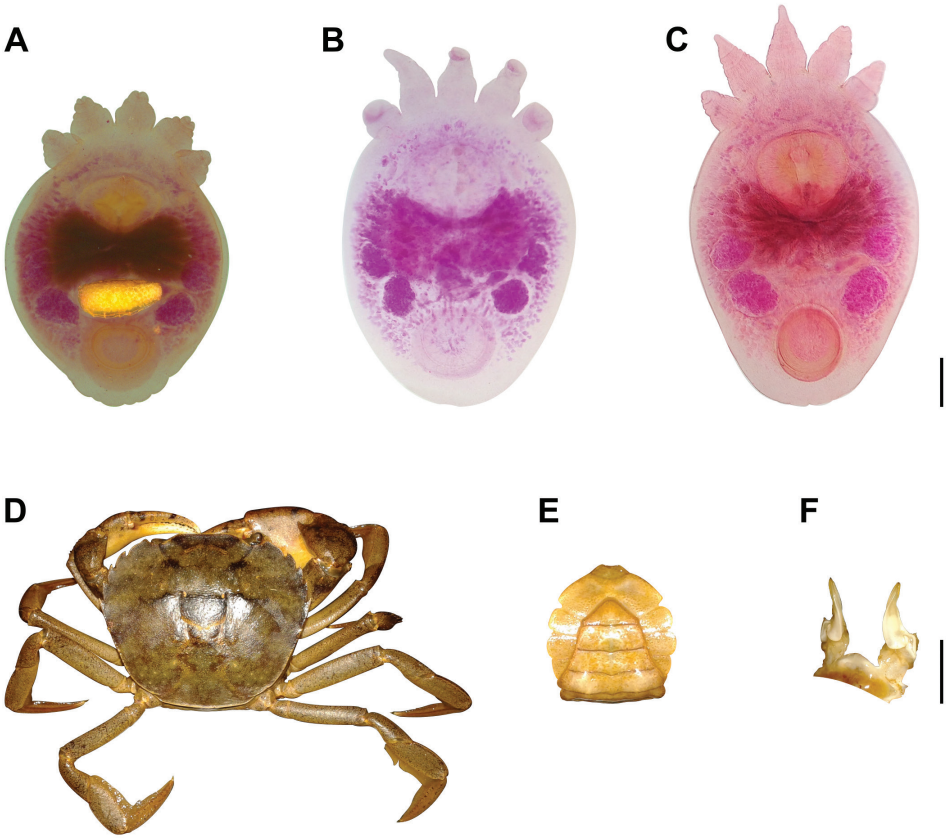

Figure 1. *Temnocephala ivandarioi* sp. nov. and *Valdivia serrata* **A** paratype of *Temnocephala ivandarioi* sp. nov. showing an egg, ventral view **B** adult paratype stained in Meyer´s paracarmine **C** holotype stained in Borax carmine **D** male specimen of *V. serrata* **E** abdomen **F** gonopods, lateral view. Scale bars: 200 µm (**A–C**); 10 mm (**D–F**).

front of the eyespots and the brain. Disk glands between the adhesive disk and the genital complex (Fig. 2A).

Female. Ovary ventral to the resorbens vesicle 57–100 (83 ± 13; *N* = 7), long by 60–145 (105 ± 26; *N* = 7) wide. Vagina elongated with strong muscular wall, connects to the genital atrium dorsally, 75–180 (125 ± 37; *N* = 4) long by 16–30 (23 ± 4; *N* = 6) wide with a widening of the distal portion; proximal vaginal sphincter symmetrical 16–34 (23 ± 6; *N* = 6) and distal vaginal sphincter symmetrical (16–20; *N* = 2) (Figs 2B, 4A, B). Resorbens vesicle ovoid 110–180 (134 ± 24, *N* = 9) long by 172–212 (194 ± 16; *N* = 9) wide. Vitellarium arborescent and thin (Fig. 2A). Eggs 557–638 (585 ± 37; *N* = 4) long by 302–331 (312 ± 13; *N* = 4) wide; filament small, subapical or apical (Fig. 3C, D, F); peduncles 146–243 (341 ± 97); the plane of fracture is oblique with respect to the longitudinal axis of the egg (Fig. 3C, D). Eggs deposited on branchial chambers of host (Fig. 3G, H).

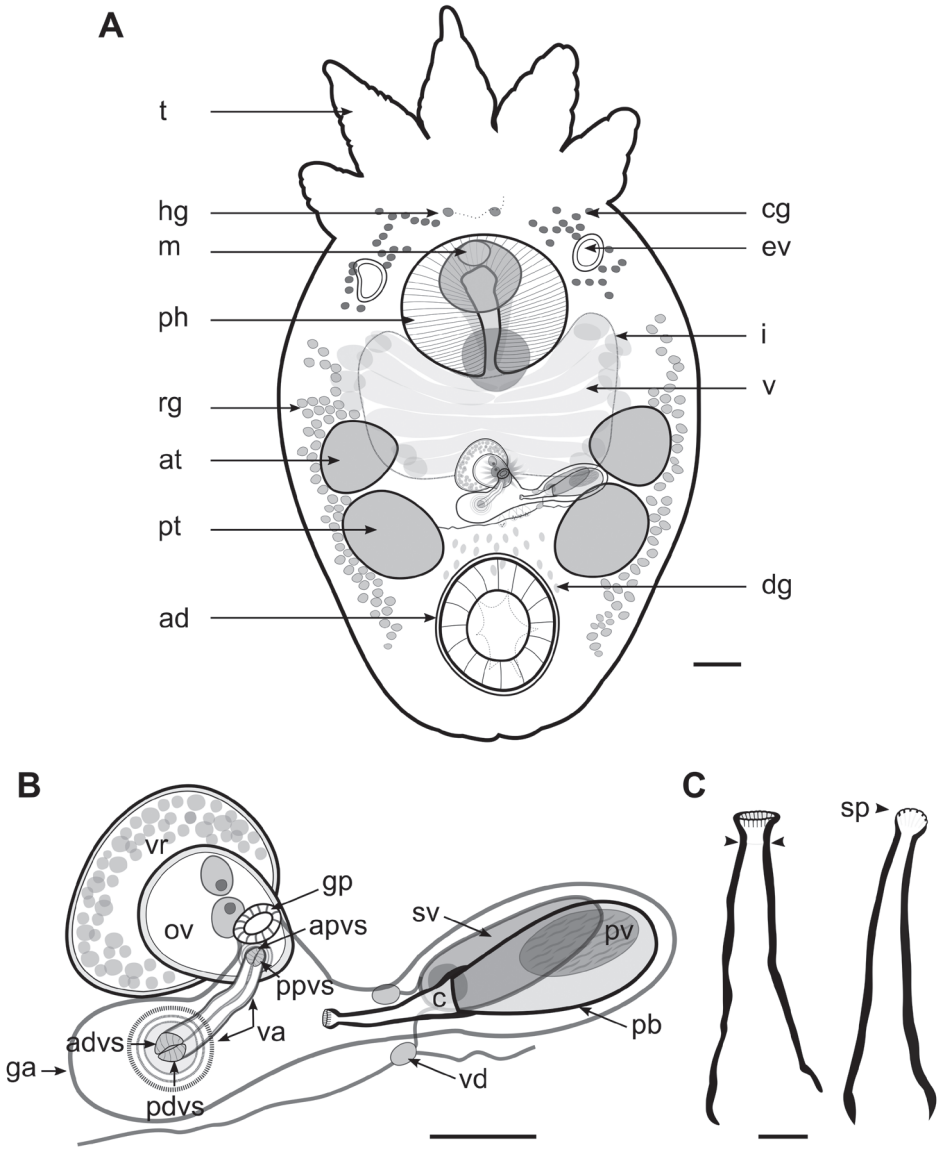

Figure 2. *Temnocephala ivandarioi* sp. nov. **A** adult specimen diagram, showing adhesive disk (ad), anterior testes (at), cyanophilous glands (cg), disk glands (dg), excretory vesicle (ev), Haswell´s glands (hg), intestinal sac (i), mouth (m), pharynx (ph), posterior testes (pt), rhabditogenic glands (rg), tentacles (t), and vitellarium (v) **B** reproductive system, showing female reproductive complex: anterior portion of the distal vaginal sphincter (advs), anterior portion of the proximal vaginal sphincter (apvs), genital atrium (ga), genital pore (gp), posterior portion of the distal vaginal sphincter (pdvs), posterior portion of the proximal vaginal sphincter (ppvs), ovary (ov), vagina (va), and resorbens vesicle (vr); and male reproductive organs: cirrus (c), prostatic bulb (pb), prostatic vesicle (pv), seminal vesicle (sv), and vasa deferentia (vd) **C** line drawing of cirrus in different focusing planes, showing the sclerites portion of the introvert (sp), and proximal limit of the introvert (arrows). Scale bars: 200 µm (**A**); 100 µm (**B**); 20 µm (**C**).

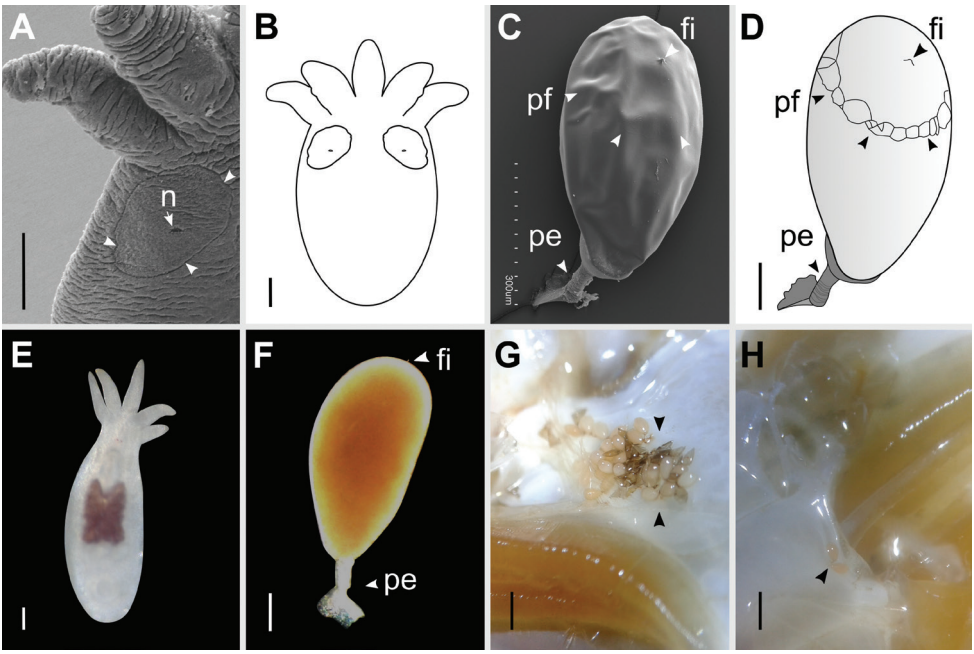

Figure 3. *Temnocephala ivandarioi* sp. nov. details of epidermal excretory syncytial plates (DLSPs) and eggs **A** antero-lateral area observed with SEM showing leftmost tentacle and left DLSP, arrow showing contour and position of excretory pores (n) **B** line drawing of entire specimen showing the DLSP$_s$ **C** egg observed with SEM showing the filament (fi), peduncle (pe), and plane of fracture of the operculum (pf) **D** line drawing of a whole egg showing the oblique fracture plane to the longitudinal axis of the egg **E** live adult specimen showing red eyespot pigment **F** unhatched egg showing the filament (fi) **G, H** live eggs deposited on branchial chambers of *V. serrata*. Scale bars: 100 µm (**A–F**); 1mm (**G, H**).

Male. Two pairs of testes, medium-sized, usually rounded, slightly oblique, anterior testes 180–310 (231 ± 34) long by 120–320 (220 ± 48) wide; posterior testes 200–400 (260 ± 60) long by 110–360 (254 ± 65) wide (Fig. 2A). Seminal vesicle dorsal and anterolateral to the prostatic bulb (Figs 2B, 4D), 52–137 (92 ± 27) long by 82–237 (168 ± 47) wide, wall 8.6 thick. Prostatic bulb 70–107 (91 ± 14) long by 155–240 (191± 30) wide (Figs 2B, 4C). Cirrus small-sized, 120–147 (129 ± 8) long; shaft cone-shaped, slightly curved up, with maximum width at base 40–47 (44 ± 2); introvert cone-shaped, not oblique, not curved, with a circle of sclerites (range 18–20) in the distal portion followed by a smooth portion without spines or ridges, 7.5–15 (10 ± 3; N = 8) long, with maximum width 15–22 (18 ± 3; N = 10) at level of the distal portion (Figs 2C, 4C, E, F, 6A). Ratio between total body length, without tentacles:total length of cirrus 14:1; ratio between total length of cirrus:width of shaft´s base 3:1; ratio between total length of cirrus:total length of introvert 13:1.

Etymology. The new species is dedicated to Dr. Iván Darío Vélez Bernal for his outstanding contributions to the study of helminthology and the understanding of tropical diseases in Colombia.

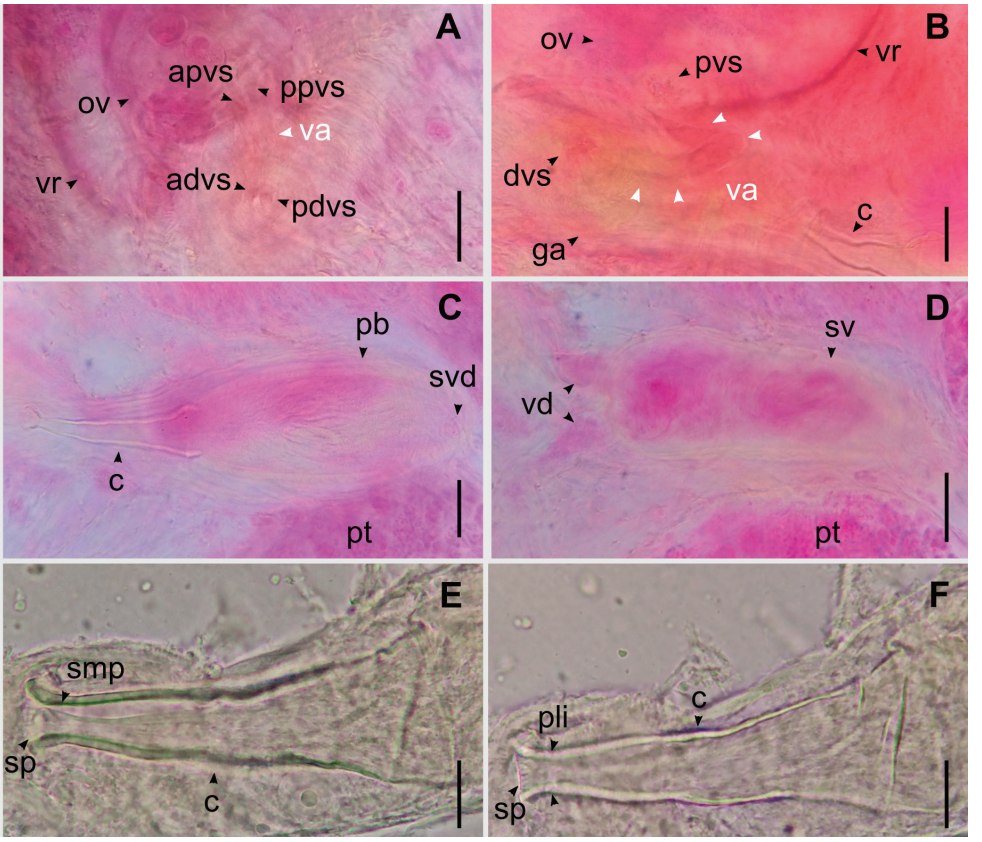

Figure 4. Details of the reproductive system of *Temnocephala ivandarioi* sp. nov. **A, B** partial female reproductive system, showing: anterior portion of the distal vaginal sphincter (advs), anterior portion of the proximal vaginal sphincter (apvs), distal vaginal sphincter (dvs), genital atrium (ga), posterior portion of the distal vaginal sphincter (pdvs), posterior portion of the proximal vaginal sphincter (ppvs), proximal vaginal sphincter (pvs), ovary (ov), vagina (va), and vesicula resorbens (vr) **C, D** partial male reproductive system, showing: cirrus (c), prostatic bulb (pb), seminal vesicle duct (svd), seminal vesicle (sv), and vasa deferentia (vd) **E, F** cirrus introvert observed in different focusing planes, view of the circle of sclerites (sp) in the distal portion of the introvert and the smooth portion (smp) in the proximal limit of the introvert (pli). Scale bars: 50 µm (**A–D**); 20 µm (**E, F**).

Discussion. Temnocephalida is a monophyletic group within the Platyhelminthes included in Lymnotyphloplanida, which is part of the Dalytyphloplanida clade, a major group of Rhabdocoela (Van Steenkiste et al. 2013). Temnocephalidae Monticelli, 1899 is the most diverse family of the Temnocephalida. Its members are distributed around Australia and the Neotropics (Martínez-Aquino et al. 2014); the type genus of Temnocephalidae, *Temnocephala* Blanchard, 1849, is exclusive to the Neotropics. Autapomorphies of the *Temnocephala* include red-pigmented eyespots, four epidermal syncytial plates, and excretory pores enclosed within the boundaries of the DLSPs (Damborenea and Cannon 2001). Major hosts to the members of the *Temnocephala* are chelonians, molluscs, insects, and

crustaceans, each hosting a particular assemblage of *Temnocephala* species. Particular host families are also specific for particular *Temnocephala* species (Martínez-Aquino et al. 2014).

Taxonomy of temnocephalans is based on morphology of adult specimens with emphasis on the reproductive system. The structure of the cirrus is the trait of greatest taxonomic value (Damborenea 1991, Damborenea and Cannon 2001, Sewell et al. 2007, Garcés et al. 2013). Other traits important for species differentiation include composition of the female reproductive complex, eggs deposit areas in the host, and the shape of the DLSPs (Damborenea and Brusa 2008, Amato et al. 2010, Volonterio 2010, Seixas et al. 2011, 2015, 2018).

Nine species of *Temnocephala* are known for their association with crabs of the Trichodactylidae family. Of these, *T. ivandarioi* sp. nov., *T. longivaginata* Seixas, Amato & Amato, 2011, and *T. lutzi* Monticelli, 1913 (Amato et al. 2005) present a similar-sized cirri and have the Amazon River basin as a biogeographical connection. *Temnocephala longivaginata* and *T. ivandarioi* sp. nov. are most similar to each other in the length of the vagina and the presence of sclerites in the distal portion of the cirrus.

Temnocephala ivandarioi sp. nov. can be distinguished by the combination of the following features: cirrus with a circle of small sclerites (range 18–20) in the distal portion of the introvert, without spines or ridges in the inner wall of the introvert (Fig. 2C). The ovary lies ventral to vesicle resorbens followed by an elongated vagina with two vaginal sphincters similar in size, one symmetric and proximal, and one symmetric and distal; the vagina connects to the genital atrium dorsally. The seminal vesicle is located anterolateral to the prostatic bulb. The DLSPs are small and 'elliptical-shape', with a partially sinuous contour.

On an ecological-level *T. ivandarioi* sp. nov., *T. longivaginata*, and *T. lutzi* inhabit the branchial chambers of trichodactylid crabs from the middle basin and lower basin of the Amazon River (Leticia, Amazonas, Colombia; Peixe-Boi, Pará State; Rio Amapá, Amapá State, northern Brazil, respectively). *Temnocephala ivandarioi* sp. nov. is the third species described from Colombia, and therefore *V. serrata* is registered as a new trichodactylid host for neotropical temnocephalans. *Valdivia serrata* is widely distributed throughout the Orinoco and Amazon River basins in Venezuela, the islands of Trinidad and Tobago, the Guianas, Colombia, Brazil, Peru and Bolivia (Cumberlidge 2008). In Colombia this species is found in the eastern region of the country (Amazonas, Arauca, Caqueta, Meta, Putumayo, and Vichada Departments) in the Putumayo and Maqueta rivers that drain into the Amazon River, and the Guaviare, Meta, and Arauca rivers that drain into the Orinoco River (Campos 2005, 2014). It is likely that *T. ivandarioi* sp. nov., *T. longivaginata*, and *T. lutzi* are closely related due to their morphological similarities and geographical proximity. The implementation of molecular studies will reveal the phylogenetic relationships between the different species of *Temnocephala* in the Neotropics.

In Colombia more than 132 species of decapod crustaceans have been recorded (Campos 2014), while only two associated species of temnocephalans have been reported to date: *T. icononcensis* (Arias-Pineda et al. 2015) and *T. ivandarioi* sp. nov. The great diversity of these potential hosts (Campos 2014) suggests that most temnocephalans remain undescribed.

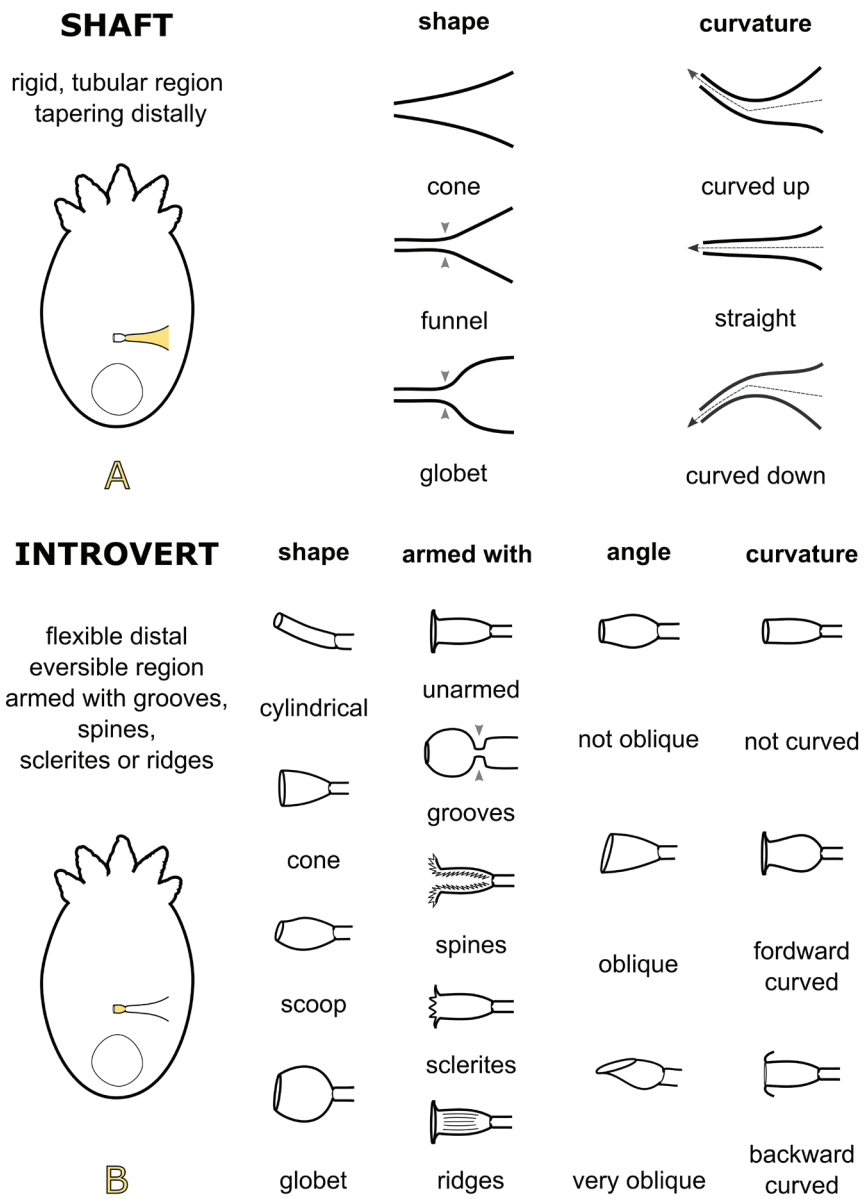

Figure 5. Diagrams showing the terminology used to describe the cirrus of the species of *Temnocephala* (terminology updated from Sewell et al. (2007), Seixas et al. (2011), Garcés et al. (2013), and Ponce de León et al. (2015); diagrams modified from Sewell et al. 2007: 205, fig. 2).

Comparative notes. The cirrus is the only rigid structure and therefore of constant general morphology in juveniles and adults (except for small intraspecific variations) for each species. The morphology of the cirrus constitutes one of the few characters used and is the most valuable taxonomic character for species identification

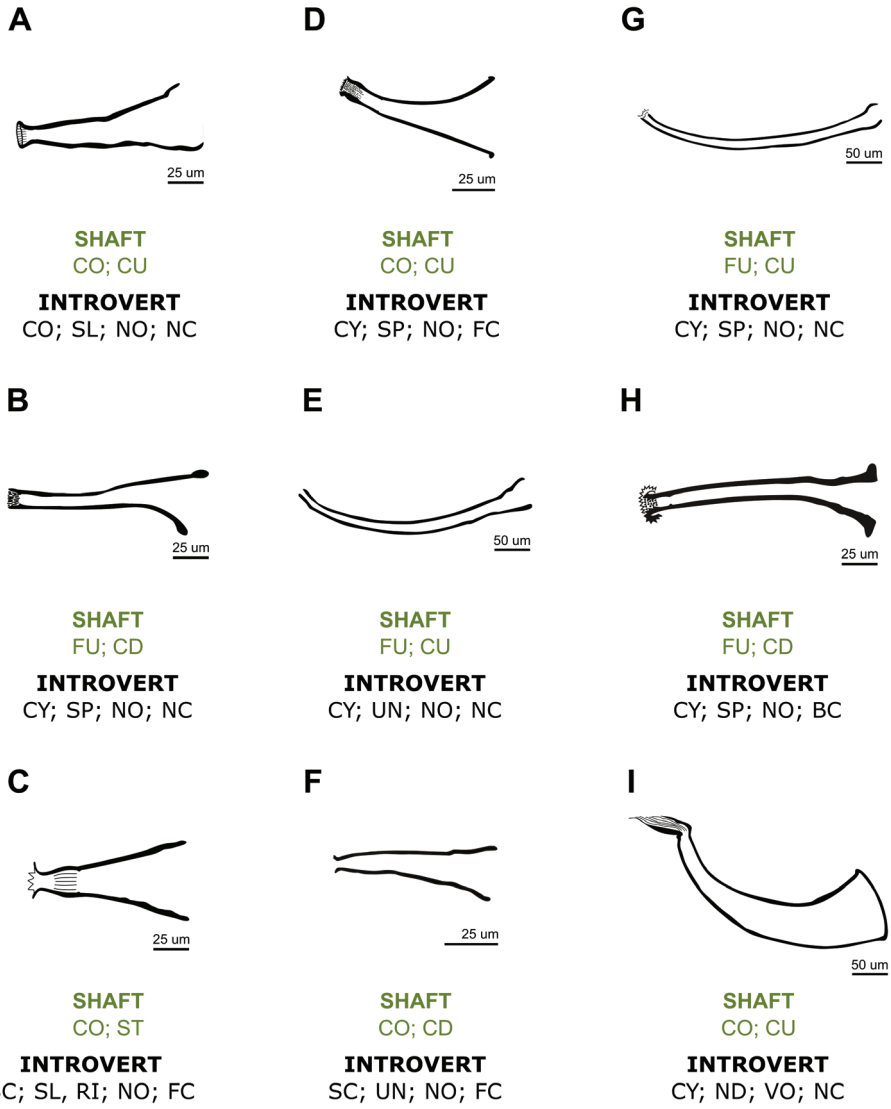

Figure 6. Diagrams of the cirrus of the species of *Temnocephala* associated with trichodactylid crabs. Terminology based on the cirrus structure (see comparative notes and Fig. 5). **A** *Temnocephala ivandarioi* sp. nov. (present study) **B** *Temnocephala lanei* Pereira & Cuocolo, 1941 **C** *Temnocephala longivaginata* Seixas, Amato & Amato, 2011 **D** *Temnocephala lutzi* Monticelli, 1913 (Amato et al. 2005) **E** *Temnocephala microdactyla* Monticelli, 1903 **F** *Temnocephala pignalberiae* Dioni, 1967 (Amato et al. 2010) **G** *Temnocephala santafesina* Dioni, 1967 **H** *Temnocephala trapeziformis* Amato, Amato & Seixas, 2006 **I** *Temnocephala travassosfilhoi* Pereira & Cuocolo, 1941. Key: **Shaft** [**shape: CO**–cone; **FU**–funnel]; [**curvature: CU**–curved up; **ST**–straight; **CD**–curved down]. **Introvert** [**shape: CY**–cylindrical; **CO**–cone; **SC**–scoop]; [**armed with: UN**–unarmed; **SP**–spines; **SL**–sclerites; **RI**–ridges; **ND**–not described]; [**angle: NO**–not oblique; **VO**–very oblique]; [**curvature: NC**–not curved; **FC**–forward curved; **BC**–backward curved].

(Seixas et al. 2015b). In the present study, terminology describing the temnocephalan cirrus is updated for neotropical species (Fig. 5), according to Sewell et al. (2007), Seixas et al. (2011), Garcés et al. (2013) and Ponce de León et al. (2015). The cirrus of the species of *Temnocephala* described from trichodactylid crabs (Fig. 6) are compared based on this terminology.

The cirrus is defined as the entire sclerotised male copulatory organ comprised of a 'shaft' (rigid, tubular region tapering distally; Fig. 5A) and an 'introvert' (flexible distal eversible region armed with grooves, spines, sclerites or ridges, Fig. 5B) (modified from Sewell et al. 2007). Furthermore, the degree of shaft curvature is a reliable taxonomic characteristic of neotropical temnocephalans (Garcés et al. 2013).

The shape of the shaft may be described as a 'funnel', 'goblet', or 'cone'. Funnel- or goblet-shaped shafts have a wide proximal region which tapers rapidly into a narrow tubular distal region (Sewell et al. 2007). The cirrus may be more or less curved, and it may be described as 'curved up', 'straight', or 'curved down'. Similarly, the position of the cirrus with respect to the body may be described as 'towards the forebody', 'horizontal', or 'towards the hindbody' (modified from Garcés et al. 2013). The position of the cirrus can or may not depend on the cirrus curvature i.e. cirrus 'curved up' directed 'towards the forebody', but cirrus 'straight' are directed towards the 'forebody', 'horizontal' or 'towards the hindbody'. The cirrus position can be examined only from a complete diagram of the temnocephalan.

The introvert shape can be described as 'cylindrical', 'cone'; 'scoop', or 'goblet'. Scoop- or goblet-shaped introvert have a wide middle region, which tapers into a narrow distal region. In addition, the introvert may be 'unarmed', armed with 'grooves' in the proximal limit of the introvert, or armed with 'spines', 'sclerites', and 'ridges' in the inner wall of the introvert. The distal opening of the introvert may be at right angles with respect to the proximal limit of the introvert i.e. 'not oblique', 'oblique', or 'very oblique' (modified from Sewell et al. 2007). Additionally, the distal region of the introvert may be curved (with or without spines, sclerites, or ridges), and described as 'forward curved', 'straight' or 'backward curved' (described as with non-spined region or without non-spined region by Sewell et al. 2007).

The morphology of the cirrus is necessary for species identification and should be clearly described based on the terminology proposed in the present study. This new terminology can be applied to species of neotropical temnocephalans described to date.

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RESEARCH ARTICLE

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A giant new species of *Enchiridium* (Polycladida, Prosthiostomidae) from southwestern Japan

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Abstract

We describe a new species of polyclad flatworm, *Enchiridium daidai* **sp. nov.**, from the rocky subtidal zone in the East China Sea along the coasts of the Kyushu and Okinawa Islands, Japan. *Enchiridium daidai* **sp. nov.** is characterized by i) the entire periphery of the dorsal surface narrowly fringed with orange, ii) a marginal-eyespot band extending to the position of the mouth (about anterior one-eighth of body), and iii) two prostatic vesicles covered by a common muscle sheath, which is penetrated by the ejaculatory duct. We performed a molecular phylogenetic analysis based on 945-bp 28S rDNA sequences of 16 species of Prosthiostomidae currently available in public databases in addition to those of *E. daidai* **sp. nov.** and *Prosthiostomum torquatum* Tsuyuki et al., 2019. In the resulting tree, our new species was nested in a clade composed of *Enchiridium* species. The tree topology was in favor of a taxonomic view that *Enchiridium* should be defined by having i) a common muscle sheath that encloses two prostatic vesicles and ii) marginal eyespots that may or may not surround the periphery of the dorsal surface.

Keywords

Cotylea, marine flatworm, phylogeny, Platyhelminthes, taxonomy

Introduction

Polyclad flatworms in the family Prosthiostomidae Lang, 1884 are characterized by i) an elongated body with a ventral sucker after the female gonopore, ii) a plicate tubular pharynx, and iii) paired prostatic ducts, each of which extends from a spherical prostatic vesicle and enters the penis or the ejaculatory duct independently, instead of

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uniting to each other before the entrance. Prosthiostomidae is composed of five genera: *Enchiridium* Bock, 1913; *Enterogonimus* Hallez, 1913; *Euprosthiostomum* Bock, 1925; *Lurymare* Du Bois-Reymond Marcus & Marcus, 1968; and *Prosthiostomum* Quatrefages, 1845 (Faubel 1984; Litvaitis et al. 2019). The genus *Enchiridium sensu* Faubel (1984) contains eight species: *E. delicatum* (Palombi, 1939); *E. evelinae* Marcus, 1949; *E. gabriellae* (Marcus, 1949); *E. japonicum* Kato, 1943; *E. magec* Cuadrado et al., 2017; *E. periommatum* Bock, 1913; *E. punctatum* Hyman, 1953; and *E. russoi* (Palombi, 1939). Members of this genus are distinguished from other prosthiostomids by having a muscle sheath (or bulb) that encloses just the two prostatic vesicles among other male reproductive organs; i.e., the seminal vesicle and the male atrium are not enclosed by the muscle sheath (Faubel 1984).

In Japan, 21 species of prosthiostomids were previously reported, but there was no record of *Enchiridium* (Kato 1944; Tsuyuki et al. 2019). In this paper, we describe a new species of *Enchiridium* from Kagoshima and Okinawa, Japan, based on morphological and molecular data. In addition, we infer the phylogenetic position of the new species within Prosthiostomidae from an analysis using partial 28S ribosomal DNA (28S rDNA) sequences.

Material and methods

Three polyclad specimens were collected subtidally from under rocks in Kagoshima and Okinawa, southwestern Japan (Fig. 1). Worms were anaesthetized in seawater containing menthol before fixation. The relaxed worms were photographed with a Nikon D5600 digital camera with external strobe lighting provided by a pair of Morris Hikaru Komachi Di flash units. For DNA extraction, a posterior piece of the body was removed and stored in 99.5% ethanol. The rest of the body was fixed in Bouin's solution for 24 h and preserved in 70% ethanol for long-term storage.

For histological examination, tissues were dehydrated in an ethanol series, cleared in xylene, embedded in paraffin wax, and sectioned at a thickness of 7 μm using a microtome. Sections were stained with hematoxylin and eosin, mounted on glass slides in Entellan New (Merck, Germany), and then observed and photographed under an Olympus BX51 compound microscope. All type slides have been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan.

Total DNA was extracted using a silica-based method (Boom et al. 1990) after specimens were homogenized. A fragment (585 bp) of the cytochrome *c* oxidase subunit I (COI) gene was amplified with the primers Pros_COIF (5'-AGGTGTTTGAGCAGGTTTTATAGGTACAGG-3') and Pros_COIR (5'-ATGGGATCTCCTCCTCCTGAAGGRTC-3') for investigating intraspecific genetic distances. PerlPrimer ver. 1.1.21 (Marshall 2003–2011) was used to design these universal primers for Prosthiostomidae *de novo*, based on complete mitochondrial genome sequences (Aguado et al. 2016) from two prosthiostomids, *Enchiridium* sp. (GenBank KT363734) and *Prosthiostomum siphunculus* (Delle Chiaje, 1822) (GenBank KT363736). A 1017-bp

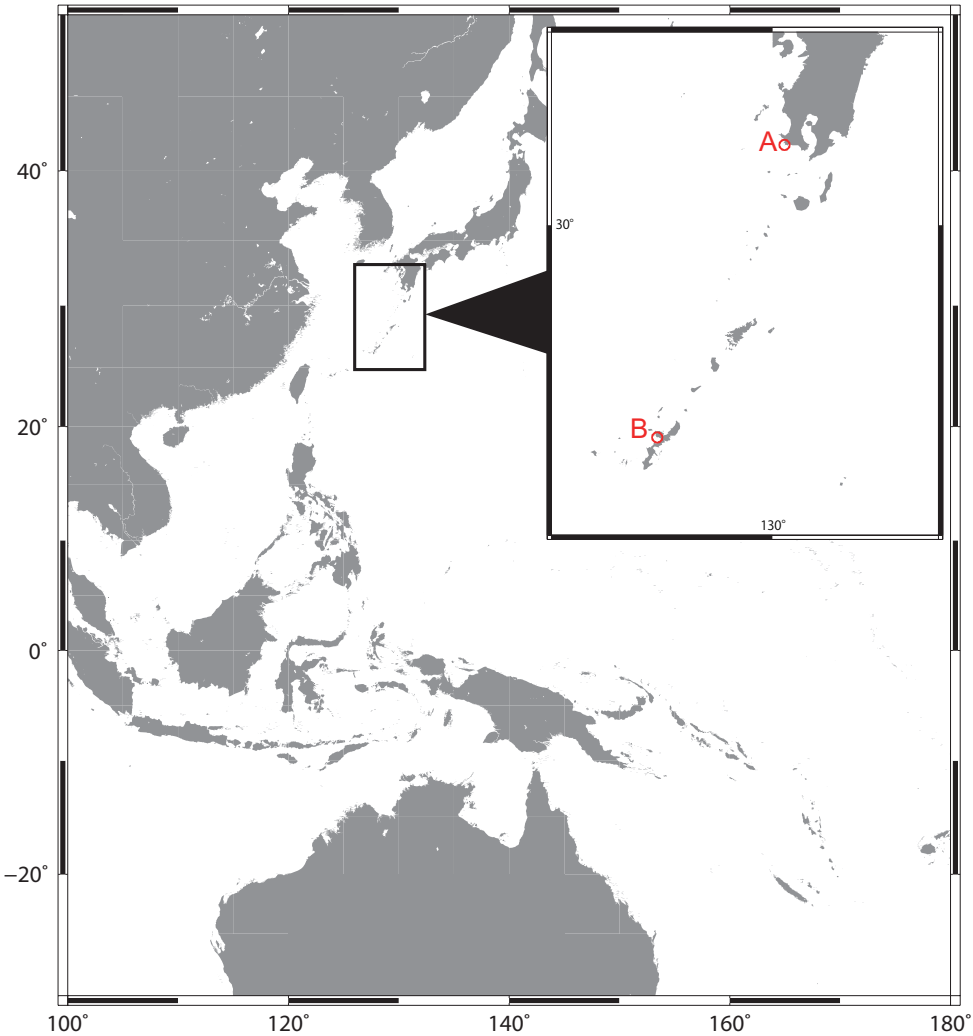

Figure 1. Map showing distribution of *Enchiridium daidai* sp. nov.: point A, off the coast of Bonomisaki, Kagoshima (type locality); point B, Nago, Okinawa Island.

fragment of 28S rDNA was amplified with the primers fw1 and rev2 (Sonnenberg et al. 2007) for molecular phylogenetic analyses. Polymerase chain reaction (PCR) amplification conditions were 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 50 °C (COI) or 52.5 °C (28S rDNA) for 30 s, 72 °C for 1.5 min (COI) or 2 min (28S rDNA); and 72 °C for 7 min. All nucleotide sequences were determined by direct sequencing with a BigDye Terminator Kit ver. 3.1 and a 3730 Genetic Analyzer (Life Technologies, California, USA); two internal primers, hrms_fw2 (Oya et al. unpublished) and rev4 (Sonnenberg et al. 2007), were used in addition to fw1 and rev2 due to failure in sequencing by the internal primer fw2. Sequences were checked and edited by us-

Table 1. List of species used for the molecular phylogenetic analysis and respective GenBank accession numbers.

Species	GenBank accession number	Reference
Prosthiostomidae		
Enchiridium daidai sp. nov.	LC504235	This study
	LC504236	
	LC504237	
Enchiridium evelinae Marcus, 1949	KY263683	Bahia et al. (2017)
Enchiridium japonicum Kato, 1943	MH700298	Litvaitis et al. (2019)
Enchiridium periommatum Bock, 1913	MH700299	Litvaitis et al. (2019)
	MH700300	
	MH700301	
Enchiridium sp. 1	MH700302	Litvaitis et al. (2019)
	MH700303	
Enchiridium sp. 2	MN384686	Dittmann et al. (2019)
Enchiridium sp. 3	KY263673	Bahia et al. (2017)
Enchiridium sp. 4	KY263679	Bahia et al. (2017)
Euprosthiostomum mortenseni Marcus, 1948	MH700304	Litvaitis et al. (2019)
Prosthiostomum acroporae (Rawlinson et al., 2011)	HQ659011	Rawlinson et al. (2011)
Prosthiostomum cynarium Marcus, 1950	MH700371	Litvaitis et al. (2019)
Prosthiostomum lobatum Pearse, 1938	MH700372	Litvaitis et al. (2019)
Prosthiostomum milcum Du Bois-Reymond Marcus & Marcus, 1968	MH700373	Litvaitis et al. (2019)
Prosthiostomum purum Kato, 1937	MH700374	Litvaitis et al. (2019)
Prosthiostomum siphunculus (Delle Chiaje, 1822)	HQ659012	Rawlinson et al. (2011)
Prosthiostomum torquatum Tsuyuki et al., 2019	LC504234	This study
Prosthiostomum trilineatum Yeri & Kaburaki, 1920	MH700376	Litvaitis et al. (2019)
Prosthiostomum utarum Marcus, 1952	MH700377	Litvaitis et al. (2019)
Prosthiostomum sp.	MH700375	Litvaitis et al. (2019)
Outgroup		
Prostheceraeus crozieri (Hyman, 1939)	HQ659013	Rawlinson et al. (2011)
Pseudobiceros splendidus (Lang, 1884)	MH700388	Litvaitis et al. (2019)

ing MEGA ver. 7.0 (Kumar et al. 2016). In addition to three specimens collected in this study, a 946-bp partial sequence of the 28S rDNA from the holotype specimen of *Prosthiostomum torquatum* Tsuyuki et al., 2019 (ICHUM 5563) was determined by the same methods described above. All the edited sequences have been deposited in DDBJ/EMBL/GenBank.

Additional 28S rDNA sequences were downloaded from GenBank; *Pseudobiceros splendidus* (Lang, 1884) (Pseudocerotidae) and *Prostheceraeus crozieri* (Hyman, 1939) (Euryleptidae) were chosen as outgroups (Table 1). Sequences were aligned using MAFFT ver. 7.427 (Katoh et al. 2017), with L-INS-i strategy selected by the "Auto" option. Ambiguous sites were removed with Gblocks ver. 0.91b (Castresana 2002) using options for a less stringent selection. The optimal substitution model selected with MEGA ver. 7.0 (Kumar et al. 2016) under the Akaike Information Criterion (AIC) (Akaike 1974) was GTR+I+G. Phylogenetic analysis was performed with the maximum likelihood (ML) method by using RAxML ver. 8.2.10 (Stamatakis 2014). Nodal support within the ML tree was assessed by analyses of 1000 bootstrap pseudorepli-

cates (Felsenstein 1985). COI uncorrected *p*-distances were calculated using MEGA ver. 7.0 (Kumar et al. 2016).

All graphical treatments were done with Adobe Photoshop CC. Illustrations were prepared with Adobe Illustrator CC.

Results

Family Prosthiostomidae Lang, 1884
Genus *Enchiridium* Bock, 1913 *sensu* Faubel (1984)

***Enchiridium daidai* sp. nov.**
http://zoobank.org/5D0FCB54-F262-4616-8790-FD210878679E
New Japanese name: daidai-hoso-hiramushi
Figures 2–4

Etymology. The new specific name *daidai* is a Japanese noun, meaning the color orange. It was named after the thin marginal orange line surrounding the entire dorsal fringe.

Material examined. Three specimens, all collected by A. Tsuyuki. ***Holotype*:** ICHUM 5993, sagittal sections through reproductive structures (22 slides), and the rest of the body, unsectioned, preserved in 70% ethanol, collected at 13–14 m depth off the coast of Bonomisaki (31.2542N, 130.2150E), Kagoshima, Japan, on 26 July 2018. ***Paratypes*:** ICHUM 5994, sagittal sections through head to reproductive structures (9 slides); ICHUM 5995, cross sections through reproductive structures (21 slides); both collected at 5 m depth in Nago (26.6013N, 127.9137E), Okinawa, Japan, on 22 May 2019.

Type locality. Off the coast of Bonomisaki (31.2542N, 130.2150E), Kagoshima, Japan.

Description. Body elongated, tapered posteriorly, 28–77 mm long (77 mm in holotype) and 4.6–14 mm maximum width (14 mm in holotype) in living state (Fig. 2A); anterior margin rounded; mid-point of posterior margin acute. Tentacles absent. Dorsal surface smooth, translucent, fringed with thin marginal orange line (Fig. 2A). Ventral surface translucent, without color pattern. Pair of cerebral-eyespot clusters, each consisting of 20–52 eyespots (left 20 and right 23 in holotype); each cluster of an antero-posteriorly elongated spindle shape (Fig. 2B). Marginal-eyespot clusters forming single marginal band, extending to position of mouth (about anterior one-eighth of body) along margins on both sides; marginal eyespots abundant along anterior margin, diminishing posteriorly (Fig. 2B). Ventral eyespots absent. Intestine highly branched, spreading all over body. Plicated pharynx tubular in shape, about one-fifth of body length, located in anterior one-third of body (Fig. 2A). Oral pore situated at anterior end of pharynx, behind brain. Male gonopore and female gonopore closely set, both situated behind posterior end of pharynx. Male copulatory apparatus consisting of large seminal vesicle, pair of prostatic vesicles, and armed penis papilla (Fig. 3A).

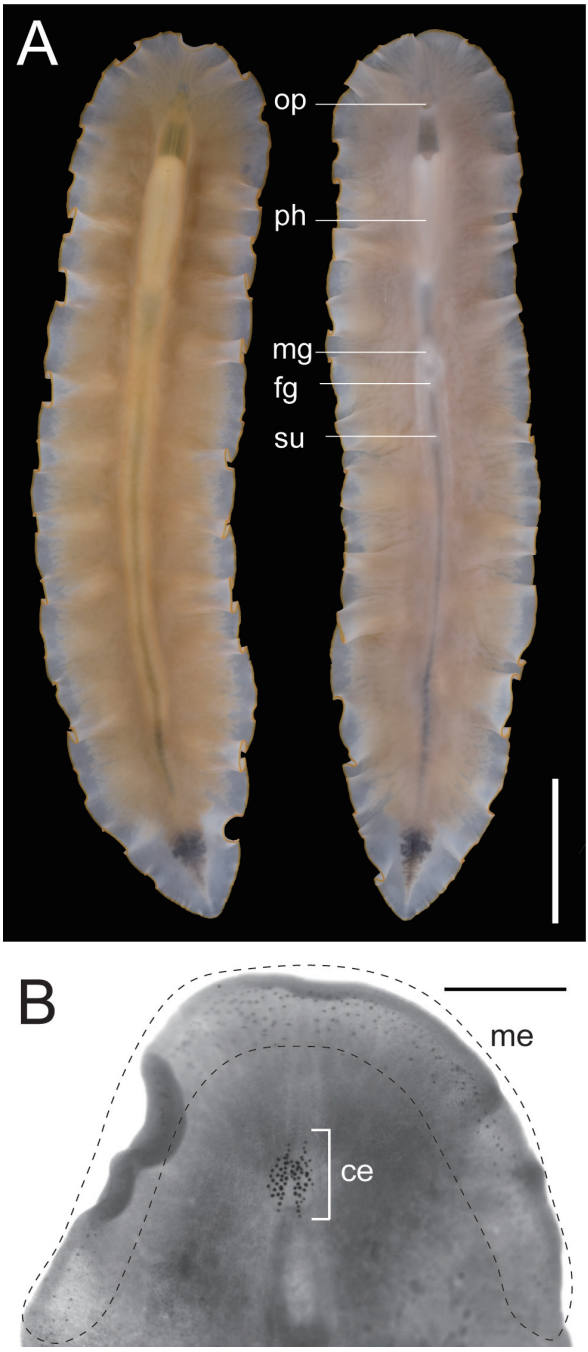

Figure 2. *Enchiridium daidai* sp. nov., photograph taken in life and eyespots observed in fixed state after being cleared in xylene. **A** ICHUM 5993 (holotype), entire animal, dorsal view (left) and ventral view (right) **B** ICHUM 5994 (paratype), magnification of anterior body, showing arrangements of cerebral and marginal eyespots. Abbreviations: **ce** cerebral eyespots **fg** female gonopore **me** marginal eyespots **mg** male gonopore **op** oral pore **ph** pharynx **su** sucker. Scale bars: 10 mm (**A**); 1 mm (**B**).

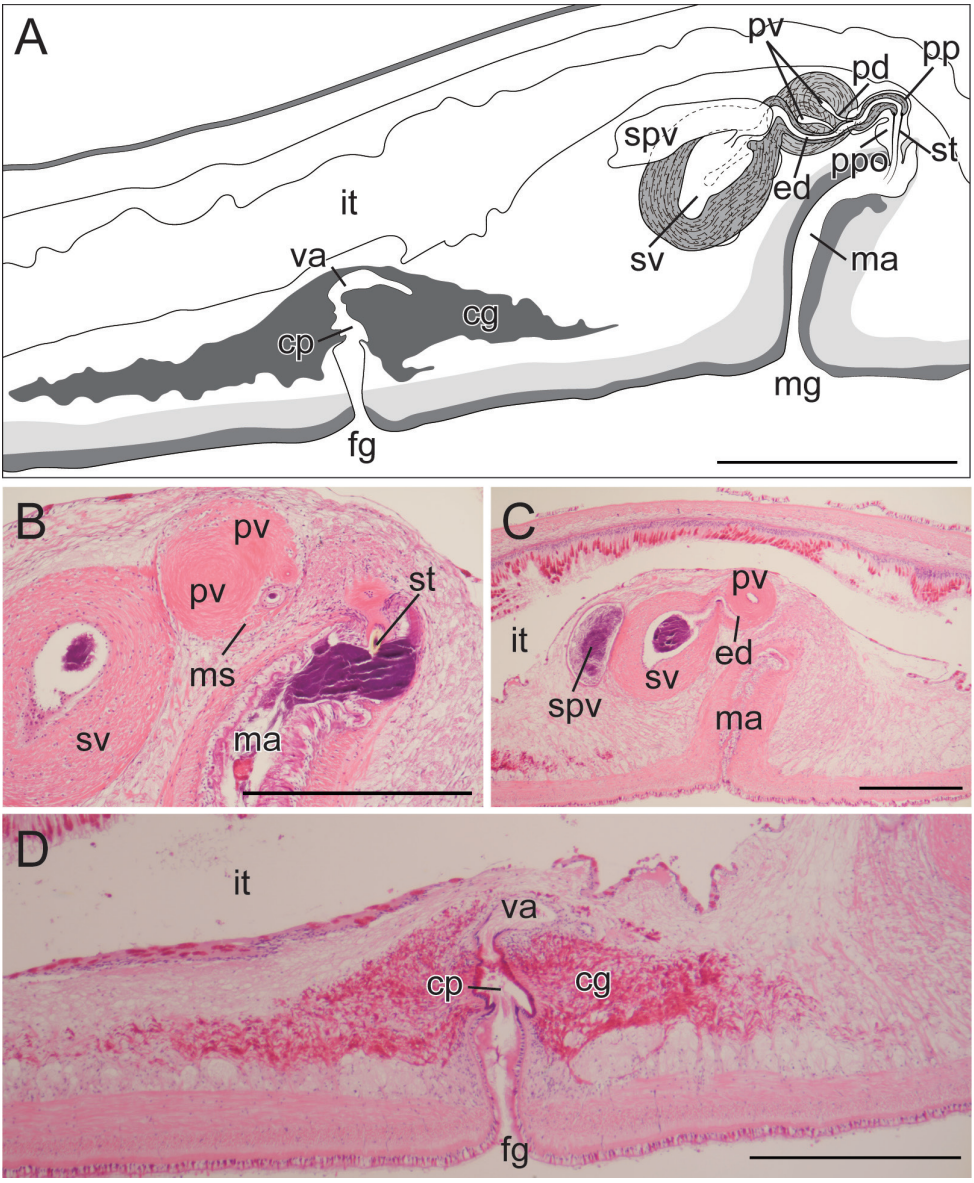

Figure 3. *Enchiridium daidai* sp. nov., ICHUM 5993 (holotype), schematic diagram (**A**) and sagittal sections (**B–D**), anterior to the right. **A** Schematic diagram of copulatory complex **B** a common muscle sheath/bulb enclosing two prostatic vesicles and penis stylet **C** ejaculatory duct penetrating a common muscle sheath/bulb **D** female copulatory apparatus. Abbreviations: **cg** cement glands **cp** cement pouch **ed** ejaculatory duct **fg** female gonopore **it** intestine **ma** male atrium **mg** male gonopore **ms** muscle sheath/bulb **pd** prostatic duct **pp** penis papilla **ppo** penis pouch **pv** prostatic vesicle **spv** spermiducal vesicle **st** stylet **sv** seminal vesicle **va** vagina. Scale bars: 500 μm.

Antero-posterior length of seminal vesicle more than twice as long as diameter of each prostatic vesicle. Spermiducal vesicles forming single row on each side of midline, separately entering into seminal vesicle. Ejaculatory duct with thick muscular layer, entering penis papilla. Prostatic ducts with muscular layer, connected to ejaculatory duct separately at proximal end of penis papilla. Pair of spherical prostatic vesicles coated within thin non-nucleated muscular wall, arranged anterodorsally to ejaculatory duct. Common muscular sheath enclosing two prostatic vesicles (Fig. 3B). Seminal vesicle oval, coated with thick muscular wall, narrowing anteriorly and forming ejaculatory duct; latter almost immediately penetrating common muscular sheath (Fig. 3C). Penis papilla armed with pointed tubular stylet, enclosed in penis pouch, protruding into male atrium (Fig. 3C). Male atrium elongated anteriorly, lined with ciliated, muscularized epithelium (Fig. 3B). Female reproductive system immediately posterior to male reproductive system. Cement glands numerous, concentrated around vagina and releasing their contents in cement pouch (Fig. 3D). Vagina curving anteriorly, leading to two narrow lateral branches of uteri. Each branch of uteri turning laterally and then running backwards. Lang's vesicle absent. Sucker set on body center (Fig. 2A).

Habitat. Subtidal (5–14 m depth), under rocks.

Variation. Specimens from Kagoshima and Okinawa differed in body size. The holotype from Kagoshima was 77 mm long and 15 mm wide, whereas the paratype specimens from Okinawa were 28–37 mm long and 4.6–7.4 mm wide (Fig. 4).

Diagnosis. Body elongated, usually rounded anteriorly; dorsal surface translucent, fringed by a thin marginal orange line; marginal eyespots present only anteriorly; plicated pharynx tubular in shape, about one-fifth of body length; pair of prostatic vesicles bound by common muscular sheath, the latter penetrated by ejaculatory duct.

Sequences. Partial COI (585 bp) and 28S rDNA (1017 bp) sequences from three individuals: LC504240 (COI), LC504235 (28S rDNA) from ICHUM 5993 (holotype); LC504238 (COI), LC504236 (28S rDNA) from ICHUM 5994 (paratype); LC504239 (COI), LC504237 (28S rDNA) from ICHUM 5995 (paratype).

Molecular phylogeny and genetic distances. In the phylogenetic tree, *Enchiridium daidai* sp. nov. was nested in a clade composed of *Enchiridium* species (Fig. 5). The genetic distances (uncorrected *p*-values) for the COI sequences among three specimens of *Enchiridium daidai* sp. nov. were 0.002–0.012. Genetic distances between individuals from different localities (Kagoshima vs. Okinawa), 0.010–0.012, were larger than that between individuals from the same locality (Okinawa), 0.002.

Remarks. In spite of the noticeable difference in body size, specimens from Kagoshima and Okinawa – all having reached sexual maturity – were identified as conspecific. They shared the following morphological characteristics: i) body dorsally fringed with a thin orange line, ii) marginal-eyespot band extending to the position of the mouth (about anterior one-eighth of the body), iii) two prostatic vesicles covered by a common muscle sheath, and iv) common muscle sheath penetrated by ejaculatory duct. In addition, the COI *p*-distances among the specimens, 0.002–0.012, fell in a range of intraspecific values, 0.000–0.020, which was observed in four species of the acotylean leptoplanoid *Notocomplana* (Oya and Kajihara 2017), thus rendering sup-

Figure 4. Difference in mature body size among *Enchiridium daidai* sp. nov. **A** ICHUM 5993 (holotype), from Kagoshima **B** ICHUM 5995 (paratype), from Okinawa **C** ICHUM 5994 (paratype), from Okinawa. Scale bar: 10 mm.

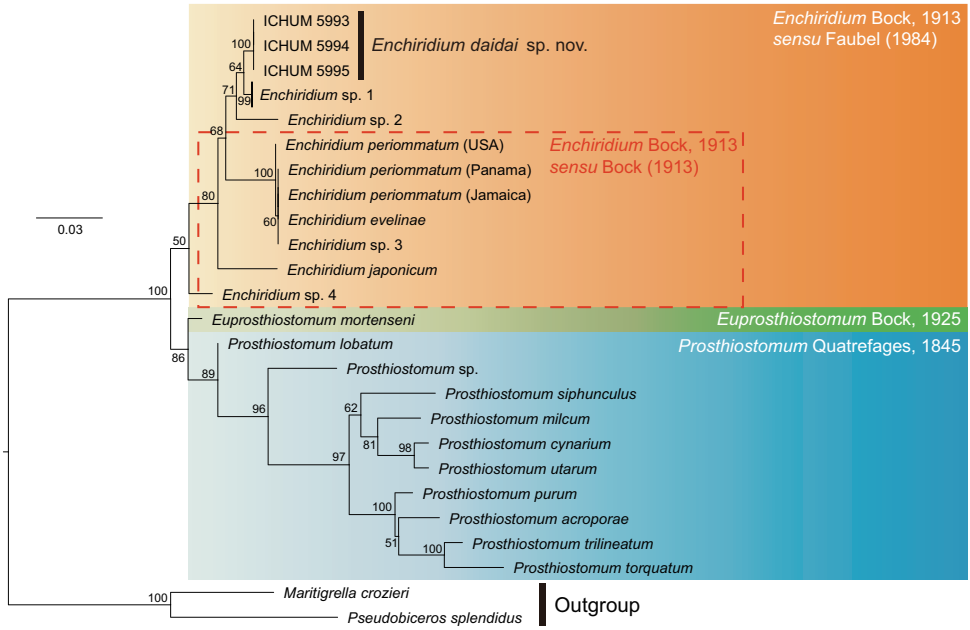

Figure 5. Maximum likelihood phylogenetic tree based on 935-bp 28S rDNA. Bootstrap support values are indicated near nodes.

port for our interpretation of conspecificity. Within Polycladida, remarkable intraspecific variation in body size has been reported for the acotylean stylochoid *Planocera reticulata* (Stimpson, 1855), which was recorded to vary by 10–80 mm in length and 6–45 mm in width (Yeri and Kaburaki 1918). Among the cotylean Proshiostomidae, sexually matured individuals of *Prosthiostomum cyclops* (Verrill, 1901) have been reported to vary a great deal (> ×10) in size by locality: 75–90 mm long × 10–15 mm wide in the Bermuda Islands (Verrill 1901), whereas 6.5 mm long × 1.7 mm wide in the islands of Bonaire and Klein Bonaire (Du Bois-Reymond Marcus and Marcus 1968). These observations may imply that these polyclads undergo an indeterminate growth, in which growth is not terminated after reaching adulthood, although other factors – such as geographical and ecological ones – must also be taken into account.

As for the taxon concept of *Enchiridium*, our results did not show a compatibility to Bock's (1913) original view on the genus. The genus *Enchiridium* was established by Bock (1913) for *E. periommatum* based on two characteristics: i) two prostatic vesicles enclosed in a common muscle sheath, and ii) marginal eyespots completely surrounding the entire periphery of the dorsal surface. Subsequently, *E. evelinae*, *E. japonicum*, and *E. punctatum* were added to the genus (Kato 1943; Marcus 1949; Hyman 1953) before Faubel (1984) re-defined *Enchiridium*. It was circumscribed so that "only the prostatic vesicles are bound into a common muscle bulb and oriented anterodorsal to the ejaculatory duct" (Faubel 1984, p. 231); namely, the encircling marginal eyespots were not regarded as a necessary condition for *Enchiridium*. At the same time, Faubel

Table 2. Comparison of characters between five *Enchiridium* species in which marginal eyespots are distributed only anteriorly.

	***E. daidai* sp. nov.**	***E. delicatum***	***E. gabriellae***	***E. magec***	***E. russoi***
Type locality	Off the coast of Bonomisaki, Kagoshima, Japan	East London, South Africa	São Sebastião Island, São Paulo, Brazil	North of El Balito, Tenerife, Canary Islands, Spain	Shelley Beach, East London, South Africa
Dorsal coloration/ pattern:					
Background color	Translucent	Light pale yellow	Transparent	Whitish to cream	Greyish yellow
Spots or maculae on dorsal surface	None	None	None	Brown caramel spots, arranged more densely in the central region	Brown pigment spots spread especially in the central part
Median line	None	Two yellow bands	None	A band composed of brown caramel spots	An ocher yellow band
Fringed line	A thin orange line	None	None	None	None
Reference	This study	Palombi (1939)	Marcus (1949)	Cuadrado et al. (2017)	Palombi (1939)

(1984) transferred three *Lurymare* species, *viz.*, *L. delicatum*, *L. gabriellae*, and *L. russoi*, into *Enchiridium*. As a result, seven species were included in *Enchiridium* in the taxonomic system of Faubel (1984). In contrast, Prudhoe (1985) supported Bock's (1913) taxon concept of *Enchiridium*, retaining four species, *E. evelinae*, *E. japonicum*, *E. periommatum*, and *E. punctatum*, in *Enchiridium* and three species, *L. delicatum*, *L. gabriellae*, and *L. russoi*, in *Lurymare*. On the other hand, Cuadrado et al. (2017) followed Faubel's (1984) redefinition when they established *E. magec*. The monophyly of *Enchidirium sensu* Faubel (1984) was strongly supported in a molecular phylogenetic analysis based on partial 28S rDNA (Litvaitis et al. 2019). In our study, *Enchidirium sensu* Faubel (1984) received 80% bootstrap support with the exclusion of *Enchiridium* sp. 4 of Bahia et al. (2017); including the latter, the branch support decreased to 50% (Fig. 5). Also, *Enchiridium* in the sense of Bock (1913) and Prudhoe (1985) – represented by *E. evelinae*, *E. japonicum*, *E. periommatum*, *Enchiridium* sp. 3, and *Enchiridium* sp. 4 (cf. Bahia et al. 2017, Table 2) in our analysis – was not monophyletic. Therefore, the taxonomy of *Enchiridium* should be revised with further molecular phylogenetic analyses as well as careful examination of morphological characters among the constituent members. At the moment, however, we adopt Faubel's (1984) redefinition and place our new species in the genus *Enchiridium* along with eight other species. We did so because our results indicated that the arrangement of the marginal eyespots should not be taken into account as generic diagnostic characters.

Enchiridium daidai sp. nov. is distinguished from *E. evelinae*, *E. japonicum*, *E. periommatum*, and *E. punctatum* by the arrangement of the marginal eyespots; the marginal-eyespot band in these four species completely encircles the periphery of the dorsal surface, whereas that of our specimens is present only along the anterior margin. Our new species is also easily distinguished from the other four congeners, *E. delicatum*,

E. gabriellae, *E. magec*, and *E. russoi*, by the thin marginal orange line surrounding the entire dorsal fringe and by the lack of spots or maculae on the dorsal surface (Table 2).

Reaching 77 mm in body length, *Enchiridium daidai* sp. nov. is the largest species in the genus over *E. punctatum* (about 40 mm in body length; Hyman 1953, p. 386). Indeed, *E. daidai* is the second largest in the Prosthiostomidae after *P. cyclops*, which reaches 90 mm (Verrill 1901). Among about 80 species of prosthiostomids, only *E. daidai* sp. nov. and *P. cyclops* are known to exceed 70 mm in body length, while most of the other species are less than 30 mm long. Therefore, our new species is considered to be unusually big in body size for a prosthiostomid.

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RESEARCH ARTICLE

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The value of a single character: the Paleogene European land snail *Ferussina* Grateloup, 1827 is likely a cyclophorid (Gastropoda, Caenogastropoda)

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Abstract

Ferussina Grateloup, 1827 is a European Paleogene land snail genus, which is currently classified in its own family, the Ferussinidae Wenz, 1923 (1915), in the superfamily Cyclophoroidea. The shell of this genus is remarkable by its last quarter whorl turning towards the apex instead of away from it, which is an unusual trait in terrestrial snails. We show, however, that this trait has evolved at least nine times in terrestrial Eupulmonata and Caenogastropoda, and it does not justify distinction at the family level in any of the reported cases. This observation suggests the systematic position of *Ferussina* should not be based on the apexward-turning last quarter whorl alone but instead on the general morphology of the shell. As a result, we re-evaluate the systematic position of the Ferussinidae and treat it as a subfamily of the Cyclophoridae.

Keywords

character evolution, Eocene, Oligocene, parallel evolution, terrestrial Gastropoda, unique trait

Introduction

Ferussina Grateloup, 1827 (and its synonym *Strophostoma* Deshayes, 1828; see Wenz 1923; Kadolsky 2008) is a genus reported from middle Eocene (Lutetian) to upper Oligocene (Chattian) deposits of France, Germany, Italy, and Switzerland (Fig. 1);

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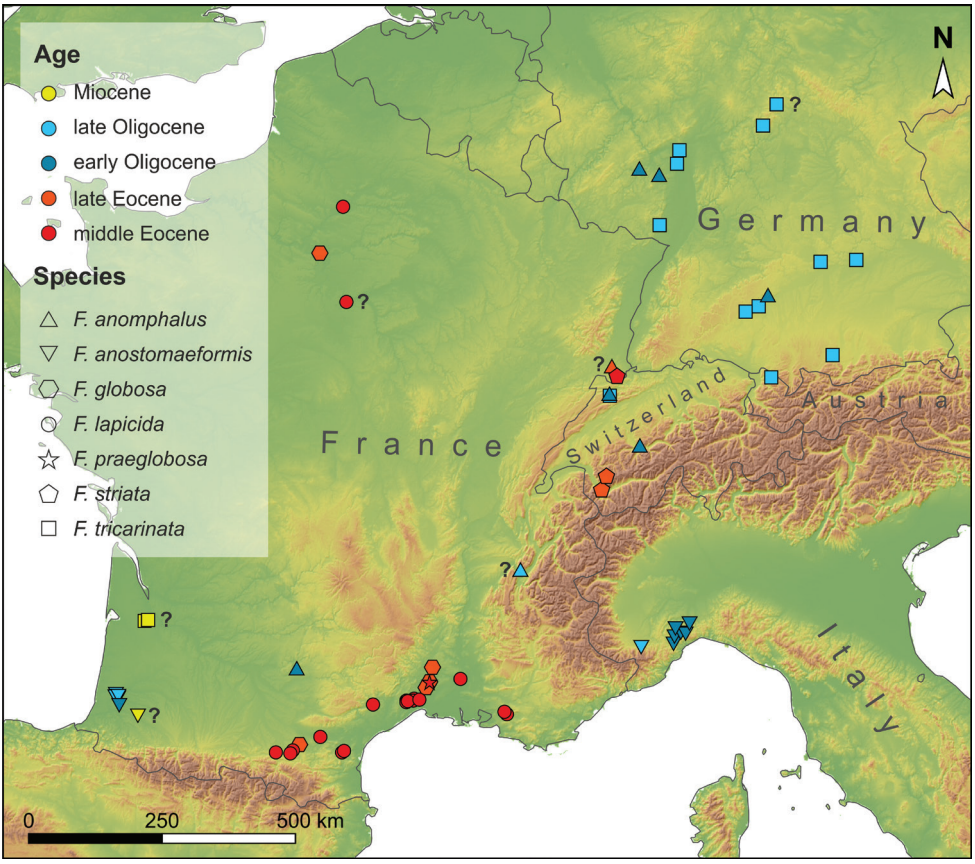

Figure 1. Geographic and stratigraphic distribution of *Ferussina* in central and western Europe. Records that are questionable in terms of stratigraphic horizon or species identification are indicated with a question mark (see Suppl. material 1: Table S1 for more information). The map was created with ESRI ArcGIS 10.4.

a dubious record comes from presumably lower Miocene strata of southern France (Degrange-Touzin 1892). It is currently classified in its own family, the Ferussinidae Wenz, 1923 (1915) (Bouchet et al. 2017) in the superfamily Cyclophoroidea Gray, 1847. *Ferussina* is characterized by a relatively large (ca 1–3 cm), depressed-globular shell with an obtusely conical spire, a round aperture, and a last quarter whorl turning towards the apex (Sandberger 1870–1875; Roman 1899; Rey 1968; Kadolsky 2008; Salvador et al. 2016). As a result, the aperture opens in the adapical direction of the shell, orientating the umbilicus of the shell upwards while the animal was crawling.

In his catalogue of fossil non-marine gastropods, Wenz (1923) included seven species in the genus *Ferussina*, i.e. *F. anomphalus* (Sandberger, 1871), *F. anostomaeformis* Grateloup, 1827 (the type species by monotypy), *F. globosa* Dumas, 1876, *F. lapicida* Leufroy, 1828, *F. praeglobosa* (Roman, 1904), *F. striata* (Deshayes, 1828), and *F. tricarinata* (Braun, 1838). These are distinguished by the relative height of the spire, presence of an inflation on the last whorl, presence of a keel or angulation on the last whorl, and

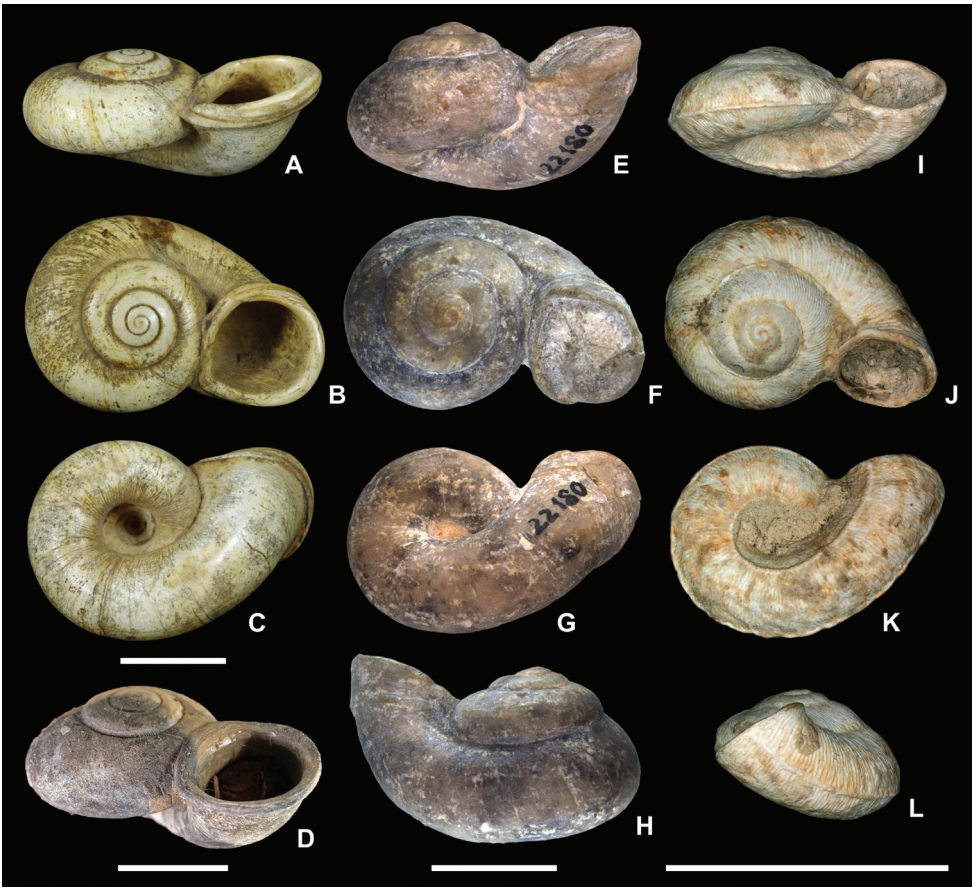

Figure 2. Representatives of the genus *Ferussina*. **A–C** *Ferussina anostomaeformis* Grateloup, 1827, Gaas (Larrat), France, lower Oligocene; syntype, UBRG, Grateloup collection, no. 65-2-150 **D** *Ferussina anostomaeformis*, St-Paul-lès-Dax (Abesse, "Château"), France, upper Oligocene; MNHN.F.A72133 **E–H** *Ferussina anomphalus capellinii* (Sandberger, 1873), Blaustein (Arnegg), Germany, lower Oligocene; syntype, SMNS 22180 **I–L** *Ferussina tricarinata* (Braun, 1838), Hochheim, Germany, upper Oligocene (Hochheim Formation, "Landschneckenkalk"); NHMW 75000/E/1778. Photos: Laurent Charles (**A–C**), Pierre Lozouet (**D**), Rodrigo Salvador (**E–H**), Barna Páll-Gergely (**I–L**). Scale bars: 1 cm.

presence and width of an umbilicus, as well as surface ornamentation (Fig. 2; compare also Deshayes 1828; Sandberger 1870–1875; Roman 1899, 1904; Kadolsky 2008). Sculpture ranges from fine to distinct, riblet-like growth lines and, in the case of *F. tricarinata*, narrow spiral keels on base and periphery.

An "upright" turning last whorl (termed "anostomy" by Nordsieck 1986) is unusual in terrestrial snails but has repeatedly evolved in both the Eupulmonata ("pulmonates") (at least six times) and Caenogastropoda (at least three times) (Schileyko 1998, 1999, 2000; Egorov 2009, 2013), and even in a Devonian marine gastropod (Braun 1838). This trait has not been considered a justification for the distinction at the family level in

any of the reported cases. This observation suggests the systematic position of *Ferussina* should not be based on the apexward-turning last quarter whorl alone but instead on the general morphology of the shell. As a result, we re-evaluate the systematic position of the Ferussinidae and treat it as a subfamily of the Cyclophoridae Gray, 1847.

Abbreviations used:

MNHN – Muséum National d'Histoire Naturelle, Paris; NHMW – Natural History Museum Vienna; SMNS – State Museum of Natural History Stuttgart; UBRG – Université du Bordeaux.

Results

While the apexward-turning last whorls are unique among fossil European land snails, we found this trait in a number of unrelated extant and fossil clades, including 12 pulmonate (Wenz 1940; Schileyko 1998, 1999, 2000) and four caenogastropod genera (Egorov 2009, 2013), representing at least nine independent events. Table 1 summarises the key information.

Table 1. Summary of key information of extant land snail genera with apexward turning body whorl. In addition, we provide information on shell shape of relatives within the same family to assess the relevance of shape traits for systematic placement. Information derives from Wenz (1940) and Schileyko (1998, 1999, 2000).

Genera	Size (mm)	Shell shape without body whorl	Habitat	Geographic region	Shell shape of relatives
Boysia, *Gyliotrachela*, *Hypselostoma*	2–4	ovoid, conic	rock surfaces	Southeast Asia to Australia	ranging from ovoid and conic to lenticular and globular
Campolaemus	2	ovoid	not rock-dwelling	Saint Helena	unknown (might be ovoid or depressed)
Anostoma, *Clinispira*, *Ringicella*	14–16	obesely lenticular (depressed-globular), elongate-conical	not rock-dwelling (caves, under stones/logs)	Brazil	mostly high-spired
Hendersoniella	11–13	flat	rock surfaces	Mexico	all high-spired
Tonkinia	4.3–5	elliptical	not rock-dwelling	Vietnam	mostly high-spired
Anostomopsis, *Enneopsis*, *Strophostomella*	11–18	cup-shaped, ovoid, depressed globular	unknown	Austria, Hungary, France	planispiral, lenticular, ovoid
Anosycolus	12	conical	unknown, probably not rock-dwelling	Madagascar	conic, ovoid, high-spired
Laotia	2.2–4.4	depressed globular	unknown, probably not rock-dwelling	Laos and Vietnam	mostly low-spired to conical
Opisthostoma, *Plectostoma*	1.0–3.7	ovoid to depressed-globular	rock surfaces	Southeast Asia	ovoid to conic

Eupulmonata

(1) Genera *Boysia* Pfeiffer, 1849, *Gyliotrachela* Tomlin, 1930, *Hypselostoma* Benson, 1856

Remarks. These genera were included in the family Hypselostomatidae by Schileyko (1998), which was recognized as a subfamily of Gastrocoptidae Pilsbry, 1918 by Bouchet et al. (2017). Other genera of the same (sub)family are variable in shape, ranging from ovoid and conic to lenticular and globular. The direction of the aperture is variable even in the same genus. Some *Hypselostoma* and *Gyliotrachela* species have even normally coiled shells. The shells are small (2–4 mm). All the species with detached last whorl inhabit limestone rock areas and spend a considerable time of their life tightly attached to rock surfaces (Panha and Burch 2005).

(2) Genus *Campolaemus* Pilsbry, 1892

Remarks. This genus was classified in the Hypselostomatidae by Schileyko (1998). However, this species more probably belongs to the Streptaxidae (Páll-Gergely 2020). Nevertheless, its position within that family is questionable. Shell height is ca 2 mm. No information on its habitat preference is known. However, it is probably not a rock-dwelling species, because streptaxids typically occur among leaf litter, in decaying plant material, and under logs and stones (Páll-Gergely pers. obs.).

(3) Genera *Anostoma* Fischer von Waldheim, 1807, *Clinispira* Simone & Casati, 2013, *Ringicella* Gray, 1847

Remarks. *Anostoma* was classified in the tribe Odontostomini (Bulimulidae, Bulimulinae) by Schileyko (1999), which was recognized as a distinct family by Bouchet et al. (2017). According to Schileyko (1999), there are 11 high-spired genera and 3 low-spired/globular genera in the Odontostomini, all of which comprise relatively large snails (30–45 mm in shell diameter). *Anostoma* inhabit the semi-arid biomes of Brazil (the Cerrado and Caatinga ecoregions), and living specimens are typically found under stones (Rodrigo Salvador, pers. comm.). The genus *Ringicella* (treated as a genus of its own by Simone 2006 and as a subgenus of *Anostoma* by Schileyko 1999) is known from the Amazon region, and animals have been found living under decaying logs (Rodrigo Salvador pers. comm.). *Clinispira* Simone & Casati, 2013 was collected in caves in the semi-dry environment of the Caatinga ecoregion (Simone and Casati 2013). Inferring from the flat profile of the peristome, *Clinispira* might live attached to rock surfaces.

(4) Genus *Hendersoniella* Dall, 1905

Remarks. This genus was classified in the Urocoptidae, Holospirinae (Schileyko 1999), where many high-spired genera belong. Shell diameter is 11–13 mm. *Hendersoniella*

are obligate rock-dwelling, as the other members of the family ("live snails were found under limestone slabs that were spalding from the underlying rock"; Thompson and Correa 1991: 15).

(5) Genus *Tonkinia* Mabille, 1887

Remarks. This genus was classified as a member of the Streptaxidae, Streptaxinae by Schileyko (2000), and in the Diapheridae in MolluscaBase (2020) following Dance (1970), who mentioned that *Tonkinia* and its probably closest relative, *Platycochlium* Laidlaw, 1950, are most similar to juvenile shells of *Diaphera* Albers, 1850 and *Sinoennea* Kobelt, 1904. With the exception of *Platycochlium* and *Tonkinia*, all other diapherids are high-spired. The shell is 4.3–5 mm wide (Schileyko 2000). We have not found any published information about its habitat preference, but it probably lives among decaying plant material and under logs and stones as other Diapheridae.

(6) Genera *Anostomopsis* Sandberger, 1871, *Enneopsis* Wenz, 1940, *Strophostomella* Fischer, 1883

Remarks. The three genera derive from upper Cretaceous (Coniacian–Maastrichtian) strata of Europe (Austria, Hungary, and France) and are currently classified in the fossil family Anostomopsidae with uncertain position in the Stylommatophora (Nordsieck 2014, 2017). *Strophostomella* has a depressed-globular shell similar to that of *Ferussina* (Tausch 1886, there as "*Strophostoma*"), *Anostomopsis* has a peculiarly cup-shaped morphology with flat apical side and narrow, tube-like aperture (Sandberger 1870–1875), and *Enneopsis* is characterized by an ovoid shape (Roule 1886, as "*Anostomopsis*"). All share a complex system of internal plicae (Nordsieck 2014; see also Wenz 1940).

Caenogastropoda

(1) Genus *Anosycolus* Fischer-Piette, C.P. Blanc, F. Blanc & Salvat, 1993

Remarks. This taxon was classified in the Hainesiidae by Egorov (2009) and in the Cyclophoridae in MolluscaBase (2020). However, a current investigation suggests it is a relative of *Boucardicus*, which includes conical-globular and high-spired species and may deserve its own family within Cyclophoroidea (Páll-Gergely unpublished information). Shell does not exceed 12 mm in maximum diameter.

(2) Genus *Laotia* Saurin, 1953

Remarks. This genus was classified in the Diplommatinidae by Egorov (2013) and in the Alycaeidae in Do et al. (2015). Recent investigations corroborate placement in Alycaeidae, where it will be classified in a separate new subfamily together with *Messageria*

Bavay & Dautzenberg, 1904 (Páll-Gergely unpublished information). Shell diameter is 2.2–4.4 mm (Páll-Gergely 2014). Nothing is known about its habitat preference, but *Laotia* is probably not an obligate rock-dwelling genus, since the aperture is not flat in front profile to allow attachment to rock surfaces.

(3) Genera *Opisthostoma* W.T. Blanford & H.F. Blanford, 1860, *Plectostoma* Adams, 1865

Remarks. Both are members of the Diplommatinidae (Webster et al. 2012; Egorov 2013) together with a number of other genera usually possessing high-spired and ovoid shells. *Plectostoma* is 1.0–3.7 mm in shell height, *Opisthostoma* is less than 1.3 mm in largest measurement, and both are obligate limestone-dwelling genera (Liew et al. 2014; Vermeulen 1991).

Discussion

The list above shows that shells with the last whorl turned apexward are present in numerous unrelated lineages of pulmonate and operculate terrestrial snails. In all cases, the species and genera with this peculiar shape have normally coiled relatives. Similarly, the fossil *Ferussina* certainly evolved from normally coiled ancestors, and we should not give too great importance to this trait when determining its systematic position. Moreover, the closest relatives of these genera are often species with high-spired shells. This suggests that we cannot exclude high-spired cyclophoroideans from the possible relatives of *Ferussina*.

We can exclude the Pomatiidae as possible relatives, as members of this family have calcareous opercula that are often found as fossils. No such opercula have been documented for *Ferussina*. The Cochlostomatinae, also with numerous extant and fossil members, are smaller than *Ferussina* and are characterized by high, conical shells, and some members have calcareous opercula (Fehér 2004; Zallot et al. 2015). The cyclophoroid family Craspedopomatidae, represented by several fossil species in Europe, comprises only very small, globular forms of only a few millimetres in diameter (Wenz 1923; Harzhauser and Neubauer 2018).

The most probable group of relatives is the Cyclophoridae. Most members of this family have broadly conical shells similar to that of *Ferussina*, except for the apexwards turn of the last quarter whorl. Extant Cyclophoridae have non-calcareous opercula, which are not preserved as fossils. So far, 14 species of Cyclophoridae are known from the Cenozoic sedimentary record of Europe (Wenz 1923; Steklov 1966; Schütt 1991, 1997; Stworzewicz 1995). The oldest records derive from the upper Paleocene (Thanetian) of France. Earlier mentions of European cyclophoroids from the Jurassic and Cretaceous belong to the families Diplommatinidae, Megalostomatidae, and Pupinidae, or are unassigned cyclophoroids (Hrubesch 1965; Bandel 1991, 1993; Neubauer et al. 2019). The genus *Ventriculus* Wenz in Fischer & Wenz, 1914 was classified in the

family Cyclophoridae, subfamily Pupinellinae by Wenz (1923), a group now included in Pupinidae (Bouchet et al. 2017). The Pupinidae presently inhabits Asia from India to the oceanic islands (Egorov 2013).

Cyclophoridae are otherwise mostly restricted to south-eastern Asia, and the European fossils represent a rare exception of biogeographic affinity between both regions. Only a few other taxa that are widespread in East Asia today are also found in the European Cenozoic fossil record, such as Diplommatinidae, Strobilopsidae, and Pupinidae (e.g. Wenz 1923; Manganelli et al. 2008; Páll-Gergely et al. 2015; Harzhauser and Neubauer 2018).

In summary, we suggest a revised systematic position of the genus *Ferussina* in the Cyclophoridae. Given the distinct biogeographic and stratigraphic setting and morphological differences to extant Cyclophoridae, we suggest to maintain the genus in a distinct subfamily, Ferussininae.

The extant genera with apexward-turning body whorl listed above inhabit various habitats, with about half of them being obligatory rock-dwellers, indicating that this peculiar trait can be developed under various environmental conditions. *Ferussina* lived in a period when the regions it occurred in central and western Europe (France, Switzerland, Germany, Austria, and northern Italy) were dominated by warm-temperate to subtropical evergreen forests (Pound and Salzmann 2017). The Late Oligocene *Ferussina tricarinata* was thriving in semiarid conditions in the Mainz Basin on the shores of a brackish to hypersaline lake (Kadolsky 1989). Other *Ferussina* species may have dwelled in more humid climates among leaf litter and under decaying logs, but we have insufficient data about the taphonomy and paleoecology of their occurrences.

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Supplementary material 1

Table S1

Authors: Barna Páll-Gergely, Thomas A. Neubauer

Data type: occurrence data

Explanation note: Geographic and stratigraphic occurrence data for species of *Ferussina*. Provided are approximate GPS coordinates of the localities, alternative spellings of locality names, indications of type localities and literature.

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RESEARCH ARTICLE

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Cryptic diversity and range extension in the big-eyed bat genus *Chiroderma* (Chiroptera, Phyllostomidae)

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Abstract

Since the last systematic review of *Chiroderma* (big-eyed bats) more than two decades ago, we report on biodiversity surveys that expand the distribution and species diversity of this Neotropical genus. The Caribbean endemic species *Chiroderma improvisum* is documented for the first time from Nevis in the northern Lesser Antilles. A broader geographic sampling for a molecular analysis identifies a paraphyletic relationship in *Chiroderma trinitatum* with respect to *Chiroderma doriae*. Cis-Andean populations of *C. trinitatum* are most closely related to the morphologically distinctive and allopatrically distributed *C. doriae* in the Cerrado and Atlantic Forest of Brazil and Paraguay. The sister taxon to this grouping includes trans-Andean populations of *C. trinitatum*, which we recommend to elevate to species status as *C. gorgasi*. This is an example of a cryptic species because *C. gorgasi* was previously considered morphologically similar to *C. trinitatum*, but more detailed examination revealed that it lacks a posterolabial accessory cusp on the lower second premolar and has a narrower breadth of the braincase. We provide an amended description of *Chiroderma gorgasi*.

Keywords

Chiroderma gorgasi, *Chiroderma improvisum*, cryptic species, cytochrome c oxidase subunit 1, Lesser Antilles

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Introduction

Cryptic species, phenotypically similar organisms that are classified as a single species but are genetically divergent lineages, are being discovered at a greater rate due to the increasing prevalence of molecular methods, such as DNA barcoding (e.g., Hebert et al. 2004). It has been estimated that Neotropical mammalian biodiversity is underestimated by one-third (Lim 2012). At typical lowland tropical forest sites, bats comprise the majority of mammal species diversity (Voss and Emmons 1996), so more species are expected to be recognized in this group as traditional taxonomic hypotheses are tested by genetic techniques. In addition, new surveying methods such as the use of triple-high netting systems to catch higher flying aerial insectivorous bats, and harp traps to target species that may be able to better detect mist nets, is decreasing the sampling bias associated with traditional mist nets set just above ground level.

The big-eyed bats in the genus *Chiroderma* Peters (Phyllostomidae) are characterized by greatly reduced nasal bones in the skull and a combination of external features including a white dorsal stripe that does not extend onto the head; legs and interfemoral membrane conspicuously hairy; and relatively large eyes (Straney 1984; Gardner 2008). The genus currently comprises six species (Simmons 2005, Taddei and Lim 2010): *C. doriae* Thomas, 1891 occurs in central-eastern Brazil and Paraguay; *C. improvisum* Baker & Genoways, 1976 is endemic to the Lesser Antillean islands of Guadeloupe, Montserrat, and Saint Kitts (Beck et al. 2016); *C. salvini* Dobson, 1878 is found from Mexico to Bolivia (recent records from Brazil are misidentifications of *C. villosum* Peters, 1860 – see Brandão et al. 2019); *C. trinitatum* Goodwin, 1958 is distributed from Honduras (Turcios-Casco et al. 2020) and Costa Rica to Amazonian Brazil and Trinidad; *C. villosum* ranges from Mexico to southeastern Brazil and Trinidad; and *C. vizottoi* Taddei & Lim, 2010 is found only in northeastern Brazil.

The systematics of *Chiroderma* was last reviewed by Baker et al. (1994) based on a phylogenetic study of the mitochondrial DNA cytochrome *b* (Cytb) gene; however, each of the five species known at the time was represented by a single specimen. With broader geographic coverage, we re-assess the distributional range, genetic diversity, and morphological differences in the genus.

Material and methods

Fieldwork

We conducted a survey of bats on the Caribbean island of Nevis from 24–29 April 2016. Live traps used included a harp trap and 6 m or 12 m mist nets set singly in the forest understory or on a triple-high telescoping pole system. Traps were regularly monitored for the first 2–3 hours after sunset when bat activity is the highest after they leave their roosts to feed. Individuals not kept as part of the representa-

tive collection documenting the species diversity were released at point of capture. A combined scientific research and export permit (F002) was issued through the authority of the Nevis Historical and Conservation Society. An Animal Use Protocol (2016-01) was obtained from the Royal Ontario Museum Animal Care Committee. An import permit (#2016-02101-4) was authorized by the Canadian Food Inspection Agency. Use of wild mammals followed the guidelines of the American Society of Mammalogists (Sikes et al. 2016).

Molecular analyses

The cytochrome c oxidase subunit 1 (CO1) gene is the best represented molecular marker for *Chiroderma* on the genetic sequence database GenBank (www.ncbi.nih.gov/genbank). There are 117 samples from nine countries in Central and South America (Brazil, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Mexico, Panama, and Suriname). We add 26 new sequences to bring the sample size to 143 sequences representing 12 countries in the Neotropics, including Venezuela, Peru, and Nevis, and five species in the genus (Appendix 1). There are no tissue samples or nucleotide sequences on GenBank of any genes for the recently described *Chiroderma vizottoi* (Taddei and Lim 2010). Outgroup taxa were other genera in the subtribe Vampyressina Baker et al., 2016 (*Platyrrhinus incarum* Thomas, 1912 and *Uroderma bilobatum* Peters, 1866) of the New World leaf-nosed bats, for direct comparison to Baker et al. (1994) in their analysis of Cytb. Alternative phylogenetic relationships within the subtribe are given by Baker et al. (2016) and Rojas et al. (2016). We also analyzed Cytb, but there are only 11 sequences on GenBank, although we did add one new sequence of *Chiroderma trinitatum gorgasi* from Panama (Appendix 2).

Molecular methods for new sequences of CO1 follow the protocol for DNA extraction, PCR amplification, and automated nucleotide sequencing outlined in Lim (2017). For Cytb, extraction, amplification, and sequencing followed Lim et al. (2008). Base calls were confirmed with bidirectional sequences and aligned using Sequencher version 4.8 (Gene Code Corporation, Ann Arbor, Michigan). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 (Tamura et al. 2013). For a robust comparison of phylogeny, we used parsimony as a method that minimizes evolutionary change without an explicit model of evolution and maximum likelihood as a probabilistic method with an explicit model of evolution. Maximum parsimony used the subtree pruning regrafting inference method with 500 bootstrap replicates to test branch supports. Maximum likelihood used the Tamura 3-parameter substitution model and gamma distributed rates with invariant sites for COI as determined by the best fit test. For Cytb, the Tamura Nei model and gamma rates were the best fit. Tree inference used nearest neighbor interchange heuristic inference with 500 bootstrap replicates. Genetic distances were calculated with the Tamura 3-parameter model with gamma distributed rates among sites for the larger COI dataset.

Morphological analyses

Morphological and morphometric comparisons included 138 specimens from five species of *Chiroderma*, including two *C. improvisum*, four *C. doriae*, seven *C. salvini*, 58 *C. trinitatum*, and 66 *C. villosum* (Appendix 3). We also analyzed the holotypes of *C. trinitatum gorgasi* Handley, 1960 and *C. trinitatum trinitatum* Goodwin, 1958, but did not have specimens of the most recently described *C. vizottoi*. Only adults (defined as having closed cranial sutures and complete epiphyseal ossification of metacarpal and phalanx joints) of both sexes were examined. Specimens are deposited in the following institutions; Royal Ontario Museum (ROM, Toronto, Canada); National Museum of Natural History (USNM, Washington, DC, USA); American Museum of Natural History (New York, USA); Texas Tech University (Lubbock, USA); and Field Museum of Natural History (Chicago, USA).

Measurements defined below were taken with digital calipers accurate to 0.01 mm following the descriptions of Handley (1960): forearm length (FA); greatest length of skull (GSL); interorbital width (IOW); postorbital width (POW); braincase width (BCW); condyloincisive length (CIL); zygomatic breadth (ZB); width across upper molars (M-M); width across upper canines (C-C); and length of maxillary toothrow (C-M). An analysis of variance (ANOVA) for each measurement and a multivariate analysis of variance (MANOVA) were performed to examine the significance of morphometric divergence among species of *Chiroderma*. The level of significance was $p = 0.05$ for all statistical tests. The homoscedasticity of each variable was tested using Bartlett's test with the R package mvoutliers. Statistical analyzes were performed using R 3.1.0 (R Core Team 2005) and PAST 2.17. Variables were log-transformed and a correlation matrix was used in a Principal Components Analysis (PCA) to assess phenetic differences in multivariate morphological space.

Results

We report the first occurrence of *Chiroderma improvisum* (Fig. 1) from Nevis in the northern Leeward Islands of the Lesser Antilles in the Caribbean. An adult male was caught at Barnes Ghaut on April 28, 2016, in a harp trap set across a dry ravine in forest bisected by a road and surrounded by residential homes (Fig. 2). Other equipment deployed included 6 m mist nets set on a triple-high telescoping pole system, a single 6 m mist net, and a single 12 m mist net from 1900–2100 h. In addition to the new distributional record for the island, one *Ardops nichollsi*, one *Noctilio leporinus*, and 12 *Artibeus jamaicensis* were captured.

Molecular analyses

For COI, the 657 basepairs (bp) at the 5' end were available for most (82%) of the specimens analyzed. The complete 1140 bp of Cytb were available, including the newly generated sequence, for most (75%) of the specimens analyzed. The topology of the *Chiroderma* COI maximum likelihood tree identified six primary terminal clades with (1) *C. salvini* as

Figure 1. First record of the big-eyed bat *Chiroderma improvisum* from Nevis (ROM 126002).

Figure 2. Collecting locality of the first record of the big-eyed bat *Chiroderma improvisum* from Nevis caught in a harp trap on April 28, 2016. The habitat is a dry ravine within forest that is bisected by a road in the residential area of Barnes Ghaut.

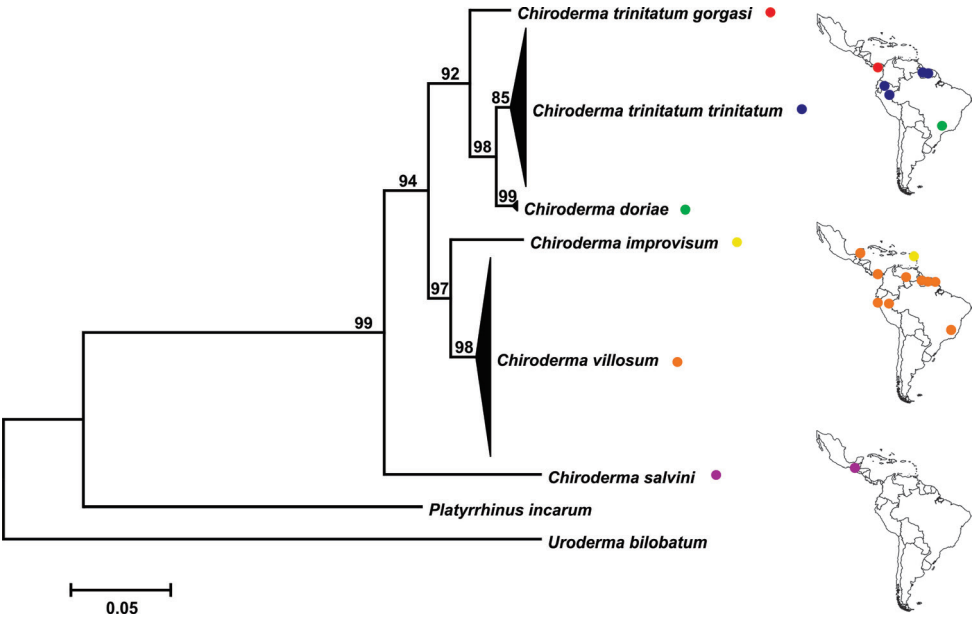

Figure 3. Maximum likelihood tree of cytochrome c oxidase subunit 1 gene for big-eyed bats *Chiroderma* and general localities of molecular sampling sites. Bootstrap percentages show support at each node.

sister species to all other taxa; (2) *C. improvisum* and (3) *C. villosum* as sister species; and (4) *C. doriae* sister to (5) *C. trinitatum trinitatum* with (6) *C. trinitatum gorgasi* sister to these taxa (Fig. 3; Suppl. material 1: Fig. S1). These phylogenetic relationships were supported by bootstrap values ≥85 and were congruent with the maximum parsimony tree (Suppl. material 2: Fig. S2), which had bootstrap values ≥73. Not surprisingly for linked mtDNA loci, the same interspecific topology was recovered by the smaller Cytb dataset analyzed by maximum likelihood (Suppl. material 3: Fig. S3) and maximum parsimony (Suppl. material 4: Fig. S4), except for lower bootstrap supports. The unexpected result was the paraphyly of *C. trinitatum* in relation to *C. doriae*. The sister-group relationship of *C. t. trinitatum* and *C. doriae* was well supported by values ≥73 in all molecular analyses.

Interspecific genetic distances of the larger COI dataset ranged from 11.3% between *C. doriae* and *C. salvini* to 2.5% between *C. doriae* and *C. t. trinitatum* (Table 1). The sequence divergence between *C. t. trinitatum* and *C. t. gorgasi* was 3.9%. Intraspecific distances were 1% within *C. villosum*, 0.9% within *C. t. trinitatum*, and 0.2% within *C. doriae*, but three taxa were represented by only one sample.

Morphological analyses

Cranial and body measurements for the six taxa of *Chiroderma* identified in the molecular analyses are shown in Table 2. *Chiroderma trinitatum gorgasi* and *C. trinitatum trinitatum* are the smallest members of the genus, whereas *C. improvisum* is the largest for most measurements. In the PCA, there are three main groups of species (Fig. 4). The

Table 1. Genetic divergence of cytochrome c oxidase subunit 1 for the big-eyed bat *Chiroderma* and outgroup taxa *Uroderma* and *Platyrrhinus*. Interspecific distances shown in the lower left matrix; intraspecific distances shown in bold in the diagonal.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
U. bilobatum (1)	–							
P. incarum (2)	0.203	–						
C. villosum (3)	0.223	0.178	**0.010**					
C. improvisum (4)	0.231	0.194	0.047	–				
C. t. trinitatum (5)	0.222	0.184	0.067	0.075	**0.009**			
C. salvini (6)	0.205	0.185	0.093	0.101	0.110	–		
C. t. gorgasi (7)	0.195	0.149	0.059	0.070	0.039	0.101	–	
C. doriae (8)	0.213	0.173	0.066	0.077	0.025	0.113	0.039	**0.002**

Table 2. Cranial and body measurements of six taxa of the big-eyed bat *Chiroderma*. See Material and methods for variable abbreviations.

	C. trinitatum gorgasi (*N* = 11)	*C. trinitatum trinitatum* (*N* = 47)	*C. villosum* (*N* = 66)	*C. salvini* (*N* = 6)	*C. doriae* (*N* = 4)	*C. improvisum* (*N* = 2)
FA	37.7 (37.0–40.5)	38.9 (37.1–42.6)	47.9 (44.6–51.0)	49.6 (49.3–50.0)	54 (53.0–55.0)	58.2 (56.2–60)
GLS	21.2 (20.6–21.7)	21.1 (20.0–22.7)	24.5 (23.2–25.7)	26.1 (24.2–26.5)	28.2 (27.6–28.9)	29.3 (28.7–29.9)
CLI	17.3 (16.3–18.1)	17.4 (16.1–18.8)	20.3 (18.7–21.58)	21.6 (21.5–22.3)	23.9 (23.1–24.5)	26.3 (25.3–27.8)
ZB	13.0 (12.5–13.5)	12.9 (11.7–14.2)	15.5 (14.4–16.7)	16.2 (15.8–16.7)	17.84 (17.7–18.1)	18.75 (18.5–19)
POW	5.3 (4.9–5.6)	5.3 (5.8–5.8)	5.9 (5.3–6.3)	6.2 (5.9–6.3)	6.4 (6.1–6.6)	6.6 (6.5–6.6)
IOW	5.6 (5.2–5.9)	5.5 (5.0–6.2)	6.0 (5.5–6.8)	6.8 (6.1–7.3)	7.6 (7.1–7.8)	7.4 (7.4–7.4)
BCW	9.4 (8.9–9.8)	9.6 (9.2–10.4)	10.7 (10.1–11.5)	11.21 (11.0–11.5)	11.9 (11.3–12.1)	12.0 (11.5–12.5)
C-M	7.0 (6.5–7.3)	7.1 (6.7–7.8)	8.7 (8.1–9.4)	9.4 (9.1–9.4)	10.3 (10.0–11.1)	11.0 (10.9–11.1)
M-M	9.6 (9.2–10.0)	9.5 (8.7–10.3)	11.3 (10.3–12.4)	12.0 (11.5–12.3)	13.3 (13.0–13.7)	13.8 (13.6–13.9)
C-C	4.7 (4.4–5.0)	4.6 (4.1–5.0)	5.8 (5.3–6.3)	6.1 (6.0–6.2)	6.5 (6.3–6.8)	7.4 (7.4–7.4)

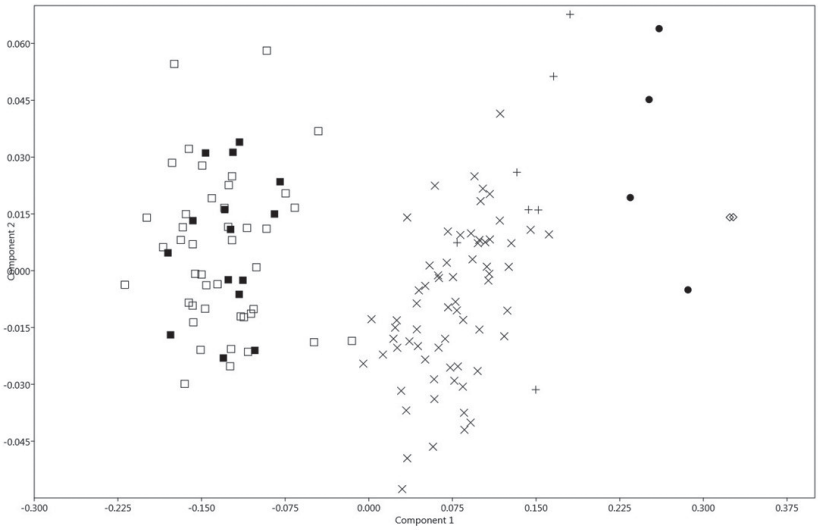

Figure 4. Principal Component Analysis (PCA) carried out using the correlation matrix of ten measurements for six taxa of the big-eyed bat *Chiroderma*. *C. trinitatum gorgasi* (■) *C. trinitatum trinitatum* (□), *C. salvini* (×), *C. villosum* (+), *C. doriae* (●), and *C. improvisum* (◊).

Table 3. Eigenvalue and loadings for the first and second components in the Principal Component Analysis (PCA) of big-eyed bats *Chiroderma*. See Material and methods for variable abbreviations.

	PC1	PC2
Eigenvalue	1.57	0.53
% Variance	91.4	3.11
IOW	0.25	0.44
POW	0.21	0.40
C-M	0.34	-0.23
GSL	0.31	-0.05
BCW	0.34	0.35
CIL	0.34	0.07
C-C	0.36	-0.23
M-M	0.36	-0.14
ZB	0.37	-0.12
FA	0.42	0.32

first group is formed by the smaller taxa *C. t. gorgasi* and *C. t. trinitatum*. The second group has species with medium size, *C. villosum* and *C. salvini*, and the third group is formed by the largest species of the genus, *C. doriae* and *C. improvisum*. The first and second principal components (PC1 and PC2) explained 94.5% of the total variation. PC1 shows a pattern in general size variation and is explained mostly by C-M, C-C, and FA. PC2 has positive loadings for most measurements, especially IOW, with the exception of C-M, C-C, M-M, and ZB that have negative loadings (Table 3). All the species seem to occupy the entire range of PC2, indicating that the contrast among measurements is negligible and it is not responsible for the separation of groups.

All variables had $p > 0.05$ for Bartlett's test of homoscedasticity, indicating constant variances (p values: FA = 0.06, GSL = 0.25, IOW = 0.59, POW = 0.3l, BCW = 0.06, CIL = 0.45, ZB = 0.08, M-M = 0.32, C-C = 0.08, and C-M = 0.06). The MANOVA and the ANOVA demonstrated that *C. t. trinitatum* and *C. t. gorgasi* are significantly different from the other taxa of *Chiroderma* (P < 0.001) for all measured variables (Appendix 3). By contrast, *C. t. gorgasi* and *C. t. trinitatum* are not significantly different from each other ($p = 0.16$, $F = 56.0$). However, the ANOVA showed that one measurement, BCW ($p = 0.01$; $F = 62.0$), was significantly larger for *C. t. trinitatum* than for *C. t. gorgasi*. All other cranial measurements had smaller mean values for *C. t. trinitatum* than for *C. t. gorgasi*.

Although similar in size, *C. t. trinitatum* has a more robust breadth of the braincase than *C. t. gorgasi*. *Chiroderma t. trinitatum* also has an accessory cusp on the second lower premolar, which is absent in *C. t. gorgasi* (Fig. 5). In the genetic analyses, *C. t. trinitatum* is well supported as the sister species to *C. doriae* and does not share a most recent common ancestor with *C. t. gorgasi*. We consider this as a previous example of a cryptic species and therefore now recognize *C. gorgasi* as a distinct species from *C. trinitatum*. Sáez and Lozano (2005: 111) considered cryptic species to be "groups of organisms that are morphologically indistinguishable from each other, yet found to belong to different evolutionary lineages". They also stated that "after detailed comparisons of morphological and non-morphological features, we can often establish key

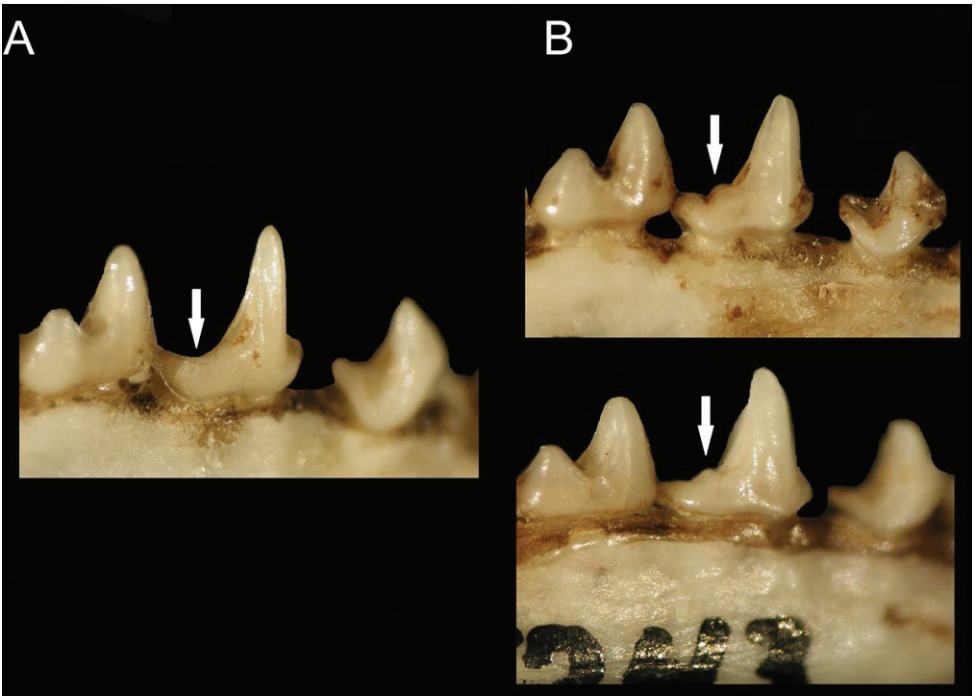

Figure 5. Lateral view of the second lower premolar on the right mandible of **A** *Chiroderma gorgasi* and **B** *C. trinitatum*. The arrow points to the accessory cusp that is absent in *C. gorgasi* and present in *C. trinitatum*. But note the variation in cusp formation in *C. trinitatum*.

morphological characters for their identification. In those cases, we can then refer to pseudo-cryptic or pseudo-sibling species". Because Handley's original description was qualitative and univariate, we offer an amended description of this taxon.

Taxonomic account

Chiroderma gorgasi Handley, 1960

Chiroderma gorgasi Handley, 1960:464
Chiroderma trinitatum gorgasi Barriga-Bonilla, 1965:246

Material examined. ***Holotype.*** – USNM 309903 (Field number COH 5436), adult male with skin, skull and partial skeleton. Collected on March 6, 1959, by C. O. Handley, Jr, and B. R. Feinstein in Tacarcuna Village (8°05'N, 77°17'W), 3200 feet [975 meters], Río Pucro, Darién, Panama.

Geographic distribution. *Chiroderma gorgasi* is distributed west of the Andes in northwestern Ecuador (Albuja 1989), western Colombia (Gardner 2008), Panama

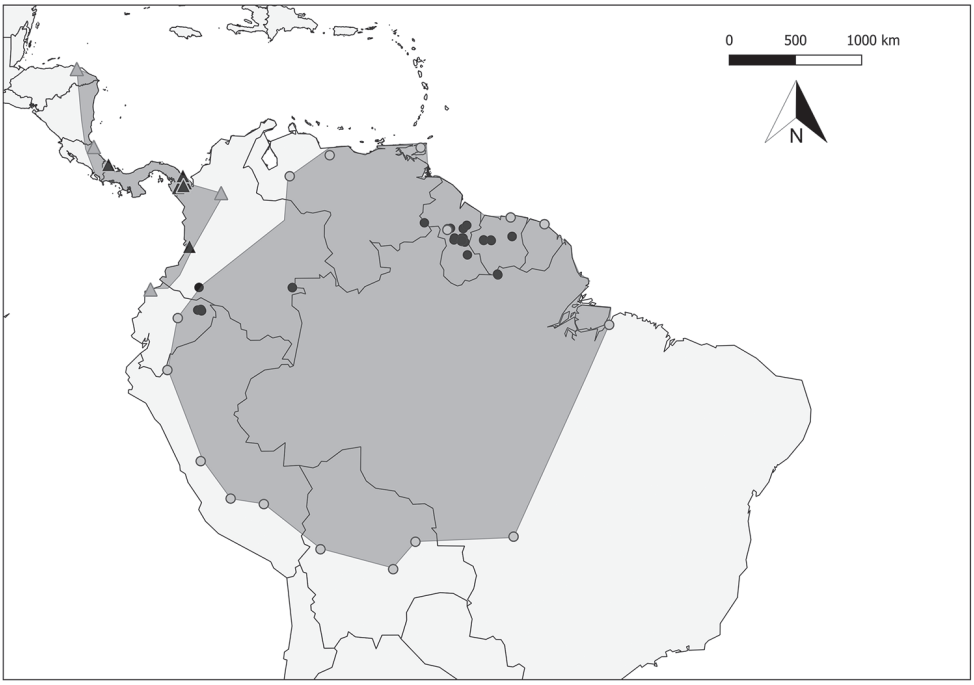

Figure 6. Geographic distribution of *Chiroderma gorgasi* (▲) and *C. trinitatum* (●) localities analyzed in our study (See Appendix 3) (◉) Represents marginal localities reported for *C. trinitatum* and (◬) *C. gorgasi* reported by previous papers (Handley 1967; Pine et al. 1970; Ojasti and Linares 1971; Gardner 1976; Albuja 1989; Timm and LaVal 1998; Lim and Engstrom 2001; Genoways et al. 1981; Webster and Fugler 1984; Anderson 1997; Ochoa et al. 1988; Simmons and Voss 1998; Gardner 2008).

(Handley 1960), Costa Rica (LaVal and Rodríguez-Herrera 2002), and Honduras (Turcios-Casco et al. 2020) (Fig. 6).

Description. *Chiroderma gorgasi* is a small species of *Chiroderma* (FA 37.0–40.5; GLS 20.2–22.5) that is similar in size to *C. trinitatum* (sensu stricto) (Table 2). Overall, the dorsal pelage is tricolor varying from light to dark brown (Fig. 7). The dorsal hairs have a dark brown band at the base, a buff coloration in the middle, and brown tips. A white medial stripe extends from the interscapular region to the base of the rump. Proximal two-thirds of forearm hairy. Basal third of uropatagium hairy. Conspicuous white facial stripes extend from the noseleaf to the inner base of the ears, and from the posterior part of the upper lip to the base of the ears. The uropatagium is medium brown. The skull has an elongated braincase with an undeveloped sagittal and lambdoidal crest. The nasal aperture is short, not extending beyond the second premolar. The occipital is rounded in posterior view. The upper incisors are thin and elongated with parallel or convergent tips, which may or may not touch apically. The second lower premolar lacks a third cusp (Fig 5). The postorbital processes are undeveloped and rounded (Fig. 8).

Comparisons. *Chiroderma gorgasi* is morphologically very similar to *C. trinitatum*. Both species have a small cranial and body size for the genus (Table 2, Fig. 4), an

Figure 7. Dorsal view of the skin of the holotype of *Chiroderma gorgasi* (USNM 309903).

Figure 8. Dorsal, ventral, and lateral views of the skull of the holotype of *Chiroderma gorgasi* (USNM 309903).

undeveloped sagittal and lambdoidal crest, a rounded occipital complex, a short nasal aperture, and undeveloped supraorbital region. However, *C. trinitatum* has a third posterior cusp on the second lower premolar, which is absent in *C. gorgasi* (Fig. 5). This cusp in *C. trinitatum* may vary from very pointed and developed to rounded and less marked, but is always present. In addition, *C. gorgasi* tends to have a broader braincase (Table 4) and a flatter supraorbital region, which tends to be deeper in *C. trinitatum*.

Chiroderma gorgasi is easily distinguished from other species of the genus by its smaller cranial and body size (Table 2). *C. villosum* shares with *C. gorgasi* an elongated braincase, rounded occipital region in dorsal view, and absence of a third cusp on the second lower premolar. However, *C. gorgasi* has an undeveloped postorbital processes, a short nasal aperture, and conspicuous white stripes on the face and back, whereas *C. villosum* has a very developed and pointed postorbital processes, a long nasal ap-

Table 4. ANOVA comparing *Chiroderma trinitatum trinitatum* to *C. doriae*, *C. improvisum*, *C. villosum*, *C. salvini*, and *C. trinitatum gorgasi*. * indicates significantly distinct variables ($p \leq 5\%$).

Variables	*C. doriae*	*C. improvisum*	*C. villosum*	*C. salvini*	*C. t. gorgasi*
GSL	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	0.1541
CI	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	0.4423
ZB	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	0.3094
POW	< 2.2e-16*	1.373e-06*	< 2.2e-16*	1.061e-12*	0.6747
IOW	< 2.2e-16*	1.347e-09*	2.366e-14*	1.355e-14*	0.6272
BWC	< 2.2e-16*	2.602e-08*	< 2.2e-16*	< 2.2e-16*	0.0339*
M-C	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	0.5257
B-M	< 2.2e-16*	1.527e-15*	< 2.2e-16*	< 2.2e-16*	0.1444
B-C	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	0.1251
FA	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	< 2.2e-16*	0.1753

erture, which extends beyond the first molar, a conspicuous posterior palatine spine, and usually incipient white stripes on the face and back. *Chiroderma salvini* resembles *C. gorgasi* in the undeveloped sagittal and lambdoidal crest and by the rounded postorbital processes, but a set of other cranial characters distinguish both species, such as a triangular occipital complex and a long nasal aperture. In the dentition, *C. gorgasi* can be readily distinguished from *C. salvini* and *C. villosum* by having a tall first lower premolar, with a crown height approximately 2/3 the height of the crown of the second lower premolar, and placed approximately in the middle of the distance between the canine and the second lower premolar. In *C. salvini* and *C. villosum*, this tooth is much smaller, usually with a low crown, shorter than the mesiodistal length of the tooth, and is nearer to the canine than to the second lower premolar.

Chiroderma doriae and *C. improvisum* are the largest species of the genus, and unlike *C. gorgasi* have a triangular occipital complex in dorsal view, a pointed and developed supraorbital region, a relatively more developed sagittal and lambdoidal crest, and a long nasal aperture. In addition, *C. doriae* also tends to have a relatively broader braincase than *C. gorgasi* and the presence of an undeveloped third cusp in the second lower premolar. We were not able to examine specimens of the more recently described *C. vizottoi*, but it is larger than *C. gorgasi* and most similar to *C. doriae* in qualitative craniodental traits.

Discussion

The only big-eyed bat species occurring in the Caribbean is *Chiroderma improvisum*, which until recently was known from Guadeloupe (Baker and Genoways 1976) and Montserrat (Jones and Baker 1979; Pierson et al. 1986) by six individuals (Larsen et al. 2007). Subsequently, it was caught on Saint Kitts by Beck et al. (2016) and we are the first to report its occurrence on Nevis. Although this species has been sporadically documented since its discovery, the distribution has broadened in the northern Lesser Antilles but this may be ephemeral depending on weather systems such as hurricanes (Larsen et al. 2007).

Chiroderma gorgasi was originally described by Handley (1960) using five specimens from the type locality in Panama. The author distinguished the new species from *C. trinitatum* by its smaller size, deeper brain case, shorter rostrum, shaper lacrimal ridge, bulging forehead, larger upper incisors, and thicker white band in the dorsal hairs. But at that time, *C. trinitatum* was only known by the holotype from Trinidad (Goodwin 1958) so the extent of variation within each species was poorly understood. Based on a specimen from Mitu in Amazonian Colombia, Barriga-Bonilla (1965) recognized the taxon as two subspecies and assigned his Colombian specimen to *C. t. gorgasi*. The subspecies were considered to be distributed from eastern Panama to western Venezuela for *C. t. gorgasi* and Trinidad to the Amazon basin for *C. t. trinitatum* (Jones and Carter 1976). However, with more geographic sampling the initial distinctions between the two taxa were less obvious due to individual and geographic variation (Simmons and Voss 1998), as also demonstrated by our morphometric analysis. But the taxonomy and distributional limits were still contentious with Gardner (2008) recognizing the Andes as the delineation of the subspecies and reassigning the specimen of Barriga-Bonilla (1965) from Mitu, Colombia, to *C. t. trinitatum*.

Our morphological review identified the presence of three cusps on the second lower premolar in cis-Andean populations referable to *C. trinitatum* and two cusps in trans-Andean populations referable to *C. gorgasi* that also match the taxonomic boundaries of Gardner (2008). Morphometrically, *C. trinitatum* averages smaller than *C. gorgasi* in all cranial measurements except for a proportionately broader braincase. Furthermore, our genetic analyses recovered *C. trinitatum* as the well-supported sister species to the larger and morphologically distinctive *C. doriae*, and not to the superficially similar *C. gorgasi*. Based on this morphological and molecular evidence, we recognize *C. gorgasi* as a distinct species and divergent lineage that does not share the most recent common ancestor with *C. trinitatum* (sensu stricto).

The overall topology of the Cytb tree proposed by Baker et al. (1994) is identical to our tree except for the recognition of *C. gorgasi*, which they did not have a sample of, as the sister species to *C. trinitatum* and *C. doriae*. The evolution of *Chiroderma* was suggested as occurring primarily by allopatric speciation (Baker et al. 1994). More specifically, *C. improvisum* arose by peripatric speciation in the Lesser Antilles after dispersing from its most recent common ancestor with *C. villosum* in South America. The Andes is an obvious geographic barrier separating *C. gorgasi* from the most recent common ancestor of *C. trinitatum* and *C. doriae*. A dated phylogeny is needed to test whether this is an older sundering event associated with the uplift of the northern Andes in the Late Miocene or a more recent dispersal event followed by isolation and the cessation of gene flow. Rojas et al. (2016) date the divergence of *Chiroderma* species to the Pliocene-Holocene, but *C. gorgasi* was not included in their dataset. The allopatric distribution of *C. trinitatum* and *C. doriae* suggests that perhaps the Cerrado Savanna in Brazil acted as a barrier after colonization of the Atlantic Forest from the Amazon, but the records of *C. doriae* for the Cerrado and the discovery of a species of *Chiroderma* in the dry deciduous forests of the Brazilian Caatinga, *C. vizottoi*, indicates that species of the genus can adapt to more harsh habitats. The speciation event that gave rise to *C. salvini* and the most recent common ancestor

of the other species of *Chiroderma* is speculative without a thorough biogeographic analysis with a dated phylogeny.

Although not an overly species-rich genus, biodiversity surveys and molecular analyses are finding new distributional and taxonomic discoveries in *Chiroderma*. However, there are still large geographic gaps in sampling throughout the Neotropics, such as the Amazon basin in Brazil and northern South America in Colombia and Venezuela. In addition, this has hindered detailed study of the biogeography of the genus and more broadly the evolution of bats in the Neotropics.

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Appendix 1

Tissue samples of *Chiroderma* used in the cytochrome c oxidase subunit 1 analysis.

Sample ID	Species	GenBank	Country	State/Department
ROM 111114	*Chiroderma doriae*	JF448016	Brazil	Sao Paulo
ROM 111141	*Chiroderma doriae*	JF446371	Brazil	Sao Paulo
ROM 111149	*Chiroderma doriae*	JF446373	Brazil	Sao Paulo
ROM 111163	*Chiroderma doriae*	JF446372	Brazil	Sao Paulo
	Chiroderma villosum	KT236232	Brazil	Espirito Santo
	Chiroderma villosum	KT236233	Brazil	Espirito Santo
ROM 105191	*Chiroderma trinitatum*	JF448017	Ecuador	Napo
ROM 105230	*Chiroderma trinitatum*	JF448810	Ecuador	Napo
ROM 105243	*Chiroderma trinitatum*	JF448806	Ecuador	Napo
ROM 105253	*Chiroderma trinitatum*	JF448805	Ecuador	Napo
ROM 105581	*Chiroderma trinitatum*	JF448811	Ecuador	Napo
ROM 105685	*Chiroderma trinitatum*	JF448807	Ecuador	Napo
ROM 105718	*Chiroderma trinitatum*	JF448809	Ecuador	Napo
ROM 105766	*Chiroderma trinitatum*	JF448808	Ecuador	Napo
ROM 106342	*Chiroderma trinitatum*	JF448812	Ecuador	Napo
ROM F40504	*Chiroderma trinitatum*	JF448813	Ecuador	Napo
ROM 104448	*Chiroderma villosum*	JF448818	Ecuador	Napo
ROM 104540	*Chiroderma villosum*	JF448829	Ecuador	Napo
ROM 104541	*Chiroderma villosum*	JF448814	Ecuador	Napo
ROM 104549	*Chiroderma villosum*	JF448828	Ecuador	Napo
ROM 105244	*Chiroderma villosum*	JF448815	Ecuador	Napo
ROM 105254	*Chiroderma villosum*	JF448816	Ecuador	Napo
ROM 105267	*Chiroderma villosum*	JF448826	Ecuador	Napo
ROM 105361	*Chiroderma villosum*	JF448825	Ecuador	Napo
ROM 105540	*Chiroderma villosum*	JF448824	Ecuador	Napo
ROM 105587	*Chiroderma villosum*	JF448830	Ecuador	Napo
ROM 105719	*Chiroderma villosum*	JF448822	Ecuador	Napo
ROM 105720	*Chiroderma villosum*	JF448821	Ecuador	Napo
ROM 105721	*Chiroderma villosum*	JF448820	Ecuador	Napo
ROM 105928	*Chiroderma villosum*	JF448817	Ecuador	Napo
ROM 105968	*Chiroderma villosum*	JF448819	Ecuador	Napo
ROM F37400	*Chiroderma villosum*	JF448827	Ecuador	Napo
ROM F37774	*Chiroderma villosum*	JF448823	Ecuador	Napo
ROM 101245	*Chiroderma villosum*	JF446499	El Salvador	Ahuachapan
	Chiroderma villosum	KU295490	French Guiana	
ROM 99703	*Chiroderma salvini*	JF446777	Guatemala	El Progreso
ROM 103486	*Chiroderma trinitatum*	JF454560	Guyana	Upper Demerara-Berbice
ROM 103503	*Chiroderma trinitatum*	JF454561	Guyana	Upper Demerara-Berbice
ROM 103504	*Chiroderma trinitatum*	MN714876	Guyana	Upper Demerara-Berbice
ROM 103505	*Chiroderma trinitatum*	JF454562	Guyana	Upper Demerara-Berbice
ROM 107205	*Chiroderma trinitatum*	EF080285	Guyana	Potaro-Siparuni
ROM 107419	*Chiroderma trinitatum*	EF080286	Guyana	Potaro-Siparuni
ROM 107476	*Chiroderma trinitatum*	MN714877	Guyana	Potaro-Siparuni
ROM 108144	*Chiroderma trinitatum*	JF454552	Guyana	Cuyuni-Mazaruni
ROM 108244	*Chiroderma trinitatum*	JF454559	Guyana	Cuyuni-Mazaruni

Sample ID	Species	GenBank	Country	State/Department
ROM 108463	*Chiroderma trinitatum*	JF454544	Guyana	Potaro-Siparuni
ROM 108554	*Chiroderma trinitatum*	JF454545	Guyana	Potaro-Siparuni
ROM 108587	*Chiroderma trinitatum*	JF454555	Guyana	Potaro-Siparuni
ROM 108588	*Chiroderma trinitatum*	JF454554	Guyana	Potaro-Siparuni
ROM 108714	*Chiroderma trinitatum*	EF080287	Guyana	Potaro-Siparuni
ROM 108763	*Chiroderma trinitatum*	MN714878	Guyana	Potaro-Siparuni
ROM 108889	*Chiroderma trinitatum*	EF080288	Guyana	Potaro-Siparuni
ROM 108950	*Chiroderma trinitatum*	JF454557	Guyana	Potaro-Siparuni
ROM 108993	*Chiroderma trinitatum*	JF454556	Guyana	Potaro-Siparuni
ROM 109026	*Chiroderma trinitatum*	MN714879	Guyana	Potaro-Siparuni
ROM 109195	*Chiroderma trinitatum*	JF454558	Guyana	Potaro-Siparuni
ROM 109271	*Chiroderma trinitatum*	JF454553	Guyana	Potaro-Siparuni
ROM 109333	*Chiroderma trinitatum*	JF454542	Guyana	Potaro-Siparuni
ROM 111627	*Chiroderma trinitatum*	JF454543	Guyana	Potaro-Siparuni
ROM 111809	*Chiroderma trinitatum*	JF454547	Guyana	Potaro-Siparuni
ROM 111844	*Chiroderma trinitatum*	MN714880	Guyana	Potaro-Siparuni
ROM 111884	*Chiroderma trinitatum*	JF454546	Guyana	Potaro-Siparuni
ROM 111946	*Chiroderma trinitatum*	JF454548	Guyana	Potaro-Siparuni
ROM 115807	*Chiroderma trinitatum*	JF454550	Guyana	Potaro-Siparuni
ROM 116630	*Chiroderma trinitatum*	JF454549	Guyana	Potaro-Siparuni
ROM 118996	*Chiroderma trinitatum*	JF454551	Guyana	Upper Takutu-Upper Essequibo
ROM 121975	*Chiroderma trinitatum*	MN714881	Guyana	Potario-Siparuni
ROM 125124	*Chiroderma trinitatum*	MN714882	Guyana	Potaro-Siparuni
ROM 103214	*Chiroderma villosum*	JF454584	Guyana	Upper Takutu-Upper Essequibo
ROM 103331	*Chiroderma villosum*	JF454585	Guyana	Upper Takutu-Upper Essequibo
ROM 106644	*Chiroderma villosum*	JF454566	Guyana	Upper Takutu-Upper Essequibo
ROM 107111	*Chiroderma villosum*	EF080290	Guyana	Potaro-Siparuni
ROM 107112	*Chiroderma villosum*	EF080291	Guyana	Potaro-Siparuni
ROM 107394	*Chiroderma villosum*	EF080292	Guyana	Potaro-Siparuni
ROM 108203	*Chiroderma villosum*	JF454565	Guyana	Cuyuni-Mazaruni
ROM 108219	*Chiroderma villosum*	JF454564	Guyana	Cuyuni-Mazaruni
ROM 108764	*Chiroderma villosum*	JF454571	Guyana	Potaro-Siparuni
ROM 108765	*Chiroderma villosum*	JF454570	Guyana	Potaro-Siparuni
ROM 108843	*Chiroderma villosum*	EF080289	Guyana	Potaro-Siparuni
ROM 108998	*Chiroderma villosum*	JF454573	Guyana	Potaro-Siparuni
ROM 109138	*Chiroderma villosum*	JF454572	Guyana	Potaro-Siparuni
ROM 109175	*Chiroderma villosum*	JF454569	Guyana	Potaro-Siparuni
ROM 109221	*Chiroderma villosum*	JF454568	Guyana	Potaro-Siparuni
ROM 109270	*Chiroderma villosum*	JF454567	Guyana	Potaro-Siparuni
ROM 109307	*Chiroderma villosum*	JF454583	Guyana	Potaro-Siparuni
ROM 109308	*Chiroderma villosum*	JF454582	Guyana	Potaro-Siparuni
ROM 109337	*Chiroderma villosum*	JF454581	Guyana	Potaro-Siparuni
ROM 111628	*Chiroderma villosum*	EF080293	Guyana	Potaro-Siparuni
ROM 111629	*Chiroderma villosum*	JF459119	Guyana	Potaro-Siparuni
ROM 111754	*Chiroderma villosum*	JF454580	Guyana	Potaro-Siparuni
ROM 111768	*Chiroderma villosum*	JF454579	Guyana	Potaro-Siparuni
ROM 111769	*Chiroderma villosum*	JF454578	Guyana	Potaro-Siparuni
ROM 111770	*Chiroderma villosum*	JF454577	Guyana	Potaro-Siparuni
ROM 111788	*Chiroderma villosum*	JF454576	Guyana	Potaro-Siparuni
ROM 111836	*Chiroderma villosum*	JF454575	Guyana	Potaro-Siparuni

Sample ID	Species	GenBank	Country	State/Department
ROM 111845	*Chiroderma villosum*	JF454574	Guyana	Potaro-Siparuni
ROM 119167	*Chiroderma villosum*	MN714883	Guyana	Upper Takutu-Upper Essequibo
ROM 119230	*Chiroderma villosum*	JF454586	Guyana	Upper Takutu-Upper Essequibo
ROM 122481	*Chiroderma villosum*	MN714884	Guyana	Potaro-Siparuni
ROM 98850	*Chiroderma villosum*	JF454563	Guyana	Barima-Waini
ROM 125179	*Chiroderma villosum*	MN714885	Guyana	East Berbice-Corentyne
ROM F38952	*Chiroderma villosum*	MN714886	Guyana	Potaro-Siparuni
ROM 98702	*Uroderma bilobatum*	JF435925	Guyana	Barima-Waini
ROM 96536	*Chiroderma villosum*	JF448018	Mexico	Campeche
ROM FN30654	*Chiroderma villosum*	JF447242	Mexico	Campeche
ROM 104342	*Chiroderma gorgasi*	MN714901	Panama	Darien
ROM 104352	*Chiroderma villosum*	JF447405	Panama	Darien
ROM F38210	*Chiroderma villosum*	JF447406	Panama	Darien
ROM 122084	*Chiroderma trinitatum*	MN714887	Peru	Loreto
ROM 122137	*Chiroderma trinitatum*	MN714888	Peru	Loreto
ROM 122149	*Chiroderma trinitatum*	MN714889	Peru	Loreto
ROM 122165	*Chiroderma villosum*	MN714890	Peru	Loreto
ROM 122260	*Chiroderma villosum*	MN714891	Peru	Loreto
ROM 125567	*Chiroderma villosum*	MN714892	Peru	Tumbes
ROM 126002	*Chiroderma improvisum*	MN714893	Nevis	Saint Thomas Lowland Parish
ROM 114170	*Chiroderma trinitatum*	JF447622	Suriname	Brokopondo
ROM 114213	*Chiroderma trinitatum*	JF447625	Suriname	Brokopondo
ROM 114233	*Chiroderma trinitatum*	JF447623	Suriname	Brokopondo
ROM 114234	*Chiroderma trinitatum*	JF447624	Suriname	Brokopondo
ROM 117003	*Chiroderma trinitatum*	JF447627	Suriname	Sipaliwini
ROM 117003	*Chiroderma trinitatum*	MN714894	Suriname	Sipaliwini
ROM 117027	*Chiroderma trinitatum*	JF447626	Suriname	Sipaliwini
ROM 117083	*Chiroderma trinitatum*	JF447628	Suriname	Sipaliwini
ROM 117376	*Chiroderma trinitatum*	EU096695	Suriname	Sipaliwini
ROM 117555	*Chiroderma trinitatum*	EU096696	Suriname	Sipaliwini
ROM 120098	*Chiroderma trinitatum*	MN714895	Suriname	Sipaliwini
ROM 120168	*Chiroderma trinitatum*	HQ545629	Suriname	Sipaliwini
ROM 120225	*Chiroderma trinitatum*	HQ545678	Suriname	Sipaliwini
ROM 120384	*Chiroderma trinitatum*	HQ919736	Suriname	Sipaliwini
ROM 114212	*Chiroderma villosum*	JF447630	Suriname	Brokopondo
ROM 114228	*Chiroderma villosum*	JF447631	Suriname	Brokopondo
ROM 117119	*Chiroderma villosum*	JF447629	Suriname	Sipaliwini
ROM 117375	*Chiroderma villosum*	EU096697	Suriname	Sipaliwini
ROM 120226	*Chiroderma villosum*	HQ545679	Suriname	Sipaliwini
ROM 120239	*Chiroderma villosum*	HQ545445	Suriname	Sipaliwini
ROM 120240	*Chiroderma villosum*	HQ545446	Suriname	Sipaliwini
ROM 120354	*Chiroderma villosum*	MN714896	Suriname	Sipaliwini
ROM 120364	*Chiroderma villosum*	HQ919717	Suriname	Sipaliwini
ROM 121027	*Chiroderma villosum*	MN714897	Suriname	Sipaliwini
ROM 121117	*Chiroderma villosum*	MN714898	Suriname	Sipaliwini
ROM 126174	*Chiroderma villosum*	MN714899	Suriname	Para
ROM 113919	*Platyrrhinus incarum*	JF435616	Suriname	Brokopondo
ACUNHC 393	*Chiroderma villosum*	MN714900	Venezuela	Amazonas

Appendix 2

Tissue samples of *Chiroderma* used in the cytochrome b analysis.

Sample ID	Species	GenBank	Country	State/Province
UNESP 16506	*Chiroderma doriae*	L28937	Brazil	Sao Paulo
TK 16379	*Chiroderma doriae*	AY169958	Brazil	
TK 15713	*Chiroderma improvisum*	L28938	Montserrat	St. Anthony
TK 25052	*Chiroderma villosum*	DQ312414	Trinidad	St. George
FMNH 174652	*Chiroderma villosum*	FJ154121	Peru	Madre de Dios
TK 17627	*Platyrrhinus helleri*	L28940	Suriname	Marowijne
TK 25256	*Uroderma bilobatum*	L28941	Trinidad	St. George
TK 22581	*Chiroderma salvini*	L28939	Panama	Darien
TK 25211	*Chiroderma trinitatum*	DQ312413	Trinidad	St. George
ASK 7799	*Chiroderma villosum*	JF442196	Ecuador	Orellana
ASK 7667	*Chiroderma villosum*	JF442139	Ecuador	Napo
MN 36375	*Chiroderma villosum*	DQ903823	Brazil	
SK-Bat-61	*Chiroderma improvisum*	JQ915203	Saint Kitts	
ROM 104342	*Chiroderma gorgasi*	MN714902	Panama	Darien

Appendix 3

Specimens of *Chiroderma* examined morphologically. Vouchers examined are arranged alphabetically by species and country. See "Material and Methods" for collection acronyms.

Chiroderma doriae – Brazil: São Paulo - ROM 111163, ROM 111141, ROM 111114, ROM 111149.

Chiroderma improvisum – Montserrat: St. Anthony Parsish – TTU 31403; St. Kitts and Nevis: Barnes Ghaut - ROM 126002.

Chiroderma salvini – El Salvador: Morazan - ROM 83365, ROM 85948, Santa Ana - ROM 101526; Guatemala: El Progreso - ROM 99703; Panama: Darien - ROM 78472, ROM 91194.

Chiroderma trinitatum gorgasi – Colombia: Valle del Cauca - USNM 483763, USNM 483765, Antioquia - USNM 499478, USNM 499476; Panama: Bocas Del Toro – USNM 319498, USNM 335295, Darien - FMNH 128132, ROM 104342, USNM 309901, USNM 309903-**holotype**, San Blas - USNM 309905.

Chiroderma trinitatum trinitatum – Colombia: Vaupes - ROM 45276, ROM 45278, ROM 45280, ROM 45281, ROM 45284, Putumayo – ROM 63236, ROM 63237, ROM 63238; Ecuador: Napo - ROM 105191, ROM 105243, ROM 105253, ROM 105685, ROM 105766, ROM 106342; Guyana: Cuyuni-Mazaruni - ROM 108144, Demerara-Berbice - ROM 57392, ROM 103486, ROM 103503, Upper, Potaro-Siparuni - ROM 107205, ROM 107419, ROM 107476, ROM 108463, ROM 108554, ROM 108587, ROM 108714, ROM 108763, ROM ROM 108889,

ROM 108950, ROM 108993, ROM 109195, ROM 109333, ROM 111627, ROM 111809, ROM 111884, ROM 111946, ROM 115807, ROM 116630; Suriname: Brokopondo - ROM 114170, ROM 114213, ROM 114233, ROM 114234, Sipaliwini - ROM 117027, ROM 117376, ROM 120168, ROM 120225, ROM 120384; Trinidad: Saint Andrew County – AMNH 175325-**holotype**.

Chiroderma villosum – Bolivia: Carrasco - ROM 78471; Colombia: Choco - ROM 85849, Vaupes - ROM 44952, ROM 44953, ROM 44954, ROM 45243, ROM 45245, ROM 45246, ROM 45247, ROM 45249, ROM 45250, ROM 45251, ROM 45252, ROM 45253, ROM 45254, ROM 45255, ROM 45257; Ecuador: Napo - ROM 104448, ROM 104541, ROM 104549, ROM 105244, ROM 105254, ROM 105361, ROM 105720, ROM 105721); Guyana: Barima-Waini - ROM 98850, Potaro-Siparuni - ROM 107111, ROM 107112, ROM 107394, ROM 108219, ROM 108764, ROM 108843, ROM 108998, ROM 109138, ROM 109175, ROM 109221, ROM 109307, ROM 109308, ROM 109337, ROM 111628, ROM 111629, ROM 111754, ROM 111768, ROM 111769, ROM 111770, ROM 111788, ROM 111836, ROM 111845, ROM 122481, Upper Demerara-Berbice - ROM 60402, ROM 60423, Upper Takutu-Upper Essequibo - ROM 35614, ROM 103214, ROM 106644, ROM 119167, ROM 119230; Panama: Darien - ROM 104352; Suriname: Brokopondo - ROM 114212, Sipaliwini - ROM 117119, ROM 117375, ROM 120226, ROM 120239, ROM 120364, ROM 121027; Trinidad and Tobago: Nariva - ROM 124684, ROM 124691.

Supplementary material 1

Maximum likelihood tree of cytochrome c oxidase subunit 1 gene for big-eyed bats *Chiroderma* as presented in Fig. 3, but expanded to show individuals

Authors: Burton K. Lim, Livia O. Loureiro, Guilherme S. T. Garbino

Data type: phylogenetic dendrogram

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Link: https://doi.org/10.3897/zookeys.918.48786.suppl1

Supplementary material 2

Maximum parsimony tree of cytochrome c oxidase subunit 1 gene for *Chiroderma*. Bootstrap percentages show support at each node

Authors: Burton K. Lim, Livia O. Loureiro, Guilherme S. T. Garbino

Data type: phylogenetic dendrogram

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Link: https://doi.org/10.3897/zookeys.918.48786.suppl2

Supplementary material 3

Maximum likelihood tree of cytochrome b gene for *Chiroderma*. Bootstrap percentages show support at each node

Authors: Burton K. Lim, Livia O. Loureiro, Guilherme S. T. Garbino

Data type: phylogenetic dendrogram

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Link: https://doi.org/10.3897/zookeys.918.48786.suppl3

Supplementary material 4

Maximum parsimony tree of cytochrome b gene for *Chiroderma*. Bootstrap percentages show support at each node

Authors: Burton K. Lim, Livia O. Loureiro, Guilherme S. T. Garbino

Data type: phylogenetic dendrogram

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CHECKLIST

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An updated annotated checklist of scale insects (Hemiptera, Sternorrhyncha, Coccomorpha) of Poland

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Abstract

A checklist of scale insects recorded to date in Poland is presented. The data provided here are based on literature records and include the latest taxonomic and nomenclatural changes and updates on Coccomorpha reported in Poland. Changes in comparison with ScaleNet and Fauna Europaea electronic databases are also discussed. A total of 185 species belonging to 98 genera and 16 families are included in the list. Of this group, 47 species are alien introduced species and live only indoors, and one species, *Pulvinaria floccifera* (Westwood), develops both indoors and outdoors.

Keywords

Coccoids, native and alien species, validation source

Introduction

Scale insects form a relatively small group of insects in the Polish fauna and represent only approximately 0.7% of the 27,000 insect species currently known in Poland. However, a few species are identified as pests of economic importance, and especially, in recent years, the invasion of alien scale insects has been observed in several parts of Poland (Łagowska et al. 2015, 2018; Golan et al. 2017).

Scale insects have been known for centuries in Poland for the carmine dye extracted from the Polish cochineal scale *Porphyrophora polonica* (Linnaeus). The presence of *P. polonica* in Poland was reported for the first time in the 16th century (Miechowita 1521) and

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information about the harmful scale insects was published in later years by some authors (Trzebiński 1916; Ruszkowski 1925, 1933; Minkiewicz 1926). Advanced studies on the scale insect fauna of Poland were initiated by Kawecki, whose results have been presented in numerous publications from 1935 to 1985. In the same period and later, Koteja and Koteja and Żak-Ogaza, in publications from1964 to 2000, extensively studied the Coccomorpha and greatly contributed to the knowledge of the scale insect fauna in Poland. Further contributions are due to the studies by Komosińska (1961-1987), Komosińska and Podsiadło (1967), Dziedzicka (1970-1990), Podsiadło (1975); Podsiadło and Komosińska (1976), Łagowska (1990-2005), Łagowska and Koteja (1996), Dziedzicka and Karnkowski (1999), Łagowska and Golan (2005) and Łagowska et al. (2015, 2017, 2018).

Finally, 90 native and greenhouse species of scale insects that were new to the Polish fauna were discovered in the years 1961–1980 (Koteja 1985; Łagowska and Golan 2005). However, the records of species new to the Polish fauna significantly decreased from 32 in the period 1971–1980 to 8 in 1991–2005 (Łagowska and Golan 2005), and only another 8 new species were recorded in Poland in 2006-2019.

The early data on the distribution of scale insects in Poland were summarized by Kawecki (1985) in a catalogue listing 170 species, including 34 indoor species, and 11 records of misidentified species or species for which no host plants or localities were given. Later, two checklists of scale insects in Poland were presented by Koteja (1996) and Łagowska (2004) who reported 184 and 185 species respectively, each including 44 indoor species. In addition, an annotated list of alien scale insects present in Poland was published by Łagowska et al. (2015).

Two electronic databases provide important world-wide information on scale insect distribution: the Fauna Europaea (FaEu) database (Burckhardt 2013), which reports 163 species of scale insects from Poland, and the ScaleNet database (García Morales et al. 2016), which lists 177 species. Since the last checklist (Łagowska 2004), several new records of scale insects from Poland have been published (Łabanowski 2009; Kalandyk and Węgierek 2010; Kozár et al. 2013; Łagowska et al. 2015, 2018). In the meantime, the nomenclature of the scale insects has also been partially changed. Moreover, several records reported in FaEu and ScaleNet databases were regarded as doubtful or erroneous and need revision. The present paper provides a comprehensive revised list of the scale insects of Poland with updated nomenclature and references to the first reliable Polish records of each species. In addition, discrepancies between the present list and the last checklist (Łagowska 2004) as well as differences from the records reported in the FaEu and ScaleNet databases are discussed.

The aim of the present checklist is to provide baseline reliable data for future faunistic and taxonomic studies.

Materials and methods

The list presented in this paper is based on the literature records of Coccomorpha in Poland available up to September 2019. A reference to the first reliable record of each species is included. Fossil species of scale insects and those that have been

intercepted only once on imported plant materials are excluded. Families and species within each family are listed in alphabetical order according to the classification used in the ScaleNet database (García Morales et al. 2016). The references to species recorded in Poland reported in FaEu and ScaleNet have been checked and, if erroneous, corrected in the present lists. Changes in systematic status and synonymies, mostly proposed by Kozár et al. (2013) and Danzig and Gavrilov-Zimin (2014, 2015), and presently accepted in ScaleNet database, have been adopted in the present list. Scale insect species recorded in Poland are listed in Table 1. They belong to four categories as follows: (i) native species; (ii) alien species established outdoors; (iii) alien species established indoors; and (iv) alien species that can live and develop both outdoors and indoors. The definition of alien species in this paper is the one proposed by Łagowska et al. (2015).

Results

At the present time the Polish scale insect fauna comprises a total of 185 species, distributed in 98 genera and 16 families. The Pseudococcidae are the most numerous family, with 50 recorded species, followed by Diaspididae (48 species), Coccidae (43 species), and Eriococcidae (sensu lato) (18 species) (Table 1). The remaining 12 families are each represented by 1–5 species. The ratio of species to genera differs between families. The highest ratio (2.6:1) is in the Eriococcidae, followed by Coccidae (2.3:1), Diaspididae (2.0:1), and Kermesidae (2.0:1) (Table 2). The ratio of species number per genus in the Pseudococcidae family is 1.8:1, which is close to the general mean ratio of 1.9:1 reported for Poland (Table 2).

Table 1. Checklist of scale insects (Hemiptera: Sternorrhyncha: Coccomorpha) of Poland (* alien established indoors only; ** alien established outdoors; ***alien established indoors and outdoors.

Taxa	Validation source
Asterolecaniidae	
1. *Asterodiaspis quercicola* (Bouché, 1851)	Boratyński 1961
2. *Asterodiaspis variolosa* (Ratzeburg, 1870)	Wünn 1919
3. *Asterolecanium epidendri* (Bouché, 1844)*	Kawecki 1985
4. *Planchonia arabidis* Signoret, 1876	Komosińska and Podsiadło 1967
Cerococcidae	
5. *Antecerococcus intermedius* (Balachowsky, 1930)	Koteja 1984
Coccidae	
6. *Ceroplastes rusci* (Linnaeus, 1758)*	Szulczewski 1926
7. *Coccus hesperidum* Linnaeus, 1758*	Brischke 1883
8. *Eriopeltis festucae* (Fonscolombe, 1834)	Szulczewski 1921
9. *Eriopeltis lichtensteini* Signoret, 1877	Szulczewski 1921
10. *Eriopeltis stammeri* Schmutterer, 1952	Komosińska and Podsiadło 1967
11. *Eucalymnatus tessellatus* (Signoret, 1873)*	Koteja 1972
12. *Eulecanium ciliatum* (Douglas, 1891)	Wünn 1919
13. *Eulecanium douglasi* (Šulc, 1895)	Żak-Ogaza 1961
14. *Eulecanium franconicum* (Lindinger, 1912)	Kawecki 1938
15. *Eulecanium sericeum* (Lindinger, 1906)	Kawecki 1938
16. *Eulecanium tiliae* (Linnaeus, 1758)	Kawecki 1935

Taxa	Validation source
17. *Lecanopsis formicarum* Newstead, 1893	Koteja 1969
18. *Lecanopsis subterranea* (Gomez-Menor Ortega, 1948)	Koteja and Żak-Ogaza 1969
19. *Luzulaspis dactylis* Green, 1928	Żak-Ogaza and Koteja 1964
20. *Luzulaspis frontalis* Green, 1928	Koteja 1964
21. *Luzulaspis grandis* Borchsenius, 1952	Żak-Ogaza and Koteja 1964
22. *Luzulaspis luzulae* (Dufour, 1864)	Kawecki 1938
23. *Luzulaspis nemorosa* Koteja, 1966	Koteja 1966
24. *Luzulaspis scotica* Green, 1926	Komosińska and Podsiadło 1967
25. *Nemolecanium graniforme* (Wünn, 1921)	Wünn 1919
26. *Palaeolecanium bituberculatum* (Signoret, 1873)	Kawecki 1935
27. *Parafairmairia bipartita* (Signoret, 1872)	Żak-Ogaza and Koteja 1964
28. *Parafairmairia gracilis* Green, 1916	Koteja and Żak-Ogaza 1969
29. *Parasaissetia nigra* (Nietner, 1861)*	Kawecki 1985
30. *Parthenolecanium corni* (Bouché, 1844)	Lindinger 1911
31. *Parthenolecanium fletcheri* (Cockerell, 1893)**	Kawecki 1935
32. *Parthenolecanium perlatum* (Cockerell, 1898)*	Dziedzicka and Madro 1999
33. *Parthenolecanium persicae* (Fabricius, 1776)	Ruszkowski 1922 (recognized as a doubtful species by Kawecki (1985) and confirmed in Poland by Łagowska (2005a))
34. *Parthenolecanium pomeranicum* (Kawecki, 1954)	Kawecki 1954
35. *Parthenolecanium rufulum* (Cockerell, 1903)	Kawecki 1957
36. *Parthenolecanium smreczynskii* (Kawecki, 1967)	Kawecki 1967
37. *Phyllostroma myrtilli* (Kaltenbach, 1874)	Kawecki 1957
38. *Physokermes hemicryphus* (Dalman, 1826)	Wünn 1919
39. *Physokermes piceae* (Schrank, 1801)	Kawecki 1935
40. *Psilococcus ruber* Borchsenius, 1952	Koteja 1969
41. *Pulvinaria floccifera* (Westwood, 1870)***	Koteja 1972
42. *Pulvinaria hydrangeae* Steinweden, 1946**	Łagowska (unpublished)
43. *Pulvinaria regalis* Canard, 1968	Łagowska et al. 2018
44. *Pulvinaria vitis* (Linnaeus, 1758)	Szulczewski 1921
45. *Saissetia coffeae* (Walker, 1852)*	Szulczewski 1926
46. *Saissetia oleae* (Olivier, 1791)*	Czyżewski 1937
47. *Sphaerolecanium prunastri* (Fonscolombe, 1834)	Kawecki 1957
48. *Vittacoccus longicornis* (Green, 1916)	Koteja 1969
Cryptococcidae	
49. *Cryptococcus fagisuga* Lindinger, 1936	Szulczewski 1921
50. *Pseudochermes fraxini* (Kaltenbach, 1860)	Szulczewski 1926
Diaspididae	
51. *Aonidia lauri* (Bouché, 1833)*	Komosińska 1968
52. *Aonidiella aurantii* (Maskell, 1879)*	Dziedzicka 1988b
53. *Aspidiotus destructor* Signoret, 1869*	Karnkowski 1993
54. *Aspidiotus nerii* Bouché, 1833*	Ruszkowski 1933
55. *Aspidiotus palmarum* Bouché, 1834*	Schander 1910
56. *Aulacaspis rosae* (Bouché, 1833)**	Trzebiński 1916
57. *Aulacaspis yasumatsui* Takagi, 1977*	Łabanowski 2009
58. *Carulaspis juniperi* (Bouché, 1851)	Szulczewski 1926
59. *Chionaspis salicis* (Linnaeus, 1758)	Wünn 1919
60. *Chrysomphalus aonidum* (Linnaeus, 1758)*	Czyżewski 1937
61. *Chrysomphalus dictyospermi* (Morgan, 1889)*	Dziedzicka 1989
62. *Comstockaspis perniciosa* (Comstock, 1881)**	Kawecki 1950
63. *Diaspidiotus alni* (Marchal, 1909)	Kawecki 1935 (recognized as a doubtful species by Kawecki (1985) and confirmed in Poland by Łagowska (2002)
64. *Diaspidiotus bavaricus* (Lindinger, 1912)	Kawecki 1948
65. *Diaspidiotus gigas* (Thiem & Gerneck, 1934)	Komosińska 1974
66. *Diaspidiotus marani* (Zahradnik, 1952)	Krzysztofowicz 1957
67. *Diaspidiotus ostreaeformis* (Curtis, 1843)	Szulczewski 1921

Taxa	Validation source
68. *Diaspidiotus pyri* (Lichtenstein, 1881)	Szulczewski 1921
69. *Diaspidiotus zonatus* (Fauenfeld, 1868)	Szulczewski 1921
70. *Diaspis boisduvalii* Signoret, 1869*	Czyżewski 1975
71. *Diaspis bromeliae* (Kerner, 1778)*	Kawecki 1985
72. *Diaspis echinocacti* (Bouché, 1883)*	Czyżewski 1937
73. *Dynaspidiotus abietis* (Schrank, 1776)	Kawecki 1935
74. *Dynaspidiotus britannicus* (Newstead, 1898)*	Szulczewski 1926
75. *Furchadaspis zamiae* (Morgan, 1890) *	Komosińska 1968
76. *Gymnaspis aechmeae* Newstead, 1898*	Komosińska 1961
77. *Hemiberlesia cyanophylli* (Signoret, 1869) *	Komosińska 1961
78. *Hemiberlesia gliwicensis* (Komosińska, 1965)*	Komosińska 1965
79. *Hemiberlesia lataniae* (Signoret, 1869)*	Czyżewski 1937
80. *Hemiberlesia palmae* (Cockerell, 1892)*	Komosińska 1961
81. *Hemiberlesia rapax* (Comstock, 1881)*	Komosińska 1961
82. *Howardia biclavis* (Comstock, 1883)*	Dziedzicka 1987
83. *Kuwanaspis pseudoleucaspis* (Kuwana, 1902)*	Komosińska 1968
84. *Lepidosaphes conchiformis* (Gmelin, 1790)	Komosińska 1969
85. *Lepidosaphes juniperi* (Lindinger, 1912)	Komosińska 1969
86. *Lepidosaphes newsteadi* (Šulc, 1895)	Komosińska 1974
87. *Lepidosaphes tokionis* (Kuwana, 1902)*	Łabanowski 2009
88. *Lepidosaphes ulmi* (Linnaeus, 1758)	Trzebiński 1916
89. *Leucaspis loewi* Colvée, 1882	Szulczewski 1921
90. *Leucaspis pini* (Hartig, 1839)	Szulczewski 1921
91. *Parlatoria parlatoriae* (Šulc, 1895)*	Żak-Ogaza and Koteja 1964
92. *Parlatoria pergandii* Comstock, 1881*	Komosińska 1964
93. *Parlatoria proteus* (Curtis, 1843)*	Szulczewski 1926
94. *Pinnaspis aspidistrae* (Signoret, 1869)*	Szulczewski 1926
95. *Pinnaspis strachani* (Cooley, 1899)*	Komosińska 1961
96. *Pseudaulacaspis pentagona* (Targioni-Tozzetti, 1886)*	Dziedzicka and Karnkowski 1999
97. *Rhizaspidiotus canariensis* (Lindinger, 1911)	Łagowska 1990
98. *Umbaspis regularis* (Newstead, 1911)*	Komosińska 1968
Eriococcidae	
99. *Acanthococcus aceris* Signoret, 1875	Kawecki 1957
100. *Acanthococcus macedoniensis* Fetykó & Kaydan, 2013	Kozár et al. 2013
101. *Anophococcus agropyri* (Borchsenius, 1949)	Koteja and Żak-Ogaza 1966
102. *Anophococcus confusus* (Danzig, 1962)	Koteja 1971a
103. *Anophococcus herbaceus* (Danzig, 1962)	Żak-Ogaza and Koteja 1964
104. *Anophococcus inermis* (Green, 1915)	Żak-Ogaza and Koteja 1964
105. *Anophococcus insignis* (Newstead, 1891)	Żak-Ogaza and Koteja 1964
106. *Anophococcus pseudinsignis* (Green, 1921)	Koteja and Żak-Ogaza 1969
107. *Gossyparia spuria* (Modeer, 1778)	Trzebiński 1916
108. *Greenisca brachypodii* Borchsenius & Danzig, 1966	Koteja and Żak-Ogaza 1966
109. *Greenisca gouxi* (Balachowsky, 1954)	Koteja and Żak-Ogaza 1983
110. *Kaweckia glyceriae* (Green, 1921)	Żak-Ogaza and Koteja 1964
111. *Neokaweckia laeticoris* (Tereznikova, 1965)	Koteja and Żak-Ogaza 1989
112. *Rhizococcus cantium* (Williams, 1985)	Łagowska and Koteja 1996
113. *Rhizococcus devoniensis* Green,1896	Koteja and Żak-Ogaza 1979
114. *Rhizococcus greeni* (Newstead, 1898)	Żak-Ogaza and Koteja 1964
115. *Rhizococcus munroi* (Boratyński, 1962)	Komosińska and Podsiadło 1967
116. *Rhizococcus palustris* Dziedzicka & Koteja, 1971	Dziedzicka and Koteja 1971
Kermesidae	
117. *Kermes quercus* (Linnaeus, 1758)	Szulczewski 1921
118. *Kermes* roboris (Fourcroy,1785)	Koteja and Żak-Ogaza 1983
Margarodidae	
119. *Neomargarodes festucae* Archangelskaja, 1935	Jakubski 1965

Taxa	Validation source
120. *Porphyrophora polonica* (Linnaeus, 1758)	Miechowita 1521
Matsucoccidae	
121. *Matsucoccus pini* (Green, 1925)	Boratyński 1960
Monophlebidae	
122. *Icerya purchasi* Maskell, 1879*	Chałańska and Łabanowski 2002
123. *Palaeococcus fuscipennis* (Burmeister, 1835)	Szulczewski 1921
Steingeliidae	
124. *Steingelia gorodetskia* Nasonov, 1908	Nasonov 1908
Xylococcidae	
125. *Xylococcus filiferus* Löw, 1883	Kawecki 1948
Ortheziidae	
126. *Arctorthezia cataphracta* (Olafson, 1772)	Kawecki 1938
127. *Newsteadia floccosa* (De Geer, 1778)	Kawecki 1938
128. *Insignorthezia insignis* (Browne, 1887)*	Ruszkowski 1933
129. *Orthezia urticae* (Linnaeus, 1758)	Nowicki 1868
130. *Ortheziola vejdovskyi* Šulc, 1895	Komosińska and Podsiadło 1967
Pseudococcidae	
131. *Atrococcus cracens* Williams, 1962	Koteja 1971a
132. *Atrococcus paludinus* (Green, 1921)	Koteja 1971a
133. *Balanococcus boratynskii* Williams, 1962	Koteja 1986
134. *Boreococcus ingricus* Danzig, 1960	Koteja 1986
135. *Brevennia pulveraria* (Newstead, 1892)	Żak-Ogaza and Koteja 1964
136. *Ceroputo pilosellae* Šulc, 1898	Kawecki 1948
137. *Chaetococcus sulcii* (Green, 1934)	Koteja and Żak-Ogaza 1969
138. *Coccura comari* (Künow, 1880)	Koteja et al. 1978
139. *Dysmicoccus angustifrons* (Hall, 1926)	Koteja and Żak-Ogaza1979
140. *Dysmicoccus walkeri* (Newstead, 1891)	Komosińska and Podsiadło 1967
141. *Fonscolombia abdita* (Borchsenius, 1949)	Koteja 1971a
142. *Fonscolombia europaea* (Newstead, 1897)	Koteja and Żak-Ogaza 1969
143. *Fonscolombia tomlinii* (Newstead, 1892)	Koteja 1972
144. *Heliococcus bohemicus* Šulc, 1912	Komosińska 1977
145. *Heliococcus destructor* Borchsenius, 1941	Koteja et al. 1978
146. *Heliococcus sulcii* Goux, 1934	Łagowska and Koteja 1996
147. *Heterococcus nudus* (Green, 1926)	Żak-Ogaza and Koteja 1964
148. *Kiritshenkella lianae* Koteja, 1988	Koteja 1988
149. *Metadenopus festucae* Šulc, 1933	Koteja and Żak-Ogaza 1969
150. *Mirococcopsis subterranea* (Newstead, 1893)	Koteja and Żak-Ogaza1969
151. *Mirococcus clarus* Borchsenius, 1949	Koteja 1971b
152. *Mirococcus festucae* Koteja, 1971	Koteja 1971b
153. *Nipaecoccus nipae* (Maskell, 1893)*	Czyżewski 1937
154. *Peliococcopsis parviceraria* (Goux, 1937)	Koteja et al. 1978
155. *Peliococcus calluneti* (Lindinger, 1912)	Koteja and Żak-Ogaza1966
156. *Peliococcus morrisoni* (Kiritchenko, 1936)	Łagowska 2005b
157. *Pelionella balteata* (Green, 1928)	Koteja 1972
158. *Pelionella manifecta* (Borchsenius, 1949)	Koteja and Żak-Ogaza1989
159. *Phenacoccus aceris* (Signoret, 1875)	Wünn 1919
160. *Phenacoccus hordei* (Lindeman, 1886)	Koteja and Żak-Ogaza 1979
161. *Phenacoccus interruptus* Green, 1923	Żak-Ogaza and Koteja 1964
162. *Phenacoccus phenacoccoides* (Kiritchenko, 1932)	Łagowska and Koteja 1996
163. *Phenacoccus piceae* (Löw, 1883)	Kawecki 1935
164. *Planococcus citri* (Risso, 1813)*	Szulczewski 1926
165. *Planococcus vovae* (Nasonov, 1908)	Kawecki 1948
166. *Pseudococcus longispinus* (Targioni Tozzetti, 1867)*	Szulczewski 1926
167. *Pseudococcus maritimus* (Ehrhorn, 1900)*	Dziedzicka 1988a
168. *Rhodania occulta* Schmutterer, 1952	Koteja and Żak-Ogaza1966

Taxa	Validation source
169. *Rhodania porifera* Goux, 1935	Koteja and Żak-Ogaza1969
170. *Saccharicoccus isfarensis* (Borchsenius, 1949)	Koteja 1969
171. *Spilococcus mamillariae* (Bouchè, 1844)*	Łabanowski 2009
172. *Trionymus aberrans* Goux, 1938	Koteja and Żak-Ogaza 1966
173. *Trionymus hamberdi* (Borchsenius, 1949)	Danzig 1985 (confirmed in Poland by Łagowska and Koteja (1996))
174. *Trionymus newsteadi* (Green, 1917)	Koteja and Żak-Ogaza1966
175. *Trionymus perrisii* (Signoret, 1875)	Koteja 1969
176. *Trionymus phalaridis* Green, 1925n	Komosińska 1980
177. *Trionymus placatus* (Borchsenius, 1949)	Koteja and Łagowska 1986
178. *Trionymus radicum* (Newstead, 1895)	Koteja 1971a
179. *Trionymus thulensis* Green, 1931	Koteja 1969
180. *Volvicoccus volvifer* Goux, 1945	Kalandyk and Węgierek 2010
Putoidae	
181. *Puto superbus* (Leonardi, 1907)	Łagowska 2000
Rhizoecidae	
182. *Rhizoecus americanus* (Hambleton, 1946)*	Łabanowski 2009
183. *Rhizoecus cacticans* (Hambleton, 1946)*	Kawecki 1985
184. *Rhizoecus dianthi* Green, 1926*	Dziedzicka and Madro 1999
185. *Ripersiella halophila* (Hardy, 1868)	Koteja 1972

Table 2. Number of scale insect species per genus in relation to families in Poland.

Family	Number of genus	Number of species	Ratio of species to genera
Asterolecaniidae	3	4	1.3:3
Cerococcidae	1	1	1:1
Coccidae	19	43	2.3:1
Cryptococcidae	2	2	1:1
Diaspididae	23	48	2.0:1
Eriococcidae	7	18	2.6:1
Kermesidae	1	2	2.0:1
Margarodidae	2	2	1:1
Matsucoccidae	1	1	1:1
Monophlebidae	2	2	1:1
Steingeliidae	1	1	1:1
Xylococcidae	1	1	1:1
Ortheziidae	5	5	1:1
Pseudococcidae	27	50	1.8:1
Putoidae	1	1	1:1
Rhizoecidae	2	4	2.0:1
All scale families	98	185	1.9:1

Of the 185 species present in Poland, 133 (71.9%) are native (Figure 1). The alien scale insect species number 52 (28.1% of total); these species clearly dominate over the native ones in the Diaspididae family, while the remaining families are represented by 1–10 alien species or only by native species (Figure 1). Of the 52 alien species known in Poland, 47 can develop only indoors, while five, namely *Aulacaspis rosae*, *Comstockaspis perniciosa*, *Parthenolecanium fletcheri*, *Pulvinaria floccifera*, and *P. hydrangeae*, overwinter and develop outdoors. *Pulvinaria floccifera* develops both indoors and outdoors (Table 1).

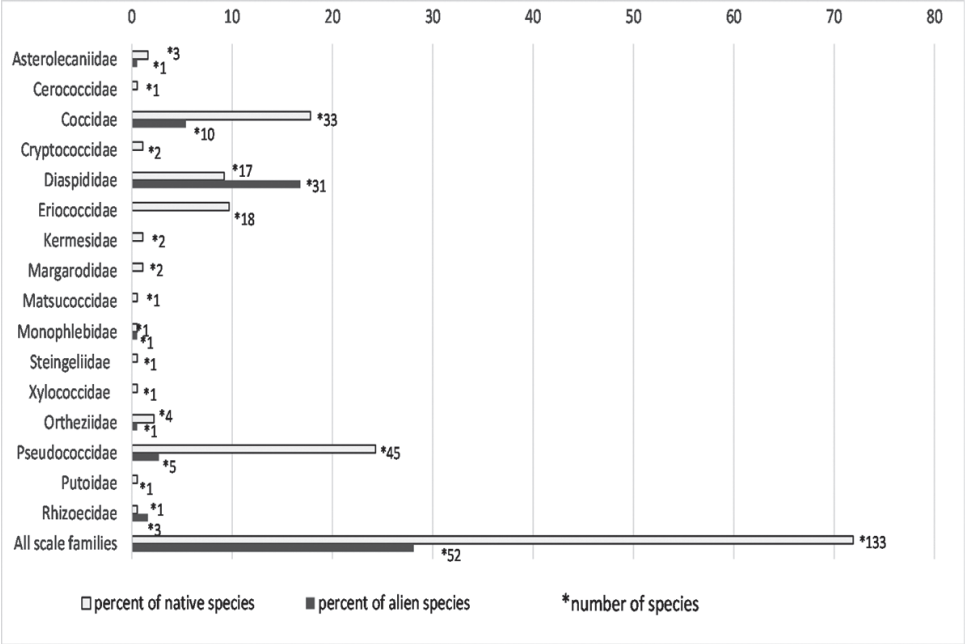

Figure 1. Native and alien scale insect species in different families in Poland.

Discussion

The scale insect species recorded in Poland represent only ca. 7.3% of the 2536 species known in the Palearctic region (García Morales et al. 2016) and ca. 41.1% of the 450 species reported in Europe (Pellizzari and Germain 2010). The previous checklist of scale insects of Poland was published 15 years ago (Łagowska 2004) and listed 185 species distributed in nine families and 94 genera. In the present list, the families Cryptococcidae, Matsucoccidae, Monophlebidae, Steingeliidae, Xylococcidae, Putoidae, and Rhizoecidae have been added, using the currently accepted classification of Coccomorpha. Moreover, eleven species new for the country have been added and ten species removed. The new entries are: *Asterodiaspis quercicola*, *Pulvinaria hydrangeae*, *P. regalis*, *Aspidiotus palmarum*, *Aulacaspis yasumatsui*, *Lepidosaphes tokionis*, *Acanthococcus macedoniensis*, *Icerya purchasi*, *Spilococcus mamillariae*, *Volvicoccus volvifer*, and *Rhizoecus americanus*. Of these *V. volvifer*, *P. hydrangeae*, *P. regalis*, and *A. macedoniensis* are established outdoors (Kalandyk and Węgierek 2010; Kozár et al. 2013; Łagowska et al. 2018), whereas *A. yasumatsui*, *L. tokionis*, *S. mamillariae*, and *R. americanus*, are indoors species (Łabanowski 2009). *Icerya purchasi* and *A. palmarum* were overlooked in the previous checklist and are therefore added to the present one. *Asterodiaspis quercicola* is here considered as a valid species (García Morales et al. 2016), despite the fact that Podsiadło (1990) and Stumpf and Lambdin (2006) considered *A. quercicola* and *A. variolosa* as synonyms.

Of the ten species removed from the list, six have been synonymized with other species (*Eulecanium slavum* (Kawecki, 1961), *Lepidosaphes oleae* Leonardi, 1908, *Heliococcus danzigae* Bazarov, 1974, *Trionymus isfarensis* Borchsenius, 1949,

T. singularis Schmutterer, 1952, and *Phenacoccus evelinae* Tereznikova, 1975). The presence in Poland of the other four species removed from the list, *Ripersia corynephori* Signoret, 1875, *Carulaspis visci* (Schrank, 1781), *Fiorinia fioriniae* (Targioni-Tozzetti, 1867) and *Oceanaspidiotus spinosus* (Comstock, 1883) is here considered as doubtful or erroneous. Kiritchenko (1940) listed *R. corynephori* as found near Warsaw, but Kawecki (1985) assumed that this record was incorrect and the species might be a misidentification of *Pseudococcus parvus* Borchsenius, 1949 (now *Mirococcopsis subterranea* (Newstead, 1893)). Similarly, the record of *C. visci* was very likely incorrect and the species may be a misidentification of *Carulaspis juniperi* (Bouchè, 1851). In addition, the records of *F. fioriniae* and *O. spinosus* are questionable because no host plants or localities were given in the list published by Czyżewski (1937). All the species mentioned above have not been collected again since they were first recorded.

Based on the distribution data reported by Łagowska (2001), the native species currently known from Poland are all Palearctic. Almost half of them are widely distributed in this region, and relatively few are known only from three or four countries. This latter group includes the following species: *Anophococcus confusus*, *Rhizococcus cantium*, *Boreococcus ingricus*, *Mirococcus festucae*, and *A. macedoniensis.* Two species, *Parthenolecanium smreczynskii* and *Kiritshenkella lianae*, are known so far only from Poland and are possibly endemic.

Recently, much attention has been paid to the alien species of scale insects that have been introduced or have spread into Poland. This group includes 47 indoor and five outdoor species. One species (*P. floccifera*) has been recorded on ornamental plants in greenhouses in Poland as well as on outdoor ornamentals, mostly on *Ilex* sp., and appears to be established (Łagowska et al. 2017). Of the 47 species established indoors, 29 (61.7%) belong to the Diaspididae. This high proportion of species from Diaspididae family introduced into Poland is similar to the results presented by Pellizzari and Germain (2010) for Europe. According to these authors, the 60 alien species belonging to the Diaspididae account for nearly half (44.6%) of the 130 alien species estimated to occur in Europe. Of the five alien species established outdoors in Poland, only *C. perniciosa* and *P. floccifera* are considered as invasive (Łagowska et al. 2017, 2018). Currently, *C. perniciosa* poses the greatest threat, affecting a number of fruit trees in Poland (Golan et al. 2017).

Some differences in the species richness were found between the data reported in the databases ScaleNet and FaEu and the present checklist. Scale insects that are erroneously recorded as present in Poland in the above recorded databases are discussed below:

Antecerococcus cistarum (Balachowsky, 1927), *A. laniger* (Goux, 1932), and *A. pocilliferus* (Neves, 1954)

The presence of these three species in Poland, cited by the ScaleNet database, is a misunderstanding of the text of Koteja (1984), which records them as present in several European countries (Portugal, France, Algeria, Cyprus) but not in Poland. These three

species were not listed by any of the researchers studying the fauna of scale insects in Poland, so they are excluded from the present list. The same species have also been incorrectly recorded in the FaEu database.

Asterodiaspis minor (Russell, 1941)

This species was recorded by Russell (1941) in Poland, but Podsiadło (1975) recognized only *Asterodiaspis quercicola* and *A. variolosa* in Poland based on extensive morphological studies. Since this time, *A. minor* was not listed in the subsequent publications pertaining to the fauna of scale insects in Poland and is excluded from the present list, although it is listed in the ScaleNet database.

Epidiaspis leperii (Signoret, 1869)

In the ScaleNet database Poland is included among the locations of distribution of this species based on the paper of Danzig and Pellizzari (1998). However, the paper does not provide any precise indication of its presence in Poland. The species was also not listed later by the researchers who studied the fauna of scale insects in Poland, so it is excluded from the present list.

Kermes bacciformis Leonardi, 1908 and *Kermes ilicis* (Linnaeus, 1758)

These two species are recorded as hosts of a parasitoid by Sugonyaev (1965), and are cited by the ScaleNet database as records of distribution of scale insects, but this is probably a misunderstanding of the text, as the distribution records concern the parasitoid species and not the scale insects. These two species have also been incorrectly included in the FaEu database.

Lecanopsis turcica (Bodenheimer, 1951)

Poland was included among the countries in which this species is distributed in the FaEu database, but the source of information is missing. As there is no published evidence for the presence of this coccid in Poland, it is excluded from the present list.

Leucaspis pusilla Löw, 1883

This species is erroneously recorded in the ScaleNet catalogue citing Danzig and Pellizzari (1998), but those authors do not mention Poland as a location of its distribution.

Matsucoccus matsumurae (Kuwana, 1905)

Poland was included among the countries where this species is present in the FaEu database, but the source of information is missing. As there is no evidence for the presence of this species in Poland, it is excluded from the present list.

Parlatoria oleae (Colvee, 1880) and *Parlatoria theae* Cockerell, 1896

These two species are recorded as present in Poland in the FaEu database with an incorrect citation of ScaleNet as the source of information. We have been unable to trace the original sources of publication concerning the presence of these species in Poland and therefore we consider these records erroneous.

Parlatoria ziziphi (Lucas, 1853)

This species is cited in ScaleNet based on Komosińska (1964). However, this species was only found on citrus fruits imported to Poland. Since *P. ziziphi* was not mentioned in subsequent papers, we assume that this species is not established in Poland.

Pseudococcus viburni (Signoret, 1875)

Poland was included among the countries in which this species is present in the FaEu database, but the source of information is missing. As there is no evidence of the presence of this mealybug in Poland, this species is excluded from the present list.

Trionymus levis (Tang, 1992)

Koteja (1974) and Koteja and Żak-Ogaza (1983) do not provide evidence of the presence of *T. levis* in Poland as cited by the ScaleNet database. Moreover, this species was not listed in the subsequent publications related to the Polish fauna of scale insects and is therefore removed from the present checklist. It has also been incorrectly included in the FaEu database.

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RESEARCH ARTICLE

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New species and new records of Trigonalyidae (Hymenoptera) from Tibet, China

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Abstract

Two new species of Trigonalyidae are described from Tibet (SW China): *Jezonogonalos nyingchiensis* Chen & van Achterberg, **sp. nov.**, and *Taeniogonalos eurysoma* Chen & van Achterberg, **sp. nov.** In total, seven species representing four genera are known from Tibet, and two of them are newly recorded from Tibet: *Taeniogonalos bucarinata* Chen, van Achterberg, He & Xu, 2014, and *Teranishia crenulata* Chen, van Achterberg, He & Xu, 2014.

Keywords

Hyperparasitoid, *Jezonogonalos*, *Orthogonalys*, parasitoid, *Taeniogonalos*, *Teranishia*, trigonalid wasp

Introduction

Trigonalyidae is a small family of Hymenoptera in its own superfamily Trigonalyoidea, with approximately 120 recognized species in 16 genera worldwide (Carmean and Kimsey 1998; Smith and Stocks 2005; Santos et al. 2012; Smith and Tripotin 2012; Smith et al. 2012; Chen et al. 2014; Yamane 2014; Smith and Tripotin 2015; Tan et al. 2017; Lelej 2019). The family name Trigonalidae and Trigonlyidae have been used by different authors, but we follow Weinstein and Austin (1991) and Lelej (2003) in

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using the family name as corrected by Krieger (1894) to Trigonalyidae; for the argumentation see Lelej (2003), and Engel and Lelej (2020).

The biology of trigonalid wasps is peculiar. Rather than laying their eggs directly on or in their host, females of these wasps lay thousands of minute eggs on foliage, which must be eventually consumed by caterpillars or sawfly larvae. Once inside the caterpillars or sawfly larvae, the wasp egg either hatches and attacks any other parasitoid larvae (wasps: Ichneumonidae or Braconidae; flies: Tachinidae) or it waits until the caterpillars or sawfly larvae are fed to a Vespidae larva, which it then attacks. Therefore, these wasps are hyperparasitoids or primary parasitoids, but extremely unusual among hymenopterans (Carmean and Kimsey 1998; Murphy et al. 2009).

The greatest diversity of this family occurs in tropical and subtropical regions. In fact, the family seems to be absent from arctic and alpine habitats (Carmean and Kimsey 1998), though they were found to be fairly common at 1300–1500 m altitude in the Qinling Mountains of NW China (Tan et al. 2017). Here we describe two new species and record two described species from the mountainous province of Tibet.

Material and methods

This work is based upon specimens in the following collections, with abbreviations used in the text: **SYSBM**, Sun Yat-sen University, The Museum of Biology, Guangzhou, China; **ZJUH**, Institute of Insect Sciences, Zhejiang University, Hangzhou, China. Morphological terminology generally follows Chen et al. (2014). Images and measurements were made using a Nikon SMZ25 microscope with a Nikon DS-Ri 2 digital camera system. Images were post-processed with Adobe Photoshop CS6 Extended. YPT stands for collected in yellow pan trap.

Taxonomy

Jezonogonalos Tsuneki, 1991

Figs 1–22

Jezonogonalos Tsuneki, 1991: 32, 2003: 4; Carmean and Kimsey 1998: 70; Chen et al. 2014: 22–44 (diagnosis, key). Type species: *Jezonogonalos marujamanae* Tsuneki, 1991 [= *J. marujamae* Tsuneki, 1991], by monotypy. Synonymized with *Pseudogonalos* Schulz, 1906, by Lelej (1995) and re-instated by Chen et al. (2014).

Diagnosis. Antenna black and with 23–27 segments; area above supra-antennal elevations flat, more or less punctate, without protuberance between elevations and inner side of supra-antennal elevations flat, smooth and black; tyloids of male antenna present on 10th–16th segments, short and nearly circular or elliptical; occipital carina widened medio-dorsally; apical segment of labial palp widened and obtuse, more or less triangu-

lar; vertex normal, at most with slight median depression dorsally; mandibles wide in anterior view and sublaterally attached to head; metanotum strongly convex and finely sculptured medially; anterior propodeal sulcus crenulate and medially widened; posterior propodeal carina curved and distinctly protruding and more or less separated from foramen medio-dorsally; fore wing with large dark patch below pterostigma; vein 1-SR of fore wing long; hind trochanter black or ivory; hind tarsus slightly or not modified; second and third sternites of female flat and moderately sclerotized and no protuberances; body without pale pattern, at most malar space and margins of basal metasomal sternites and tergites narrowly ivory, remainder black (Chen et al. 2014).

Biology. Unknown. Collected in June–November.

Distribution. China, Japan. Before this study, eight species of this genus had been described from China, with only one species recorded from Tibet. We describe here another species new to science from Tibet.

Jezonogonalos jiangliae Chen, van Achterberg, He & Xu, 2014
Figs 1–11

Jezonogonalos jiangliae Chen et al., 2014: 29–32 (diagnosis, description, distribution).

Material examined. 1 male, China: Tibet, Nyingchi, Yigong, 225 6m, 30°10'53"N, 94°54'30"E, 3.viii.2018, sweep net, SCAU 3040486 (SYSBM); 3 males, China: Tibet, Nyingchi, Yigong, Tongjiacun, 2214 m, 30°14'12"N, 94°53'48"E, 6.viii.2018, YPT, SCAU 3040489, SCAU 3040187, SCAU 3040188 (SYSBM).

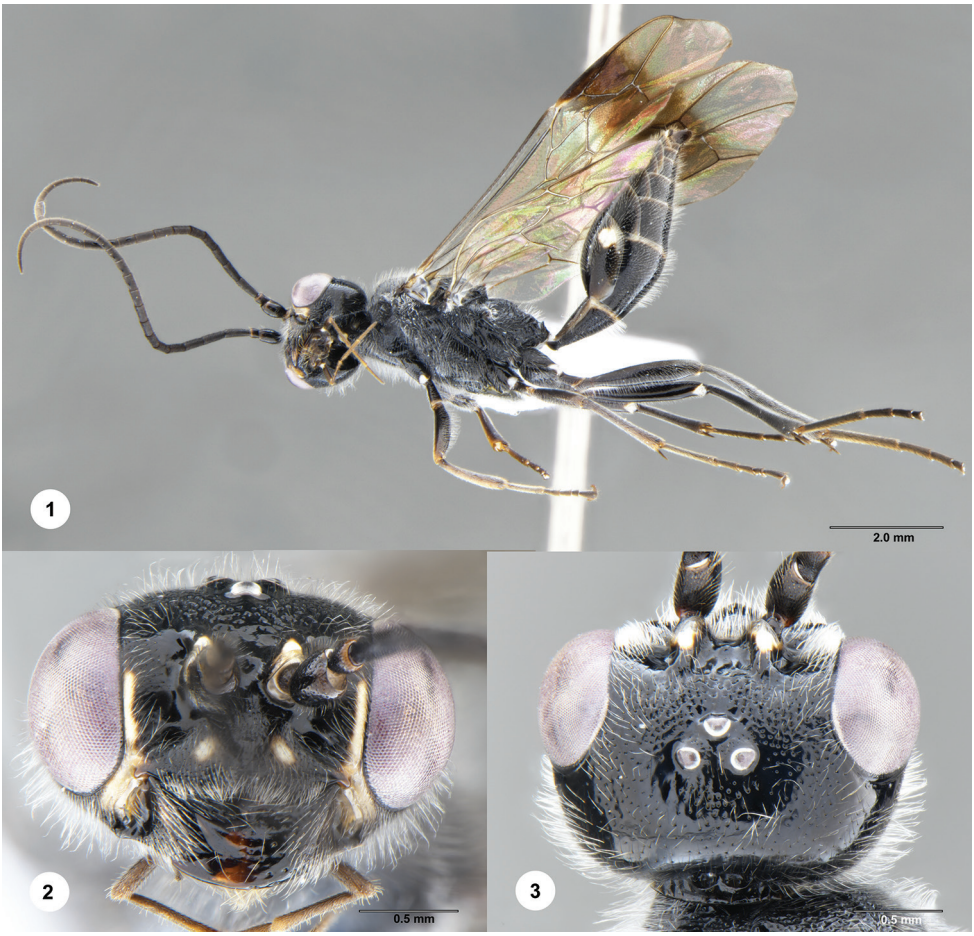
Distribution. China (Tibet). Collected at 2214–2256 m.

Notes. *Jezonogonalos jiangliae* was first described by Chen et al. (2014) based on a single male without complete antennae from Tibet. Based on the additional material, this species shows the following variations: male antenna with 25 or 26 segments, with tyloids present on 10th–15th or 11th–16th segments; clypeus usually entirely black, but sometimes partly ivory; second tergite sometimes with ivory spots latero-posteriorly. The female of this species is still unknown. As Chen et al. (2014) suggested, collection at the type locality and the use of COI ("barcoding") will recover the conspecific female.

Jezonogonalos nyingchiensis Chen & van Achterberg, sp. nov.
http://zoobank.org/C47806BA-246D-4131-B4F4-8B632009CD26
Figs 12–22

Material examined. ***Holotype***, female, China: Tibet, Nyingchi, Yigong, 2256 m, 30°10'53"N, 94°54'30"E, 3.viii.2018, sweep net, SCAU 3040487 (deposited in SYSBM). ***Paratypes:*** 2 females, same data as holotype.

Diagnosis. Occipital carina very wide medio-dorsally, with pair of curved lamellae separated by a carina (Fig. 14); outer side of supra-antennal elevations subvertical,



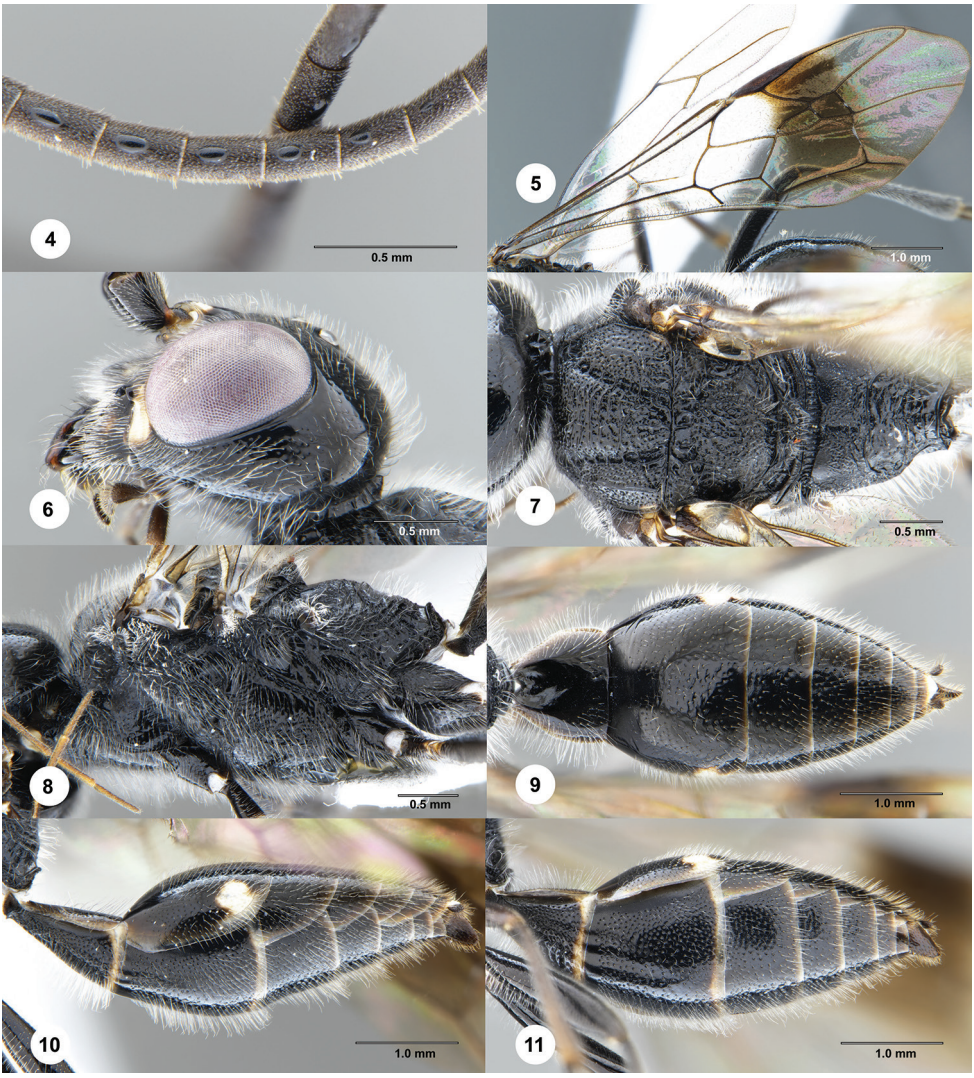
Figures 1–3. *Jezonogonalos jiangliae* Chen, van Achterberg, He & Xu**,** male (SCAU 3040188). **1** Habitus, lateral aspect **2** head, anterior aspect **3** head, dorsal aspect.

smooth, and elevations approximately 0.6 × as long as scapus (Fig. 14); frons densely punctate dorsally and laterally, largely smooth ventrally and medially (Fig. 13); supra-antennal elevations largely ivory dorsally (Fig. 14); mandible mainly black, except dark brown base of teeth (Fig 13); metasoma dorsally largely smooth and largely black (Fig. 20); first tergite approximately 0.7 × as long as its apical width (Fig. 20); third sternite approximately 0.4 × as long as second sternite (Fig. 22).

Comments. This species is similar to *J. shaanxiensis* from Shaanxi (NW China) and it would run to that taxon (couplet 7) in the key of Tan et al. (2017), but can be distinguished by having the frons largely smooth medially and the mesopleuron mainly punctate-rugose, but narrowly smooth posteriorly.

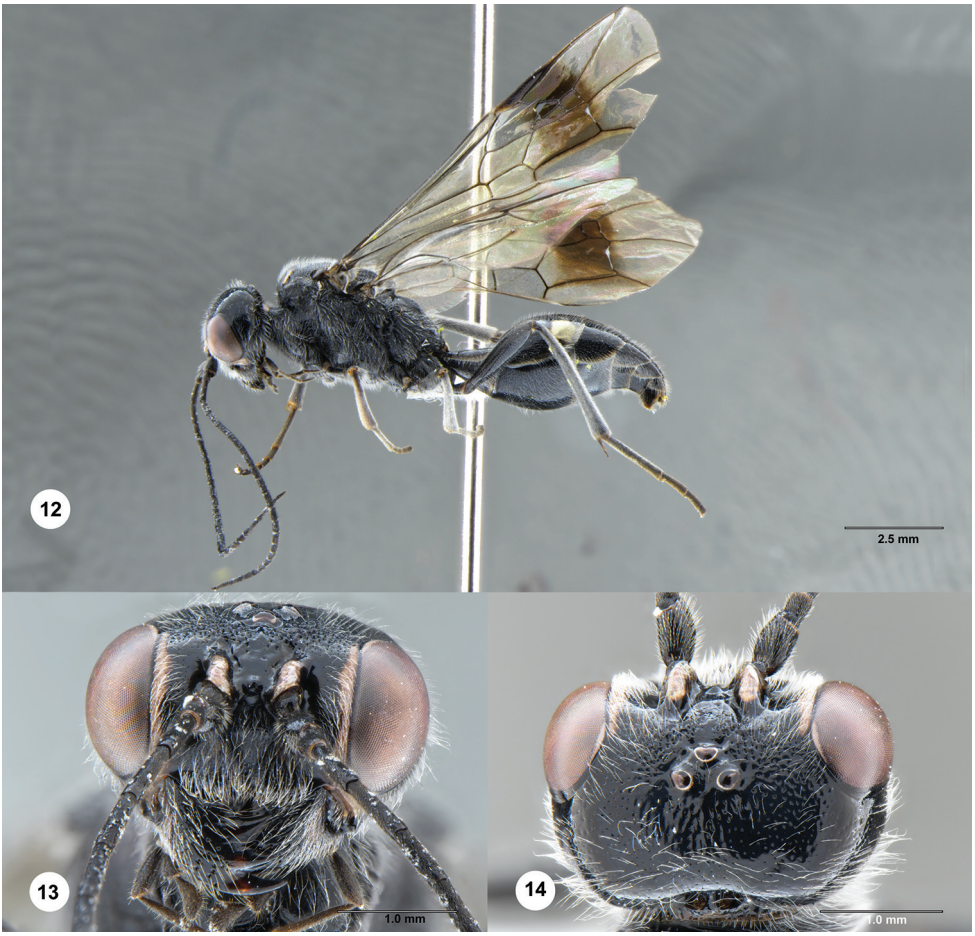
Description. Holotype, ♀, length of body 11.8 mm (of fore wing 9.8 mm).

Head. Antenna with 25 segments; frons densely punctate dorsally and laterally, with medium-sized whitish setae, largely smooth ventrally and medially (Fig. 13); ver-



Figures 4–11. *Jezonogonalos jiangliae* Chen, van Achterberg, He & Xu**,** male (SCAU 3040188). **4** Tyloids on 11th–15th segments of antenna **5** wings **6** head, lateral aspect **7** mesosoma, dorsal aspect **8** mesosoma, lateral aspect **9** metasoma, dorsal aspect **10** metasoma, lateral aspect **11** metasoma, latero-ventral aspect.

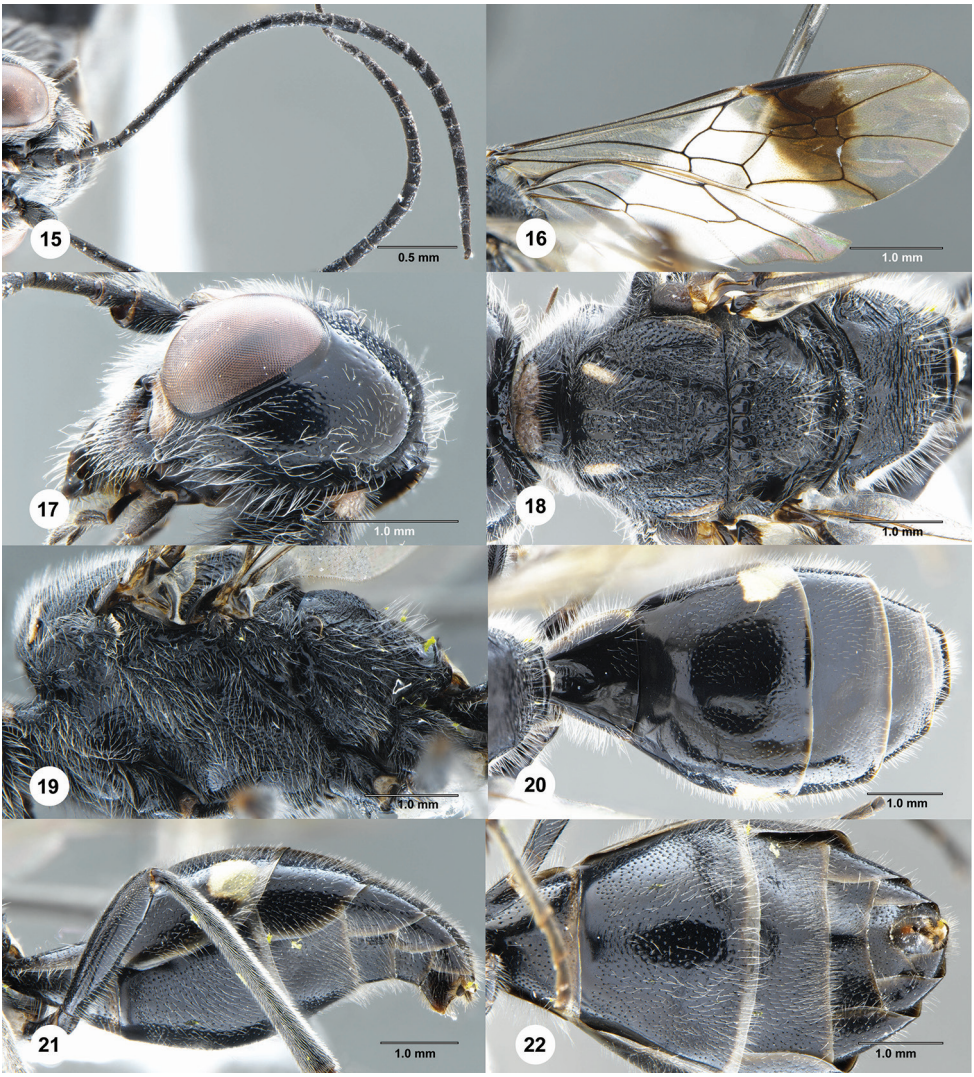
tex largely smooth, moderately punctulate behind posterior ocellus (Fig. 14); temple largely smooth, punctulate (Fig. 17); head hardly narrowed behind eyes, eye in dorsal view 1.1 × as long as temple (Fig. 14); occipital carina strongly widened and pair of circular lamellae medio-dorsally, separated by a distinct carina (Figs 14, 17), laterally distinctly crenulate; supra-antennal elevations distinctly enlarged (approximately 0.6 × as long as scapus), smooth and outer side subvertical; clypeus concave and thick medio-ventrally and area above it convex and obtusely protruding (Fig. 13).



Figures 12–14. *Jezonogonalos nyingchiensis* Chen & van Achterberg, sp. nov., holotype, female (SCAU 3040487). **12** Habitus, lateral aspect **13** head, anterior aspect **14** head, dorsal aspect.

Mesosoma. Mesosoma 1.7 × as long as its dorsoventral height (Fig. 19); mesopleuron mainly punctate-rugose, narrowly smooth posteriorly; notauli wide, deep and coarsely crenulate; middle lobe of mesoscutum smooth antero-medially, otherwise transversely punctate-rugose, lateral lobes mainly punctate except for a smooth line (Fig. 18); scutellar sulcus wide and coarsely crenulate; scutellum coarsely punctate and laterally with smooth spaces between longitudinal rugae, flattened, in lateral view below level of metanotum (Fig. 18); metanotum medially protruding, obtuse and densely and finely punctate (Fig. 18); propodeum antero-laterally irregularly rugulose to smooth, remainder coarsely transversely rugose and shiny medially, and smooth posteriorly (Fig. 18); posterior propodeal carina thick lamelliform (foramen approximately 4 × as wide as high medially).

Wings. Fore wing: length of vein 1-M 1.4 × as long as vein 1-SR; third submarginal cell much wider anteriorly than petiolate second cell (Fig. 16).



Figures 15–22. *Jezonogonalos nyingchiensis* Chen & van Achterberg, sp. nov., holotype, female (SCAU 3040487). **15** Antenna **16** wings **17** head, lateral aspect **18** mesosoma, dorsal aspect **19** mesosoma, lateral aspect **20** metasoma, dorsal aspect **21** metasoma, lateral aspect **22** metasoma, ventral aspect.

Metasoma. First tergite 0.7 × as long as its apical width, smooth but basal depression anteriorly with some crenulae (Fig. 20); second and following tergites shiny and smooth except for punctulation; sternites rather sparsely finely punctate, with wide smooth interspaces; second sternite weakly curved in lateral view; third sternite approximately 0.4 × as long as second sternite (Fig. 22); hypopygium triangularly protruding in ventral view (Fig. 21).

Colour. Black; inner orbita narrowly ivory and connected to ivory malar space; pair of faint patches on clypeus, basal patch of mandible, large patch on supra-antennal

elevations, large patch on anterior margin of pronotum, pair of elongate patches on middle lobe of mesoscutum anteriorly, pair of narrow lines near tegulae, epipleura of tergites, large patch apico-laterally on second tergite and narrow apical bands of sternites ivory; mandible teeth dark brown basally (Fig. 13); tegulae mainly dark brown; palpi dark brown; legs mainly black, but fore femur apico-ventrally brownish; pterostigma basally yellow, and remainder dark brown; large area below pterostigma dark brown and remainder of wing membrane subhyaline (Fig. 18).

Variations. Length of body 10.8–11.2 mm, of fore wing 8.9–9.4 mm; metanotum black or with pair of faint ivory spots medially; ivory patches of clypeus and mesoscutum rather small to large; length of vein 1-M of fore wing 1.3–1.5 × as long as vein 1-SR.

Male. Unknown.

Biology. Unknown.

Distribution. China (Tibet). Collected at 2256 m.

Etymology. Named after Nyingchi County, where it was collected. Treat as an adjective in apposition.

Orthogonalys Schulz, 1905

Orthogonalys Schulz, 1905: 76; Weinstein and Austin 1991: 421; Carmean and Kimsey 1998: 52; Smith and Tripotin 2012: 3; Chen et al. 2014: 60–87 (synonymy, diagnosis, key to Chinese species). Type species: *Orthogonalys boliviana* Schulz, 1905, by monotypy.

Diagnosis. Antenna with 21–32 segments, often with a pale band in apical third of antenna and slender medially; male antenna without tyloids; supra-antennal elevations smooth and shiny, usually comparatively large, without depression dorsally and moderately to widely separated; vertex normal, at most with slight median depression dorsally; apical segment of labial palp widened and obtuse, more or less triangular; mandibles wide in anterior view and sublaterally attached to head; occipital carina usually narrow and smooth; mesoscutum and scutellum often smooth or sparsely punctulate, at most moderately punctate with wide smooth interspaces; metanotum concave latero-dorsally and often sculptured, matt and distinctly convex medially; anterior propodeal sulcus distinctly crenulate, rarely partly reduced; posterior propodeal carina curved and lamelliform; vein 1-SR of fore wing medium-sized to long; fore wing subhyaline, at most slightly infuscate below pterostigma in female; triangular dorso-apical part of hind trochanter separated by an oblique groove; fore trochanter subparallel-sided and distinctly longer than hind trochanter; hind tarsus slightly or not modified; second metasomal sternite and tergite flat in lateral view, weakly sclerotized and smooth; second sternite in ventral view flat medially or weakly convex and no medial elevation or teeth posteriorly; basal half of third sternite flat, without a distinct ledge anteriorly; fifth sternite of female straight or slightly emar-

ginate medio-posteriorly; body often slender (including metasoma) and sometimes ichneumonid-like (Chen et al. 2014).

Biology. Reared as hyperparasitoid of Tachinidae in caterpillars of the family Limacodidae (Carmean and Kimsey 1998; Murphy et al. 2009). Collected in May–August.

Distribution. Mainly East Palaearctic and Northeast Oriental regions, with a few species in East Afrotropical (including Madagascar), Neotropical and Nearctic regions. Chen et al. (2014) and Tan et al. (2017) reported eight species of *Orthogonalys* from China, with only one species from Tibet.

Orthogonalys elongata (Teranishi, 1929)

Orthogonalos elongata Teranishi, 1929: 146; Marshakov 1981: 105; Tsuneki 1991: 20; Weinstein and Austin 1991: 424.
Satogonalos elongata; Weinstein and Austin 1991: 424.
Orthogonalys elongata; Carmean and Kimsey 1998: 54; Bennett and Lelej 2003: 8; Chen et al. 2014: 62, 72–80 (key, synonymy, diagnosis, description, distribution); Tan et al. 2017: 37, 39 (key, distribution).

Material examined. 4 females, China: Tibet, Motuo, 13.vii.2013, Zhen Liu, 201300022–201300025 (ZJUH).

Distribution. China (Henan, Shaanxi, Sichuan, Tibet); Russia (South Sakhalin, South Kurils); Japan (Hokkaido, Honshu).

Taeniogonalos Schulz, 1906

Figs 23–33

Taeniogonalos Schulz, 1906: 212; Weinstein and Austin 1991: 416; Tsuneki 1991: 59; Carmean and Kimsey 1998: 65; Chen et al. 2014: 95–193 (synonymy, references, diagnosis, key to Chinese species). Type species: *Trigonalys maculata* Smith, 1851, by monotypy.

Diagnosis. Antenna with 21–26 segments, without pale band and slender medially; male antenna with linear tyloids (= elevated elongate areas) on 11th–16th antennal segments; supra-antennal elevations smooth or punctate, without depression dorsally, remain far separated from each other medially and without horizontal "shelf" between antennal bases; temple usually punctate or reticulate-punctate and moderately shiny; occipital carina ending at hypostomal carina at level of mandibular base; vertex flattened, without median depression dorsally; apical segment of labial palp widened and obtuse, more or less triangular; mandibles wide in anterior view and sublaterally attached to head; mesoscutum and scutellum distinctly punctate or rugose; metanotum at least partly convex latero-dorsally and often sculptured; vein 1-SR of fore wing me-

dium-sized to long; fore wing often with subapical dark patch or large part of fore wing dark brown; triangular dorso-apical part of hind trochanter separated by an oblique groove; fore trochanter subparallel-sided and distinctly longer than hind trochanter; hind tarsus slightly or not modified; propodeal foramen more or less arched dorsally and often with a lamelliform carina; second sternite convex in lateral view (but less so in males), strongly sclerotized and frequently densely punctate, sometimes with a medio-posterior elevation but without pair of small teeth; basal half of third sternite flat, without a distinct ledge anteriorly; hypopygium of female pointing anteriorly toward second sternite or straight down or pointing posteriad (Chen et al. 2014).

Biology. Reared as hyperparasitoid of parasitoid wasps (Ichneumonidae and Braconidae) and parasitoid flies (Tachinidae) in caterpillars, but some species are primary parasitoids of pergid sawflies in Australia (Raff 1934; Carne 1969; He and Chen 1986; Weinstein and Austin 1995; Carmean and Kimsey 1998). Collected mainly in April–October, rarely in November or January.

Distribution. This genus occurs in all major regions, but is unknown from Europe and western Nearctic region. Most of the species occur in the East Palaearctic, Northeast Oriental, and Neotropical regions (Carmean and Kimsey 1998). Chen et al. (2014) reported two species (*Taeniogonalos formosana* (Bischoff 1913) and *T. taihorina* (Bischoff 1914)) from Tibet. Here we describe a third species new to science and report a fourth species from this region.

Taeniogonalos bucarinata Chen, van Achterberg, He & Xu, 2014

Taeniogonalos bucarinata Chen, van Achterberg, He & Xu, 2014: 108–113 (description, diagnosis, distribution); Tan et al. 2017: 52–53 (distribution).

Material examined. 1 male, China: Tibet, Yadong County, Renqinggang Village, 3083 m, 18.vii.2013, Zhen Liu, 201300035 (ZJUH); 1 male, China: Tibet, Nielamu County, 26.vii.2013, Zhen Liu, 201300123 (ZJUH).

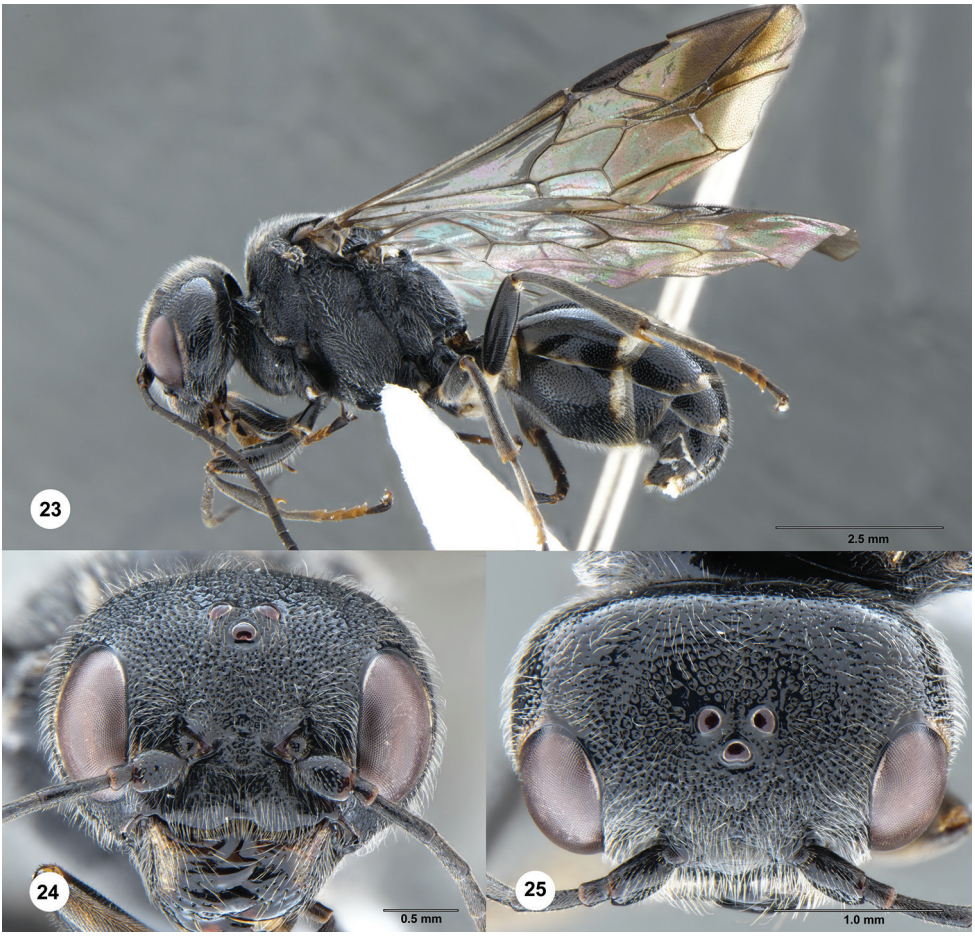
Distribution. China (Fujian, Gansu, Henan, Ningxia, Shaanxi, Sichuan, Tibet, Yunnan, Zhejiang). Collected at 1200–3083 m.

Note. This species is newly recorded from Tibet.

Taeniogonalos eurysoma Chen & van Achterberg, sp. nov.

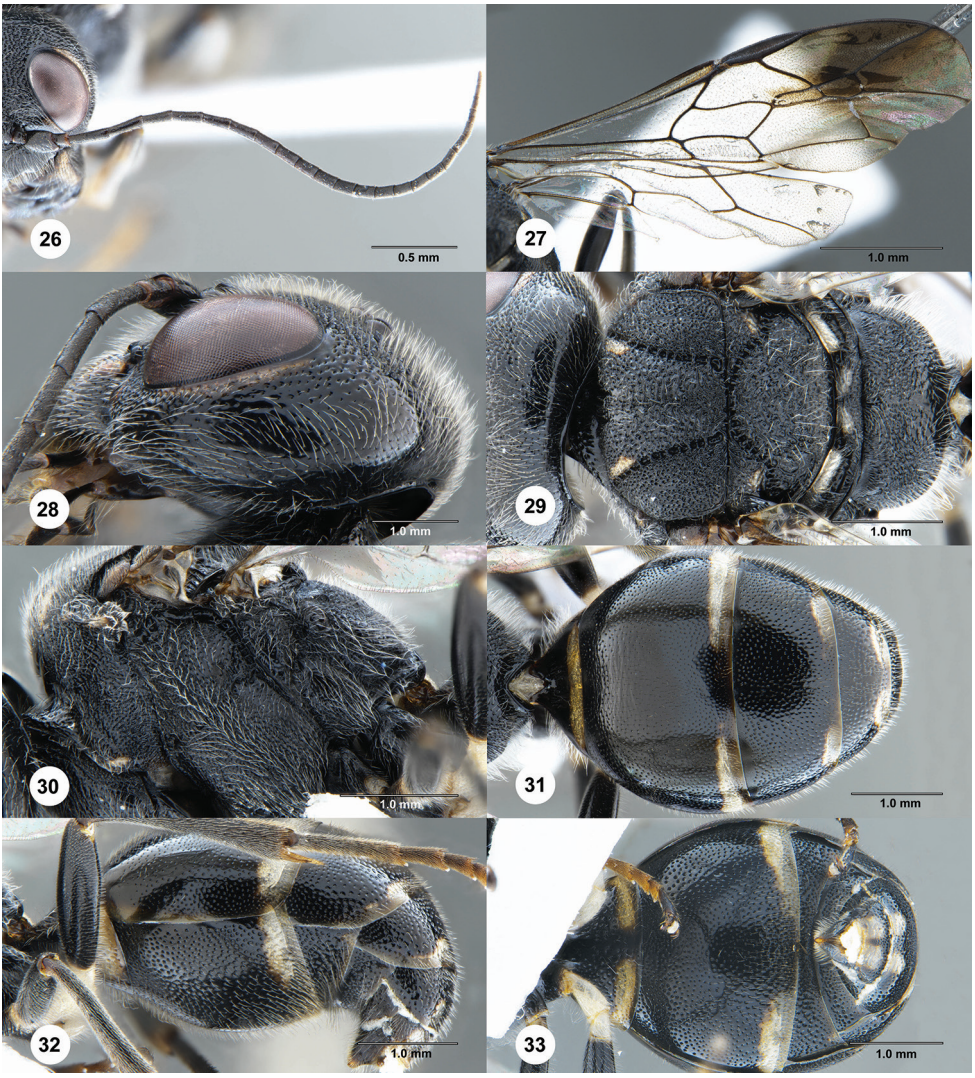
http://zoobank.org/8806BD55-3128-4E08-942D-44F6FD92D04A
Figs 23–33

Material examined. ***Holotype***, female, China: Tibet, Nyingchi, Yigong, 2268 m 30°15'10"N, 94°48'24"E, 5.viii.2018, sweep, SCAU 3040488 (deposited in SYSBM). ***Paratype:*** 1 female China: Tibet, Nyingchi, Yigong, 2256 m, 30°10'53"N, 94°54'30"E, 3.viii.2018, sweep net (SYSBM).



Figures 23–25. *Taeniogonalos eurysoma* Chen & van Achterberg, sp. nov., holotype, female (SCAU 3040488). **23** Habitus, lateral aspect **24** head, anterior aspect **25** head, dorsal aspect.

Diagnosis. Supra-antennal elevations medium-sized (approximately 0.2 × as long as scapus) and their outer side oblique (Fig. 25); occipital carina narrow, non-lamelliform, smooth (Fig. 25); head anteriorly and posteriorly, and pronotum laterally entirely black (Figs 24, 25, 28); vertex reticulate-punctate behind stemmaticum and near eyes, becoming spaced punctate (with interspaces much wider than width of punctures) posteriorly (Fig. 25); mesoscutum coarsely sculptured (Fig. 29); notauli wide and crenulate (Fig. 29); scutellum coarsely rugose, convex laterally and shallowly concave medially (Fig. 29); metanotum slightly convex, rugose (Fig. 29); posterior propodeal carina distinctly arched, narrow lamelliform, foramen comparatively narrow (Fig. 29); posterior margin of tergites, 1st and 2nd sternites with ivory stripes (Figs 31–33); second sternite slightly convex (Fig. 32); third sternite without depression (Fig. 33).



Figures 26–33. *Taeniogonalos eurysoma* Chen & van Achterberg, sp. nov., holotype, female (SCAU 3040488). **26** Antenna **27** wings **28** head, lateral aspect **29** mesosoma, dorsal aspect **30** mesosoma, lateral aspect **31** metasoma, dorsal aspect **32** metasoma, lateral aspect **33** metasoma, ventral aspect.

Comments. This species is close to *T. alticola* and it would run to that taxon (couplet 17) in the revised key of Tan et al. (2017), but can be distinguished by having smaller supra-antennal elevations, notauli wide, deep and coarsely crenulate, and wide metasomal segments.

Description. Holotype, female, length of body 8.7 mm (of fore wing 7.8 mm).

Head. Antenna with 25 segments; frons reticulate-punctate (Fig. 24); vertex reticulate-punctate behind stemmaticum and near eyes, becoming spaced punctate (interspaces much wider than width of punctures) posteriorly (Fig. 25); temple largely

smooth with few punctures at orbita and densely punctate near mandible (Fig. 28); head gradually narrowed behind eyes, eye in dorsal view 0.9 × as long as temple (Fig. 25); occipital carina narrow, non-lamelliform, smooth (Fig. 25); supra-antennal elevations medium-sized (approximately 0.2 × as long as scapus) and their outer side oblique (Fig. 25); clypeus distinctly concave and thick medio-ventrally (Fig. 24).

Mesosoma. Mesosoma 1.5 × as long as its dorso-ventral height (Fig. 30); mesopleuron largely punctate-rugose, becoming densely punctate posteriorly; transverse mesopleural groove narrow, crenulate; notauli wide, deep and coarsely crenulate; middle lobe of mesoscutum smooth antero-medially, otherwise transversely punctate-rugose, lateral lobes densely punctate anteriorly, becoming punctate-rugose posteriorly (Fig. 29); scutellar sulcus complete, moderately narrow and crenulate; scutellum coarsely rugose, convex laterally and shallowly concave medially, anteriorly distinctly above level of mesoscutum; metanotum slightly convex, rugose (Fig. 29); propodeum largely punctate-rugose, becoming smooth posteriorly (Fig. 29); posterior propodeal carina distinctly arched, narrow lamelliform, foramen comparatively narrow (Fig. 29) and as high as wide basally.

Wings. Fore wing: vein 1-M 1.1 × as long as vein 1-SR (Fig. 27); second submarginal cell 1.3 × as long as third cell.

Metasoma. First tergite 0.4 × as long as apically wide, smooth and with shallow but wide depression medially (Fig. 31); second tergite largely smooth and shiny medially, moderately punctate laterally; following tergites moderately punctate (Fig. 31); second sternite slightly convex, densely punctate (Fig. 32); third sternite without depression, densely punctate; following sternites densely punctate.

Colour. Black; outer orbita with pale yellow stripes, inner orbita with small patches near malar space (Figs 13, 14); mandibles largely dark brown, with basal patches; pair of elongate patches on middle lobe of mesoscutum anteriorly, pair of patches on antero-lateral margin of scutellum, two pairs of transverse patches on metanotum; palpi, and tegulae dark brown; posterior margin of tergites, 1st and 2nd sternites with ivory stripes (Fig. 33); legs mainly black with tarsi dark brown; pterostigma nearly black; apical half of marginal cell of fore wing largely infuscate as area below it, remainder of wing membrane subhyaline (Fig. 27).

Male. Unknown.

Biology. Unknown.

Distribution. China (Tibet). Collected at 2256–2268 m.

Etymology. The specific epithet originates from Greek "eurys", wide, with reference to the wide terga of metasoma. Treat as a noun in apposition.

Taeniogonalos taihorina (Bischoff, 1914)

Nanogonalos taihorina Bischoff, 1914: 93; Tsuneki 1991: 58; Weinstein and Austin 1991: 421.

Taeniogonalos taihorina: Carmean and Kimsey 1998: 68; Chen et al. 2014: 171–179 (synonymy, diagnosis, distribution).

Poecilogonalos maga Teranishi, 1929: 148; Marshakov 1981: 106; Tsuneki 1991: 51; Weinstein and Austin 1991: 423; Lelej 1995: 14; Tan et al. 2017: 54–55 (synonymy, distribution)

Taeniogonalos maga: Chen et al. 2014: 146–150 (synonymy, diagnosis, distribution).

Taiwanogonalos claripennis Tsuneki, 1991: 38. Synonymized by Carmean and Kimsey 1998 with *T. maga*; Tan et al. 2017: 54–55 (synonymy, distribution)

Material examined. 1 male, China: Tibet, Motuo, 2954 m, 9.vii.2013, Zhen Liu, 201300003 (ZJUH); 2 males, China: Tibet, Bomi, 3083 m, 12.vii.2013, Zhen Liu, 201300014, 201300015 (ZJUH).

Distribution. China (Fujian, Gansu, Guangxi, Heilongjiang, Ningxia, Shaanxi, Sichuan, Taiwan, Tibet, Yunnan, Zhejiang). Japan (Hokaido, Honshu). Collected at 1200–3083 m.

Teranishia Tsuneki, 1991

Teranishia Tsuneki, 1991: 15–18; Lelej 1995: 12, 2003: 3; Carmean and Kimsey 1998: 73; Chen et al. 2014: 193–201 (diagnosis, key to species). Type species (by monotypy): *Teranishia nipponica* Tsuneki, 1991.

Diagnosis. Antenna black and with 24–27 segments; male antenna without tyoloids; area above supra-antennal elevations flat, more or less punctate, with protuberance between elevations and inner side of supra-antennal elevations flat, smooth and black; occipital carina widened medio-dorsally; apical segment of labial palp widened and obtuse, more or less triangular; vertex normal, at most with slight median depression; mandibles wide in anterior view and sublaterally attached to head; anterior propodeal sulcus distinctly crenulate; metanotum strongly convex and finely sculptured medially; anterior propodeal sulcus crenulate and medially widened; posterior propodeal carina curved and distinctly protruding and more or less separated from foramen medio-dorsally; fore wing with large dark patch below pterostigma; vein 1-SR of fore wing long; hind trochanter black, dark brown or ivory; hind tarsus slightly or not modified; second and third sternites of female flat and moderately sclerotized and no protuberances; body without pale pattern, at most malar space and margins of basal metasomal sternites and tergites narrowly ivory, remainder black (Chen et al. 2014).

Biology. Unknown. Collected in June–September.

Distribution. China, Japan. Chen et al. (2014) reported two species from China. *Teranishia crenulata* Chen, van Achterberg, He & Xu, is newly recorded from Tibet in this study.

Teranishia crenulata Chen, van Achterberg, He & Xu, 2014

Teranishia crenulata Chen et al., 2014: 194–197 (diagnosis, description).

Material examined. 3 males, China: Tibet, Motuo, 12.vii.2013, Zhen Liu, 201300011–201300013 (ZJUH).

Distribution. China (Gansu, Ningxia, Sichuan, Tibet). Collected at 1800–2539 m.

Acknowledgments

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RESEARCH ARTICLE

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Checklist of aquatic Diptera (Insecta) of Plitvice Lakes National Park, Croatia, a UNESCO world heritage site

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Abstract

Studies on aquatic Diptera in the Plitvice Lakes National Park (Croatia) conducted in the last 50 years have produced 157 species and 7 taxa of aquatic Diptera placed in 13 families. Samples were collected at 25 sampling sites representing the four main types of karst aquatic habitats: spring, stream, tufa barriers and lakes. All records of all the aquatic families of Diptera in Plitvice Lakes NP are summarized, including previously unpublished data. Twelve species new for Plitvice Lakes NP are recorded for the first time, belonging to the families: Chironomidae – *Labrundinia longipalpis* (Goetghebuer, 1921), *Nilothauma brayi* (Goetghebuer, 1921), *Potthastia longimanus* Kieffer, 1922, *Polypedilum* (*Polypedilum*) *nubeculosum* (Meigen, 1804), *Tanytarsus brundini* Lindeberg, 1963; Dixidae – *Dixella autumnalis* (Meigen, 1838); Scathophagidae – *Acanthocnema latipennis* Becker, 1894 and Stratiomyidae – *Oxycera pardalina* Meigen, 1822, *Oxycera limbata* Loew, 1862, *Oxycera turcica* Ustuner & Hasbenli, 2004, *Nemotelus pantherinus* (Linnaeus, 1758), *Oplodontha viridula* (Fabricius, 1775). The most species-rich family was the Chironomidae with 62 species (and an additional seven taxa), followed by the Empididae with 22 species and

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Limoniidae with 19 species. The highest number of species was recorded in springs. The relatively low number of species in certain families and the complete absence of some aquatic families shows that further research into the aquatic Diptera in Plitvice Lakes NP is needed.

Keywords
Barrage lake system, Chironomidae, Empididae, Limoniidae, new records, *Oxycera* spp.

Introduction

Most people probably know true flies (Diptera) mainly as a nuisance and as disease-carrying blood-sucking insects, but Diptera are also key players in the recycling of organic material in ecosystems, from the sewage of our urban communities to the leaf litter of the forest floor. In addition, Diptera provide other general ecosystem services such as pollination and pest control, but are also vectors of disease as terrestrial adults (Pape 2009; Marshall 2012; Adler and Courtney 2019).

More than any other group of macro-organisms, Diptera dominate the freshwater environment and are the most numerous group in terms of described species in freshwaters. Nearly one-third of all described fly species, roughly 46,000 species, have some connection with an aquatic environment during development process (Adler and Courtney 2019). Their abundance, omnipresence, and diversity of adaptations to the aquatic environment, position them as major drivers of ecosystem processes (Hölker et al. 2015). Fly larvae are well represented as ecosystem engineers and keystone species that alter the abiotic and biotic environments through activities such as burrowing, grazing, suspension feeding, and predation (Wotton et al. 1998; Adler and Courtney 2019). The enormous populations sometimes achieved by aquatic flies can provide the sole or major dietary component for other organisms, and as both predators and herbivores, they can serve as biological control agents (Collins and Blackwell 2000; Werner and Pont 2003; Adler and Courtney 2019). They serve as indicators of historical and future ecological and climate change while at the same time they have played a role as indicators of water quality from the earliest years of bioassessment (Walker 1987; Mihaljević et al. 1998; Larocque et al. 2001; Adler and Courtney 2019).

As holometabolous insects that undergo complete metamorphosis, all aquatic Diptera have a life cycle that includes a series of distinct stages or instars. A typical life cycle consists of a brief egg stage (usually a few days or weeks, but sometimes much longer), three or four larval instars (usually three in Brachycera, four in lower Diptera, and more in Simuliidae, Tabanidae, Thaumaleidae, some Chironomidae, and a few others), a pupal stage of varying length, and an adult stage lasting from less than two hours (Deuterophlebiidae) to several weeks or even months (Courtney et al. 2017; Lackmann and Butler 2018; Adler and Courtney 2019).

From all types of aquatic habitats, including tree holes to open oceans, and glacial meltwaters to hot springs, Diptera are the true conquerors of the aquatic environment. They have been found at elevations up to 5600 m in the Himalayas and at depths of

more than 1000 m in Lake Baikal. Furthermore, the presence of Diptera species in mainland Antarctica (e.g., *Belgica antarctica* Jacobs, 1900) makes them the only group of insects inhabiting all of the world's continents (Allegrucci et al. 2006; Ferrington et al. 2008; Adler and Courtney 2019). Aquatic Diptera are free-living insects that require a wet environment in at least one life stage (Adler and Courtney 2019) or, more strictly, aquatic Diptera are considered as those associated with water bodies (Courtney et al. 2017). Out of 158 dipteran families worldwide, 41 have aquatic representatives (Adler and Courtney 2019), and in Europe there are 130 dipteran families of which about 25 are related to aquatic habitats (Oosterbroek 2006).

Plitvice Lakes form the oldest National Park in the Balkan region and is probably one of the most famous National Parks in Europe because of its exquisite beauty. Plitvice Lakes NP was established as a National Park in 1949, and from 1979 Plitvice lakes NP has been a UNESCO world natural heritage site (Stilinović and Božičević 1998). Its importance is not only scientific, as a unique karstic phenomenon, but also as a place of huge economic importance for the local community as more than a million people per year come to visit it.

Materials and methods

Study site

Plitvice Lakes National Park (NP) is a 295 km^2 forest reserve located in the karst region of the Dinaric Mountains in Croatia. The Plitvice Lakes barrage lake system consists of 16 oligotrophic, dimictic and fluvial lakes divided by tufa barriers that form an approximately 8.2 km long barrage system. The lakes are characterised by a low organic solute concentration, supersaturation with calcium salts, pH > 8.0, and the presence of algae and mosses mediating tufa barriers formation (Srdoč et al. 1985; Stilinović and Božičević 1998). After the confluence of the Bijela rijeka and Crna rijeka Rivers, they form the Matica River which is the main surface-water supplier of the lakes (Stilinović and Božičević 1998). According to the Köppen climate classification, this area is influenced by temperate and boreal climates (Šegota and Filipčić 2003).

Specimen records

This paper is based on unpublished data from our own research and on published data gleaned from the literature. Each record was georeferenced using ArcGIS software. The names of taxa presented in this checklist reflect current nomenclature and classifications (Yang et al. 2007; Ashe and O'Connor 2009, 2012; Pape and Beuk 2012; Adler and Crosskey 2018; Ivković et al. 2019; Oosterbroek 2019; Starý 2019). Locality records are listed for each species. A list of locality names including latitude, longitude, altitude, and number code for each locality is given in Table 1, and a map with all sites

Table 1. Sampling sites in National Park Plitvice Lakes, Croatia.

Site Name	Site ID	Latitude / Longitude	Elevation (m)
Spring of Bijela rijeka, Plitvice Lakes NP	1	44°49'58"N, 15°33'25"E	720
Upper reach of Bijela rijeka, Plitvice Lakes NP	2	44°50'04"N, 15°33'33"E	715
Plitvički Ljeskovac, Plitvice Lakes NP	3	44°50'27"N, 15°35'40"E	668
Spring of Crna rijeka, Plitvice Lakes NP	4	44°50'14"N, 15°36'28"E	680
Upper reach of Crna rijeka, Plitvice Lakes NP	5	44°50'10"N, 15°36'30"E	670
Crna rijeka by the bridge, Plitvice Lakes NP	6	44°50'22"N, 15°35'59"E	665
Lake Prošće, Plitvice Lakes NP	7	44°51'33"N, 15°36'09"E	635
Tufa barrier Labudovac, Plitvice Lakes NP	8	44°52'17"N, 15°35'59"E	630
Lake Okrugljak, Plitvice Lakes NP	9	44°52'23"N, 15°35'56"E	626
Lake Batinovac, Plitvice Lakes NP	10	44°52'16"N, 15°36'11"E	624
Tufa barrier Batinovac-Crno Lake-Malo Lake-Vir, Plitvice Lakes NP	11	44°52'25"N, 15°36'10"E	603
Tufa barrier Batinovac-Galovac, Plitvice Lakes NP	12	44°52'21"N, 15°36'15"E	605
Tufa barrier Galovac-Milino, Plitvice Lakes NP	13	44°52'32"N, 15°36'29"E	594
Lake Gradinsko, Plitvice Lakes NP	14	44°52'39"N, 15°36'37"E	565
Tufa barrier Burget-Kozjak, Plitvice Lakes NP	15	44°52'47"N, 15°36'53"E	547
Riječica, Plitvice Lakes NP	16	44°52'27"N, 15°36'47"E	555
Lake Kozjak, Plitvice Lakes NP	17	44°52'40"N, 15°37'07"E	535
Tufa barrier Kozjak-Milanovac,Plitvice Lakes NP	18	44°53'39"N, 15°36'32"E	545
Tufa barrier Milke Trnine, Plitvice Lakes NP	19	44°53'53"N, 15°36'39"E	540
Tufa barrier Gavanovac-Kaluđerovac, Plitvice Lakes NP	20	44°53'58"N, 15°36'39"E	537
Lake Kaluđerovac, Plitvice Lakes NP	21	44°54'02"N, 15°36'40"E	535
Tufa barrier Novakovića Brod, Plitvice Lakes NP	22	44°54'08"N, 15°36'38"E	505
Stream Sartuk, Plitvice Lakes NP	23	44°55'57"N, 15°33'10"E	765
Stream Plitvica, Plitvice Lakes NP	24	44°54'08"N, 15°36'27"E	555
Korana Village, Plitvice Lakes NP	25	44°55'33"N, 15°37'09"E	390

plotted is provided as Figure 1. Photographs of several studied sites are also given (Figs 2–7). Adult specimens were collected using emergence traps (details in Ivković et al. 2013a), sweep nets, yellow pan traps and aspirators, whereas larvae were collected by Surber sampler (25 × 25 cm) and kick-net sampler (25 × 25 cm, 500 µm mesh size). Specimens were preserved in 80% or 96% ethanol (EtOH). We identified the specimens to species level using Thomas (1997) for Athericidae; Reiss and Fittkau (1971); Bitušík (2000), Langton and Pinder (2007a, 2007b), Andersen et al. (2013), Bitušík and Hamerlík (2014), Vallenduuk (2017) for Chironomidae; Disney (1999) for Dixidae; Engel (1938–1946) for Empididae; Gorodkov (1988) for Scathophagidae; and Rozkošný and Kniepert (2000) for Stratiomyidae.

Results and discussion

List of aquatic Diptera of National Park Plitvice Lakes

The following format is used for the distribution data: literature references (name of the site and in parentheses the citation of site ID and the reference); new records (life stage in which the identifications were made, i.e., adult ♂, ♀ and larvae, name of the site and in parentheses the site ID, date of collection and the collector if possible). New

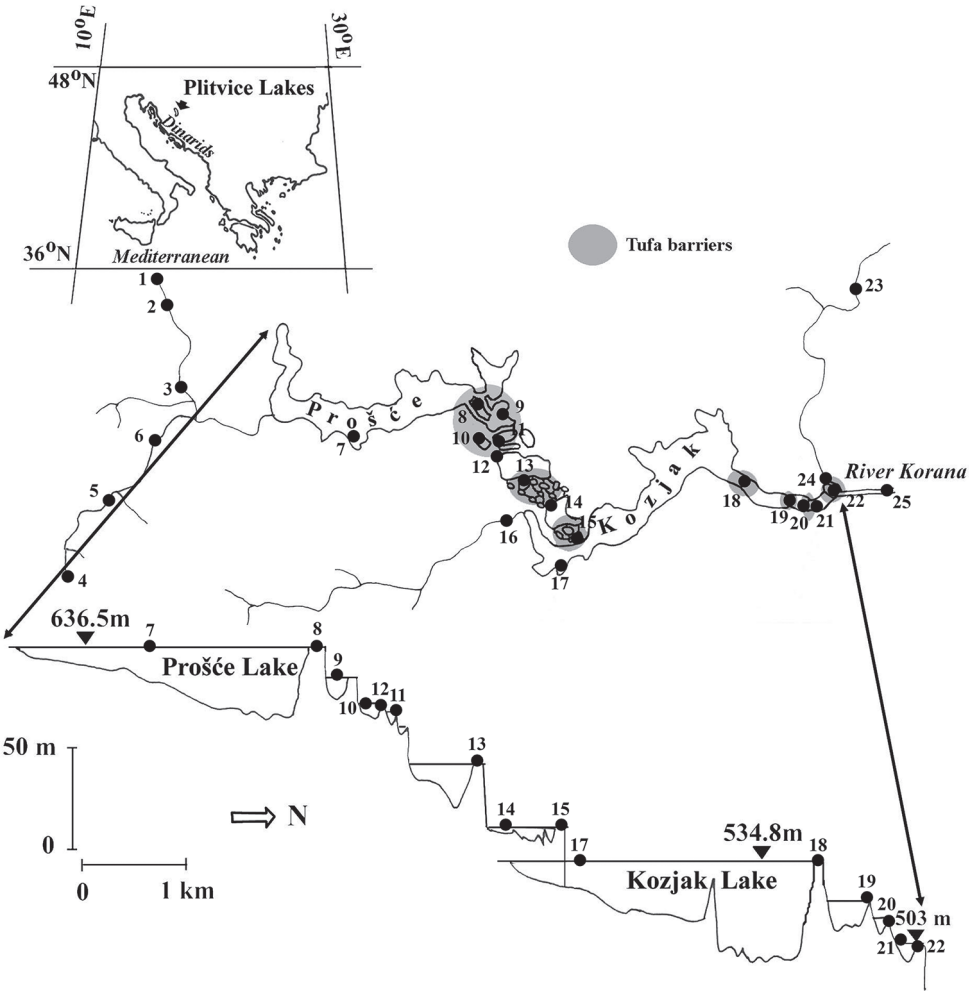

Figure 1. Sampling sites in Plitvice Lakes National Park (see Table 1 for codes).

species for Plitvice Lakes NP are listed with an asterisk before the name of the species. All the sites and site ID are listed in Table 1.

Family Athericidae

Ibisia marginata (Fabricius, 1781)

Literature reference. • tufa barrier Burget-Kozjak, Plitvice Lakes NP (15) (Sertić Perić et al. 2014).

New records. • 1♂; tufa barrier Labudovac, Plitvice Lakes NP (8); 26 Jul. 2010; M. Ivković leg. • 1♂; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 28 Jun.

2012; M. Ivković leg. • 1♀; same site; 25 Jul. 2014; M. Ivković leg. • 3♂, 6♀; tufa barrier Novakovića Brod, Plitvice Lakes NP (22); 25 Jul. 2007; M. Ivković leg. • 10♂, 21♀; same site; 30 Aug. 2007; M. Ivković leg. • 1♂, 6♀; Korana Village, Plitvice Lakes NP (25); 29 Aug. 2008; M. Ivković leg.

Family Chironomidae
Subfamily Chironominae

***Cryptochironomus* (*Cryptochironomus*) *albofasciatus* (Staeger, 1839)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Dicrotendipes nervosus* (Staeger, 1839)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Limnochironomus nervosus* (Staeger, 1839) in Kostić-Brnek and Brnek-Kostić (1971), an accepted synonym of *D. nervosus* in Spies and Saether (2013).

***Endochironomus* gr. *dispar sensu* Moller Pillot, 2009**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Einfeldia dissidens* (Walker, 1856)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Harnischia fuscimanus* Kieffer, 1921**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Cryptochironomus fuscimanus* Kieffer, 1921 in Kostić-Brnek and Brnek-Kostić (1971) a synonym of *H. fuscimanus* in Moller Pillot (2009).

Figure 2. Spring of Crna Rijeka, Plitvice Lakes National Park.

Micropsectra notescens (Walker, 1856)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Micropsectra uva Giłka, Zakrzewska, Baranov & Dominiak, 2013

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Giłka et al. 2013, Ivković et al. 2015) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Microtendipes pedellus (De Geer, 1776)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Microtendipes tarsalis* (Walker, 1856)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

****Nilothauma brayi* (Goetghebuer, 1921)**

New record. • 1 larva; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

***Paracladopelma camptolabis* (Kieffer, 1913)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Paratanytarsus lauterborni* (Kieffer, 1909)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Paratendipes albimanus* (Meigen, 1818)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Phaenopsectra flavipes* (Meigen 1818)**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).
New record. • 24 larvae; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

***Polypedilum* (*Pentapedilum*) *exsectum* (Kieffer, 1916)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Pentapedilum exsectum* Kieffer, 1913 in Kostić-Brnek and Brnek-Kostić (1971), an accepted synonym of *P. exsectum* in Spies and Saether (2013).

Figure 3. Crna Rijeka by the bridge, Plitvice Lakes National Park.

**Polypedilum* (*Polypedilum*) *nubeculosum* (Meigen, 1804)

New record. • 6 larvae; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

Polypedilum (*Tripodura*) *scalaenum* (Schrank, 1803)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Polypedilum breviantennatum* Chernovskij, 1949 in Kostić-Brnek and Brnek-Kostić (1971), an accepted synonym of *P. scalaenum* in Spies and Saether (2013).

Rheotanytarsus nigricauda Fittkau, 1960

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Rheotanytarsus pentapoda (Kieffer, 1909)

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Galovac-Milino, Plitvice Lakes NP (13) • tufa barrier Burget-Kozjak, Plitvice Lakes NP (15) • tufa barrier Milke Trnine, Plitvice Lakes NP (19) (Matoničkin et al. 1971, Matoničkin 1987).

Remark. Mentioned as *Rheotanytarsus lapidicola* Kieffer, 1909 in Matoničkin et al. (1971) and Matoničkin (1987), an accepted synonym of *R. pentapoda* in Spies and Saether (2013).

Stempellina bausei (Kieffer, 1911)

Literature references. • Plitvički Ljeskovac, Plitvice Lakes NP (3) (Matoničkin 1987) • Lake Batinovac, Plitvice Lakes NP (10) (Matoničkin et al. 1971) • tufa barrier Batinovac-Crno Lake-Malo Lake-Vir, Plitvice Lakes NP (11) (Matoničkin 1987) • Lake Gradinsko, Plitvice Lakes NP (14) • tufa barrier Burget-Kozjak, Plitvice Lakes NP (15) (Matoničkin et al. 1971).

New records. • 50 larvae; Lake Prošće, Plitvice Lakes NP (7); 17 Sep. 2018 • 92 larvae; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

**Tanytarsus brundini* Lindeberg, 1963

New record. • 2♂; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 30 Jun. 2009; M. Ivković leg.

Tanytarsus heusdensis Goetghebuer, 1923

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Zavrelia pentatoma Kieffer & Bause, 1913

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Zavreliella marmorata (van der Wulp, 1859)

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Subfamily Diamesinae

***Diamesa* (*Diamesa*) *thomasi* Serra-Tosio, 1970**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Baranov et al. 2012, Ivković et al. 2015).

***Diamesa* (*Diamesa*) *tonsa* (Haliday in Walker, 1856)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

****Potthastia longimanus* Kieffer, 1922**

New record. • 1 larva; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

Subfamily Prodiamesinae

***Monodiamesa bathyphila* (Kieffer, 1918)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Prodiamesa olivacea* (Meigen, 1818)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015) • Lake Prošće, Plitvice Lakes NP (7) (Kostić-Brnek and Brnek-Kostić 1971) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Matoničkin et al. 1971, Matoničkin 1987) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971) • tufa barrier Gavanovac-Kaluđervoac, Plitvice Lakes NP (20) (Matoničkin 1987) • Lake Kaluđerovac, Plitvice Lakes NP (21) (Matoničkin et al. 1971).

Subfamily Orthocladiinae

***Acricotopus lucens* (Zetterstedt, 1850)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Acricotopus lucidus* Brundin, 1949 in Kostić-Brnek and Brnek-Kostić (1971), mentioned as a synonym of *A. lucens* in Moller Pillot (2013).

Brillia bifida **(Kieffer, 1909)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Brillia longifurca **Kieffer, 1921**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Chaetocladius dentiforceps **(Edwards, 1929)**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Chaetocladius melaleucus **(Meigen, 1818)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

Cricotopus **(*Cricotopus*)** ***bicinctus*** **(Meigen, 1818)**

Literature reference. • Stream Plitvica, Plitvice Lakes NP (24) (Matoničkin, 1971).

Remark. Mentioned as *Trichocladius bicinctus* (Meigen, 1818) in Kostić-Brnek and Brnek-Kostić (1971).

Cricotopus **(*Cricotopus*)** ***fuscus*** **(Kieffer, 1909)**

Literature reference. • Stream Plitvica, Plitvice Lakes NP (24) (Matoničkin, 1971).

Remark. Mentioned as *Cricotopus* (*Cricotopus*) *biformis* Edwards, 1929 in Matoničkin et al. (1971), known as a questionable synonym of *C.* (*Cricotopus*) *fuscus* in Ashe and O'Connor (2012).

Corynoneura lobata **Edwards, 1924**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Eukiefferiella devonica* (Edwards, 1929)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Eukiefferiella gracei* (Edwards, 1929)**

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) (Matoničkin et al. 1971).

Remark. Mentioned as *Eukiefferiella longicalcar* Kieffer in Matoničkin et al. (1971), an accepted synonym of *E. gracei* in Spies and Saether (2013).

***Eukiefferiella ilkleyensis* (Edwards, 1929)**

Literature reference. • Stream Plitvica, Plitvice Lakes NP (24) (Matoničkin 1987).

Remark. Mentioned as *Plectrocladius eukiefferiella quadridentata* Chernovskij, 1949 in Matoničkin (1987); see Discussion for more details.

***Eukiefferiella minor* (Edwards, 1929)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

***Epoicocladius ephemerae* (Kieffer, 1924)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Heterotrissocladius marcidus* (Walker, 1856)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Limnophyes gurgicola* (Edwards, 1929)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

***Limnophyes* cf. *minimus sensu* Langton & Pinder, 2007**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Metriocnemus* cf. *albolineatus sensu* Langton & Pinder, 2007**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Metriocnemus eurynothus* (Holmgren, 1883)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

***Metriocnemus intergerivus* Sæther, 1995**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

***Orthocladius* (*Mesorthocladius*) *frigidus* (Zetterstedt, 1838)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Paracladius conversus* (Walker, 1856)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Paratrichocladius inserpens* Pankratova, 1970 in Kostić-Brnek and Brnek-Kostić (1971), a synonym of *P. conversus* in Moller Pillot (2013).

***Parametriocnemus stylatus* (Spaerck, 1923)**

Literature references. • Plitvički Ljeskovac, Plitvice Lakes NP (3) (Matoničkin 1987) • tufa barrier Burget-Kozjak, Plitvice Lakes NP (15) (Matoničkin et al. 1971, Matoničkin 1987) • tufa barrier Gavanovac-Kaluđerovac, Plitvice Lakes NP (20) • Stream Plitvica, Plitvice Lakes NP (24) (Matoničkin 1987).

New records. • 11 larvae; Lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018 • 2♂; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 30 Nov. 2009; M. Ivković leg.

Remark. Mentioned as *Limnophyes transcaucasicus* Chernovskij, 1949 in Matoničkin et al. (1971) and Matoničkin (1987), a synonym of *P. stylatus* in Moller Pillot (2013).

***Parametriocnemus* cf. *stylatus sensu* Moller Pillot, 2013**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Paraphaenocladius* cf. *exagitans sensu* Moller Pillot, 2013**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Paraphaenocladius impensus* (Walker, 1856)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

***Paraphaenocladius* cf. *irritus sensu* Moller Pillot, 2013**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Paratrichocladius skirwithensis* (Edwards, 1929)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Psectrocladius* (*Psectrocladius*) *barbimanus* (Edwards, 1929)**

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) (Matoničkin et al. 1971, Matoničkin 1987) • Crna Rijeka by the bridge, Plitvice Lakes NP (6) • Stream Plitvica, Plitvice Lakes NP (24) (Matoničkin 1987).

***Psectrocladius* (*Psectrocladius*) *psilopterus* (Kieffer, 1906)**

Literature reference. • Crna Rijeka by the bridge, Plitvice Lakes NP (6) (Matoničkin 1987).

***Rheocricotopus effusus* (Walker, 1856)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Synorthocladius semivirens **(Kieffer, 1909)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

New record. • 1 larva; Lake Prošće, Plitvice Lakes NP (7); 26 Jul. 2019.

Thienemannia gracilis **Kieffer, 1909**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

Tvetenia veralli **(Edwards, 1929)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

Subfamily Tanypodinae

Ablabesmyia **(*Ablabesmyia*) *monilis*** **(Linnaeus, 1758)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Pentaneura monilis* Linnaeus, 1758 in Kostić-Brnek and Brnek-Kostić (1971).

Apsectrotanypus trifascipennis **(Zetterstedt, 1838)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Psectrotanypus trifascipennis* Zetterstedt, 1838 in Kostić-Brnek and Brnek-Kostić (1971) which is probably a misspelling.

****Labrundinia longipalpis*** **(Goetghebuer, 1921)**

New record. • 9 larvae; Lake Prošće, Plitvice Lakes NP (7); 26 Jul. 2019.

Krenopelopia binotata **(Wiedemann, 1817)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

***Macropelopia* cf. *fehlmanni sensu* Kieffer, 1912**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Procladius* (*Holotanypus*) *choreus* (Meigen, 1804)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

***Thienemannimyia carnea* (Fabricius, 1805)**

Literature reference. • Lake Prošće, Plitvice Lakes NP (7) • Lake Kozjak, Plitvice Lakes NP (17) (Kostić-Brnek and Brnek-Kostić 1971).

Remark. Mentioned as *Pentaneura carnea* Fabricius, 1805 in Kostić-Brnek and Brnek-Kostić (1971).

Family Dixidae

***Dixa dilatata* Strobl, 1900**

Literature reference. • Stream Sartuk, Plitvice Lakes NP (23) (Ivković and Ivanković 2019).

***Dixa maculata* Meigen, 1818**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) (Ivanković et al. 2019).

***Dixa nebulosa* Meigen, 1830**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Ivanković et al. 2019) • Lake Kozjak, Plitvice Lakes NP (17) (Ivković and Ivanković 2019).• tufa barrier Kozjak- Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivanković et al. 2019).

Figure 4. Tufa barrier Labudovac, Plitvice Lakes National Park.

Dixa nubilipennis Curtis, 1832

Literature reference. • Korana Village, Plitvice Lakes NP (Ivanković et al. 2019) (25).

Dixa puberula Loew, 1849

Literature reference. • spring of Bijela rijeka stream, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Ivanković et al. 2019) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) (Ivanković et al. 2019) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak- Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivanković et al. 2019, Ivković and Ivanković 2019) • Korana Village, Plitvice Lakes NP (25) (Ivanković et al. 2019).

Dixa submaculata Edwards, 1920

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • upper reach of Crna rijeka, Plitvice Lakes NP (5)

• Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) (Ivanković et al. 2019).

***Dixella aestivalis* (Meigen, 1818)**

Literature reference. • Lake Okrugljak, Plitvice Lakes NP (9) • Riječica, Plitvice Lakes NP (16) (Matoničkin 1987).

***Dixella amphibia* (De Geer, 1776)**

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) (Matoničkin 1987).
New record. • 1 larva; lake Kozjak, Plitvice Lakes NP (17); 11 Jul. 2019.

****Dixella autumnalis* (Meigen, 1838)**

New record. • 1 larva; lake Kozjak, Plitvice Lakes NP (17); 18 Jul. 2018.

Family Empididae
Subfamily Clinocerinae

***Clinocera stagnalis* (Haliday, 1833)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a).
New record. • 1♂; tufa barrier Labudovac, Plitvice Lakes NP (8); 26 Jul. 2013; M. Ivković leg.

***Clinocera wesmaeli* (Macquart, 1835)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2010, 2012a).

***Dolichocephala guttata* (Haliday, 1833)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) • up-

per reach of Crna rijeka, Plitvice Lakes NP (5) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2010, 2012a).

Dolichocephala ocellata **(Costa, 1854)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Ivković et al. 2010, 2012a).

Kowarzia barbatula **(Mik, 1880)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna rijeka, Plitvice Lakes NP (4) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a).

Kowarzia bipunctata **(Haliday, 1833)**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Ivković et al. 2010).

Wiedemannia aquilex **(Loew, 1869)**

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) (Ivković et al. 2010, 2012a).

Wiedemannia lamellata **(Loew, 1869)**

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2010, 2012a).

Wiedemannia zetterstedti **(Fallén, 1826)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2010).

Figure 5. Tufa barrier Galovac-Milino, Plitvice Lakes National Park.

Subfamily Hemerodromiinae

***Chelifera concinnicauda* Collin, 1927**

Literature references. • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a) • Korana Village, Plitvice Lakes NP (25) (Horvat 1990, Ivković et al. 2010, 2012a).

***Chelifera flavella* (Zetterstedt, 1838)**

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) • upper reach of Crna rijeka, Plitvice Lakes NP (5) (Ivković et al. 2010, 2012a).

***Chelifera precabunda* Collin, 1961**

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) •

upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) (Ivković et al. 2010, 2012a).

Chelifera precatoria (Fallén, 1816)

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) (Ivković et al. 2010, 2012a).

Chelifera pyrenaica Vaillant, 1981

Literature references. • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2010, 2012a).

Chelifera siveci Wagner, 1984

Literature references. • spring of Bijela Rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna Rijeka, Plitvice Lakes NP (4) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a).

Chelifera stigmatica (Schiner, 1862)

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a).

Chelifera trapezina (Zetterstedt, 1838)

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes (4) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2010, 2012a).

Hemerodromia laudatoria **Collin, 1927**

Literature references. • Crna rijeka by the bridge, Plitvice Lakes NP (6) • Lake Prošće, Plitvice Lakes NP (7) (Ivković et al. 2010, 2012a).

Hemerodromia melangyna **Collin, 1927**

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a) • Korana Village, Plitvice Lakes NP (25) (Horvat 1990, Ivković et al. 2010, 2012a).

New record. • 1♂, 43♀; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 29 Jun. 2015.

Hemerodromia oratoria **(Fallén, 1816)**

Literature references. • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2010, 2012a).

Hemerodromia raptoria **Meigen, 1830**

Literature references. • Lake Kozjak, Plitvice Lakes NP (17) (Ivković et al. 2010, 2012a) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Ivković et al. 2013b).

Hemerodromia unilineata **Zetterstedt, 1842**

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2010, 2012a).

Family Limoniidae
Subfamily Chioneinae

Ellipteroides **(*Ellipteroides*)** ***lateralis*** **(Macquart, 1835)**

Literature reference. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Kolcsár et al. 2015).

***Gonomyia* (*Gonomyia*) *tenella* (Meigen, 1818)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

***Molophilus* (*Molophilus*) *bifidus* Goetghebuer, 1920**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Kolcsár et al. 2015).

***Molophilus* (*Molophilus*) *repentinus* Starý, 1971**

Literature reference. • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

***Ormosia* (*Oreophila*) *bergrothi* (Strobl, 1895)**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Kolcsár et al. 2015).

***Rhabdomastix* (*Rhabdomastix*) *edwardsi* Tjeder, 1967**

Literature reference. • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

***Rhypholophus phryganopterus* Kolenati, 1860**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Kolcsár et al. 2015).

Subfamily Limnophilinae

***Eloeophila apicata* (Loew, 1871)**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Kolcsár et al. 2015).

***Eloeophila maculata* (Meigen, 1804)**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • Crna rijeka by the bridge, Plitvice Lakes NP (6) (Kolcsár et al. 2015).

***Epiphragma* (*Epiphragma*) *ocellare* (Linnaeus, 1760)**

Literature reference. • spring of Crna rijeka, Plitvice Lakes NP (4) (Kolcsár et al. 2015).

Remark. This species is not aquatic. Larvae develop in forests, woodlands, larvae associated with woody debris.

***Hexatoma* (*Eriocera*) *chirothecata* (Scopoli, 1763)**

Literature reference. • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

***Paradelphomyia senilis* (Haliday, 1833)**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) (Kolcsár et al. 2015).

Subfamily Limoniinae

***Antocha* (*Antocha*) *vitripennis* (Meigen, 1830)**

Literature reference. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

***Dicranomyia* (*Dicranomyia*) *chorea* (Meigen, 1818)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Kolcsár et al. 2015).

Remark. Larvae associated with rotting woody debris, but sometimes also reared from semiaquatic habitats; larvae possibly feeding in partially submerged wood.

***Dicranomyia* (*Dicranomyia*) *didyma* (Meigen, 1804)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • Stream Plitvica, Plitvice Lakes NP (24) (Kolcsár et al. 2015).

Figure 6. Lake Kaluđerovac and Tufa barrier Novakovića Brod, Plitvice Lakes National Park.

Dicranomyia (*Dicranomyia*) *imbecilla* Lackschewitz, 1941

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Kolcsár et al. 2015).

Dicranomyia (*Dicranomyia*) *mitis* (Meigen, 1830)

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Kolcsár et al. 2015).

Remarks. Mentioned as *Dicranomyia* (*Dicranomyia*) *mitis* (Meigen, 1830) complex by Kolcsár et al. (2015). After re-identification of specimens by Kolcsár L.-P., using the identification key published by Starý and Stubbs (2015), it was confirmed that the specimens belong to *Dicranomyia* (*Dicranomyia*) *mitis* (Meigen, 1830).

Limonia hercegovinae (Strobl, 1898)

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) (Kolcsár et al. 2015).

Remark. Larvae unknown, but perhaps not associated with aquatic habitats as other *Limonia* species have terrestrial larvae.

***Lipsothrix nobilis* Loew, 1873**

Literature reference. • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Kolcsár et al. 2015).

Family Muscidae
Subfamily Coenosiinae

***Limnophora croatica* Pont & Ivković, 2013**

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Pont and Ivković 2013, Ivković and Pont 2016) • spring of Crna Rijeka, Plitvice Lakes NP (4) (Pont and Ivković 2013) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Pont and Ivković 2013, Ivković and Pont 2016) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Pont and Ivković 2013).

***Limnophora olympiae* Lyneborg, 1965**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Pont and Ivković 2013, Ivković and Pont 2016).

***Limnophora pulchriceps* (Loew, 1860)**

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Pont and Ivković 2013, Ivković and Pont 2016) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Pont and Ivković 2013).

***Limnophora riparia* (Fallén, 1824)**

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Pont and Ivković 2013) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Matoničkin et al. 1971, Pont and Ivković 2013, Ivković and Pont 2016) • tufa barrier Batinovac-Galovac, Plitvice Lakes NP (12) • tufa barrier Galovac-Milino, Plitvice Lakes NP (13) • tufa barrier Burget-Kozjak, Plitvice Lakes NP

(15) (Matoničkin et al. 1971) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Pont and Ivković 2013, Ivković and Pont 2016) • tufa barrier Milke Trnine, Plitvice Lakes NP (19) (Matoničkin et al. 1971) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Pont and Ivković 2013).

Limnophora setinerva Schnabl, 1911

Literature references. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Pont and Ivković 2013) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Pont and Ivković 2013, Ivković and Pont 2016) • Stream Plitvica, Plitvice Lakes NP (24) (Pont and Ivković 2013).

Limnophora tigrina (Am Stein, 1860)

Literature reference. • Korana Village, Plitvice Lakes NP (25) (Pont and Ivković 2013).

Limnophora triangula (Fallén, 1825)

Literature reference. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Ivković and Pont 2016) • Korana Village, Plitvice Lakes NP (25) (Pont and Ivković 2013).

Lispe tentaculata (De Geer, 1776)

Literature reference. • Korana Village, Plitvice Lakes NP (25) (Ivković and Pont 2015).

Lispocephala brachialis (Rondani, 1877)

Literature reference. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Ivković and Pont 2015).

Lispocephala spuria (Zetterstedt, 1838)

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković and Pont 2015).

Figure 7. Stream Plitvica, Plitvice Lakes National Park.

Family Pediciidae
Subfamily Pediciinae

***Dicranota* (*Dicranota*) *bimaculata* (Schummel, 1829)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Kolcsár et al. 2015).

***Dicranota* (*Paradicranota*) *pavida* (Haliday, 1833)**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Kolcsár et al. 2015).

***Pedicia* (*Amalopis*) *occulta* (Meigen, 1830)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) • Crna rijeka by the bridge, Plitvice Lakes NP (6) (Kolcsár et al. 2015).

***Tricyphona* (*Tricyphona*) *immaculata* (Meigen, 1804)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • spring of Crna rijeka, Plitvice Lakes NP (4) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Kolcsár et al. 2015).

Family Psychodidae
Subfamily Sycoracinae

***Sycorax feuerborni* Jung, 1954**

Literature reference. • spring of Crna Rijeka, Plitvice Lakes NP (4) (Ivković et al. 2015).

***Sycorax tonnoiri* Jung, 1953**

Literature reference. • spring of Crna Rijeka, Plitvice Lakes NP (4) (Kvifte et al. 2013, Ivković et al. 2015).

Subfamily Psychodinae

***Berdeniella keroveci* Kvifte, Ivković & Klarić, 2013**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) • spring of Crna Rijeka, Plitvice Lakes NP (4) (Kvifte et al. 2013, Ivković et al. 2015).

***Pericoma blandula* Eaton, 1893**

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) (Kvifte et al. 2013).

***Pericoma miljenkoi* Kvifte & Ivković, 2018**

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Kvifte and Ivković 2018).

***Pericoma pseudocalcilega* Krek, 1972**

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) (Kvifte et al. 2013).

***Psychoda* (*Logima*) *albipennis* Zetterstedt, 1850 complex**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Kvifte et al. 2013).

***Psychoda* (*Psychodocha*) *gemina* (Eaton, 1904)**

Literature reference. • spring of Crna Rijeka, Plitvice Lakes NP (4) (Kvifte et al. 2013, Ivković et al. 2015).

***Jungiella valachia* (Vaillant, 1963)**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Kvifte et al. 2013, Ivković et al. 2015).

Family Scathophagidae
Subfamily Scathophaginae

****Acanthocnema latipennis* Becker, 1894**

New records. • 4♂; spring of Bijela rijeka, Plitvice Lakes NP (1); 26 Jul. 2016; M. Ivković leg. • 2♂; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 29 Apr. 2015; M. Ivković leg.

Family Simuliidae
Subfamily Simuliinae

***Prosimulium tomosvaryi* (Enderlein, 1921)**

Literature references. • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Eusimulium*) *angustipes* Edwards, 1915**

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Eusimulium*) *rubzovianum* (Sherban, 1961)**

Literature reference. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) (Ivković et al. 2016).

***Simulium* (*Nevermannia*) *angustitarse* (Lundström, 1911)**

Literature reference. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) (Ivković et al. 2016).

***Simulium* (*Nevermannia*) *costatum* Friederichs, 1920**

Literature references. • spring of Bijela Rijeka, Plitvice Lakes NP (1) • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • upper reach of Crna rijeka, Plitvice Lakes NP (5) • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Simulium*) *monticola* Friederichs, 1920**

Literature references. • upper reach of Bijela rijeka, Plitvice Lakes NP (2) • Crna rijeka by the bridge, Plitvice Lakes NP (6) • tufa barrier Labudovac, Plitvice Lakes NP (8) (Ivković et al. 2012b, 2014).

New record. • 7♂; tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18); 28 Jun. 2007; M. Ivković leg.

***Simulium* (*Simulium*) *ornatum* Meigen, 1818 (complex)**

Literature references. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

New records. • 4♂; tufa barrier Labudovac, Plitvice Lakes NP (8); 29 May 2009; M. Ivković leg. • 3♂; same site; 30 Jun. 2009; M. Ivković leg.

***Simulium* (*Simulium*) *trifasciatum* Curtis, 1839**

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) (Ivković et al. 2012b, 2014).

***Simulium* (*Simulium*) *tuberosum* (Lundström, 1911)**

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Simulium*) *variegatum* Meigen, 1818**

Literature references. • tufa barrier Labudovac, Plitvice Lakes NP (8) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Stream Plitvica, Plitvice Lakes NP (24) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Trichodagmia*) *auricoma* Meigen, 1818**

Literature references. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) • Korana Village, Plitvice Lakes NP (25) (Ivković et al. 2012b, 2014).

***Simulium* (*Wilhelmia*) *pseudequinum* Séguy, 1921**

Literature references. • tufa barrier Kozjak-Milanovac, Plitvice Lakes NP (18) • tufa barrier Novakovića Brod, Plitvice Lakes NP (22) (Ivković et al. 2012b, 2014).

Remark. Formerly this was misidentified as *Simulium* (*Wilhelmia*) *equinum* (Linnaeus, 1758) in Ivković et al. (2012, 2014, 2016).

Family Stratiomyidae

****Oxycera pardalina* Meigen, 1822**

New records. • 1 larva; spring of Bijela rijeka, Plitvice Lakes NP (1); 30 May 2008; M. Ivković leg. • 6 larvae; upper reach of Bijela rijeka, Plitvice Lakes NP (2); 29 May

2007; M. Ivković leg. • 1♀; same site; 26 Jul. 2010; M. Ivković leg. • 1 larva; upper reach of Crna rijeka, Plitvice Lakes NP (5); 30 Apr. 2007; M. Ivković leg. • 4 larvae; same site; 29 May 2007; M. Ivković leg. • 1 larva, 1♀; same site; 30 Jun. 2007; M. Ivković leg. • 2 larvae; same site; 30 Apr. 2008; M. Ivković leg. • 1 larva; same site; 30 May 2008; M. Ivković leg. • 1♀; tufa barrier Labudovac, Plitvice Lakes NP (8); 30 Jun. 2008; M. Ivković leg. • 1♂; same site; 30 Jun. 2011; M. Ivković leg. • 1♀; same site; 28 Jun. 2012; M. Ivković leg.

****Oxycera limbata*** **Loew, 1862**

New records. • 1♀; tufa barrier Labudovac, Plitvice Lakes NP (8); 25 Jul. 2011; M. Ivković leg. • 3♂; Korana Village, Plitvice Lakes NP (25); 29 Jun. 2007; M. Ivković leg.

****Oxycera turcica*** **Ustuner & Hasbenli, 2004**

New records. • 3♂, 2♀; Korana Village, Plitvice Lakes NP (25); 29 Jun. 2007; M. Ivković leg. • 1♂, 2♀; same site; 26 Jul. 2007; M. Ivković leg.

****Nemotelus pantherinus*** **(Linnaeus, 1758)**

New record. • 1♀; upper reach of Bijela rijeka, Plitvice Lakes NP (1); 24 Jul. 2009; M. Ivković leg.

****Oplodontha viridula*** **(Fabricius, 1775)**

New record. • 1♀; tufa barrier Labudovac, Plitvice Lakes NP (8); 26 Jul. 2010; M. Ivković leg.

Family Tabanidae
Subfamily Chrysopsinae

Chrysops caecutiens **(Linnaeus, 1758)**

Literature reference. • Plitvički Ljeskovac, Plitvice Lakes NP (3) (Krčmar et al. 2008).

Chrysops viduatus **(Fabricius, 1794)**

Literature reference. • Plitvički Ljeskovac, Plitvice Lakes NP (3) (Krčmar et al. 2008).

Family Tipulidae
Subfamily Tipulinae

***Tipula* (*Savtshenkia*) *rufina rufina* Meigen, 1818**

Literature reference. • spring of Bijela rijeka, Plitvice Lakes NP (1) (Ivković et al. 2015).

New records. • 2♂; spring of Bijela rijeka, Plitvice Lakes NP (1); 1 Oct. 2009; M. Ivković leg. • 1♂; spring of Bijela rijeka, Plitvice Lakes NP (1); 2 Nov. 2011; M. Ivković leg. • 1♀; spring of Bijela rijeka, Plitvice Lakes NP (1); 27 Jun. 2013; M. Ivković leg.

Species richness and assemblage composition

In total, 157 species and 7 taxa of aquatic Diptera (Table 2) belonging to 13 families, collected from 25 sites (Table 1, Figure 1) are recorded in the Plitvice Lakes NP, with twelve species new for the dipteran fauna of the National Park. New species belonging to the family Chironomidae are *Labrundinia longipalpis* (Goetghebuer, 1921), *Nilothauma brayi* (Goetghebuer, 1921), *Potthastia longimanus* Kieffer, 1922, *Polypedilum* (*Polypedilum*) *nubeculosum* (Meigen, 1804) and *Tanytarsus brundini* Lindeberg, 1963; to the family Dixidae is *Dixella autumnalis* (Meigen, 1838), and Scathophagidae *Acanthocnema latipennis* Becker, 1894. New species found in the Plitvice Lakes NP belonging to Stratiomyidae family are *Oxycera pardalina* Meigen, 1822, *Oxycera limbata* Loew, 1862, *Oxycera turcica* Ustuner & Hasbenli, 2004, *Nemotelus pantherinus* (Linnaeus, 1758), and *Oplodontha viridula* (Fabricius, 1775).

Overall, the greatest species richness was recorded within the family Chironomidae, with 62 species (and additional seven taxa) recorded so far in Plitvice Lakes NP (Table 2, Figure 8). This was expected since the Chironomidae are an extremely diverse group with more than 8000 described species (Marshall 2012), and with many more undescribed or waiting to be discovered. Some chironomid species have been mentioned in the literature, but are not included in the formal list for various reasons that are considered here. The species *Micropsectra curvicornis* (Chernovskij, 1949) listed in Kostić-Brnek and Brnek-Kostić (1971) and *Thienemanniella flaviforceps* Kieffer, 1925, mentioned from Plitvice in Matoničkin (1987), are considered to be *nomina dubia* in Ashe and Cranston (1990), as well as in Ashe and O'Connor (2012). *Ablabesmyia* cf. *tetrasticta* could possibly be a misinterpretation of the name *Pelopia tetrasticta* Kieffer, 1916 and as such is considered a *nomen dubium* in the subfamily Tanypodinae (Ashe and O'Connor 2009). Furthermore, the species *Cricotopus latidentatus* (Chernovskij, 1949), published in Matoničkin (1987) and Matoničkin et al. (1971), is considered a questionable synonym within the genus *Cricotopus* according to the Ashe and O'Connor (2012). *Cricotopus filiformis* Edwards, mentioned in Matoničkin (1987), is considered an unidentifiable error since Edwards never described a chironomid species with "fili" in the name (Ashe and Cranston 1990). Chironomidae species that are part of the formal species list, but should be addressed with caution, are also considered.

Table 2. Aquatic Diptera at different types of karstic habitats in National Park Plitvice Lakes.

Species/Habitat type	Spring	Stream	Tufa barrier	Lake
Athericidae				
Ibisia marginata (Fabricius, 1781)		•	•	
Chironomidae				
Ablabesmyia (*Ablabesmyia*) *monilis* (Linnaeus, 1758)				•
Acricotopus lucens (Zetterstedt, 1850)				•
Apsectrotanypus trifascipennis (Zetterstedt, 1838)				•
Brillia bifida (Kieffer, 1909)	•			
Brillia longifurca Kieffer, 1921				•
Chaetocladius dentiforceps (Edwards, 1929)	•			
Chaetocladius melaleucus (Meigen, 1818)	•			
Corynoneura lobata Edwards, 1924	•			
Cricotopus (*Cricotopus*) *bicinctus* (Meigen, 1818)				•
Cricotopus (*Cricotopus*) *fuscus* (Kieffer, 1909)		•		
Cryptochironomus (*Cryptochironomus*) *albofasciatus* (Staeger, 1839)				•
Diamesa (*Diamesa*) *thomasi* Serra-Tosio, 1970	•			
Diamesa (*Diamesa*) *tonsa* (Haliday in Walker, 1856)	•			
Dicrotendipes nervosus (Staeger, 1839)				•
Einfeldia dissidens (Walker, 1856)				•
Endochironomus cf. *dispar sensu* Moller Pillot, 2009				•
Epoicocladius ephemerae (Kieffer, 1924)	•			
Eukiefferiella devonica (Edwards, 1929)	•			
Eukiefferiella ilkleyensis (Edwards, 1929)	•			
Eukiefferiella minor (Edwards, 1929)	•			
Eukiefferiella gracei (Edwards, 1929)	•			
Harinischia fuscimanus Kieffer, 1921				•
Heterotrissocladius marcidus (Walker, 1856)				•
Krenopelopia binotata (Wiedemann, 1817)	•			
Labrundinia longipalpis (Goetghebuer, 1921)				•
Limnophyes cf. *minimus sensu* Langton & Pinder, 2007	•			
Limnophyes gurgicola (Edwards, 1929)	•			
Macropelopia cf. *fehlmanni sensu* Kieffer, 1912				•
Metriocnemus cf. *albolineatus sensu* Langton & Pinder, 2007	•			
Metriocnemus eurynothus (Holmgren, 1883)	•			
Metriocnemus intergerivus Sæther, 1995	•			
Micropsectra notescens (Walker, 1856)	•			
Micropsectra uva Giłka, Zakrzewska, Baranov & Dominiak, 2013	•			
Microtendipes pedellus (De Geer, 1776)				•
Microtendipes tarsalis (Walker, 1856)				•
Monodiamesa bathyphila (Kieffer, 1918)				•
Nilothauma brayi (Goetghebuer, 1921)				•
Orthocladius (*Mesorthocladius*) *frigidus* (Zetterstedt 1838)	•			
Paracladius conversus (Walker, 1856)				•
Paracladopelma camptolabis (Kieffer, 1913)				•
Parametriocnemus cf. *stylatus sensu* Moller Pillot, 2013	•			
Parametriocnemus stylatus (Spaerck, 1923)		•	•	
Paraphaenocladius cf. *exagitans sensu* Moller Pillot, 2013	•			
Paraphaenocladius impensus (Walker, 1856)	•			
Paraphaenocladius cf. *irritus sensu* Moller Pillot, 2013	•			
Paratanytarsus lauterborni (Kieffer, 1909)				•
Paratendipes albimanus (Meigen, 1818)				•
Paratrichocladius skirwithensis (Edwards, 1929)	•			
Phaenopsectra flavipes (Meigen 1818)	•			•
Polypedilum (*Pentapedilum*) *exsectum* (Kieffer, 1916)				•
Polypedilum (*Polypedilum*) *nubeculosum* (Meigen, 1804)				•
Polypedilum (*Tripodura*) *scalaenum* (Schrank, 1803)				•
Potthastia longimanus Kieffer, 1922				•

Species/Habitat type	Spring	Stream	Tufa barrier	Lake
Procladius (*Holotanypus*) *choreus* (Meigen, 1804)				•
Prodiamesa olivacea (Meigen, 1818)	•		•	•
Psectrocladius (*Psectrocladius*) *barbimanus* (Edwards, 1929)		•	•	
Psectrocladius (*Psectrocladius*) *psilopterus* (Kieffer, 1906)		•		
Rheocricotopus effusus (Walker, 1856)	•			
Rheotanytarsus nigricauda Fittkau, 1960	•			
Rheotanytarsus pentapoda (Kieffer, 1909)			•	
Stempellina bausei (Kieffer, 1911)		•	•	•
Synorthocladius semivirens (Kieffer, 1909)	•			
Tanytarsus brundini Lindeberg, 1963			•	
Tanytarsus heusdensis Goetghebuer, 1923				•
Thienemannia gracilis Kieffer, 1909	•			
Thienemannimyia carnea (Fabricius, 1805)				•
Tvetenia veralli (Edwards, 1929)	•			
Zavrelia pentatoma Kieffer & Bause, 1913				•
Zavreliella marmorata (van der Wulp, 1859)				•
Dixidae				
Dixa dilatata Strobl, 1900		•		
Dixa maculata Meigen, 1818		•	•	
Dixa nebulosa Meigen, 1830		•	•	•
Dixa nubilipennis Curtis, 1832		•		
Dixa puberula Loew, 1849	•	•	•	
Dixa submaculata Edwards, 1920	•	•	•	
Dixella aestivalis (Meigen, 1818)		•		•
Dixella amphibia (De Geer, 1776)			•	
Dixella autumnalis (Meigen, 1838)				•
Empididae				
Chelifera concinnicauda Collin, 1927		•	•	
Chelifera flavella (Zetterstedt, 1838)	•	•		
Chelifera precabunda Collin, 1961	•	•		
Chelifera precatoria (Fallén, 1816)	•	•		
Chelifera pyrenaica Vaillant, 1981		•	•	
Chelifera siveci Wagner, 1984	•	•		
Chelifera stigmatica (Schiner, 1862)		•	•	
Chelifera trapezina (Zetterstedt, 1838)	•	•		
Clinocera stagnalis (Haliday, 1833)	•	•		
Clinocera wesmaeli (Macquart, 1835)	•			
Dolichocephala guttata (Haliday, 1833)	•	•		
Dolichocephala ocellata (Costa, 1854)	•	•		
Hemerodromia laudatoria Collin, 1927		•		•
Hemerodromia melangyna Collin, 1927		•	•	
Hemerodromia oratoria (Fallén, 1816)		•	•	
Hemerodromia raptoria Meigen, 1830			•	•
Hemerodromia unilineata Zetterstedt, 1842		•	•	
Kowarzia barbatula (Mik, 1880)	•	•	•	
Kowarzia bipunctata (Haliday, 1833)		•		
Wiedemannia aquilex (Loew, 1869)	•	•		
Wiedemannia lamellata (Loew, 1869)	•	•	•	
Wiedemannia zetterstedti (Fallén, 1826)	•			
Limoniidae				
Antocha (*Antocha*) *vitripennis* (Meigen, 1830)		•	•	
Dicranomyia (*Dicranomyia*) *chorea* (Meigen, 1818)	•	•	•	
Dicranomyia (*Dicranomyia*) *didyma* (Meigen, 1804)	•	•	•	
Dicranomyia (*Dicranomyia*) *imbecilla* Lackschewitz, 1941	•		•	
Dicranomyia (*Dicranomyia*) *mitis* (Meigen, 1830) complex		•		
Ellipteroides (*Ellipteroides*) *lateralis* (Macquart, 1835)			•	
Eloeophila apicata (Loew, 1871)		•		
Eloeophila maculata (Meigen, 1804)		•		

Species/Habitat type	Spring	Stream	Tufa barrier	Lake
Epiphragma (*Epiphragma*) *ocellare* (Linnaeus, 1760)	•			
Gonomyia (*Gonomyia*) *tenella* (Meigen, 1818)	•	•		
Hexatoma (*Eriocera*) *chirothecata* (Scopoli, 1763)		•	•	
Limonia hercegovinae (Strobl, 1898)	•	•		
Lipsothrix nobilis Loew, 1873		•	•	
Molophilus (*Molophilus*) *bifidus* Goetghebuer, 1920	•			
Molophilus (*Molophilus*) *repentinus* Starý, 1971		•		
Ormosia (*Oreophila*) *bergrothi* (Strobl, 1895)	•			
Paradelphomyia (*Oxyrhiza*) *senilis* (Haliday, 1833)	•	•		
Rhabdomastix (*Rhabdomastix*) *edwardsi* Tjeder, 1967		•		
Rhypholophus phryganopterus Kolenati, 1860	•			
Muscidae				
Limnophora croatica Pont & Ivković, 2013	•	•	•	
Limnophora olympiae Lyneborg, 1965	•		•	
Limnophora pulchriceps (Loew, 1860)		•	•	
Limnophora riparia (Fallén, 1824)	•	•	•	
Limnophora setinerva Schnabl, 1911	•	•	•	
Limnophora tigrina (Am Stein, 1860)		•		
Limnophora triangula (Fallén, 1825)		•	•	
Lispe tentaculata (De Geer, 1776)		•		
Lispocephala brachialis (Rondani, 1877)			•	
Lispocephala spuria (Zetterstedt, 1838)	•			
Pediciidae				
Dicranota (*Dicranota*) *bimaculata* (Schummel, 1829)	•			
Dicranota (*Paradicranota*) *pavida* (Haliday, 1833)		•		
Pedicia (*Amalopis*) *occulta* (Meigen, 1830)	•	•		
Tricyphona (*Tricyphona*) *immaculata* (Meigen, 1804)	•	•	•	
Psychodidae				
Sycorax feuerborni Jung, 1954	•			
Sycorax tonnoiri Jung, 1953	•			
Berdeniella keroveci Kvifte, Ivković & Klarić, 2013	•			
Pericoma blandula Eaton, 1893			•	
Pericoma miljenkoi Kvifte & Ivković, 2018			•	
Pericoma pseudocalcilega Krek, 1972			•	
Psychoda (*Logima*) *albipennis* Zetterstedt, 1850 complex	•			
Psychoda (*Psychodocha*) *gemina* (Eaton, 1904)	•			
Jungiella valachia (Vaillant, 1963)	•			
Scathophagidae				
Acanthocnema latipennis Becker, 1894	•		•	
Simuliidae				
Prosimulium tomosvaryi (Enderlein, 1921)		•		
Simulium (*Eusimulium*) *angustipes* Edwards, 1915		•	•	
Simulium (*Eusimulium*) *rubzovianum* (Sherban, 1961)		•	•	
Simulium (*Nevermannia*) *angustitarse* (Lundström, 1911)		•		
Simulium (*Nevermannia*) *costatum* Friederichs, 1920	•	•	•	
Simulium (*Simulium*) *monticola* Friederichs, 1920		•	•	
Simulium (*Simulium*) *ornatum* Meigen, 1818 complex		•	•	
Simulium (*Simulium*) *trifasciatum* Curtis, 1839			•	
Simulium (*Simulium*) *tuberosum* (Lundström, 1911)		•	•	
Simulium (*Simulium*) *variegatum* Meigen, 1818		•	•	
Simulium (*Trichodagmia*) *auricoma* Meigen, 1818		•	•	
Simulium (*Wilhelmia*) *pseudequinum* Séguy, 1921			•	
Stratiomyidae				
Oxycera pardalina Meigen, 1822	•	•	•	
Oxycera limbata Loew, 1862		•	•	
Oxycera turcica Ustuner & Hasbenli, 2004		•		
Nemotelus pantherinus (Linnaeus, 1758)		•		
Oplodontha viridula (Fabricius, 1775)			•	

Species/Habitat type	Spring	Stream	Tufa barrier	Lake
Tabanidae				
Chrysops caecutiens (Linnaeus, 1758)		•		
Chrysops viduatus (Fabricius, 1794)		•		
Tipulidae				
Tipula (*Savtshenkia*) *rufina rufina* Meigen, 1818	•			
Number of taxa	**75**	**71**	**53**	**38**

In Kostić-Brnek and Brnek-Kostić (1971), the authors did not clearly state whether their identifications of *Endochironomus* cf. *dispar* and *Macropelopia* cf. *fehlmanni* were based on adults or larvae. If the identifications were made based on adults, then these reports can be considered valid. On the other hand, if larvae were identified then these reports are doubtful records since these species cannot be identified based on larval morphology alone. The species listed in the formal species list as *Eukiefferiella ilkleyensis* (Edwards, 1929) is mentioned as *Plectrocladius eukiefferiella quadridentata* Chernovskij, 1949 in Matoničkin (1987). This could be a typing error since a genus *Plectrocladius* does not exist within the family Chironomidae. On the other hand, *Eukiefferiella quadridentata* Chernovskij, 1949 is a synonym pro parte of *E. ilkleyensis* (Moller Pillot 2013). If this is not a typing error, then this species name is an unidentifiable error.

Families following Chironomidae by number of species are Empididae, Limoniidae, Simuliidae and Muscidae (only aquatic species identified) with 22, 19, 12 and 10 species, respectively (Table 2, Figure 8). Dixidae and Psychodidae are both present with nine species, while Stratiomyidae, Pediciidae and Tabanidae are present with 5, 4 and 2 species, respectively (Table 2, Figure 8). Families with only one recorded species are Athericidae, Scathophagidae and Tipulidae (Table 2, Figure 8).

The families Dixidae, Empididae and Simuliidae have been dealt with in detail in previous publications (Ivković et al. 2010, 2012a, b, 2016; Ivanković et al. 2019; Ivković and Ivanković 2019) so the numbers presented here might be the final species numbers for Plitvice Lakes NP. For these particular families, only a few additional species might be recorded in the future studies. Other families of aquatic Diptera have been studied sporadically and, in many cases, only a few sites have been completely processed, such as Chironomidae in Kostić-Brnek and Brnek-Kostić (1971) and Ivković et al. (2015). Aquatic Diptera families that are present in the Plitvice Lakes NP but are still unidentified to the species level are Ceratopogonidae, Culicidae, Ephydridae, Sciomyzidae and Syrphidae.

Springs and streams have higher numbers of species recorded than tufa barriers and lakes (Table 2). One of the reasons for this is because the springs were studied in greater detail than other sites, especially when it comes to Chironomidae (Ivković et al. 2015). On the other hand, many aquatic Diptera families have in fact a higher diversity at spring habitats and the upper reaches of streams, such as Empididae, Psychodidae, Limoniidae, etc., because most environmental parameters at those sites remain constant (Ivković et al. 2010, 2012a, 2015; Kvifte et al. 2013; Kolcsár et al. 2015).

The species list of aquatic Diptera of Plitvice Lakes NP is still not complete since many families are either dealt with partially or not at all due to the lack of available

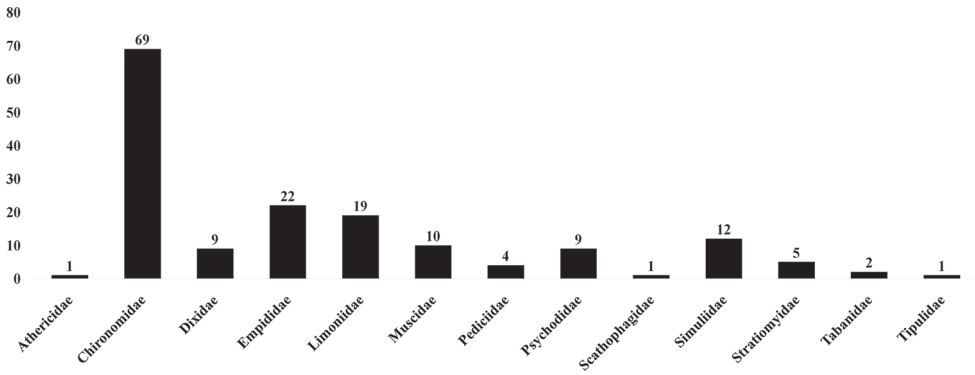

Figure 8. Species richness of Diptera families in Plitvice Lakes National Park.

experts. There is still a lot of work in front of us since we belive that about 250 species of aquatic Diptera can be expected in the unique karstic area of Plitvice Lakes NP.

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RESEARCH ARTICLE

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One new species and one newly recorded species of the genus *Lasiochira* Meyrick, 1931 (Lepidoptera, Oecophoridae) from China

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Abstract

Lasiochira wuzhishanensis Yin, **sp. nov.** is described herein from the island province of Hainan, China. It is diagnosed from a similar species, *Lasiochira jianfengensis* Yin, Wang & Park, 2014. Both species are sympatric in Hainan province, but the latter is also known in northern Vietnam. *Lasiochira pallidiptera* Yin, Wang & Park, 2014 is recorded for the first time from China. Color images of the adults and genitalia of the above three species are provided. In addition, a checklist and a geographical distribution map of all species of *Lasiochira* Meyrick are included.

Keywords

Checklist, key, morphology, moth, taxonomy

Introduction

Lasiochira Meyrick, 1931 is a small genus included in the Oecophoridae, with unknown biology. The type species, *Lasiochira camaropa* Meyrick, 1931, was diagnosed based on two male syntype specimens from Kwanhsien, China. Clarke (1963) designated a lectotype from Meyrick's original material and transferred a second species, *L. xanthacma* (Meyrick, 1938), from *Allotalanta* Meyrick, 1913. No further taxonomic work was published on the genus until Wang (2006) treated the Oecophoridae of

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China, including the two known species. Yin et al. (2014) described six new species, but the entire fauna is known only from China, Korea, and Vietnam.

The genus *Lasiochira* is mainly characterized by having a pale, N-shaped pattern consisting of three joined stripes and several small tufts of erect scales on the forewing (Figs 1–3); the gnathos is often pointed and hooked terminally (Figs 4, 5, 8); the valva usually has dense fine hairs on the distal half (Figs 4, 5, 8); the cornuti are comprised of multiple short, stout spines or plates (Figs 4, 5, 8; arrows); the apophyses posteriores are longer than the apophyses anteriores (Figs 6, 7, 9); the ductus bursae is with sclerotization (Figs 6, 7, 9); and the signum is transverse, dentate, with a projecting semicircular lobe posteriorly (Figs 6a, 7a, 9) (Yin et al. 2014).

Recent collecting efforts in the Hainan and Hubei provinces produced a new species, described herein, and the first record of *L. pallidiptera* Yin, Wang & Park, 2014 in China. The objectives of this study are to describe the new species and update the distributions of the known species.

Material and methods

All specimens for this study were collected in 2018 from the Hainan and Hubei provinces of China. The descriptive terminology of the anatomical structures follows Wang (2006), Yin et al. (2014) and Kristensen (2003). Photographs of adults were taken using a Canon EOS 6D Mark II camera with an EF 100 mm f/2.8L MACRO IS USM lens assisted by the EOS Utility 3.10.20 software. Stacked images of the genitalia were captured using a Leica DM4 B upright microscope. Photomontage was performed with the Leica Application Suite X imaging software. Species distribution data were compiled within Microsoft Excel using both published records and specimen label data. The distribution map was produced with the aid of DIVA-GIS 7.5 (Hijmans et al. 2011).

All type specimens are deposited in the Morphological Laboratory, Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou, China.

Taxonomy

***Lasiochira* Meyrick, 1931: 71.**

Type species. *Lasiochira camaropa* Meyrick, 1931, by monotypy.

Checklist of *Lasiochira* Meyrick species

1 *Lasiochira camaropa* Meyrick, 1931: 71
Distribution: China (Sichuan province).

2 *Lasiochira flaviterminata* Yin, Wang & Park, 2014: 33
Distribution: China (Chongqing City).

3 *Lasiochira jianfengensis* Yin, Wang & Park, 2014: 25
Distribution: China (Hainan province), Vietnam (North).
4 *Lasiochira jiulongshana* Yin, Wang & Park, 2014: 27
Distribution: China (Zhejiang province).
5 *Lasiochira pallidiptera* Yin, Wang & Park, 2014: 30
Distribution: China (Hubei province), Korea (Central).
6 *Lasiochira rosataenia* Yin, Wang & Park, 2014: 32
Distribution: Vietnam (North).
7 *Lasiochira taiwanensis* Yin, Wang & Park, 2014: 31
Distribution: China (Taiwan province).
8 *Lasiochira wuzhishanensis* Yin, sp. nov.
Distribution: China (Hainan province).
9 *Lasiochira xanthacma* (Meyrick, 1938: 10)
Distribution: China (Guangdong, Guizhou, Henan, Shaanxi, Shanxi and Yunnan provinces).

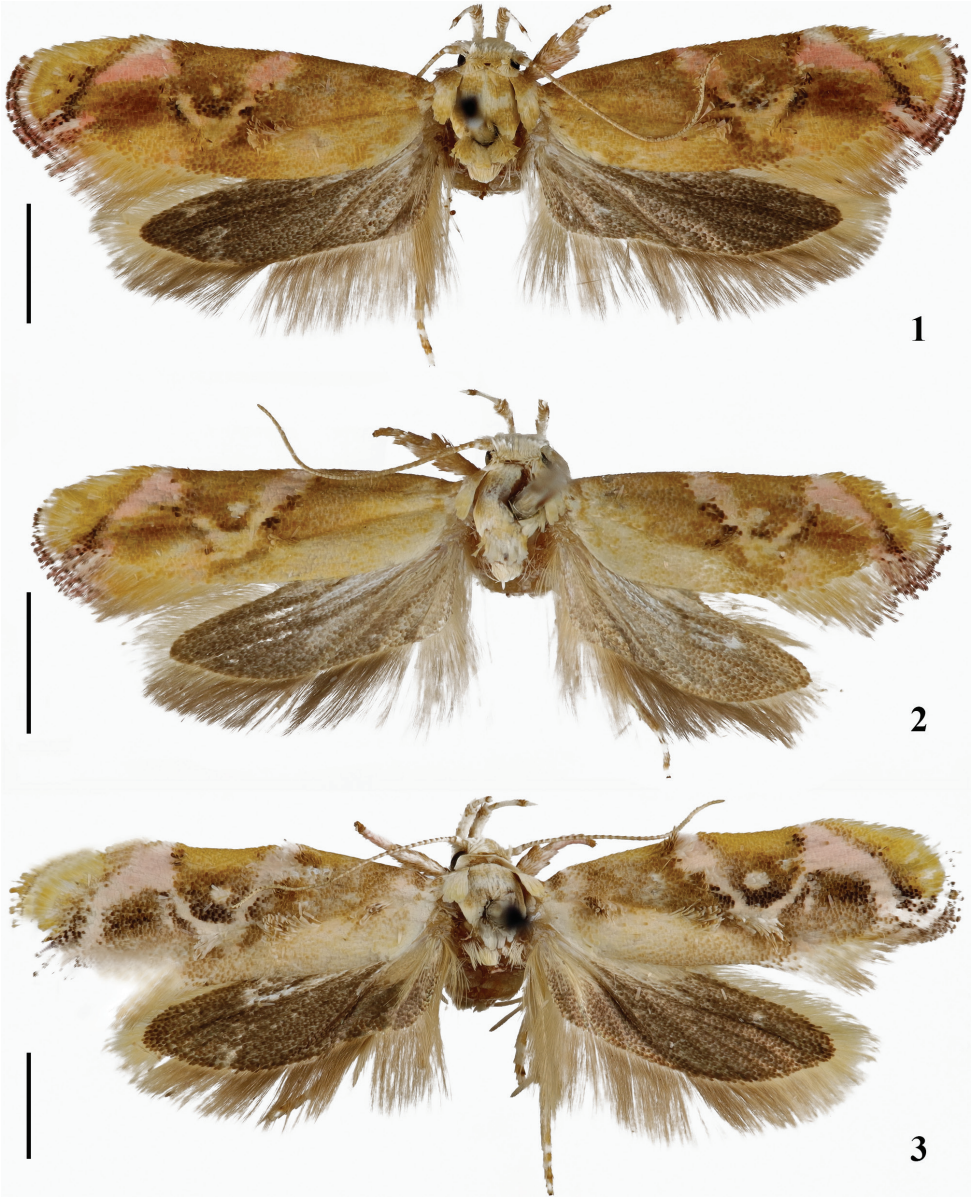
***Lasiochira wuzhishanensis* Yin, sp. nov.**
http://zoobank.org/4001CC9D-E7DA-4374-9BF1-28A607ADAD91
Figs 1, 4, 6, 6a

Material examined. ***Holotype*:** ♂; China: Hainan province, Wuzhishan City, Wuzhishan National Nature Reserve; alt. 650 m; 18°54'36"N, 109°40'48"E; 10 May 2018; Zhengyong Wang leg.; YC00025. ***Paratypes*:** 1 ♂, 3 ♀♀; same locality as holotype; alt. 650 m; 18°54'36"N, 109°40'48"E; 10–15 May 2018; Zhengyong Wang leg.; YC00026 ♂, YC00022 ♀, YC00023 ♀, YAH19072 ♀.

Diagnosis. *Lasiochira wuzhishanensis* Yin, sp. nov. can be distinguished from its congeners by the following two character states: forewing ocherous brown in ground color (Fig. 1); phallus with seven cornuti (Fig. 4, arrows).

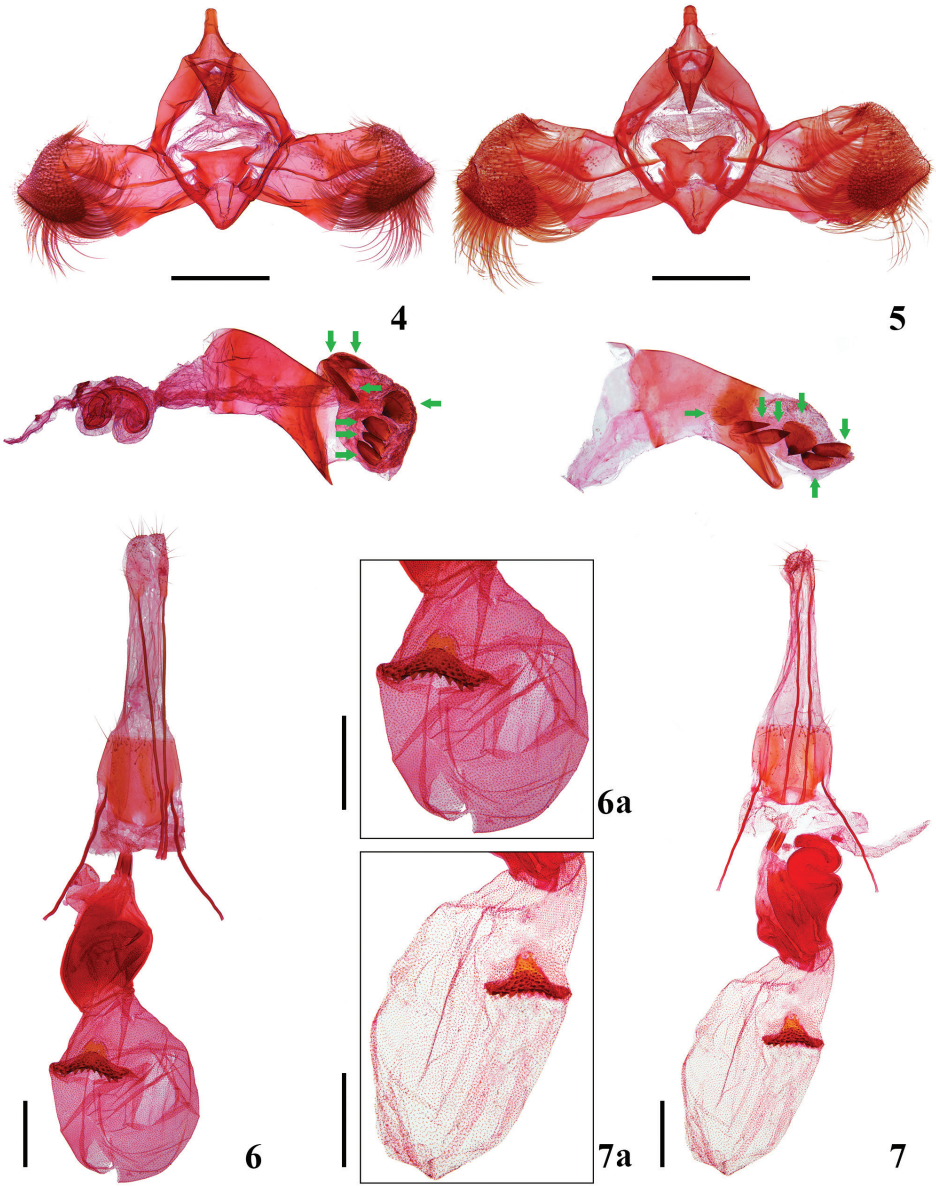
Description. Head: Vertex and front white, tinged with pale ocherous yellow on cervical area; labial palpi white, first segment with dark brown scales dorsally, second segment somewhat tinged with pale ocherous brown, denser ventrally, third segment with broad pale ocherous brown ring at distal 1/4; antenna with scape white, covered with pale ocherous yellow scales on dorsal surface, pecten pale yellow, flagellum pale ocherous yellow, ringed with white; proboscis white.

Thorax: Tegula and mesonotum white, with three transverse pale ocherous brown bands; legs pale yellowish white, tibiae and tarsi pale ocherous brown on outer surface and pale ocherous brown and pale yellowish white on inner surface. Forewing (Fig. 1): length 5.5–7.0 mm (N = 5), about 3.3 × as long as wide, ocherous brown, slightly paler between CuP and dorsum; an N-shaped pinkish pattern running from basal 2/5 of costa diagonally outward to posterior angle of cell, obliquely to subapical part of costa, enlarged on costal margin, and diagonally narrowed to ventroapical part of termen; innermost stripe edged with two erect scale tufts, tufts black or ocherous brown on inner margin, angle between innermost and middle stripes diffused with blackish brown



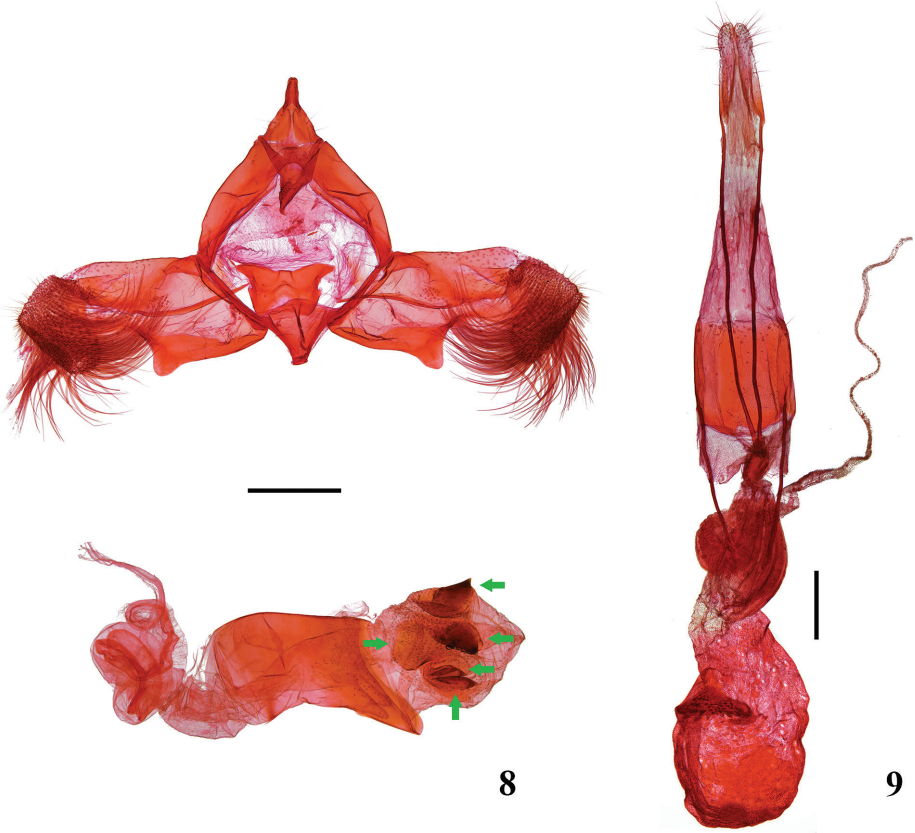
Figures 1–3. Adults of *Lasiochira* species **1** *L. wuzhishanensis* Yin, sp. nov., paratype, female, gen. slide no. YAH19072 **2** *L. jianfengensis* Yin, Wang and Park, female, gen. slide no. YC00028 **3** *L. pallidiptera* Yin, Wang and Park, male, gen. slide no. YAH18154. Scale bars: 2.00 mm.

scales, and anteriorly with an erect pale yellowish white scale tuft, middle and outmost stripes both edged with erect scale tufts, tufts black or ocherous brown on outer margins; CuP with an erect scale tuft near distal 1/3; cilia with basal 3/5 pale ocherous brown, distal 2/5 pinkish white, edged with dark brown; ventral surface grayish brown. Hindwing (Fig. 1): dark gray; cilia yellowish gray.



Figures 4–7. Male and female genitalia of *Lasiochira* species **4** *L. wuzhishanensis* Yin, sp. nov., holotype, male genital capsule and phallus, gen. slide no. YC00025 **5** *L. jianfengensis* Yin, Wang and Park, male genital capsule and phallus, gen. slide no. YC00024 **6** *L. wuzhishanensis* Yin, sp. nov., paratype, female genitalia, gen. slide no. YC00023 **6a** *L. wuzhishanensis* Yin, sp. nov., enlargement of corpus bursae, gen. slide no. YC00023 **7** *L. jianfengensis* Yin, Wang and Park, female genitalia, gen. slide no. YC00028 **7a** *L. jianfengensis* Yin, Wang and Park, enlargement of corpus bursae, gen. slide no. YC00028. Scale bars: 0.40 mm.

Male genitalia (Fig. 4): Uncus broad at base, gradually narrowed to about 1/2, apical half parallel bilaterally, apex rounded; gnathos obtriangular, with minute granules on apical half; tegumen with nearly trapezoid posterior margins fused with uncus, anterior



Figures 8, 9. Male and female genitalia of *L. pallidiptera* Yin, Wang and Park **8** male genital capsule and phallus, gen. slide no. YAH19075 **9** female genitalia, gen. slide no. YAH18160. Scale bars: 0.40 mm.

margin deeply emarginate; valva short and broad, sub-rectangular, heavily setose on triangular area apically; costa nearly straight; ventral margin triangularly concave inward beyond end of sacculus; sacculus narrow, slightly arched ventrally, distally with a triangular process directing downward; vinculum narrowly banded; saccus short, triangular, apex bluntly rounded; juxta obtrapezoidal, posterior margin straight, anterior margin concave mesially, lateral margins deeply emarginate at middle, forming two opposable triangular arms; phallus stout, distal 1/4 protuberant, extending to a sharp point; vesica with six irregularly-shaped, plate-like cornuti, and one small cornutus (Fig. 4, arrows).

Female genitalia (Fig. 6a): Papillae anales setose, broadly rounded posteriorly; apophyses posteriores about 2.5 times length of apophyses anteriores; eighth sternite with posterior margin straight, setose on posterior half, darkly pigmented mesolaterally, paler mesolongitudinally; antrum wide, membranous; ductus bursae narrow and sclerotized posteriorly, elongate, with undulating internal sclerotization anteriorly, bearing inception of ductus seminalis on anterior part; ductus bursae subspherical, with a spiculate inner wall; signum a transverse, dentate band, with a posteriorly projecting semicircular lobe.

Remarks. *Lasiochira wuzhishanensis* Yin, sp. nov. is very similar in wing pattern to *L. jianfengensis* Yin, Wang and Park (Figs 1, 2), but differs by having juxta with pos-

terior margin straight (Fig. 4); phallus with seven cornuti (Fig. 4, arrows); and corpus bursae small, nearly spherical in shape (Fig. 6a). *Lasiochira jianfengensis* has juxta with posterior margin emarginate mesially (Fig. 5); phallus with six cornuti (Fig. 5, arrows); and corpus bursae ovate in shape and larger (Fig. 7a).

Biology. Unknown. Adults were attracted to white light in May.

Distribution. Known only from the type locality (China: Hainan province).

Etymology. The species epithet is derived from the name of the type locality, Wuzhishan National Nature Reserve.

Lasiochira pallidiptera Yin, Wang & Park, 2014

Figs 3, 8, 9

Lasiochira pallidiptera Yin, Wang & Park, 2014: 30 (*Lasiochira*; type locality: Mt. Godae-san, Yeoncheon-gun, Gyeongbuk province, Korea).

Material examined. 3 ♂♂, 1 ♀; China, Hubei province, Huanggang City, Yingshan County, Taohuachong Forest Park; alt. 700 m; 30°59'04"N, 115°56'15"E; 5 Jun. 2018; Zhengyong Wang leg.; YAH18154 ♂, YAH18157 ♂, YAH18160 ♀, YAH19075 ♂.

Remarks. This species is recorded from China for the first time.

Biology. Unknown. Adults were attracted to white light in June.

Distribution. China (Hubei province), Korea (Central).

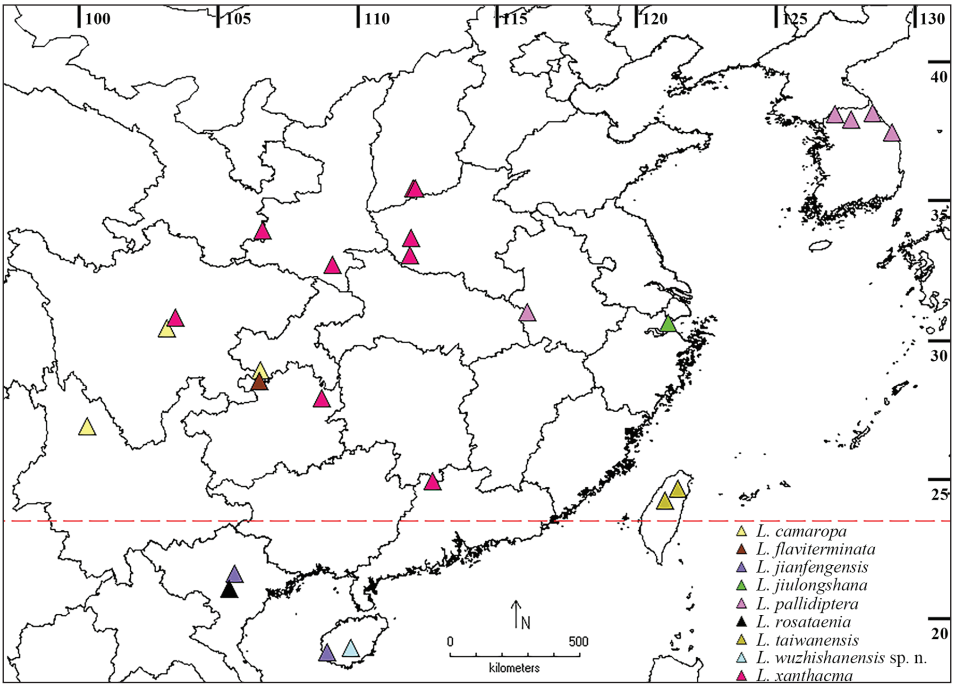


Figure 10. Distribution map of *Lasiochira* species.

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SHORT COMMUNICATION

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First record of a freshwater bryozoan species in Cuba: *Plumatella repens* (Linnaeus, 1758) (Phylactolaemata, Bryozoa)

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Abstract

The discovery of *Plumatella repens* floatoblasts in wetlands of the La Niña Bonita Reservoir and the Ciénaga de Zapata Swamp, Cuba, constitutes the first record of a freshwater bryozoan species on the island and extends the distribution range of the species in the insular Caribbean. Unlike the inland waters of the Lesser Antilles the greater availability of water and lower salinity are likely the main factors that determine the distribution of *P. repens* in the Greater Antilles.

Keywords

Caribbean Islands, Cuba, floatoblast, Phylactolaemata, salinity, water chemistry

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Introduction

The freshwater bryozoan fauna of the insular Caribbean has been mainly studied in the Leeward Islands (Aruba, Bonaire, Klein Bonaire and Curaçao), with three known species from the study of colonies and floatoblasts (statoblast buoyant with the annulus composed of gas chambers): *Plumatella agilis* (Marcus, 1942), *Plumatella casmiana* Oka, 1907 and *Plumatella longigemmis* Annandale, 1915 (Lacourt 1955, 1968); the latter was also reported in Jamaica (Lacourt 1968). In addition, *Plumatella repens* (Linnaeus, 1758) has been reported in Puerto Rico (Osburn 1940; Rogick and Brown 1942; Lacourt 1968). Unidentified *Plumatella* colonies and floatoblasts were reported on the islands of Cuba and Trinidad (Osburn 1940; Rogick and Brown 1942; Lacourt 1968; Collado et al. 1984).

Knowledge of the distribution of freshwater bryozoans in the Caribbean is scarce despite the great biogeographical interest of this area. It constitutes a complex island system located between two large continental biogeographic regions: Nearctic and Neotropical (Wood 2002; Massard and Geimer 2008a, 2008b). New records of *P. repens* from floatoblasts on the island of Cuba contribute to the understanding of the ecology and distribution of freshwater bryozoan species in the Caribbean Islands.

Material and methods

Study area

The La Niña Bonita Reservoir is located in the council of Bauta (Artemisa Province, Cuba) (Fig. 1a, b). This water body is a freshwater wetland with an area of 1.20 ha and a maximum depth of 10 m. This reservoir dams the Jaimanitas River, with a basin of 9.2 km^2 dominated by limestone rocks, and is used mainly for irrigation and fish farming (Valdés et al. 1996).

Ciénaga de Zapata Swamp is located in the Zapata Peninsula (Matanzas Province, Cuba) (Fig. 1a, c). This wetland is the largest (2600 km^2) and best conserved marsh swamp in the insular Caribbean, as well as the one with the greatest biodiversity. It has been declared a national conservation area by the government of Cuba and has been internationally recognized as a Ramsar Site (http://www.snap.cu/index.php/ct-menu-item-15/ct-menu-item-67/ct-menu-item-68). The shallow marshes show an important accumulation of organic matter and the lithology is dominated by limestones and dolomites with seeping underground waters (cenotes) (Ferrera et al. 1996; Morell et al. 1997). The waters are bicarbonated-calcic with a great spatial heterogeneity depending on the input of groundwater seeps or marine intrusions. Also, there is a great salinization of groundwater as a consequence of the exploitation of freshwater aquifers (Fagundo et al. 1992b; Rodríguez et al. 1992; Ferrera et al. 1996, 1999; Molerio-León and Parise 2008).

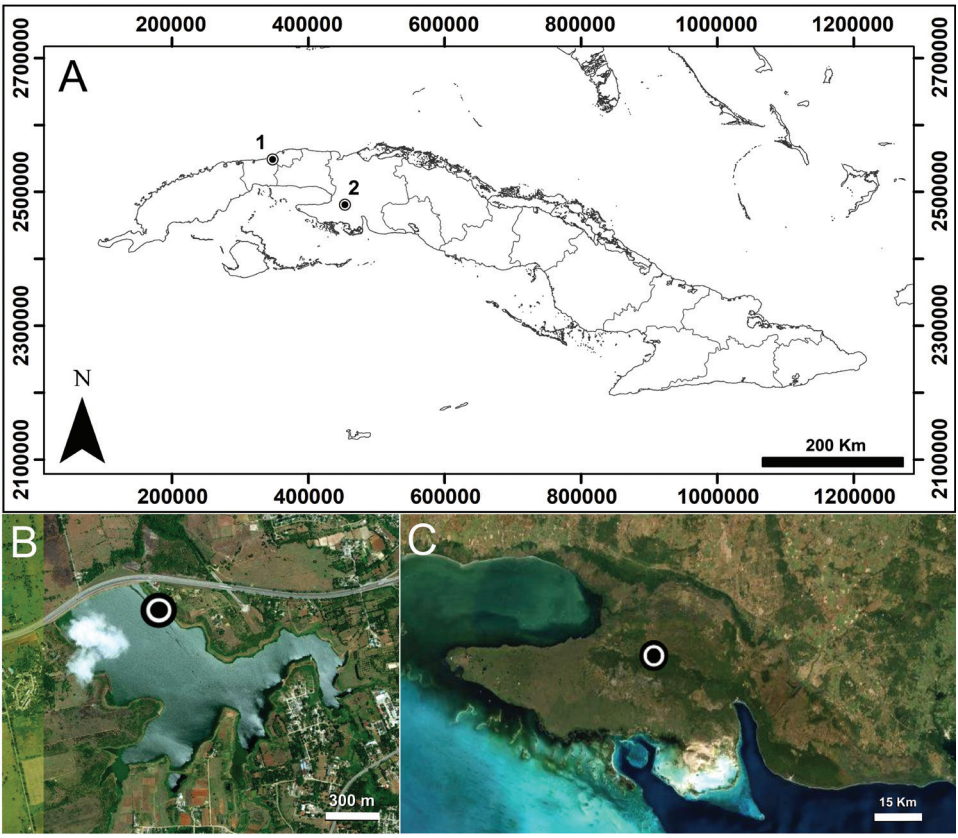

Figure 1. Presence of *Plumatella repens* (Linnaeus, 1758), in the wetlands of Cuba: **A** Location map of localities (1) La Niña Bonita Reservoir and (2) Ciénaga de Zapata Swamp **B** Aerial photograph of the La Niña Bonita Reservoir (ESRI World Imagery, ArcGIS 10.0) **C** Aerial photograph of the Ciénaga de Zapata Swamp (ESRI World Imagery, ArcGIS 10.0).

Sample collection and processing

In shallow wetland areas, samples of 2 cm^3 of surface sediment were collected in the La Niña Bonita Reservoir (23°02'24.53"N, 82°29'37.09"W; 42 m a.s.l.) (Fig. 1b) and in the Ciénaga de Zapata Swamp (22°25'44.55"N, 81°27'25.26"W; 5 m a.s.l.) (Fig. 1c). Sediment samples were screened through a 50 µm mesh; the larger fractions were examined under a stereoscopic microscope, and floatoblasts were collected with a pipette. Floatoblasts were treated with 2% NaOH for 1 min under agitation at room temperature, then subjected to an ultrasonic bath for 15 seconds, and finally washed in deionized water. Floatoblasts for scanning electron microscopy (SEM) were mounted on aluminium stubs, sputtered with platinum/palladium (15 nm) for 1 min using a Cressington Sputter Coater 208HR SEM, and studied with a JEOL Field Emission SEM JSM 7200F operated at 15 kV in the University of A Coruña's Research Support Service (Servizo de Apoio a Investigación, S.A.I.).

Results

The morphometry of the examined floatoblasts showed that they belong to the species *Plumatella repens*. The shape of the floatoblast is broadly oval, both valves are equally convex in lateral view, and the floatoblast annulus is smooth, without tubercles (Fig. 2a, b). Floatoblast measurements were 332.2 ± 14.11 (318.5–350.2) µm in total length and 220.8 ± 15.42 (217.3–252.6) µm in total width (N = 10). The fenestra of floatoblasts is rounded oval in dorsal view and oval in ventral view, covered with rounded tubercles and a relatively intense reticulation (Fig. 2a, b).

The length of the dorsal fenestra is larger than half the total length of the floatoblast. The annulus is smooth, without tubercles, occasionally with moderate nodulation and some large tubercles on the periphery, around the fenestrae especially on the ventral side (Fig. 2a, b). The measurements of the dorsal fenestra are 158.3 ± 12.40 (130.9–176.4) µm in length and 137.5 ± 10.18 (105.5–163.7) µm in width, while the ventral fenestra measures 216.4 ± 14.56 (187.5–247.3) µm in length and 179.2 ± 12.45 (141.2–188.7) µm in width. The suture between the valves is a single cord with tubercles on both sides (Fig. 2c). A section of the annulus shows circular pores with filiform projections connecting the gas chambers (Fig. 2d).

Discussion

These new records of *P. repens* are the first certain record of a freshwater bryozoan species in Cuba; only *Plumatella* sp. was reported on the island previously, without specifying a locality (Collado et al. 1984). This extends the distribution range of the species in the Caribbean area (Table 1; Fig. 3), with a single record in Puerto Rico so far (Osburn 1940; Rogick and Brown 1942; Lacourt 1968). The presence of *P. repens* on the island of Cuba is consistent with the existence of records of the species in the insular Caribbean and the cosmopolitan distribution of this species (Wood 2002; Massard and Geimer 2008a, 2008b).

The ecology of *P. repens* in Cuba and Puerto Rico is associated with permanent freshwater ecosystems and coastal wetlands with highly mineralized waters caused by the predominant limestone lithology, and influenced by seawater mixing within an oligohaline range (0.5–5‰). The species also experiences a wide range of nutrient concentrations (nitrates, phosphates) and high levels of dissolved organic matter (Ferrera et al. 1996, 1999; Kwak et al. 2007; Molerio-León and Parise 2008).

Plumatella repens is present in the La Niña Bonita Reservoir, which has waters of 798 µS cm^{-1} conductivity, pH 8.5, oxic conditions – with dissolved oxygen levels of 80.9 mg l^{-1} – and low concentration of nutrients including both orthophosphates (< 0.10 mg l^{-1}) and inorganic nitrogen (< 0.10 mg l^{-1}) (Valdés et al. 1996). However, a decrease in water quality in the reservoir was documented during the 1990s due to an increase in organic and sewage discharges (Valdés et al. 1996). The Piedras River in Puerto Rico, where *P. repens* was also recorded (Osburn 1940; Rogick and Brown 1942; Lacourt 1968), is a limestone-

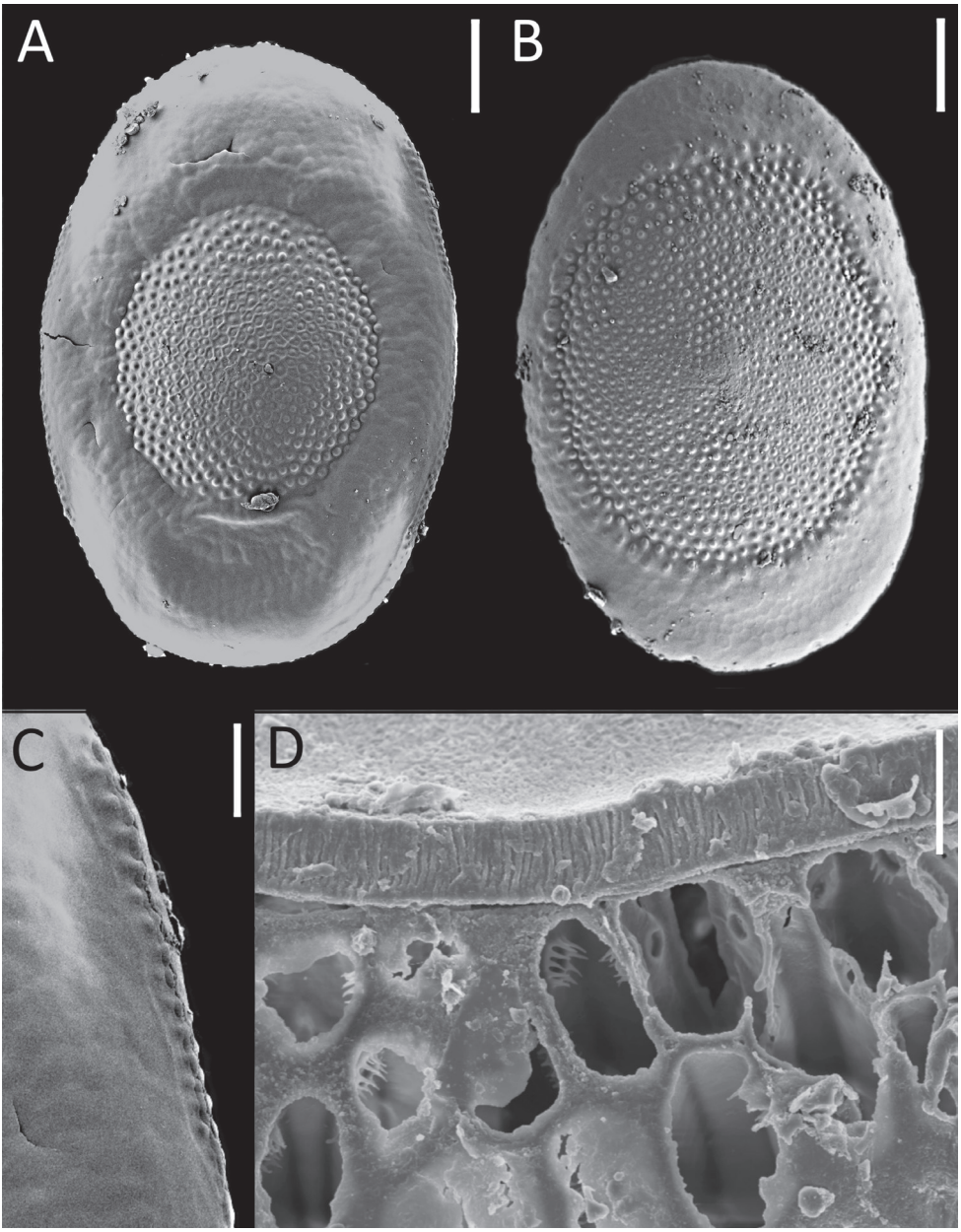

Figure 2. *Plumatella repens* (Linnaeus, 1758), floatoblast from La Niña Bonita Reservoir and the Ciénaga de Zapata Swamp (Cuba), SEM: **A** View of dorsal valve **B** View of ventral valve **C** Suture between valves is a single cord with a row of low tubercles on either side **D** Section of the annulus showing the connection between gas chambers, with circular pores with filiform extensions along the border. Scale bars: 50 µm (**A**, **B**); 10 µm (**C**); 5 µm (**D**).

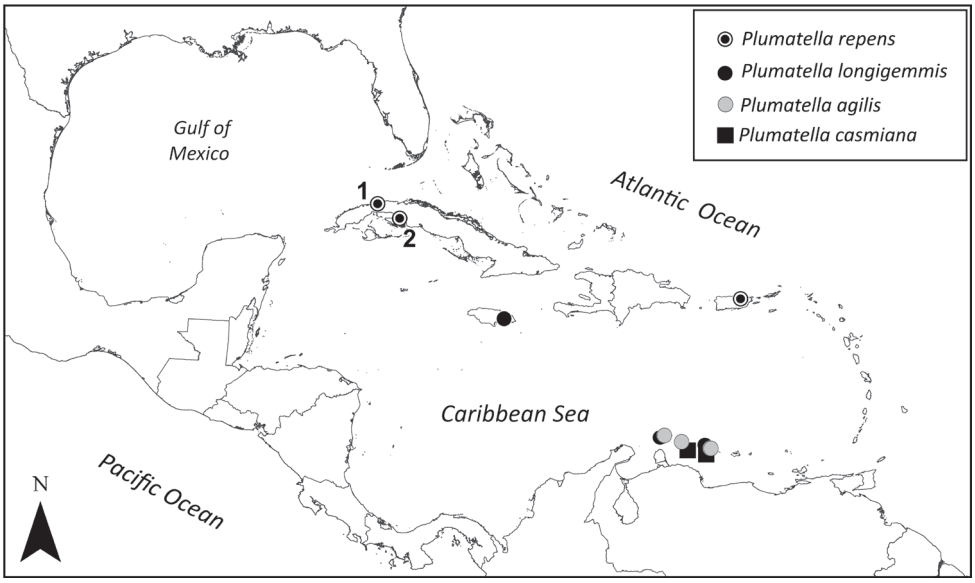

Figure 3. Geographical distribution of *Plumatella* species in the insular Caribbean area, indicating the new records of *P. repens* in Cuba (**1**) La Niña Bonita Reservoir and (**2**) Ciénaga de Zapata Swamp.

dominated basin with 452 µS cm^{-1} conductivity, pH 7.50, and 168 mg l^{-1} total hardness, although with low oxygen concentration (7.49 mg l^{-1}), average nitrate levels (0.2 mg l^{-1}), and high phosphate levels (0.49 mg l^{-1}) derived from organic contamination of anthropogenic origin (Kwak et al. 2007). *Plumatella repens* is also present in the Ciénaga de Zapata Swamp – this coastal wetland has great spatial heterogeneity due to marine intrusion and freshwater springs, and as a result the conductivity range fluctuates between 600 and 2400 µS cm^{-1} from the innermost zones towards the coastal zones (Fagundo et al. 1992a; Rodríguez et al. 1992; Ferrera et al. 1996; Morell et al. 1997).

The conductivity ranges of *Plumatella repens* in the island of Cuba are similar to those documented for continental populations in the north coast of the Gulf of Mexico (Dendy 1963; McCullough and Reed 1987) (Fig. 3), such as Lake Griffin (Florida, U.S.A.), with 290 µS cm^{-1} (Putnam et al. 1972; Taticchi et al. 2009, 2011) and Lake Texoma (Texas, U.S.A.), with 750–1200 µS cm^{-1} (Sublette 1953, 1955, 1957; Gido and Matthews 2000). In addition, populations of P. re*pens* have been recorded in the mouths of the Sabine and Neches rivers, in the coast of Louisiana, in salinities fluctuating between 38 and 4000 µS cm^{-1}, sometimes forming small-sized colonies with the brackish-water bryozoan *Victorella pavida* Saville-Kent, 1870 (Wurtz and Roback 1955; Everit 1975; Curry et al. 1981). Similar conductivity ranges have also been documented for *P. repens* in Europe, in the Mediterranean coasts of the Iberian Peninsula, where its presence has been cited in brackish coastal rivers and wetlands with conductivity values reaching up to 2519 µS cm^{-1} and even 3620 µS cm^{-1} (Margalef Mir 1953; Rueda et al. 2001; Rueda et al. 2013; Rueda et al. 2016). Also, Wood and Okamura (2005) mention that *Plumatella repens* can tolerate wide ranges of salinity, from freshwater to oligohaline in the British Isles and continental Europe.

Table 1. Records of *Plumatella* species in the Caribbean Islands area including a description of the localities.

Species	Locality	Island	Reference
Plumatella agilis (Marcus, 1942) (= *Hyalinella agilis* (Marcus, 1942))	Tanki di Cas Klein St. Joris, rather few algae. Date: 06/09/1936. Chlorinity: 1980 mg Cl l^{-1}.	Curaçao	Lacourt (1955, 1968)
	Tanki Monpos, Hato, algae temporary or semi-permanent pools. Date: 11/09/1936. Chlorinity: 310 mg Cl l^{-1}.	Curaçao	Lacourt (1955, 1968)
	Pos di Wanga, Middle Curaçao, few algae temporary or semi-permanent pools. Date: 09/11/1936. Chlorinity: 260 mg Cl l^{-1}.	Curaçao	Lacourt (1955, 1968)
	Tanki Martha Koosje, Middle Curaçao, some algae temporary or semi-permanent pools. Date: 24/07/1948. Chlorinity: 320 mg Cl l^{-1}.	Curaçao	Lacourt (1955, 1968)
	Pos Ariba, Dokterstuin, many algae temporary or semi-permanent pools. Date: 27/10/1937. Chlorinity: 710 mg Cl l^{-1}.	Curaçao	Lacourt (1955, 1968)
	Tanki Martha Koosje, Middle Curaçao, some algae temporary or semi-permanent pools. Date: 24/08/1948. Chlorinity: 320 mg Cl l^{-1}.	Curaçao	Lacourt (1955, 1968)
	Tanki Leendert, few algae pond, semi-permanent. Date: 16/12/1936. Chlorinity: 35 mg Cl l^{-1}.	Aruba	Lacourt (1955, 1968)
	Pos Bronswinkel, overflowing pool, possibly permanent, crowded with algae. Date: 27/03/1937. Chlorinity: 350 mg Cl l^{-1}.	Bonaire	Lacourt (1955, 1968)
	Pos Frances, Punt Vierkant, small well in rock crevice, semi-permanent, some algae. Date: 31/03/1937. Chlorinity: 540 mg Cl l^{-1}.	Bonaire	Lacourt (1955, 1968)
	Tanki Onima (Sta. 46), on shore of muddy pond, temporary, few algae. Date: 13/11/1936. Chlorinity: 40 mg Cl l^{-1}.	Bonaire	Lacourt (1955, 1968)
Plumatella casmiana Oka, 1907 (= *Plumatella annulata* (Howata & Toriumi, 1940))	Pos Europa, Dokterstuin, pool, semi-permanent, many algae. Date: 27/10/1936. Chlorinity: 470 mg Cl l^{-1}.	Curaçao	Lacourt (1955, 1968)
	Pos di Cas, well, permanent, many algae. Date: 15/11/1936. Chlorinity: 400 mg Cl l^{-1}.	Klein Bonaire	Lacourt (1955, 1968)
Plumatella longigemmis Annandale, 1915 (= *Hyalinella osburni* (Rogick & Brown, 1942))	Tanki Mon Plaisir, Oranjestad, pool, temporary. Date: 15/12/1936. Chlorinity: 60 mg Cl l^{-1}.	Aruba	Lacourt (1955, 1968)
	Tanki di Westpunt, pool, temporary, algae. Date: 09/12/1936. Chlorinity: 80 mg Cl l^{-1}.	Aruba	Lacourt (1955, 1968)
	Tanki di Goudmijn Tibushi, Westpunt, puddle, temporary, very few algae. Date: 09/12/1936. Chlorinity: 170 mg Cl l^{-1}.	Aruba	Lacourt (1955, 1968)
	Tanki Onima, muddy pond, temporary, few algae. Date: 13/11/1936. Chlorinity: 40 mg Cl l^{-1}.	Bonaire	Lacourt (1955, 1968)
	Waterworks of Kingston. Date: 15/06/1946.	Jamaica	Lacourt (1968)
Plumatella repens (Linnaeus, 1758)	Stones in the stream Las Piedras.	Puerto Rico	Osburn (1940); Rogick and Brown (1942); Lacourt (1968)
	La Niña Bonita reservoir, freshwater, permanent. Date: 1-12/05/2019. Chlorinity: 66 mg Cl l-1.	Cuba	This study
	Ciénaga de Zapata swamp, freshwater to brackish, permanent. Date: 1-12/05/2019. Chlorinity: 305 mg Cl l^{-1}.	Cuba	This study
Plumatella sp.	Without specify locality	Cuba	Collado et al. (1984)
	Without specify locality	Trinidad	Collado et al. (1984)

The semi-arid Lesser Antilles, unlike the Greater Antilles, have ephemeral wetlands of small extension, subjected to strong marine salinization and organic discharges due to high anthropic pressure (Van Sambeek et al. 2000; Scalley 2012). Lacourt (1955)'s study on freshwater bryozoan species in the Leeward Islands, despite the scarce ecological data, showed that the temporal stability of aquatic ecosystems and the degree of salinization are factors of great importance for the distribution of freshwater bryozoan species (Fig. 3). In the Leeward Islands, *P. longigemmis* and *P. agilis* inhabit ephemeral pools, while *P. casmiana* is found only in permanent pools (Table 1).

Plumatella longigemmis appears in freshwater environments with little saline influence (87.5 ± 57.37 (40–170) mg Cl l^{-1}), generally under 115 mg Cl l^{-1} (fresh water), while *P. casmiana* is present in waters with mild saline influence (435.0 ± 49.50 (400–470) mg Cl l^{-1}), and *P. agilis* is present in waters with a wide range of saline influence (486.5 ± 562.05 (35–1980) mg Cl l^{-1}) (Table 1).

Plumatella repens is a generalist species with a wide ecological range that can tolerate mild salinization levels; however, the ephemeral nature of these wetlands could constitute the main limitation for its distribution in the Lesser Antilles and could explain the greater affinity between the Greater Antilles and the Nearctic zone of the Gulf of Mexico in the distribution of this species (Fig. 3) (Wood 2002; Massard and Geimer 2008b).

Conclusions

These new findings of populations of *P. repens* in Cuba constitute the first record of a freshwater bryozoan species on the island, expanding the geographical distribution of this species to the Greater Antilles. The existence of permanent freshwater wetlands in Cuba, unlike in the Lesser Antilles, provides a stable habitat for the species.

Acknowledgements

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CORRIGENDA

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Corrigenda: *Hanah*, a replacement name for *Hana* Lau, Stokvis, Ofwegen & Reimer, 2018 (preoccupied name)

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Abstract

This is an addendum to the availability of the generic name *Hana* Lau, Stokvis, Ofwegen & Reimer, 2018 within the octocoral family Arulidae. Here, the replacement name *Hanah* is proposed, as *Hana* Lau, Stokvis, Ofwegen & Reimer, 2018 is a junior homonym to senior homonym *Hana* Kukalova-Peck, 1975, a genus within the family Hanidae of the Palaeozoic insect order Megasecoptera.

Keywords

Arulidae, *Hana*, homonymy, Octocorallia, replacement name, Stolonifera

Class Anthozoa
Subclass Octocorallia
Order Alcyonacea
Suborder Stolonifera
Family Arulidae

Genus *Hanah* nom. nov.

Hana Lau, Stokvis, Ofwegen & Reimer, 2018. ZooKeys 790: 1–19. (Anthozoa: Octocorallia: Alcyonacea: Stolonifera: Arulidae). Preoccupied by *Hana* Kukalova-Peck, 1975. Psyche 82: 1–19. (Insecta: Pterygota: Palaeoptera: Palaeodictyopteroidea: Megasecoptera: Hanidae).

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Following the publication of Lau et al. (2018), it has been brought to our attention that the genus name *Hana* is a preoccupied genus name within the Palaeozoic insect order Megasecoptera (Kukalova-Peck 1975) as documented in Nomenclator Zoologicus (1975: 205). The name *Hana* Lau, Stokvis, Ofwegen & Reimer, 2018 is thus invalid under the laws of homonymy, being a junior homonym of *Hana* Kukalova-Peck, 1975.

In accordance with article 60 of the International Code of Zoological Nomenclature, fourth edition (1999), the junior homonym *Hana* Lau, Stokvis, Ofwegen & Reimer, 2018 will be replaced by *Hanah* nom. nov.

According to this application, the following new combinations within *Hanah* (Lau, Stokvis, Ofwegen & Reimer, 2018) are *Hanah hanagasa* (Lau, Stokvis, Ofwegen & Reimer, 2018), comb. nov. and *Hanah hanataba* (Lau, Stokvis, Ofwegen & Reimer, 2018), comb. nov.

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