CHECKLIST



Checklist of the helminth parasites of the genus Profundulus Hubbs, 1924 (Cyprinodontiformes, Profundulidae), an endemic family of freshwater fishes in Middle-America

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Abstract

From December 2012 to November 2014, 267 fish belonging to the family Profundulidae (representing nine of the 11 species of the genus *Profundulus*) were collected in 26 localities of Middle-America, across southern Mexico, Guatemala, and Honduras, comprising the distribution range of the genus, and analyzed for helminth parasites. Additionally, a database with all ten available published accounts of the helminth parasite fauna of this genus (the only genus within the family) was assembled. Based on both sources of information, a checklist containing all the records was compiled as a tool to address future questions in the areas of evolutionary biology, biogeography, ecology and phylogeography of this host-parasite association. The helminth parasite fauna of this fish group consists of 20 nominal species, classified in 17 genera and 14 families. It includes six species of adult digeneans, five metacercariae, two monogeneans, one adult cestode, three adult nematodes and three larval nematodes. The profundulid

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fishes are parasitized by a specialized group of helminth species (*e.g. Paracreptotrema blancoi sensu* Salgado-Maldonado et al. (2011b), *Paracreptotrema profundulusi* Salgado-Maldonado, Caspeta-Mandujano & Martínez Ramírez, 2011, *Phyllodistomum spinopapillatum* Pérez-Ponce de León, Pinacho-Pinacho, Mendoza-Garfias & García-Varela, 2015, *Spinitectus humbertoi* Mandujano-Caspeta & Moravec, 2000, *S. mariaisabelae* Caspeta-Mandujano Cabañas-Carranza & Salgado-Maldonado, 2007 and *Rhabdochona salgadoi* Mandujano-Caspeta & Moravec, 2000), representing the core helminth fauna that are not shared with other Middle-American fish species.

Keywords

Killifish, Profundulidae, Middle-America, Digenea, Monogenea, Cestoda, Nematoda

Introduction

The information gathered regarding the composition of the helminth parasites of freshwater fishes of Mexico has increased in recent years (Pérez-Ponce de León and Choudhury 2010). The large number of published papers contributing to the inventory of the helminth parasite fauna of fish hosts in the last decades allowed Luque and Poulin (2007) to suggest that Mexico stands out as a hotspot of parasite diversity in freshwater fishes. The species composition of the helminth fauna of some freshwater fish families, such as the Cichlidae and Goodeidae, is well known (Vidal-Martínez et al. 2001; Martínez-Aquino et al. 2014).

The distribution of the Profundulidae extends along the Atlantic and Pacific Ocean slopes of southern Mexico, Guatemala, El Salvador and Honduras (Miller 1955; Miller et al. 2005; Doadrio et al. 1999; Matamoros and Schaeffer 2010; Matamoros et al. 2012); from the Río Aguacatillo (a tributary of the Laguna Tres Palos) in Guerrero, Mexico to the Río Nacaome in Honduras, and on the Atlantic slope from the Río Quiotepec (the Río Papaloapan drainage basin) in Oaxaca, to the Río Ulúa, Honduras (Martínez-Ramírez et al. 2004; Matamoros et al. 2012). The family contains a single genus (Profundulus Hubbs, 1924), the current species composition of which is a matter of debate, since some authors recognize only eight valid species (Matamoros and Schaeffer 2010; Matamoros et al. 2012), whereas others (see Doadrio et al. 1999: Martínez-Ramírez et al. 2004) also recognize P. balsanus Ahl, 1935 as a valid species, as well as two undescribed taxa, Profundulus sp. 1, and Profundulus sp. 2, which are currently being described by one of us (EMR). In addition to this, a molecular analysis of nuclear and mitochondrial genes, which will be published elsewhere, corroborates the validity of these three species (Ornelas-García, pers. comm.). Irrespective of the species composition, all Profundulus species represent an endemic lineage in Middle-America that has probably inhabited this region since the Pliocene and perhaps even the Miocene (Miller 1955; Doadrio et al. 1999; González-Diaz et al. 2005; Matamoros and Schaeffer 2010).

Records of the helminth parasite fauna of this family began with Caspeta-Mandujano and Moravec (2000), who described two nematode species, *Spinitectus humbertoi* Mandujano-Caspeta & Moravec, 2000 and *Rhabdochona salgadoi* Caspeta-Mandujano & Moravec, 2000, from the intestine of *Profundulus labialis* (Günther, 1866) in Inzcuinatoyac, Guerrero, Mexico. To date, ten studies have been published regarding some aspects of the helminth parasite fauna of profundulids, including descriptions of new species, inventories in particular localities and analyses of the parasite community structure of particular host species (Caspeta-Mandujano et al. 2007; Velázquez-Velázquez et al. 2011; Salgado-Maldonado et al. 2011a, b; Pinacho-Pinacho et al. 2014; Salgado-Maldonado et al. 2015; Velazquez-Velazquez et al. 2015; Pérez-Ponce de León et al. 2015).

As a continuation of effort to provide a more complete inventory of the helminth parasite fauna of freshwater fishes in this geographical region, intensive samplings were conducted during the last few years of these killifishes throughout their ranges of distribution in an attempt to obtain empirical and robust data to enable further studies of this host-parasite association from a phylogenetic, phylogeographical and biogeographical perspective, with the aim of understanding the mechanisms that have shaped the evolutionary and biogeographical history of these fishes and their parasites in Middle-America. The main objectives of this research were (1) to compile all the available published accounts on the helminth fauna of profundulid fishes, and (2) to incorporate new data derived from our own survey work of the last few years.

Material and methods

Data compilation. A dataset of all published records of the helminth species reported from members of the family Profundulidae in Middle-America was compiled. The keywords "Parasit(e)*AND *Profundulus*" were used to conduct searches through the ISI Web of Science. All those studies whose datasets provided taxonomic information on the helminth taxa found in a sample of individual hosts were considered.

Current research. Original data from our own studies of the last two years were included. From December 2012 through to November 2014, 267 individual fish belonging to nine species of Profundulus, i.e. P. balsanus; P. candalarius Hubbs, 1924; P. guatemalensis (Günther, 1866); P. hildebrandi Miller, 1950; P. kreiseri Matamoros, Schaefer, Hernández & Chakrabarty, 2012; P. labialis; P. portillorum Matamoros & Schaefer, 2010; P. punctatus (Günther, 1866); and Profundulus sp. 2 (sensu Doadrio et al. 1999), were examined for helminth parasites. Fish were collected with seine nets and electrofishing in 26 localities of southern Mexico, Guatemala and Honduras (Fig. 1; Table 1). Fish were kept alive and examined for helminths no more than 4 h after their capture. Fish were killed with an overdose of anesthetic and placed in Petri dishes, and immediately examined for helminths. All the external surfaces, viscera and musculature of each host were examined under a stereomicroscope, and the helminths found were counted in situ. Adult digeneans and metacercariae, monogeneans, cestodes and nematodes were fixed in hot 4% neutral formalin, and additional specimens were fixed in 100% ethanol for future molecular studies. Some monogeneans were mounted in a mixture of glycerine-ammonium picrate (Ergens 1969) and in Gray-Wess medium (Vidal-Martínez et al. 2001) to study their sclerotized structures. Digeneans, cestodes and monogeneans used for morphological studies



Figure 1. A Map of Middle-America indicating the localities where specimens of Profundulidae have been have been examined for helminth parasites. Colors and symbols correspond for each species of *Pro-fundulus* **B** Zoom of an endorrheic basin in San Cristóbal de la Casas, Chiapas, Mexico showing localities where the endemic fish *P. hildebrandi* was collected.

were stained with Mayer's paracarmine or iron acetocarmine, dehydrated using a graded alcohol series, cleared in methyl salicylate and mounted as permanent slides in Canada balsam. Nematodes were cleared with glycerine for light microscopy and stored in 70% ethanol. Voucher specimens of some helminth species were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City, Mexico. Additionally, vouchers of *Bothriocephalus acheilognathii* Yamaguti, 1934 were deposited in the Helminthological Collection of the Institute of Parasitology (IPCAS), Biology Centre ASCR, České Budějovice, Czech Republic (accession numbers IPCAS C-15). Parameters of infection such as prevalence (% infected) and mean intensity of infection (the average number of a particular species of parasite among the infected members of a particular host species) were calculated following Bush et al. (1997).

Results

The data analysis of both the bibliographic information and the survey work shows that 11 species of *Profundulus* (including undescribed species) studied for helminths,

Table 1. Localities in Mexico, Guatemala, El Salvador and Honduras where at least one helminth species has been recorded as a parasite of *Profundulus*. Localities marked with an asterisk (*) were sampled in this study. Collection sites (CS), locality (four letters code), geographical coordinates, country sampled and references are included. The collection site (CS) and locality code correspond with the localities referred in Table 2.

CS	Locality (code)	Geographica	l coordinates	Country	References
		Ν	W		
(1)	Arroyo Inzcuinatoyac (Inzc)	17°21'39"	99°44'00"	México	Caspeta-Mandujano and Moravec (2000)
(2)	Río Suchiapa (Such)	16°20'06"	93°27'19"	México	Caspeta-Mandujano et al. (2007)
(3)	Ecosur (Ecos)	16°42'55"	92°37'28"	México	Velázquez-Velázquez et al. (2011)
(4)	La Albarrada (Alba)	16°42'37"	92°37'32"	México	Velázquez-Velázquez et al. (2011)
(5)	5 de Marzo (5mar)	16°42'34"	92°38'14"	México	Velázquez-Velázquez et al. (2011)
(6)	El Puente (Puen)	16°43'59"	92°36'54"	México	Velázquez-Velázquez et al. (2011)
(7)	Arroyo Chamula (Cham)	16°44'52"	92°39'22"	México	Velázquez-Velázquez et al. (2011)
(8)	Peje de Oro (Peor)	16°44'48"	92°37'00"	México	Velázquez-Velázquez et al. (2011)
(9)	El Arcotete (Arco)	16°45'57"	92°31'43"	México	Velázquez-Velázquez et al. (2011)
(10)	Arenal (Aren)	16°43'31"	92°34'53"	México	Velázquez-Velázquez et al. (2011)
(11)	Agua de Pajarito (Paja)	16°43'43"	92°34'44"	México	Velázquez-Velázquez et al. (2011)
(12)	Laguna Soyul (Lsoy)	16°46'01"	92°31'39"	México	Velázquez-Velázquez et al. (2011)
(13)	Río Ocotlán (Ocot)		-	México	Salgado-Maldonado et al. (2011a)
(14)	Río Chicomosuelo (Chic)	15°44'38"	92°16'50"	México	Salgado-Maldonado et al. (2011a)
(15)	Río Suchiapa, José María Garza (Rsuc)	16°36'36"	93°05'03"	México	Salgado-Maldonado et al. (2011a)
(16)	Río San Juan, puente El Tablón, Villa Flores (Saju)	16°21'01"	93°30'56"	México	Salgado-Maldonado et al. (2011a)
(17)	Piedra Labrada (Labr)	18°58'54"	99°14'12"	México	Salgado-Maldonado et al. (2011b)
(18)	Río La Soledad Carrizo (Carr)	16°25'0.4"	97°40'12.9"	México	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014)
(19)	Río San José de las Flores (Flor)	16°24'21.5"	97°44'22.6"	México	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014)
(20)	Río Santa Cruz Flores Magón (Fmag)*	16°21'6.1"	97°45'38.3"	México	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014) Pérez-Ponce de León et al. (2015) This study
(21)	Río Pichuaca (Pich)*	16°05'34.2"	97°24'18.1"	México	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014) This study
(22)	Río La Reforma (Refo)	16°08'33.5"	97°08'41.6"	México	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014)
(23)	Río Pueblo Viejo (Viej)*	16°06'22.3"	97°03'47.8"	México	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014) Pérez-Ponce de León et al. (2015) This study
(24)	Río Santa María Huatulco (Huat)*	15°50'14.2"	96°19'30.8"	México	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014) This study
(25)	Río Macuta (Macu)	-	-	México	Salgado-Maldonado et al. (2011b)
(26)	Río Templo, San Juan del Río (Sjri)*	16°53'56.3"	96°09'57.3"	México	Salgado-Maldonado et al. (2011b) Pérez-Ponce de León et al. (2015) This study
(27)	Arroyo Ojo de Agua (Ojag)	16°13'38.6"	95°49'36.6"	México	Salgado-Maldonado et al. (2011b)

CS	Locality (code)	Geographica	l coordinates	Country	References
		N	W		
(28)	Río La Laca (Rlac)	17°14'09.3"	98°39'55.7"	México	Salgado-Maldonado et al. (2014)
(29)	Río Cahoapan (Caho)	17°16'37.8"	99°35'04.7"	México	Salgado-Maldonado et al. (2014)
(30)	Río Tamarindo (Tama)	17°00'36.5"	99°06'0.8"	México	Salgado-Maldonado et al. (2014)
(31)	Río del Aguacate (Agua)	16°07'19"	97°8'22.8"	México	Salgado-Maldonado et al. (2014)
(32)	Arroyo los Sabinos (Sabi)	16°25'39.9"	97°4'28.9"	México	Salgado-Maldonado et al. (2014)
(33)	Río Chacalapa (Chac)	15°55'54.8"	95°56'00.3"	México	Salgado-Maldonado et al. (2014)
(34)	Río Chicaguaxtla (Chic)	17°03'30.30"	97°51'32.52"	México	Salgado-Maldonado et al. (2014)
(35)	Río Chico (Rchi)	16°55'34.50"	96°12'27.42"	México	Salgado-Maldonado et al. (2014)
(36)	Amatenango del Río (Amri)	16°31'22.2"	92°25'10.7"	México	Salgado-Maldonado et al. (2014)
(37)	Río Grande de Comitán (Rcom)	16°16'49.9"	92°07'21.1"	México	Salgado-Maldonado et al. (2014)
(38)	Arroyo ECOSUR (Aeco)	16°42'27.3"	92°36'54.8"	México	Salgado-Maldonado et al. (2014)
(39)	Arroyo Teopisca (Ateo)	16°33'13.7"	92°28'34.9"	México	Salgado-Maldonado et al. (2014) Velazquez-Velazquez et al. (2015)
(40)	Río Rancho San Antonio (Rsan)	16°58'30.9"	93°03'44.7"	México	Salgado-Maldonado et al. (2014)
(41)	Arroyo Tres Picos (Atpi)	17°03'28.3"	93°11'50.7"	México	Salgado-Maldonado et al. (2014)
(42)	Río Nandalumi (Rnan)	16°43'18.4"	92°58'52.4"	México	Salgado-Maldonado et al. (2014)
(43)	Arroyo Ojo de Agua, El Canelar (Cane)	16°32'08"	92°55'02.5"	México	Salgado-Maldonado et al. (2014)
(44)	Río Nil (Rnil)	14°33'54.4"	91°43'25.4"	Guatemala	Salgado-Maldonado et al. (2014)
(45)	Río el Cantil (Cant)	14°21'22.6"	90°48'30.4"	Guatemala	Salgado-Maldonado et al. (2014)
(46)	Arroyo El Platanar (Apla)	14°36'58.6"	90°46'37.9"	Guatemala	Salgado-Maldonado et al. (2014)
(47)	Río Cauca (Rcau)	13°46'42.6"	89°51'40.8"	El Salvador	Salgado-Maldonado et al. (2014)
(48)	Río Cauca (Cauc)	13°46'41.6"	89°46'41.67"	El Salvador	Salgado-Maldonado et al. (2014)
(49)	Quebrada Los Tecomates (Qtec)	14°18'11.3"	89°09'40.8"	El Salvador	Salgado-Maldonado et al. (2014)
(50)	Río Nonoalpa (Nono)	14°17'23.2"	89°09'10.7"	El Salvador	Salgado-Maldonado et al. (2014)
(51)	Río Ojojona (Ojoj)	13°55'43.7"	87°17'40"	Honduras	Salgado-Maldonado et al. (2014)
(52)	Lepaterique (Lepa)	14°03'42.9"	87°27'58.6"	Honduras	Salgado-Maldonado et al. (2014)
(53)	Lepaterique (1Lep)	14°04'14.4"	87°25'56.9"	Honduras	Salgado-Maldonado et al. (2014)
(54)	El Platanar, Putla de Guerrero (Plat)*	16°44′55″	97°59′32″	México	This study
(55)	Río San José, Santiago Jamiltepec (Jami)*	16°24′19″	97°44′20″	México	This study
(56)	Río San Juan, Cristobal Obregón (Obre)*	16°21′00″	93°30′54″	México	This study
(57)	Río Suchiapan, La Esperanza (Espe)*	16°23′27.60″	93°17′24″	México	This study
(58)	Río Pedregal, Tres Picos (Tpic)*	15°55′1.2″	93°32′45.6″	México	This study
(59)	Río Huixtla (Huix)*	15°10′18″	92°25′24″	México	This study
(60)	El Triunfo (Triu)*	15°20′44″	92°32′30″	México	This study
(61)	Río Nueva Francia (Fran)*	15°22′7.58″	92°35′20.2″	México	Pérez-Ponce de León et al. (2015) This study
(62)	Río Nahualate (Nahu)*	14°26′44″	91°22′56″	Guatemala	This study
(63)	Río Primavera (Prim)*	14°22′19.20″	91°09′60″	Guatemala	This study
(64)	Río Escuintla (Escu)*	14°19′41.51″	91°42′57.35″	Guatemala	This study
(65)	Río Las Cabezas, Saranate (Sara)*	14°44′23″	90°04′52″	Guatemala	This study
(66)	Puente Sansare (Sans)*	14°44′52″	90°06′33″	Guatemala	This study
(67)	Río Hondo (Rhon)*	15°03′55.50″	89°35′48.28″	Guatemala	This study

CS	Locality (code)	Geographica	l coordinates	Country	References
		N	W		
(68)	Arroyo en Hidroeléctrica Chamelecón (Cham)*	15°11′51.60″	89°36′57.60″	Honduras	This study
(69)	Quebrada El Paraiso (Qpar)*	15°01′26″	88°59′32″	Honduras	This study
(70)	Los Potrerillos (Lpot)*	14°32′31″	87°52′55″	Honduras	This study
(71)	Río San Carlos (Rcar)*	16°19′10″	91°58′06″	México	This study
(72)	Río La Gloria (Lglo)*	16°30′01″	92°26′01″	México	This study
(73)	Arroyo Moxviquil (Moxv)*	16°54′9.00″	92°37′50″	México	This study
(74)	Arroyo Peje de Oro (Poro)*	16°44′48″	92°36′60″	México	This study

and that the list of helminth parasites of fish of this genus consists of 20 species classified in two taxonomic groups: Platyhelminthes (six adult digeneans, five metacercariae, two monogeneans and one adult cestode) and Nematoda (three adults and three larvae). Interestingly, no acanthocephalans and no larval cestodes are part of the helminth fauna of this fish group across its geographical distribution. Most taxa were identified to species level, except for larval stages which lacked the diagnostic characteristics present only in adult forms which are found in fish-eating birds (or freshwater turtles in the case of *Spiroxys* sp.). To better visualize the information from the checklist, the results are presented in two tables. Table 2 shows a parasite-host list. Species of parasites are organized by developmental stage, either as adults or larvae, and ordered alphabetically by family name. Species within each family are then listed alphabetically followed by their authority. The host-parasite list (Table 3) is organized alphabetically. Within each fish species, helminth parasites are listed alphabetically by taxonomic group, with their developmental stage indicated in parentheses.

Digeneans exhibit the highest species richness (11 species), followed by nematodes (six species) and monogeneans (two species) (Table 2). Based on the observed hostspecificity, at least six of the 12 adult helminth taxa listed in this work, i.e. Paracreptotrema blancoi sensu Salgado-Maldonado et al. (2011b), P. profundulusi Salgado-Maldonado, Caspeta-Mandujano & Martínez Ramírez, 2011, Phyllodistomum spinopapillatum Pérez-Ponce de León, Pinacho-Pinacho, Mendoza-Garfias & García-Varela, 2015, Spinitectus humbertoi Mandujano-Caspeta & Moravec, 2000, S. mariaisabelae Caspeta-Mandujano Cabañas-Carranza & Salgado-Maldonado, 2007 and Rhabdochona salgadoi Mandujano-Caspeta & Moravec, 2000, have only been recorded as parasites of profundulids and can be considered as members of the 'core' helminth fauna (in an historical biogeographical sense, not to be confused with the ecological "coresatellite" species concept, see Pérez-Ponce de León and Choudhury 2002). The cestode Bothriocephalus acheilognathi Yamaguti, 1934 has successfully infected some species of *Profundulus*; this is an introduced species that is commonly found in several freshwater fishes in North and Central America as a result of the introduction of cyprinids (carps) for aquaculture (see Choudhury et al. 2013). The digenean Centrocestus formosanus Nishigori, 1924 also represents a species that was introduced in North America, and perhaps Middle-America, through the introduction of its snail host, Melanoides tuber-

correspond to those in Table 1. Key: N = number of examined	viation, CNHE = catalog numbers of specimens deposited in the	
Table 2. Parasite-host list of <i>Profundulus</i> in Middle-America. Locality abbreviations (CS-Code) correspond to	hosts in each study, Site(s) of infection, P = Prevalence, MI ± SD = Mean Intensity ± standard deviation, CNH	collection.

elminth taxa	Host (s)	Locality (CS-Code)	z	Site (s) of infection	P (%)	MI±SD	CNHE (Number of specimens)	Reference
ult Trematoda								
mily Allocreadiidae Looss, 02								
<i>tracreptotrema blancoi sensu</i> lgado-Maldonado et al. 011b). (Fig. 2A).	P. balsanus	(17-Labr)	29	Intestine	50	4.8 ± 4.2	7687 (15)	Salgado-Maldonado et al. (2011b)
		(18-Carr)	25	Intestine	8	1.0 ± 0	7688 (1)	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014)
		(19-Flor)	20	Intestine	25	1.0 ± 0	7689 (3)	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014)
		(20-Fmag)	18	Intestine	44.4	2.2 ± 1.03	7690 (4)	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014)
			8	Intestine	75	1.5 ± 0.5		This study
		(21-Pich)	22	Intestine	59	2.0 ± 1.3	7691 (12)	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014)
			4	Intestine	100	1.5 ± 0.5		This study
		(22-Refo)	20	Intestine	20	1.7 ± 0.9	7692 (3)	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014)
		(23-Viej)	20	Intestine	10.0	1.5 ± 0.7	7686 (1)	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014)
			10	Intestine	70	6.4 ± 8.8		This study
		(24-Huat)	7	Intestine	71.4	1.8 ± 0.4	7694 (3)	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014)
			5	Intestine	١	١		This study
		(29-Caho)		Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(30-Tama)		Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(31-Agua)		Intestine	NR	NR		Salgado-Maldonado et al. (2014)

Helminth taxa	Host (s)	Locality (CS-Code)	z	Site (s) of infection	P (%)	MI±SD	CNHE (Number of specimens)	Reference
		(54-Plat)	4	Intestine	100	4 ± 4.2		This study
		(55-Jami)	7	Intestine	57.14	1.2 ± 0.5		This study
	P. oaxacae	(25-Macu)	37	Intestine	37.8	2.6 ± 2.1	7693 (5)	Salgado-Maldonado et al. (2011b)
		(32-Sabi)		Intestine	NR	NR	9286 (2)	Salgado-Maldonado et al. (2014)
	Profundulus sp. 2	(26-Sjri)	43	Intestine	30.2	2.5 ± 2.2	7684 (7)	Salgado-Maldonado et al. (2011b)
			2	Intestine	100	3.5 ± 0.7		This study
		(35-Rchi)		Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	P. punctatus	(27-Ojag)	30	Intestine	6.6	5.5 ± 4.9	7685 (4)	Salgado-Maldonado et al. (2011b)
		(33-Chac)		Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(42-Rnan)		Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(56-Obre)	8	Intestine	50	3 ± 1.4		This study
		(58-Tpic)	15	Intestine	13.33	3.5 ± 0.7		This study
		(59-Huix)	20	Intestine	20	2.25 ± 1.8	9803 (2)	This study
		(60-Triu)	6	Intestine	33.33	1.5 ± 0.7	9804 (2)	This study
		(61-Fran)	15	Intestine	6.66	NR		This study
		(62-Nahu)	1	Intestine	100	1 ± 0		This study
		(63-Prim)	6	Intestine	66.66	6.25 ± 3.4		This study
	P. guatemalensis	(44-Rnil)		Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(45-Cant)		Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(47-Rcau)		Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(64-Escu)	19	Intestine	50	2.1 ± 1.5		This study
		(65-Sara)	-	Intestine	100	1 ± 0		This study
		(66-Sans)	6	Intestine	100	4.8 ± 4.6		This study
	P. kreiseri	(49-Qtec)		Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(69-Qpar)	28	Intestine	14.28	1.75 ± 1.5		This study
	P. labialis	(57-Espe)	15	Intestine	6.66	NR		This study
	P. portillorum	(70-Lpot)	6	Intestine	11.11	1 ± 0		This study
	P. candalarius	(71-Rcar)	14	Intestine	78.57	4.3 ± 4.9		This study
Remarks: Specimens of Paracreptot.	trema blancoi sensu S	algado-Maldor	nado et	al. (2011b) re	epresent ai	n undescribed	species, but they requ	ire further taxonomic work.

Helminth taxa	Host (s)	Locality (CS-Code)	z	Site (s) of infection	P (%)	MI±SD	CNHE (Number of specimens)	Reference
<i>Paracreptotrema profundulusi</i> Salgado-Maldonado, Caspeta- Mandujano & Martínez Ramírez, 2011. (Fig. 2B).	Profundulus sp. 2	(26-Sjri)	43	Intestine	55.8	4 ± 5.7	7680 (1) 7681 (23)	Salgado-Maldonado et al. (2011b)
			2	Intestine	100	3.5 ± 0.7	9805 (1)	This study
		(35-Rchi)	NR	Intestine	NR	NR	9287 (1)	Salgado-Maldonado et al. (2014)
	P. punctatus	(27-Ojag)	30	Intestine	20	6.8 ± 13.8	7682 (6)	Salgado-Maldonado et al. (2011b)
		(33-Chac)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	P. balsanus	(19-Flor)	20	Intestine	2	4 ±0	7683 (4)	Salgado-Maldonado et al. (2011b) Pinacho-Pinacho et al. (2014)
		(29-Caho)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	P. oaxacae	(32-Sabi)	NR	Intestine	NR	NR	9288 (1)	Salgado-Maldonado et al. (2014)
Allocreadiidae gen. sp.	P. portillorum	(70-Lpot)	6	Intestine	11.11	1 ± 0		This study
Remarks: A single specimen was co	ollected for future m	nolecular studie	ss.					
Family Gorgoderidae Looss, 1901								
<i>Phyllodistomum inecoli</i> Razo- Mendivil, Pérez-Ponce de León & Rubio-Godoy, 2013. (Fig. 2C).	Profundulus sp. 2	(26-Sjri)	2	Urinary bladder	50	NR		Pérez-Ponce de León et al. (2015)
	P. punctatus	(56-Obre)	15	Urinary bladder	NR	NR		Pérez-Ponce de León et al. (2015)
		(61-Fran)	15	Urinary bladder	6.66	NR	9302 (1)	Pérez-Ponce de León et al. (2015)
	P. candalarius	(71-Rcar)	14	Urinary bladder	7.14	1 ± 0	9802 (1)	This study
		(72-Lglo)	22	Urinary bladder	31.81	1.5 ± 0.7	9661 (1)	This study
Remarks: This species was originall	y recorded by Pérez	-Ponce de Leó	n et al.	(2015) in fish	ies of genu	s Profundulus.		

Helminth taxa	Host (s)	Locality (CS-Code)	Z	Site (s) of infection	P (%)	MI±SD	CNHE (Number of specimens)	Reference
<i>Phyllodistomum</i> <i>spinopapillatum</i> Pérez-Ponce de León, Pinacho-Pinacho, Mendoza-Garfias & García- Varela, 2015. (Fig. 2D).	P balsanus	(18-Carr)	25	Urinary bladder	20	1 ±0	9667 (5)	Pinacho-Pinacho et al. (2014)
		(20-Fmag)	18	Urinary bladder	5.55	1 ±0	9666 (1)	Pinacho-Pinacho et al. (2014) Pérez-Ponce de León et al. (2015)
			8	Urinary bladder	25	1 ± 0	9671 (1)	This study
		(21-Pich)	22	Urinary bladder	4.54	1 ±0	9668 (1)	Pinacho-Pinacho et al. (2014)
		(22-Refo)	20	Urinary bladder	10	1 ±0		Pinacho-Pinacho et al. (2014)
		(23-Viej)	20	Urinary bladder	40	1.12 ± 0.35	9299 (1) 9300 (7)	Pinacho-Pinacho et al. (2014) Pérez-Ponce de León et al. (2015)
			10	Urinary bladder	70	1.8 ± 01.5		This study
Remarks: This species was recorded described as a new taxon by Pérez-F	l as <i>Phyllodistomum</i> Ponce de León et a	ı sp. by Pinachc I. (2015).	-Pinac	cho et al. (2014	4). Posteric	orly, based on	morphological and m	olecular evidence this species was
Family Haploporidae Nicoll, 1914								
Saccocoelioides lamothei Aguirre-Macedo & Violante- González, 2008. (Fig. 2E).	P. balsanus	(18-Carr)	25	Intestine	80	3.15 ±2.15	9806 (1)	Pinacho-Pinacho et al. (2014)
		(19-Flor)	20	Intestine	30	1.5 ± 0.83		Pinacho-Pinacho et al. (2014)
		(20-Fmag)	18	Intestine	11.11	1 ± 0		Pinacho-Pinacho et al. (2014)
			8	Intestine	12.5	1 ± 0		This study
		(55-Jami)	7	Intestine	42.85	5.3 ± 2.5		This study
Remarks: Pinacho-Pinacho et al. (2 deposited in the CNHE and molec	2014) recorded orig cular data indicate	ginally this spec that this specim	ies as (ens co	<i>Juluwiya</i> cf. <i>ci</i> rresponding w	chlidorum, iith Saccoco	but detailed t <i>velioides lamot</i>	che morphological eva bei (Andrade-Gómez)	uation of voucher specimens 2015).

Helminth taxa	Host (s)	Locality (CS-Code)	z	Site (s) of infection	P (%)	MI±SD	CNHE (Number of specimens)	Reference
Larval Trematoda								
Family Clinostomidae Lühe, 1901								
Clinostomum sp. (Fig. 2F).	P. punctatus	(13-Ocot)	12	Mesentery	8.3	1 ± 0.0	7442 (1)	Salgado-Maldonado et al. (2011a)
	1	(60-Triu)	6	Mesentery	16.66	NR		This study
	P. balsanus	(18-Carr)	25	Mesentery, Gills, eyes, Body cavity	40	2.7 ±1.94	9202 (5)	Pinacho-Pinacho et al. (2014)
		(20-Fmag)	18	Mesentery, Gills, eyes, Body cavity	5.55	1 ±0		Pinacho-Pinacho et al. (2014)
		(22-Refo)	20	Mesentery, Gills, eyes, Body cavity	10	1 ±0		Pinacho-Pinacho et al. (2014)
		(23-Viej)	20	Mesentery, Gills, eyes, Body cavity	10	1.5 ±0.70		Pinacho-Pinacho et al. (2014)
		(24-Huat)	7	Mesentery, Gills, eyes, Body cavity	42.85	1 ±0		Pinacho-Pinacho et al. (2014)
			5	Mesentery	NR	NR		This study
		(54-Plat)	4	Mesentery	25	3 ± 0		This study
		(55-Jami)	7	Mesentery	14.28	1 ± 0	9660 (1)	This study
	P. candalarius	(71-Rcar)	14	Mesentery	21.42	2 ± 0		This study
Remarks: This species was recorded (2013), the species <i>C. complanatum</i> further molecular work.	l as <i>Clinostomum c</i> n is most likely not	<i>mplanatum</i> by found in Mexid	Salgad co, anc	o-Maldonado l instead they v	et al. (201 vould corr	1a). However espond with 0	, based on recent findi <i>Clinostomum</i> sp. but th	ngs by Sereno-Uribe et al. iis needs to be determined by

Helminth taxa	Host (s)	Locality (CS-Code)	z	Site (s) of infection	P (%)	MI±SD	CNHE (Number of specimens)	Reference
Family Diplostomidae Poirier, 1886								
Diplostomidae gen. sp. (Fig. 2G).	P. balsanus	(18-Carr)	25	Mesentery	4	1 ±0		Pinacho-Pinacho et al. (2014)
		(22-Refo)	20	Mesentery	5	4 ± 0		Pinacho-Pinacho et al. (2014)
Posthodiplostomum minimum MacCallum, 1921. (Fig. 2H).	P. balsanus	(22-Refo)	20	Mesentery	25	2.6 ±1.34		Pinacho-Pinacho et al. (2014)
0		(23-Viej)	20	Mesentery	5	1 ± 0		Pinacho-Pinacho et al. (2014)
			10	Mesentery	10	2 ± 0	9807 (1)	This study
		(24-Huat)	7	Mesentery	14.28	4 ± 0		Pinacho-Pinacho et al. (2014)
			5	Mesentery	NR	NR		This study
Family Heterophyidae Leiper, 1909								
Ascocotyle (Ascocotyle) felippei Travassos, 1928. (Fig. 21).	P. balsanus	(18-Carr)	25	Heart	28	165.42 ±72.39	9199 (10)	Pinacho-Pinacho et al. (2014)
		(19-Flor)	20	Heart	20	23.75 ±21.96		Pinacho-Pinacho et al. (2014)
		(20-Fmag)	18	Heart	83.33	16.73 ±15.07		Pinacho-Pinacho et al. (2014)
		(21-Pich)	22	Heart	86.36	58.94 ±43.31	9200 (6)	Pinacho-Pinacho et al. (2014)
		(22-Refo)	20	Heart	60	7.25 ±10.48		Pinacho-Pinacho et al. (2014)
		(23-Viej)	20	Heart	95	61.84 ±77.81		Pinacho-Pinacho et al. (2014)
		(24-Huat)	7	Heart	14.28	6 ±0		Pinacho-Pinacho et al. (2014)
	P. punctatus	(63-Prim)	6	Heart	11.11	NR		This study
Centrocestus formosanus Nishigori, 1924. (Fig. 2]).	P. balsanus	(20-Fmag)	18	Gills	72.22	12.15 ±21.57		Pinacho-Pinacho et al. (2014)
		(21-Pich)	22	Gills	100	821.63 ± 417.59	9201 (3)	Pinacho-Pinacho et al. (2014)

Helminth taxa	Host (s)	Locality (CS-Code)	Z	Site (s) of infection	P (%)	MI±SD	CNHE (Number of specimens)	Reference
		(22-Refo)	20	Gills	100	42.45 ±33.39		Pinacho-Pinacho et al. (2014)
		(23-Viej)	20	Gills	5.88	31 ±0		Pinacho-Pinacho et al. (2014)
		(24-Huat)	7	Gills	50	1.66 ± 1.15		Pinacho-Pinacho et al. (2014)
	P. punctatus	(62-Nahu)	1	Gills	100	3 ± 0		This study
		(63-Prim)	6	Gills	11.11	NR		This study
Monogenea								
Family Gyrodactylidae van Beneden & Hesse, 1863								
Gyrodactylus sp. (Fig. 3A–D).	P. balsanus	(19-Flor)	20	Fins	2	1 ±0		Pinacho-Pinacho et al. (2014)
		(22-Refo)	20	Fins	5	2 ±0		Pinacho-Pinacho et al. (2014)
		(23-Viej)	20	Fins	10	1 ± 0		Pinacho-Pinacho et al. (2014)
Remarks: The limited number of slot Gwodactylus.	pecimens found pr	ecluded the spe	scific ic	lentification o	f this spec	ies; however, ŀ	ased on their morpho	logy they clearly represent members
Family Dactylogyridae Bychowsky, 1937								
Urocleidoides sp. (Fig. 3E, F).	P. balsanus	(19-Flor)	20	Gills	5	1 ± 0		Pinacho-Pinacho et al. (2014)
		(20-Fmag)	18	Gills	72.22	7.15 ±6.37		Pinacho-Pinacho et al. (2014)
		(21-Pich)	22	Gills	13.63	3 ± 1.73		Pinacho-Pinacho et al. (2014)
		(22-Refo)	20	Gills	75	7 ±5.45		Pinacho-Pinacho et al. (2014)
		(23-Viej)	20	Gills	82.35	5.85 ±5.27		Pinacho-Pinacho et al. (2014)
		(24-Huat)	7	Gills	83.33	7.4 ±4.44		Pinacho-Pinacho et al. (2014)
	P. punctatus	(62-Nahu)	1	Gills	100	1 ±		This study
	P. guatemalensis	(64-Escu)	19	Gills	5.26	4 ±		This study
		(65-Sara)	1	Gills	100	$1 \pm$		This study
Remarks: Pinacho-Pinacho et al. (2 to <i>Uvocleidoides</i> Mizelle & Price, 15	2014) recorded this 964 (sensu Kritsky e	specie as <i>Salsu</i> _s et al. 1986).	ginus sl	o.; however, a	detailed m	orphological e	evaluation of these spe	cimens confirmed that they belong

Helminth taxa	Host (s)	Locality (CS-Code)	z	Site (s) of infection	P (%)	MI±SD	CNHE (Number of specimens)	Reference
Adult Cestoda								
Family Bothriocephalidae Blanchard, 1849								
Bothriocephalus acheilognathi Yamaguti, 1934. (Fig. 3G, H).	P. hildebrandi	(3-Ecos)	234	Intestine	54	4.62±2.38	7617 (2)	Velázquez-Velázquez et al. (2011)
1		(4-Alba)	168	Intestine	61	13.10 ± 8.57		Velázquez-Velázquez et al. (2011)
		(5-5mar)	173	Intestine	59	4.35 ± 2.51		Velázquez-Velázquez et al. (2011)
		(6-Puen)	85	Intestine	2	1 ± 0.00		Velázquez-Velázquez et al. (2011)
		(7-Cham)	126	Intestine	41	1.88 ± 0.55		Velázquez-Velázquez et al. (2011)
		(8-Peor)	128	Intestine	71	6.34±2.48		Velázquez-Velázquez et al. (2011)
		(9-Arco)	64	Intestine	11	2.57±4.48		Velázquez-Velázquez et al. (2011)
		(10-Aren)	64	Intestine	9	1 ± 0.00		Velázquez-Velázquez et al. (2011)
		(11-Paja)	141	Intestine	5	1.14 ± 0.76		Velázquez-Velázquez et al. (2011)
		(12-Lsoy)	4	Intestine	100	4.50 ± 4.04		Velázquez-Velázquez et al. (2011)
		(73-Moxv)	20	Intestine	20	11 ± 9.9		This study
		(74-Poro)	\sim	Intestine	42.85	NR		This study
	P. portillorum	(51-Ojoj)	30	Intestine	NR	NR	9368	Salgado-Maldonado et al. (2015)
	P. guatemalensis	(66-Sans)	6	Intestine	16.66	$4 \pm$	9670 (1)	This study
	P. candalarius	(72-Lglo)	22	Intestine	22.72	1.6 ± 1.1	9669 (1)	This study
		(39- Ateo)	NR	Intestine	NR	NR		Velazquez-Velazquez et al. (2015)
Remarks: Velázquez-Velázquez et al for the first time in <i>P. guatemalensis</i>	l. (2011) first recor	ded species of t	apewoi	rm in <i>P. hildel</i>	<i>vandi</i> . In t	he present stu	dy, the Asian fish tape	worm <i>B. acheilognathi</i> was recorded
Adult Nematoda								
Family Cystidicolidae Skrjabin, 1946								
<i>Spinitectus humbertoi</i> Caspeta- Mandujano & Moravec, 2000. (Fig. 4A, B).	P. balsanus	(1-Inzc)	1	Intestine	100	NR	4028 (1) 4030 (2)	Caspeta-Mandujano and Moravec (2000)
		(23-Viej)	20	Intestine	10	6.5 ±3.53		Pinacho-Pinacho et al. (2014)
		(24-Huat)	~	Intestine	14.28	17 ± 0		Pinacho-Pinacho et al. (2014)

Helminth taxa	Host (s)	Locality	z	Site (s) of	P (%)	MI±SD	CNHE (Number	Reference
		(2000-00)	v	Turtection	UIV	MIM	01 specimens)	
			\sim	Intestine	NK	NK	9445 ())	Ihis study
		(28-Rlac)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(29-Caho)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(30-Tama)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	P. punctatus	(33-Chac)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(40-Rsan)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	Profundulus sp. 1	(34-Chic)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	P. labialis	(40-Rsan)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(41-Atpi)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(42-Rnan)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	P. guatemalensis	(44-Rnil)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(45-Cant)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(47-Rcau)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(48-Cauc)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(66-Sans)	9	Intestine	50	3.3 ± 2	9639 (5)	This study
	P. kreiseri	(50-Nono)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	P. portillorum	(70-Lpot)	6	Intestine	22.22	NR	9638 (5)	This study
	P. candalarius	(71-Rcar)	14	Intestine	57.14	2.6 ± 2.5		This study
Remarks: This nematode was origir Caspeta-Mandujano and Moravec (was recorded in two new host speci	nally described fron (2000) because <i>P. li</i> sies.	n the intestine o <i>ibialis</i> is not dis	of <i>Profi</i> stribute	<i>undulus labial</i> ed in Guerrerc	<i>is</i> in Guerr 5 state, and	ero, Mexico . most like th	Apparently, the type h ese authors examined .	ost was erroneously identified by <i>P balsanus</i> . In this study this species
Spinitectus mariaisabelae Caspeta-Mandujano Cabañas-Carranza & Salgado- Maldonado, 2007	P. punctatus	(2-Such)	NR	Intestine	NR	NR	5781 (1) 5783 (6)	Caspeta-Mandujano et al. (2007)
		(16-Saju)	3	Intestine	100	3.3 ± 2.0		Salgado-Maldonado et al. (2011a)
		(13-Ocot)	12	Intestine	100	5.7±2.9		Salgado-Maldonado et al. (2011a)
	P. labialis	(14-Chic)	3	Intestine	100	4.0 ± 3.0		Salgado-Maldonado et al. (2011a)
		(15-Rsuc)	24	Intestine	79.2	4.3 ± 3.2		Salgado-Maldonado et al. (2011a)
		(13-Ocot)	3	Intestine	100	2.6 ± 2.8		Salgado-Maldonado et al. (2011a)

Helminth taxa	Host (s)	Locality (CS-Code)	z	Site (s) of infection	P (%)	MI±SD	CNHE (Number of specimens)	Reference
Family Rhabdochonidae Travassos, Artigas & Pereira, 1928								
Rhabdochona salgadoi Caspeta- Mandujano & Moravec, 2000. (Fig. 4C, D).	P. balsanus	(1-Inzc)	-	Intestine	100	NR	4031 (1) 4033 (32)	Caspeta-Mandujano and Moravec (2000)
		(18-Carr)	25	Intestine	60	4.4 ±4.15		Pinacho-Pinacho et al. (2014)
		(19-Flor)	20	Intestine	70	5.71 ±4.95		Pinacho-Pinacho et al. (2014)
		(20-Fmag)	18	Intestine	83.33	4.46 ±3.11		Pinacho-Pinacho et al. (2014)
		(21-Pich)	22	Intestine	54.54	2.75 ±1.86		Pinacho-Pinacho et al. (2014)
		(22-Refo)	20	Intestine	95	8.05 ±3.99		Pinacho-Pinacho et al. (2014)
		(23-Viej)	20	Intestine	90	7.66 ±4.95		Pinacho-Pinacho et al. (2014)
		(24-Huat)	7	Intestine	100	18.57 ± 10.84		Pinacho-Pinacho et al. (2014)
		(28-Rlac)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(29-Caho)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(30-Tama)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(31-Agua)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	P. oaxacae	(32-Sabi)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	P. punctatus	(33-Chac)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(40-Rsan)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(13-Ocot)	12	Intestine	66.7	3.2 ± 1.9		Salgado-Maldonado et al. (2011a)
		(43-Cane)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(59-Huix)	20	Intestine	30	NR	9637 (5)	This study
		(62-Nahu)	1	Intestine	100	NR		This study
		(63-Prim)	6	Intestine	22.22	NR		This study
	P. labialis	(15-Rsuc)	24	Intestine	58.3	2.6 ± 1.7		Salgado-Maldonado et al. (2011a)
		(40-Rsan)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(41-Atpi)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	Profundulus sp. 2	(35-Rchi)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(13-Ocot)	3	Intestine	33.3	6.0±0		Salgado-Maldonado et al. (2011a)

Helminth taxa	Host (s)	Locality (CS-Code)	z	Site (s) of infection	P (%)	MI±SD	CNHE (Number of specimens)	Reference
	Profundulus sp. 1	(34-Chic)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	P. candalarius	(37-Rcom)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(71-Rcar)	14	Intestine	7.14	$1 \pm$	9640 (5)	This study
		(72-Lglo)	22	Intestine	4.54	1 ±		This study
	P. guatemalensis	(44-Rnil)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
		(64-Escu)	19	Intestine	21.05	NR	9642 (5)	This study
		(65-Sara)	-	Intestine	100	NR		This study
		(67-Rhon)	9	Intestine	16.66	NR		This study
	P. kreiseri	(49-Qtec)	NR	Intestine	NR	NR	9290 (5)	Salgado-Maldonado et al. (2014)
		(68-Cham)	9	Intestine	83.33	NR	9641 (5)	This study
		(69-Qpar)	28	Intestine	71.42	NR		This study
		(50-Nono)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
	P. portillorum	(52-Lepa)	NR	Intestine	NR	NR		Salgado-Maldonado et al. (2014)
Larval Nematodes								
Family Anisakidae Railliet & Henry, 1912								
Contracaecum sp.	P. punctatus	(13-Ocot)	12	Intestine	8.3	1.0 ± 0		Salgado-Maldonado et al. (2011a)
		(59-Huix)	20	Mesentery	5	NR		This study
		(60-Triu)	6	Mesentery	16.66	NR	9808 (1)	This study
Family Dioctophymatidae Railliet, 1915								
Eustrongylides sp. (Fig. 4E, F).	P. punctatus	(13-Ocot)	12	Intestine	41.6	1.6 ± 0.5		Salgado-Maldonado et al. (2011a)
	P. balsanus	(19-Flor)	20	Mesentery	5	1 ± 0		Pinacho-Pinacho et al. (2014)
		(20-Fmag)	18	Mesentery	16.66	2 ± 1		Pinacho-Pinacho et al. (2014)
	P. candalarius	(71-Rcar)	14	Mesentery	7.14	NR	9809 (1)	This study
Family Gnathostomatidae Railliet, 1895								
Spiroxys sp.	P. portillorum	(70- Lpot)	6	Intestine	11.11	NR	9810 (1)	This study



Figure 2. Species of trematodes found in *Profundulus* spp. A *Paracreptotrema blancoi* sensu Salgado-Maldonado et al. (2011b) B *Paracreptotrema profundulusi* C *Phyllodistomum inecoli* D *Phyllodistomum spinopapillatum* E *Saccocoelioides lamothei* F *Clinostomum* sp. G Diplostomidae gen. sp. H *Posthodiplostomum minimum* I *Ascocotyle (Ascocotyle) felippei* J *Centrocestus formosanus*.

culata (Müller, 1774), from Asia (Scholz and Salgado-Maldonado 2000). Of the 20 taxa found, eight (40%) were larval forms of generalist species that use freshwater fish as intermediate or paratenic hosts. In seven of the eight species, fish-eating birds are the definitive hosts, and only one, *Spiroxys* sp., requires a different vertebrate to complete its life cycle. Adult nematodes of the genus *Spiroxys* Schneider, 1866 commonly occur in the digestive tract of freshwater turtles, but can also parasitize frogs, salamanders and snakes (Li et al. 2014). Larval forms have been reported from a wide spectrum of hosts in various localities in Middle-America (e.g. Aguirre-Macedo et al. 2001; Sandlund et al. 2010).

The most widely distributed parasites among profundulids are the nematodes *R. salgadoi* and *S. humbertoi*, and the digenean *P. blancoi sensu* Salgado-Maldonado et al. (2011b), which are found in ten, eight and nine species of profundulids, in 38, 38 and 20 localities across Middle-America, respectively. Among these localities, prevalence and mean intensity values are quite variable (see Table 2). Prevalence varies between 2 and 100% for the different helminth species, although mean intensity values are usually very low (between one and five helminths per infected host), except for two

Host	Helminth parasite	Reference
Profundulus balsanus	Digenea	
Ahl, 1935	Ascocotyle (Ascocotyle) felippei (M)	Pinacho-Pinacho et al. (2014)
,	Centrocestus formosanus (M)	Pinacho-Pinacho et al. (2014)
	<i>Clinostomum</i> sp. (M)	Pinacho-Pinacho et al. (2014)
		This study
	Diplostomidae gen. sp. (M)	Pinacho-Pinacho et al. (2014)
	Phyllodistomum spinopapillatum (A)	Pinacho-Pinacho et al. (2014)
		Pérez-Ponce de León et al. (2015)
		This study
	Posthodiplostomum minimum (M)	Pinacho-Pinacho et al. (2014)
		This study
	Paracreptotrema blancoi (A)	Salgado-Maldonado et al. (2011b)
		Pinacho-Pinacho et al. (2014)
		Salgado Maldonado et al. (2014)
		This study
	Dangement to the one of the dulusi (A)	Salando Maldonado et al. (2011b)
	Furucrepioirema projunauiusi (A)	Dinacho Dinacho et al. (2017)
		Salando Maldonado et al. (2014)
	C	Disarde Disarde et al. (2014)
	Saccocoellolaes lamothel (A)	This at the
	Management	This study
	Monogenea	\mathbf{D}^{*}_{i} \mathbf{D}^{*}_{i} (2014)
	<i>Gyrodactylus</i> sp. (A)	Pinacho-Pinacho et al. (2014)
	Urocleidoides sp. (A)	Pinacho-Pinacho et al. (2014)
	Nematoda	
	<i>Eustrongylides</i> sp. (L)	Pinacho-Pinacho et al. (2014)
	Rhabdochona salgadoi (A)	Caspeta-Mandujano and Moravec (2000)
		Pinacho-Pinacho et al. (2014)
		Salgado-Maldonado et al. (2014)
	Spinitectus humbertoi (A)	Caspeta-Mandujano and Moravec (2000)
		Pinacho-Pinacho et al. (2014)
		Salgado-Maldonado et al. (2014)
		This study
Profundulus	Digenea	
candalarius Hubbs,	<i>Clinostomum</i> sp. (M)	This study
1924	Phyllodistomum inecoli (A)	This study
	Paracreptotrema blancoi (A)	This study
	Cestoda	
	Bothriocephalus acheilognathi (A)	Velazquez-Velazquez et al. (2015)
		This study
	Nematoda	
	<i>Eustrongylides</i> sp. (L)	This study
	Spinitectus humbertoi (A)	This study
Profundulus	Digenea	
guatemalensis	Paracreptotrema blancoi (A)	Salgado-Maldonado et al. (2014)
(Günther, 1866)		This study
	Monogenea	
	<i>Urocleidoides</i> sp. (A)	This study
	Cestoda	
	Bothriocephalus acheilognathi (A)	This study
	Nematoda	Salgado-Maldonado et al. (2014)
	Rhabdochona salgadoi (A)	This study
	_	
	Spinitectus humbertoi (A)	Salgado-Maldonado et al. (2014)
		This study

Table 3. Host-parasite list. Key: A = Adult, M = Metacercariae, L = Larvae.

Prefinalulus Cestods Velázquez-Velázquez et al. (2011) bildebrandi Miller, 1950 Digenea This study Prefinalulus kreizeri, Maramoros, Schafer, Hernández & Digenea Salgado-Maldonado et al. (2014) Maramoros, Schafer, Hernández & Rhabdochona salgadoi (A) Salgado-Maldonado et al. (2014) Profinalulus labialis (Günther, 1866) Digenea Rhabdochona salgadoi (A) Salgado-Maldonado et al. (2014) Profinalulus labialis (Günther, 1866) Digenea This study Salgado-Maldonado et al. (2014) Profinalulus contract Rhabdochona salgadoi (A) Salgado-Maldonado et al. (2014) Salgado-Maldonado et al. (2014) Spinitectus humbertoi (A) Salgado-Maldonado et al. (2014) Salgado-Maldonado et al. (2014) Spinitectus numbertoi (A) Salgado-Maldonado et al. (2014) Salgado-Maldonado et al. (2014) Spinitectus numbertoi (A) Salgado-Maldonado et al. (2014) Salgado-Maldonado et al. (2014) Profinalulus ozacaze (Meek, 1902) Profinalulus (A) Salgado-Maldonado et al. (2014) Profinalulus Profinalulus (A) Salgado-Maldonado et al. (2014) Profinalulus Profinalulus (A) Salgado-Maldonado et al. (2014) <td< th=""><th>Host</th><th>Helminth parasite</th><th>Reference</th></td<>	Host	Helminth parasite	Reference
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		Spinitectus mariaisabelae (A)	Salgado-Maldonado et al. (2007)

Host	Helminth parasite	Reference
Profundulus sp. 1	Nematoda	
	Rhabdochona salgadoi (A)	Salgado-Maldonado et al. (2014)
	Spinitectus humbertoi (A)	Salgado-Maldonado et al. (2014)
Profundulus sp. 2	Digenea	
	Phyllodistomum inecoli (A)	Pérez-Ponce de León et al. (2015)
	Paracreptotrema blancoi (A)	Salgado-Maldonado et al. (2014)
		Salgado-Maldonado et al. (2011b)
		This study
	Paracreptotrema profundulusi (A)	Salgado-Maldonado et al. (2014)
		Salgado-Maldonado et al. (2011b)
		This study
	Nematoda	
	Rhabdochona salgadoi (A)	Salgado-Maldonado et al. (2014)
		Salgado-Maldonado et al. (2011a)

larval forms, the heterophyids *C. formosanus* and *Ascocotyle (Ascocotyle) felippei* Travassos, 1928. These two species reached mean intensity levels as high as 821.6 and 165.4 larvae per infected host, respectively. Among adults, the nematodes *S. humbertoi* and *R. salgadoi* reached mean intensity values usually higher than five worms per infected host among the various localities.

In terms of the species richness of the helminths in relation to the host species, *Profundulus balsanus* is the species with the highest diversity, since it is parasitized by 14 species, followed by *P. punctatus* with 12, and *P. candalarius* and *P. portillorum* with six (Table 3). Finally, *P. oaxacae*, *P. kreiseri* and *P. hildebrandi* possess a depauperate fauna, with only three, three and one species, respectively.

Discussion

Fish were collected at 26 localities in southern Mexico, Guatemala and Honduras, and a total of 267 individual fish belonging to nine species of Profundulus was examined for helminth parasites. The inventory was completed by adding these records to the previous parasite surveys conducted on members of this fish group endemic to Middle-America. Interestingly, the number of individual hosts studied for helminths of this fish group has increased significantly during a two-year period across the entire distributional range, and it seems that only two new species were found. Firstly, a detailed morphological evaluation of the specimens recorded herein as Urocleidoides sp. indicate they represent an undescribed species, which will be formally described in a separate paper. A thorough revision of the morphology of the specimens identified as the trematode Paracreptotrema blancoi by Salgado-Maldonado et al. (2011b), along with the new samples obtained in this study, allowed us to determine that they in fact represent not only a new species but a new genus. The new species is readily distinguished by the size of the ventral sucker and by having a more restricted vitellarium, a shorter cirrus sac and caeca that bifurcate at the level of the ventral sucker and end at the level of the testes. Since information was also gathered from sequences



Figure 3. Species of monogeneans and the single cestode found in *Profundulus* spp. **A–D** *Gyrodactylus* sp. **E–F** *Urocleidoides* sp. **G–H** *Bothriocephalus acheilognathi.*



Figure 4. Species of nematodes found in *Profundulus*. **A–B** Spinitectus humbertoi **C–D** Rhabdochona salgadoi **E–F** Eustrongylides sp.

of the 28S rRNA gene and scanning electron microscopy micrographs, the new species will be formally described in a separate paper. The record in this checklist is presented provisionally, under the original designation of the species, as *P. blancoi sensu* Salgado-Maldonado et al. (2011b).

Six adult helminth species are considered to be part of the biogeographical 'core' helminth fauna of profundulids. As discussed by Pérez-Ponce de León and Choudhury (2002), for a parasite taxon to be considered part of a biogeographical core, it

must not only be widely distributed but must also be characteristically associated with and restricted to a monophyletic group of host species (see also Choudhury and Dick 1998), even if it is not present in all host species of that group. This concept was actually used to describe the pattern of host-specificity among the helminth parasite fauna of freshwater fishes in Mexico (Pérez-Ponce de León and Choudhury 2005); this was based on the premise that particular host-groups are characteristically associated with a biogeographical 'core' helminth fauna and that such host specificity strongly influences their biogeography. These authors tested three predictions based on that fundamental hypothesis of 'core' parasite faunas: (1) that the parasite fauna is largely circumscribed by higher levels of monophyletic host taxa (families, orders, etc.), and that this pattern is independent of areas; (2) that areas within a certain biogeographical region, and consequently with a similar fish composition, will have more similar parasite faunas compared with areas with a less similar fish faunal composition; and (3) that 'core' parasite faunas persist to some extent in transitional areas (areas where Nearctic and Neotropical species are sympatric) with limited host-sharing. The current results on the helminth fauna of Profundulus spp. along its distributional range in Middle-America further corroborate the three predictions.

This represents the second complete inventory of a freshwater fish group. Martínez-Aquino et al. (2014) recently published the inventory of the helminth parasites of goodeines, an endemic subfamily from central and a few areas of northern Mexico. Both groups belong in the order Cyprinodontiformes, and molecular phylogenetic analyses show that they are sister taxa (Webb et al. 2004, Doadrio and Domínguez 2004). Based on the premise that comprehensive data on the inventory of a particular host group is fundamental to a better understanding of the historical biogeography and evolutionary history of host-parasite associations, the information presented in this paper, along with the one for the goodeines, will allow us to discuss factors that have shaped the biogeographical and diversification patterns of parasites and hosts within a phylogenetic framework, and, to the best of our knowledge, this is the first time that these types of data have been produced.

There are, however, some notable differences between the biogeography of the Goodeinae and Profundulidae. The Goodeinae is an endemic fish component of central and northern Mexico which experienced an important adaptive radiation and contains 45 species (Domínguez-Domínguez et al. 2010). The helminth fauna of extant species (some of them have gone extinct recently due to habitat degradation) includes 51 species, according to the examination of almost 8,300 individual fish representing 36 species allocated to 18 genera, studied in 113 localities across central and northwestern Mexico (Martínez-Aquino et al. 2014). In contrast, *Profundulus* possesses only 11 species and is the only genus within the Profundulidae. These fish did not experience the same level of diversification as goodeines, and their distributional range comprises an area of Middle-America from central Mexico southwards to Honduras. Apparently, the Balsas depression establishes the distributional limit for both fish groups, since goodeines have the southernmost distribution in the same basin. However, these fish families do not occur sympatrically at any location. Since the Balsas River basin is the result of a geological event known as the Balsas Portal, which represents a marine transgression that occurred during the Mid-Cretaceous period (see Domínguez-Domínguez and Pérez-Ponce de León 2009, and references therein), it cannot be ruled out that this was the geological event that caused the divergence between goodeines and profundulids from a common ancestor, despite a molecular clock analysis showing that the ancestral split occurred during the Mid-Miocene, approximately 16 million years ago (see Doadrio and Domínguez 2004).

Irrespective of the biogeographical history of the ancestor of both profundulids and goodeids, and the subsequent radiation of the latter, the former did not diversify in the same way as goodeids did. Adaptive radiation of goodeines in central Mexico, following a complex geological and hydrological history (see Domínguez-Domínguez et al. 2010), resulted in a higher species richness, and this may have influenced their parasite fauna, contrasting the 51 helminth species that parasitize goodeines with only 20 species in profundulids. Interestingly, the helminth species composition in both host groups is relatively similar. The core helminth parasite fauna includes members of the Allocreadiidae Looss, 1902, Gorgoderidae Looss, 1901 and Haploporidae Nicoll, 1914 among the digeneans, members of the monogenean genus Gyrodactylus von Nordmann, 1832 and members of the nematode genus Rhabdochona Railliet, 1916. For instance, while goodeines are infected by two species of the allocreadiid genus Margotrema Lamothe-Argumedo, 1970, profundulids are infected by two species of the allocreadiid genus Paracreptotrema Choudhury, Pérez-Ponce de León, Brooks & Daverdin, 2006. Both host groups are parasitized by two species of *Phyllodistomum* Braun, 1899, and by two species of Rhabdochona. Likewise, scarce phylogenetic information is available to make strong comparisons, and a robust pattern cannot be established in the absence of a phylogenetic framework. However, the few available data show that the presence of congeners of different helminth groups in goodeines and profundulids is not the result of a historical association but of colonization (Brooks and McLennan 1993). In the morphological phylogenetic analysis of species of *Rhabdochona* by Mejía-Madrid et al. (2007), R. lichtenfelsi Sánchez-Alvarez, García-Prieto & Pérez-Ponce de León, 1998 (a common and widely distributed parasite of goodeines) and R. salgadoi (a common and widely distributed species in Profundulus) are not close relatives, although, needless to say, the phylogenetic analysis was not fully resolved and the morphology-based phylogeny may not be robust. In contrast, in the case of the allocreadiids, recently published molecular phylogenetic analyses clearly indicate that Margotrema spp. (in goodeines) and Paracreptotrema (in profundulids) are not sister taxa, since Margotrema clusters with species usually found in Nearctic fishes, whereas Paracreptotrema is the sister taxon to other allocreadiids that parasitize characids (Razo-Mendivil et al. 2014, a group of fish with a Neotropical origin, precluding any speculation about the speciation event that may have caused their diversification either in goodeines or profundulids).

As suggested by Pérez-Ponce de León and Choudhury (2010), molecular data are fundamental to better understanding patterns of diversity among the freshwater fish parasite fauna, but also to establishing sister group relationships among newly discovered species with respect to those already described. At present, it seems plausible to propose that the helminth fauna of goodeines was secondarily acquired from Nearctic fishes, whereas profundulids obtained their helminths from other Neotropical freshwater fishes, i.e. their parasites are the result of host-switching events following colonization from other, most probably unrelated, hosts. But this needs to be determined by proper molecular co-phylogenetic analyses. The data generated thus far will enable us to conduct such analyses in the near future and to contribute to a better understanding of the evolution and biogeography of the freshwater fish helminth parasite fauna.

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RESEARCH ARTICLE



Seven new hypselostomatid species from China, including some of the world's smallest land snails (Gastropoda, Pulmonata, Orthurethra)

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Abstract

Seven new species of Hypselostomatidae are described from the Chinese province Guangxi: Angustopila dominikae Páll-Gergely & Hunyadi, **sp. n.**, A. fabella Páll-Gergely & Hunyadi, **sp. n.**, A. subelevata Páll-Gergely & Hunyadi, **sp. n.**, A. szekeresi Páll-Gergely & Hunyadi, **sp. n.**, Hypselostoma socialis Páll-Gergely & Hunyadi, **sp. n.**, H. lacrima Páll-Gergely & Hunyadi, **sp. n.** and Krobylos sinensis Páll-Gergely & Hunyadi, **sp. n.** The latter species is reported from three localities. All other new species are known only from the type locality. Specimens nearly identical to the type specimens of Angustopila huoyani Jochum, Slapnik & Páll-Gergely, 2014 were found in a cave in northern Guangxi, 500 km from the type locality. Adult individuals of Angustopila subelevata **sp. n.** (shell height = 0.83–0.91 mm, mean = 0.87 mm) and A. dominikae **sp. n.** (shell height of the holotype = 0.86 mm) represent the smallest known members of the Hypselostomatidae, and thus are amongst the smallest land snails ever reported. We note that Pyramidula laosensis Saurin, 1953 might also belong to Krobylos. Paraboysidia neglecta van Benthem Jutting, 1961, which was previously included in Angustopila, is classified in Hypselostoma.

Keywords

Taxonomy, land snail, dwarfism, Pupillidae, Vertiginidae, apertural barriers

Introduction

The term "microsnail" usually refers to gastropods with shells smaller than 5 mm (Panha and Burch 2005). Species within this size range do not form a monophyletic unit. Hence, the term "microsnail" is used in the practical sense only. Microgastropods represent a large portion of worldwide and tropical land snail diversity. Knowledge about their biodiversity is scant due to two main reasons: i) many microsnails are reported from caves only or known to inhabit rock outcrops, meaning that they can only be effectively collected using special techniques, such as sieving from soil samples; ii) many microsnails are reported from small ranges and often from the type locality only (e.g. Neubert and Bouchet 2015). However, microsnails can also tend to inhabit the broadest ranges known for land snails (e.g. Vertiginidae, *Carychium*; Nekola and Coles 2010, Weigand et al. 2013, Nekola 2014).

High rates of endemism amongst tropical microsnails requires researchers to perform detailed samplings over large geographic areas in order to find the narrow range endemic species. Superordinate systematics (genus and above) of small-shelled gastropods confronts similar difficulties. Since finding live populations is a challenging endeavour, classification is largely conchologically driven.

One of the families known to contain particularly tiny species is the family Hypselostomatidae, introduced by Zilch (1959) as a subfamily of Chondrinidae. The subfamily Aulacospirinae was also erected in the same work. Schileyko (1998a) synonymized these two taxa because no diagnostic characters were designated by Zilch (1959). The family Hypselostomatidae sensu Schileyko (1998a) inhabits Indochina, Indonesia, Australia and the Philippines, and contains the following genera: Boysidia Ancey, 1881 (with the subgenera Paraboysidia Pilsbry, 1917 and Dasypupa Thompson & Dance, 1983), Anauchen Pilsbry, 1917, Bensonella Pilsbry & Vanatta, 1900, Aulacospira Möllendorff, 1890, Pseudostreptaxis Möllendorff, 1890, Gyliotrachela Tomlin, 1930, Hypselostoma Benson, 1856, Campolaemus Pilsbry, 1892, Boysia L. Pfeiffer, 1849 and Acinolaemus Thompson & Upatham, 1997 (Schileyko 1998a). These genera, together with Systemostoma Bavay & Dautzenberg, 1909 are sometimes included in the Pupillidae (e. g. Panha and Burch 1999) or in theVertiginidae (e.g. Thompson and Upatham 1997). Schileyko (1998b) concluded that Systenostoma probably does not belong to Hypselostomatidae, but likely belongs to the Helicodiscidae due to the characteristic spiral sculpture. Later, he postulated that the genus is possibly related to *Aulacospira* as considered by Pilsbry (1917) or to Pupisoma Stoliczka, 1873 (Valloniidae) (Schileyko 2011). Jochum et al. (2014) renamed Systenostoma Bavay & Dautzenberg, 1909 (non Systenostoma Marsson, 1887, Bryozoa) as Tonkinospira Jochum, Slapnik & Páll-Gergely, 2014, and erected a new genus (Angustopila Jochum, Slapnik & Páll-Gergely, 2014) for some species which were previously classified within Systenostoma. Jochum et al. (2014) claimed that Angustopila probably belongs to the Hypselostomatidae, but the taxonomic position of *Tonkinospira* within the family remained uncertain. We include all genera in question (Angustopila, Hypselostoma, Krobylos Panha & Burch, 1999, Tonkinospira) in Hypselostomatidae.

In the present work, seven new species recently collected in Guangxi Province, China are described, belonging to the genera *Angustopila*, *Hypselostoma* and *Krobylos*. We also highlight some difficulties in the pre-existing practice of ranking species into genera based on conchological characters.

Materials and methods

Shells were first wetted in a dish of water and then manually brushed clean of mud using fine, tapered brushes, whereby each specimen was gently rotated back and forth between the brushes until it was sediment free. The shells were viewed without coating under a low vacuum SEM (Miniscope TM-1000, Hitachi High-Technologies, Tokyo). Shell whorl number was counted to the nearest quarter whorl according to Kerney and Cameron (1979).

Measurements of *Angustopila* and *Hypselostoma* specimens were taken from images obtained by a Nikon Digital Sight DS-FI1 microscope camera attached to a Nikon SMZ 800 Zoom Stereomicroscope. *Krobylos* specimens were measured using digital Vernier callipers. For the species descriptions, shell measurements are expressed as ratios such as SW/SH and AW/AH.

Abbreviations

HA	Collection András Hunyadi (Budapest, Hungary)
HNHM	Magyar Természettudományi Múzeum (Budapest, Hungary)
MNHN	Muséum National d'Histoire Naturelle (Paris, France)
NMBE	Naturhistorisches Museum der Burgergemeinde Bern, (Bern, Switzerland)
NHMUK	The Natural History Museum (London, UK)
PGB	Collection Barna Páll-Gergely (Mosonmagyaróvár, Hungary)
SMF	Senckenberg Forschungsinstitut und Naturmuseum (Frankfurt am Main,
	Germany)

Taxonomic descriptions

Genus Angustopila Jochum, Slapnik & Páll-Gergely, 2014

Angustopila Jochum, Slapnik & Páll-Gergely, 2014; Jochum et al. 2014: 410: 26.

Type species. Systenostoma tamlod Panha & Burch, 1999, by original designation. Including. concava (Thompson & Upatham, 1997), dominikae Páll-Gergely & Hunyadi, sp. n., elevata (Thompson & Upatham, 1997), huoyani Jochum, Slapnik & Páll-Gergely, 2014, *fabella* Páll-Gergely & Hunyadi, sp. n., *subelevata* Páll-Gergely & Hunyadi, sp. n., *szekeresi* Páll-Gergely & Hunyadi, sp. n., *tamlod* (Panha & Burch, 1999).

Remarks. *Paraboysidia neglecta* van Benthem Jutting, 1961 was classified within the genus *Systenostoma* by Panha and Burch and in *Angustopila* by Jochum et al. (2014) due to the presence of only two teeth in the aperture. The wide umbilicus and the detached peristome are, however, very similar to the members of the genus *Hypselostoma* (material examined: Caves near Biserat, state of Jalor, Malay Peninsula, NHMUK 1901.07.19.24–27, syntypes). Therefore we reclassify *P. neglecta* in *Hypselostoma*.

Angustopila dominikae Páll-Gergely & Hunyadi, sp. n.

http://zoobank.org/6C7AF4AA-D0FF-4CB5-BD7F-ADD52654945C Figure 1, 12

Type material. China, Guangxi (广西), Hechi Shi (河池市), Bama Xian (巴马县), cliffs at the southern edge of Jiaole Cun (交乐村), 590 m, 24°7.045'N, 107°7.847'E, leg. Hunyadi, A. & Szekeres, M., 10.09.2013., HNHM 99435 (holotype).

Diagnosis. A tiny, corpulent species with elongated aperture having a parietal and a single palatal tooth.

Description of the holotype. Shell minute, light grey, corpulent, almost globular, the penultimate whorl is the widest from apertural view; protoconch consists of 1.5 whorls, protoconch microstructure finely pitted and granular with a powdery superficial texture, the granular microstructure collectively radiates from the nuclear whorl and ceases at the second; teleoconch finely ornamented with irregularly-spaced radial growth lines crossed by fine rows of equidistantly spaced microscopic spiral threads; the 4.75 whorls are separated by a deep suture; whorls shouldered; aperture slightly oblique to shell axis; umbilicus deep, very narrow; aperture elliptical; the sinulus is narrow; peristome slightly expanded, not reflected; the mid section comprising the parietal tooth is sinuous and slightly protruding (in side view); parietal callus well developed, its portion of the callus between the parietal tooth and the upper right sinulus edge is detached; parietal tooth well developed with a very small additional tubercle (may be homologous with the angular tooth), the palatal tooth is positioned deeper in the shell and directly opposite the parietal tooth.

Measurements (in mm): SH = 0.86, SW = 0.8, AH = 0.3, AW = 0.37, SW/SH \times 100 = 93.02, AW/AH \times 100 = 123.33 (n = 1).

Differential diagnosis. Angustopila tamlod from Thailand also possesses two teeth (parietal and palatal), but it has a conical shell, which is nearly globular in *A. domini-kae* sp. n. Moreover, *A. tamlod* has a narrower umbilicus and a more rounded aperture. Angustopila huoyani is larger than *A. dominikae* sp. n. It has a rather conical shell, more whorls, a narrower umbilicus, two apertural denticles and lacks the spiral thread-like lines (or has much weaker spiral striae) on the whole shell. The sympatric Angustopila subelevata sp. n. has a conical shell and lacks apertural dentition. See also under *A. fabella* sp. n. and *A. szekeresi* sp. n.



Figure I. Holotype of *Angustopila dominikae* Páll-Gergely & Hunyadi, sp. n. (HNHM 99435). All images: B. Páll-Gergely.

Etymology. The new species is named after Mrs. Dominika Páll-Gergely, the wife of the first author.

Type locality. China, Guangxi (广西), Hechi Shi (河池市), Bama Xian (巴马县), cliffs at the southern edge of Jiaole Cun (交乐村), 590 m, 24°7.045'N, 107°7.847'E.

Distribution. The new species is known from the type locality only (Figure 13).

Ecology. The single empty shell of this new species was found in a soil sample at the base of limestone rocks. It likely lives on limestone walls as do other similar hypselostomatid species recorded by Panha and Burch (2005).

Conservation status. A single empty shell has been collected from a soil sample at the type locality. Therefore, knowledge is very limited for evaluating its conservation status. Since the species is known from one site only, it is evaluated as Critically Endangered (CR) under IUCN criteria (IUCN 2014). Quarrying is quoted as the main threat to similar limestone habitats. However, no ongoing threats to the type locality are known at the moment.

Angustopila fabella Páll-Gergely & Hunyadi, sp. n. http://zoobank.org/E5FDAE89-5B6F-419D-BABE-2A10F0144622

Figure 2

Type material. China, Guangxi (广西), Chongzuo Shi (崇左市), Longzhou Xian (龙州 县), cliffs north of Lenglei (楞垒), north of the Nonggang Nature Reserve (弄岗国家 级自然保护区), 220 m, 22°29.161'N, 106°57.357'E, leg. Hunyadi, A. & Szekeres, M., 23.09.2013., HNHM 99436 (holotype), HNHM 99437/2 (figured paratypes), SMF 346520/1 paratype, HA/38 paratypes + 2 juvenile shells (not paratypes), PGB/1 paratype.

Diagnosis. A tiny, trigonal-shaped species with a rather rounded, slightly beanshaped aperture bearing a well-developed parietal tooth.

Description. Shell minute, light grey, bluntly trigonal; protoconch consists of slightly more than 1.25 whorls, protoconch microstructure finely pitted and granular with a powdery superficial texture, the granular microstructure collectively radiates from the nuclear whorl and ceases at the second; teleoconch finely reticulate with irregularly-spaced radial growth lines crossed by rows of microscopic spiral threads; the 4.5–4.75 whorls are separated by a deep suture; whorls shouldered; aperture slightly oblique to shell axis; umbilical zone highly reticulate, umbilicus deep, relatively narrow; aperture heart-shaped; peristome slightly expanded, not reflected; parietal callus well-developed, very slightly adnate to the penultimate whorl; parietal tooth prominent, thick and long; no other dentition is present. Body whorl bulges beyond aperture (side view) by ca. 1/7 the max. breadth of the shell. Apertural lip tilted slightly back with fine creases behind the peristome (side view).

Measurements (in mm): SH = 0.86–1.02, SW = 0.88–1, AH = 0.34–0.4, AW = 0.36–0.41 (n = 20). See also Tables 1 and 2.

Differential diagnosis. Angustopila fabella sp. n. is most similar to A. tamlod in shape and form. However, in addition to the parietal denticle, A. tamlod has a small, low palatal plica just opposite the parietal denticle. Angustopila dominikae sp. n. is smaller, has a globular shell (conical in A. fabella sp. n.) and possesses two apertural denticles with an additional tubercle on the parietal denticle. A single parietal denticle is present in A. fabella sp. n. See also A. subelevata sp. n. and A. szekeresi sp. n.


Figure 2. *Angustopila fabella* Páll-Gergely & Hunyadi, sp. n. Holotype: (HNHM 99436: **A, B, D, F**), Paratype1 (HNHM 99437: **E**), Paratype2 (HNHM 99437: **C, G, H**). All images: B. Páll-Gergely.

Etymology. The name, fabella, (Latin: little bean) refers to the bean-shaped aperture. **Type locality.** China, Guangxi (广西), Chongzuo Shi (崇左市), Longzhou Xian (龙州县), cliffs north of Lenglei (楞垒), north of the Nonggang Nature Reserve (弄岗 国家级自然保护区), 220 m, 22°29.161'N, 106°57.357'E.

Table 1. Shell measurements (mm) for *Angustopila fabella* sp. n. from the type locality. SH: shell height, SW: shell width, AH: aperture height, AW: aperture width, SW/SH×100: shell width shared with shell height and multiplied by 100, AW/AH×100: aperture width shared with aperture height and multiplied by 100.

Specimen	SH	SW	AH	AW	SW/SH×100	AW/AH×100
holotype	0.97	1	0.37	0.4	103.09	108.11
paratype1	0.96	0.98	0.39	0.41	102.08	105.13
paratype2	0.96	0.92	0.37	0.38	95.83	102.7
paratype3	1.01	0.94	0.37	0.38	93.07	102.7
paratype4	0.92	0.94	0.36	0.39	102.17	108.33
paratype5	0.86	0.98	0.4	0.4	113.95	100
paratype6	0.93	0.94	0.38	0.39	101.08	102.63
paratype7	0.97	0.93	0.39	0.39	95.88	100
paratype8	0.96	0.94	0.39	0.39	97.92	100
paratype9	0.99	0.89	0.36	0.39	89.9	108.33
paratype10	1.02	0.94	0.4	0.38	92.16	95
paratype11	0.92	0.93	0.37	0.4	101.09	108
paratype12	0.97	0.94	0.37	0.38	96.91	102.7
paratype13	0.97	0.93	0.37	0.4	95.88	108.11
paratype14	0.94	0.91	0.36	0.38	96.81	105.56
paratype15	0.93	0.88	0.34	0.37	94.61	108.82
paratype16	0.95	0.95	0.39	0.39	100	100
paratype17	0.89	0.89	0.35	0.36	100	102.86
paratype18	0.95	0.93	0.38	0.38	97.89	100
paratype19	0.93	0.91	0.37	0.39	97.85	105.41

Table 2. Average, minimum value (min), maximum value (max), variance of values (var) and standard deviation of a set of values (stdev) for *Angustopila fabella* sp. n. (n = 20).

	SH	SW	AH	AW	SW/SH×100	AW/AH×100
Average	0.95	0.9335	0.374	0.3875	98.4085	103.7195
Min	0.86	0.88	0.34	0.36	89.9	95
Max	1.02	1	0.4	0.41	113.95	108.82
Var	0.0014	0.0009	0.0003	0.0001	25.7841	14.9414
stdev	0.0376	0.0301	0.016	0.0116	5.0778	3.8654

Distribution. *Angustopila fabella* sp. n. is known from the type locality only (Figure 13). **Ecology.** Empty shells of this new species were found in a soil sample at the base of large limestone rocks. It likely lives on limestone walls as do other similar hypselostomatid species recorded by Panha and Burch (2005).

Conservation status. Empty shells have been collected from a soil sample at the type locality. Therefore, knowledge is very limited for evaluating its conservation status. Since the species is known from one site only, it is evaluated as Critically Endangered (CR) under IUCN criteria (IUCN 2014). Quarrying is quoted as the main threat to similar limestone habitats. However, no ongoing threats to the type locality are known at the moment.

Angustopila huoyani Jochum, Slapnik & Páll-Gergely, 2014 Figure 3

Angustopila huoyani Jochum, Slapnik & Páll-Gergely, 2014: Jochum et al. 2014: 410: 27–29, Video 1, Figs 4–5.

Material examined. MNHN Expedition Nr. GX07.23.07, China, Guangxi (广西), Hechi (河池市), Huanjiang Xian (环江县), Midong village (米洞), Shui Dong (cave, 水洞), 23.05.2007, river sediment, alt. 332 m, 24.7485°N, 108.27191°E, leg. Franck Bréhier 12 shells (2 broken), NMBE 535121/3, SMF 341637/3, MNHN 2012-27046/4 + 2 broken shells).

Conservation status. This study reveals that *A. huoyani* inhabits two caves that are geographically far from each other. The typical threats to such habitats is quarrying and human disturbance through tourism.

Remarks. Angustopila huoyani has been described from a single cave in northeastern Hunan Province. Nearly identical shells have been found in another cave in northern Guangxi Province, which is situated ca. 500 km south from the type locality. The only difference is that the new shells have some very faint spiral striae on the teleoconch, which were not detected in the original population. This difference is, however, insufficient to distinguish these two populations on either specific or subspecific level. Therefore, we refer to the population collected in Guangxi as a disjunct population of *A. huoyani*. This finding underscores the need to explore more cave systems in order to make inferences about subterranean biodiversity in China, and specifically here for the distribution of minute troglobitic land snails.

Angustopila subelevata Páll-Gergely & Hunyadi, sp. n.

http://zoobank.org/74DACAA7-B195-459F-B39E-B11D875FD015 Figure 4

Type material. China, Guangxi (广西), Hechi Shi (河池市), Bama Xian (巴马县), cliffs at the southern edge of Jiaole Cun (交乐村), 590 m, 24°7.045'N, 107°7.847'E, leg. Hunyadi, A. & Szekeres, M., 10.09.2013., HNHM 99438 (holotype), HNHM 99439/1 (paratype), HA/10 paratypes.

Diagnosis. A tiny, conical species with rounded or almost quadrangular aperture without dentition.

Description. Shell minute, light grey, conical with obtuse apex; spire tilted slightly left; protoconch consists of 1.25–1.5 whorls, microstructure finely pitted and granular with a powdery superficial texture, collectively radiating from the nuclear whorl; a prominent protoconch/teleoconch boundary is present (p/t), which is preceded by very faint rows of finely threaded microstructure; teleoconch finely reticulate with regularly-spaced radial growth lines crossed by rows of microscopic spiral threads.; on the last whorl, every 5th-6th radial line is stronger; the 4.25 whorls are separated by a deep



Figure 3. Angustopila huoyani Jochum, Slapnik & Páll-Gergely, 2014. Locality: Guangxi (广西), Hechi (河池市), Huanjiang Xian (环江县), Midong village (米洞), Shui Dong (cave, 水洞), 23.05.2007, river sediment, alt. 332 m, 24.7485°N, 108.27191°E. MNHN 2012-27046). All images: B. Páll-Gergely.

suture; whorls shouldered; body whorl tumid; aperture slightly oblique to shell axis; umbilicus deep, relatively wide; aperture rounded or almost quadrangular, toothless; peristome slightly expanded, not reflected; parietal margin extends forward as a slight



Figure 4. Holotype of *Angustopila subelevata* Páll-Gergely & Hunyadi, sp. n. (HNHM 99438). All images: B. Páll-Gergely.

tongue-like projection along the columellar curvature; outer lip (side view) arched slightly and drawn back below suture.

Measurements (in mm): SH = 0.83–0.91, SW = 0.77–0.81, AH = 0.27–0.3, AW = 0.29–0.32 (n = 8). See also Tables 3 and 4.

Specimen	SH	SW	AH	AW	SW/SH×100	AW/AH×100
holotype	0.88	0.8	0.3	0.31	90.91	103.33
paratype1	0.87	0.81	0.29	0.32	93.1	110.34
paratype2	0.86	0.77	0.3	0.32	89.53	106.67
paratype3	0.88	0.79	0.28	0.29	89.77	103.57
paratype4	0.85	0.78	0.3	0.32	91.76	106.67
paratype5	0.91	0.79	0.27	0.31	86.81	114.81
paratype6	0.86	0.79	0.3	0.31	91.86	103.33
paratype7	0.83	0.81	0.3	0.3	97.59	100

Table 3. Shell measurements (mm) for *Angustopila subelevata* sp. n. from the type locality. For abbreviations see Table 1.

Table 4. Average, minimum value (min), maximum value (max), variance of values (var) and standard deviation of a set of values (stdev) for *Angustopila subelevata* sp. n. (n= 8). For abbreviations see Table 1.

	SH	SW	AH	AW	SW/SH×100	AW/AH×100
Average	0.8675	0.7925	0.2925	0.31	91.4163	106.09
Min	0.83	0.77	0.27	0.29	86.81	100
Max	0.91	0.81	0.3	0.32	97.59	114.81
Var	0.0006	0.0002	0.0001	0.0001	9.8582	21.9211
stdev	0.0238	0.0139	0.0116	0.0107	3.1398	4.682

Differential diagnosis. The most similar species is the Thai *Angustopila elevata*, which has a more slender shell, a deeper umbilicus and lacks the spiral striae on its base. *A. fabella* sp. n. has a wider shell, a stronger peristome and a well-developed parietal tooth, whereas *A. subelevata* sp. n. is toothless. See also the two sympatric species, *A. dominikae* sp. n. and *A. szekeresi* sp. n.

Etymology. The name, subelevata, refers to the similarity to the Thai *Angustopila elevata*. **Type locality.** China, Guangxi (广西), Hechi Shi (河池市), Bama Xian (巴马县),

cliffs at the southern edge of Jiaole Cun (交乐村), 590 m, 24°7.045'N, 107°7.847'E.

Distribution. The new species is known from the type locality only (Figure 13). **Ecology.** As for *Angustopila fabella* sp. n.

Conservation status. As for Angustopila fabella sp. n.

Remarks. *Angustopila elevata*, which is known from approx. 1,000 km from the type locality of *A. subelevata* sp. n., is strikingly similar to the new species, although the general shell shape and the sculpture seem to be reliably different. See also Discussion.

Angustopila szekeresi Páll-Gergely & Hunyadi, sp. n. http://zoobank.org/D9845392-BD63-4253-89F5-B1F89FC779A8 Figure 5

Type material. China, Guangxi (广西), Hechi Shi (河池市), Bama Xian (巴马县), cliffs at the southern edge of Jiaole Cun (交乐村), 590 m, 24°7.045'N, 107°7.847'E,



Figure 5. *Angustopila szekeresi* Páll-Gergely & Hunyadi, sp. n. Holotype (HNHM 99440: A, C, D, E, F, G, H), Paratype (HNHM 99441: B). All images: B. Páll-Gergely.

leg. Hunyadi, A. & Szekeres, M., 10.09.2013., HNHM 99440 (holotype), HNHM 99441/2 (one of them is a figured paratype), HA/6 paratypes.

Diagnosis. A tiny, trigonal species with rounded aperture having a weak parietal tooth.

Description. Shell minute, light grey, blunt trigonal; protoconch consists of 1.25 whorls, microstructure finely pitted and granular with a powdery superficial texture,

Specimen	SH	SW	AH	AW	SW/SH×100	AW/AH×100
Holotype	0.91	0.8	0.34	0.36	87.91	105.88
paratype1	0.93	0.77	0.33	0.35	82.8	106.06
paratype2	1.03	0.89	0.36	0.39	86.41	108.33
paratype3	0.88	0.81	0.37	0.35	92.05	94.59
paratype4	1.03	0.85	0.36	0.39	82.52	108.33
paratype5	0.95	0.8	0.34	0.36	84.21	105.88

Table 5. Shell measurements (mm) for *Angustopila szekeresi* sp. n. from the type locality. For abbreviations see Table 1.

Table 6. Average, minimum value (min), maximum value (max), variance of values (var) and standard deviation of a set of values (stdev) for *Angustopila szekeresi* sp. n. (n = 6). For abbreviations see Table 1.

	SH	SW	AH	AW	SW/SH×100	AW/AH×100
Average	0.955	0.82	0.35	0.3667	85.9833	104.845
Min	0.88	0.77	0.33	0.35	82.52	94.59
Max	1.03	0.89	0.37	0.39	92.05	108.33
Var	0.0039	0.0018	0.0002	0.0003	13.1943	26.6148
stdev	0.0625	0.0429	0.0155	0.0186	3.6324	5.159

collectively radiating from the nuclear whorl; spiral threads of microstructure transverse the protoconch as well as the teleoconch, a prominent protoconch/teleoconch boundary is present (p/t), which interrupts the very faint rows of finely threaded microstructure; teleoconch finely reticulate with regularly-spaced radial growth striations crossed by rows of microscopic spiral threads; every 8th-10th radial line is stronger and visible as growth ridges; the 4–4.25 whorls are separated by a deep suture; whorls rounded; aperture oblique to shell axis; umbilicus deep, relatively narrow; aperture rounded; peristome slightly expanded, not reflected; laterally viewed, the middle section is slightly protruding; parietal callus weak, adnate; parietal tooth weak but present in all specimens.

Measurements (in mm): SH = 0.88-1.03, SW = 0.77-0.89, AH = 0.33-0.37, AW = 0.35-0.39 (n = 6). See also Tables 5 and 6.

Differential diagnosis. Sympatric species. Angustopila subelevata sp. n. lacks a parietal tooth, it has a wider umbilicus, a smaller aperture, and its peristome is not adnate. Moreover, the spiral lines on the embryonic whorls are much weaker in *A. subelevata* sp. n. Angustopila dominikae sp. n. is smaller, has a much more corpulent shell and two teeth in the aperture. Hypselostoma socialis sp. n. is much larger and has four teeth in its aperture.

Non-sympatric species. Angustopila fabella sp. n. has a wider shell, a wider umbilicus, weaker spiral lines on its umbilicus, a stronger parietal tooth and a strong parietal callus (its peristome is not adnate).

Etymology. *Angustopila szekeresi* sp. n. is named after Miklós Szekeres, our friend and partner in the field work resulting in all new species reported in this paper.

Type locality. China, Guangxi (广西), Hechi Shi (河池市), Bama Xian (巴马县), cliffs at the southern edge of Jiaole Cun (交乐村), 590 m, 24°7.045'N, 107°7.847'E.

Distribution. The new species is known from the type locality only (Figure 13). **Ecology.** As for *Angustopila fabella* sp. n.

Conservation status. As for Angustopila fabella sp. n.

Remarks. The spiral threading on the protoconch is common in the Hypselostomatidae (Panha and Burch 2005). Noteworthy, is the transition with the p/t boundary in that the microstructure continues in sync with the subsequent whorls. Normally, this phase of ontogenetic development in gastropods [p/t boundary] indicates the transition from the protoconch embryonal stage, whereby the shell structure changes and continues in the teleoconch constructional phase. The continuous protoconchteleoconch microstructural condition here suggests likely progenesis in these snails.

Genus Hypselostoma Benson, 1856

- Hypselostoma Benson, 1856b; The Annals and Magazine of Natural History, ser. 2, no.
 17: 342. (nomen novum pro *Tanystoma* Benson 1856a, non Motschulsky, 1845, Carabidae, Coleoptera).
- **Type species.** *Tanystoma tubiferum* Benson, 1856a, by monotypy. **Remarks.** See under the genus *Angustopila*.

Hypselostoma lacrima Páll-Gergely & Hunyadi, sp. n.

http://zoobank.org/F2872829-97AF-49E6-B3FC-CB787EBF8F10 Figures 6, 8F–K

Type material. China, Guangxi (广西), Chongzuo Shi (崇左市), Longzhou Xian (龙州县), cliffs N of Lenglei (楞垒), N of the Nonggang Nature Reserve (弄岗国家级自然保护区), 220 m, 22°29.161'N, 106°57.357'E, leg. Hunyadi, A. & Szekeres, M., 23.09.2013., HNHM 99444 (holotype), HNHM 99445 (figured paratype), HA/2 paratypes.

Diagnosis. Shell conical, with tumid body whorl and deep umbilicus; aperture with sinulus vertically oriented; tubus detached; aperture with one parietal lamella, one columellar and two palatal teeth; parietal lamella long and nearly straight.

Description. Shell minute, whitish/light grey, conical with enlarged body whorl; protoconch consists of 1.5 or slightly less whorls, finely granulated, with at least six fine spiral striations; teleoconch reticulated and regularly spirally striated with strong, irregular radial lines; the 5.5 or slightly less whorls are separated by a deep suture; whorls sloping and rounded; aperture oblique to shell axis; base of shell broadly umbilicate due to lateral expansion of last whorl; aperture detached from the penultimate whorl; aperture with sinulus vertically oriented (from apertural view); peristome expanded,



Figure 6. *Hypselostoma lacrima* Páll-Gergely & Hunyadi, sp. n. Holotype (HNHM 99442: **A, C–H**), Paratype (HNHM 99445: **B**). All images: B. Páll-Gergely.

not reflected, with relatively sharp edge; four apertural barriers; only the angulo-parietal lamella reaches the peristome; angulo-parietal lamella very long and high, not interrupted; it is lowest near the peristome; its posterior (inner) end is not visible in frontal view; its anterior end (closest to the peristome) is bent toward the upper palatal

Specimen	SH	SW	AH	AW	SW/SH×100	AW/AH×100
holotype	1.33	1.35	0.45	0.44	101.5	125.71
paratype	1.35	1.34	0.51	0.5	99.26	98.04

Table 7. Shell measurements (mm) for *Hypselostoma lacrima* sp. n. from the type locality. For abbreviations see Table 1.

Table 8. Average, minimum value (min), maximum value (max), variance of values (var) and standard deviation of a set of values (stdev) for *Hypselostoma lacrima* sp. n. (n = 2). For abbreviations see Table 1.

	SH	SW	AH	AW	SW/SH×100	AW/AH×100
Average	1.34	1.345	0.48	0.47	100.38	111.875
Min	1.33	1.34	0.45	0.44	99.26	98.04
Max	1.35	1.35	0.51	0.5	101.5	125.71
Var	0.0002	0.0001	0.0018	0.0018	2.5088	382.8144
stdev	0.0141	0.0071	0.0424	0.0424	1.5839	19.5656

plica, and its posterior end is bent toward the lower palatal plica; columellar and upper palatal folds elevated but short; the posterior end of the upper palatal fold curls toward the lower palatal fold; the lower palatal fold is also well-developed, and shorter than the others.

Measurements (in mm): SH = 1.33-1.35, SW = 1.34-1.35, AH = 0.45-0.51, AW = 0.44-0.5 (n = 2). See also Tables 7 and 8.

Differential diagnosis. See under Hypselostoma socialis sp. n.

Etymology. The name lacrima (Latin: tear) refers to the shape of the aperture.

Type locality. China, Guangxi (广西), Chongzuo Shi (崇左市), Longzhou Xian (龙州县), cliffs N of Lenglei (楞垒), N of the Nonggang Nature Reserve (弄岗国家 级自然保护区), 220 m, 22°29.161'N, 106°57.357'E.

Distribution. The new species is known from the type locality only (Figure 13). **Ecology.** As for *Angustopila fabella* sp. n.

Conservation status. As for Angustopila fabella sp. n.

Remarks. The subdivision of Hypselostomatidae is strongly based on the morphology of the apertural barriers ("teeth"). The main characters used for delimiting some of the major genera include the formation of the two teeth on the parietal region of the aperture, namely the parietal tooth (lamella) or parietalis and the angular tooth (lamella) or angularis. *Gyliotrachela, Paraboysidia* and *Acinolaemus* are said to possess separate parietal and angular lamellae. The former two have a more prominent parietal lamella rather than angular lamella, but in *Acinolaemus*, the angular is the dominant tooth. The angular lamella is entirely missing in the genus *Anauchen*. In the genera *Hypselostoma* and *Boysidia* these two lamellae are fused (Pilsbry 1917, Thompson and Upatham 1997, Panha and Burch 2005). Sometimes it is challenging to ascertain whether we are dealing with a single lamella (homologous with the parietal lamella) having a bifid anterior end or two lamellae (parietal and angular), which are concrescent. Moreover, the genera *Hypselostoma* and *Gyliotrachela* did not form monophyletic

units in the molecular phylogeny presented by Tongkerd et al. (2004), suggesting that the key characters used in classic taxonomy have developed phenotypically plastic forms. In this case of the two new species (*Hypselostoma lacrima* sp. n. and *H. socialis* sp. n.), we interpret the lamella on the parietal apertural wall as a congruent angulo-parietal lamella. Hence, both species are placed in *Hypselostoma*.

Hypselostoma socialis Páll-Gergely & Hunyadi, sp. n.

http://zoobank.org/49F4FD5C-C1E9-4B34-970C-C9B62072329D Figures 7, 8A–E

Type material. China, Guangxi (广西), Hechi Shi (河池市), Bama Xian (巴马县), cliffs at the southern edge of Jiaole Cun (交乐村), 590 m, 24°7.045'N, 107°7.847'E, leg. Hunyadi, A. & Szekeres, M., 10.09.2013., HNHM 99442 (holotype), HNHM 99443/3 (figured paratypes), SMF 346521/1 paratype, HA/15 paratypes + 4 juvenile shells (not paratypes), PGB/1.

Diagnosis. Shell spire conical, shell turban-shaped with tumid body whorl and broadly set, deep umbilicus; tubus detached; aperture rounded with wide sinulus, the upper parietal lamella dips to the right; aperture with a parietal lamella, one columellar and two palatal teeth; parietal lamella long and depressed, Z-shaped.

Description. Shell minute, whitish/light grey, conical with enlarged body whorl; protoconch consists of 1.5 whorls, finely pitted, with very slight indication of spiral lines; teleoconch reticulated with fine, regularly spirally striate microstructure intersected with irregular radial lines; the 5.5 whorls are separated by deep suture; whorls horizontally positioned, rounded; aperture oblique to shell axis; umbilicus deep, wide, especially at the last whorl; aperture free from the penultimate whorl, rounded with wide sinulus (area isolated by the parietal and upper palatal lamellae); sinulus horizontally oriented (apertural view); peristome slightly expanded, not reflected, with relatively sharp edge; (side view), the horizontally directed tuba is deflected downwards in alignment with the body whorl; four teeth recessed within aperture; only the ridge-like angulo-parietal lamella reaches the peristome, the others are situated deeper; angulo-parietal lamella moderately long, its end is visible from a straight view into the aperture; it is interrupted, consisting of an anterior section (situated closer to the peristome) and a slightly longer posterior section (situated deeper in the aperture); the anterior section is strongly bent toward the sinulus, its tip nearly touches the tip of the upper palatal fold; the posterior part of the angulo-parietal lamella is less strongly bent than the anterior portion, only its anterior part is bent toward the upper palatal lamella; the angulo-parietal and the upper palatal lamellae follow each other; the angulo-parietal lamella has a depressed Z-shape when observed after breaking off the lower part of the aperture; the anterior part of the angulo-parietal lamella is possibly homologous with the parietal lamella of other hypselostomatid taxa, while the second portion might be homologous with the angular lamella, or vice versa; columellar and lower palatal lamellae are elevated, blunt and short, they are about the same length and are visible through the semi-transparent shell; the upper palatal fold is also of similar

Specimen	SH	SW	AH	AW	SW/SH×100	AW/AH×100
holotype	1.34	1.36	0.46	0.51	101.49	110.87
paratype1	1.25	1.31	0.43	0.5	104.8	116.28
paratype4	1.22	1.28	0.46	0.5	104.92	108.7
paratype5	1.21	1.27	0.47	0.5	104.96	106.38
paratype6	1.22	1.26	0.45	0.49	103.28	108.89
paratype7	1.23	1.26	0.48	0.51	102.44	106.25
paratype8	1.18	1.22	0.45	0.49	103.39	108.89
paratype9	1.14	1.28	0.45	0.51	112.28	113.33
paratype10	1.26	1.31	0.5	0.53	103.97	106
paratype11	1.21	1.3	0.47	0.53	107.44	112.77

Table 9. Shell measurements (mm) for *Hypselostoma socialis* sp. n. from the type locality. For abbreviations see Table 1.

Table 10. Average, minimum value (min), maximum value (max), variance of values (var) and standard deviation of a set of values (stdev) for *Hypselostoma socialis* sp. n. (n = 10). For abbreviations see Table 1.

	SH	SW	AH	AW	SW/SH×100	AW/AH×100
Average	1.226	1.285	0.462	0.507	104.897	109.836
Min	1.14	1.22	0.43	0.49	101.49	106
Max	1.34	1.36	0.5	0.53	112.28	116.28
Var	0.0028	0.0014	0.0004	0.0002	9.3754	11.7788
stdev	0.0526	0.0378	0.0193	0.0142	3.0619	3.432

length, its posterior end runs parallel with the lower palatal fold; the tip of the upper palatal fold nearly touches the tip of the angulo-parietal lamella.

Measurements (in mm): SH = 1.14–1.34, SW = 1.22–1.36, AH = 0.43–0.5, AW = 0.49–0.53 (n = 10). See also Tables 9 and 10.

Differential diagnosis. *Hypselostoma lacrima* sp. n. and *H. socialis* sp. n. are the only species of *Hypselostoma* known from China. Some Chinese species formerly included in *Hypselostoma* have been reassigned to other genera (Yen 1939). *Hypselostoma dilatatum* Benthem Jutting 1962, *H. rupestre* Benthem Jutting 1962 and *H. annamiticum* Möllendorff, 1900 are approximately two times larger than *H. lacrima* sp. n. and *H. socialis* sp. n., and have more (5–8) apertural barriers. *Hypselostoma laidlawi* from Malaysia is similar in size to *H. lacrima* sp. n. and *H. socialis* sp. n., but it has a much narrower umbilicus and five apertural barriers.

Hypselostoma lacrima sp. n. has a much wider umbilicus than *H. socialis* sp. n. Moreover, the spiral lines on the protoconch of *H. socialis* sp. n. are weaker than those of the other species. The aperture of *H. lacrima* sp. n. is heart-shaped with the sinulus vertically oriented, whereas the aperture of *H. socialis* sp. n. is semi-quadrate and rounded with its sinulus positioned horizontally. The parietal lamella of *Hypselostoma socialis* sp. n. is interrupted and short (depressed Z-shaped), whereas that of *H. lacrima* sp. n. is longer and straighter, lacking the conspicuous blade-like ridge visible in *H. socialis* sp. n.

Etymology. The name, socialis, (Latin: social) refers to the fact that this new species has been found together with three *Angustopila* species.



Figure 7. Holotype of *Hypselostoma socialis* Páll-Gergely & Hunyadi, sp. n. (HNHM 99442). All images: B. Páll-Gergely.

Type locality. China, Guangxi (广西), Hechi Shi (河池市), Bama Xian (巴马县), cliffs at the southern edge of Jiaole Cun (交乐村), 590 m, 24°7.045'N, 107°7.847'E.

Distribution. *Hypselostoma socialis* sp. n. is known from the type locality only (Figure 13).



Figure 8. Aperture and apertural barriers of *Hypselostoma* species. **A–E** *Hypselostoma socialis* sp. n.: Holotype (HNHM 99442: **A**) Paratype1 (HNHM 99443: **B**, **E**), Paratype2 (HNHM 99443: **C**), Paratype3 (HNHM 99443: **D**); **F–K** *Hypselostoma lacrima* sp. n.: Holotype (HNHM 99444: **F–I**), Paratype (HNHM 99445: **J–K**). All images: B. Páll-Gergely.

Ecology. As for *Angustopila fabella* sp. n. **Conservation status.** As for *Angustopila fabella* sp. n. **Remarks.** See under *Hypselostoma lacrima* sp. n.

Genus Krobylos Panha & Burch, 1999

1999 Krobylos Panha & Burch, Walkerana 10 (24): 127.

Type species. Krobylos pomjuk Panha & Burch, 1999, by original designation.

Krobylos sinensis Páll-Gergely & Hunyadi, sp. n. http://zoobank.org/A2630E1E-5259-4D3F-9C05-BB769B5EAFC3 Figures 9–10

Type material. China, Guangxi (广西), Bose Shi (百色市), Leye Xian (乐业县), Chuandong Tiankeng Scenic Area (穿洞天坑景区), inner cliffs of the dolina, 1290 m, 24°48.430'N, 106° 29.277'E, leg. Hunyadi, A. & Szekeres, M., 09.09.2013., HNHM 99446 (holotype), HNHM 99447/1 (paratype), SMF 346522/1 paratype, HA/12 paratypes + 2 juvenile shells (not paratypes), PGB/1; China, Guangxi (广西), Hechi Shi (河池市), Tiane Xian (天峨县), Qimu Xiang (豈暮乡), cross towards Lahao Yan (拉号岩), 600 m, 24°51.130'N, 107°11.670'E, leg. Hunyadi, A. & Szekeres, M., 12.09.2013., HA/3 paratypes; China, Guangxi (广西), Hechi Shi (河池市), Huanjiang Xian (南丹县), cliffs above Dongning (峒宁) Village S of the Mulun Nature Reserve (木论国家级自然保护区), 530 m, 25°5.970'N, 107°57.639'E, leg. Hunyadi, A. & Szekeres, M., 17.09.2013., HA/3 paratypes.

Diagnosis. A large *Krobylos* species with conical spire, rounded, regularly coiled whorls, large oval-shaped aperture, adnate parietal side and very weak indication of spiral striae on its dorsal surface.

Description. Shell small, usually wider than high, only a single specimen from the Mulun Nature Reserve had the shell height and the shell diameter both measuring 2.7 mm; the 3.75–4.25 whorls are separated by a well-defined deep suture; whorls weakly angular, especially the penultimate whorl; protoconch light brownish purple, glossy, no notable sculpture visible; teleoconch light to dark purple, or pinkish, with blunt, irregularly course wrinkles; no spiral lines are visible under the microscope, but the SEM images revealed a hint of spiral striation on the lower half of each whorl (except for the last one); umbilicus open, narrow, (from ventral view), only its edge is covered by the peristome; aperture wide with its parietal part adnate to the penultimate whorl; peristome sharp, not thickened, not expanded nor reflexed; aperture reflected at columellar margin such that it covers the edge of the umbilicus.

Measurements (in mm): SH = 2.2-2.7, SW = 2.5-3 (n = 13 from all populations).

Differential diagnosis. Krobylos sinensis sp. n. differs from Tonkinospira depressa (Jaeckel 1950) by the larger size, rounded whorls and the absence of spiral sculpture on the upper sides of the whorls. The aperture of Tonkinospira defixa (Bavay & Dautzenberg, 1912) is not adnate, and its shell is much smaller than K. sinensis sp. n. Tonkinospira pulverea (Bavay & Dautzenberg, 1909) has more rounded whorls and the entire surface is regularly spirally striated. Tonkinospira pauperrima (Bavay



Figure 9. Holotype of *Krobylos sinensis* Páll-Gergely & Hunyadi, sp. n. (HNHM 99446). All images: B. Páll-Gergely.

& Dautzenberg, 1909) has a much more elevated spire, narrower umbilicus and stronger spiral striae.

Krobylos maehongsonensis Panha & Burch, 1999 has a higher spire, a relatively larger aperture, sharper keel, weaker radial growth lines and more bulging whorls



Figure 10. Sculpture of the holotype of *Krobylos sinensis* sp. n. (HNHM 99446). Abbreviations: NS: no spiral lines; S: spiral lines present. All images: B. Páll-Gergely.

from dorsal view (in *K. sinensis* sp. n. the whorls are ventrally more flat). *Krobylos kangkoy* Panha & Burch, 2004 (in Panha et al. 2004) has a much narrower umbilicus than the new species. *Krobylos pomjuk* Panha & Burch, 1999 also has a narrower umbilicus and a more depressed shell with a wider aperture. It is much smaller than *K. sinensis* sp. n. Similarly as small, *Krobylos takensis* Panha & Burch, 2004 (in Panha et al. 2004) has a higher spire and more angled whorls. *Krobylos tampla* is even smaller bearing a narrower umbilicus. The aperture of *Krobylos veruwan* Panha & Burch, 2004 (in Panha et al. 2004) has a low palatal ridge, which is missing in *K. sinensis* sp. n. Moreover, *K. veruwan* is much smaller than *K. sinensis* sp. n. and has a narrower

umbilicus. *Pyramidula laosensis* Saurin 1953, which also likely also belongs to *Kroby-los*, shows increased bulging whorls and a more pronounced closure of the umbilicus by the peristome.

Etymology. The species is named after China, the country of its type locality.

Type locality. China, Guangxi (广西), Bose Shi (百色市), Leye Xian (乐业县), Chuandong Tiankeng Scenic Area (穿洞天坑景区), inner cliffs of the dolina, 1290 m, 24°48.430'N, 106° 29.277'E.

Distribution. *Krobylos sinensis* sp. n. has been found in three different localities in northern Guangxi Province (Figure 13). See also remarks on the distinctness of *Krobylos* and *Tonkinospira*.

Ecology. Empty shells of this new species have been found in a soil sample at the base of large limestone rocks. It probably lives under stones and inside crevices.

Conservation status. *Krobylos sinensis* sp. n. is reported from three sites in this study. This species may inhabit similar habitats in the same geographic area. At the moment, on a global scale, its distribution is likely limited to less than 5 sites, therefore these vulnerable narrow range endemics warrant conservation priority (Vu D2) in conjunction with the Guidelines for the IUCN Red List (IUCN Standards and Petitions Subcommittee 2014).

Remarks. *Krobylos* was described as a group of toothless snails entirely lacking superficial microstructure (Panha and Burch 1999). *Tonkinospira*, on the other hand, has prominent spiral microsculpture over the entire surface. In this respect, *Krobylos sinensis* sp. n. is intermediate, because it has only very slight indication of spiral striae on the lower half of the whorls. This spiral sculpture is very faint or not visible under the microscope, but detectable using SEM images. We provisionally place *K. sinensis* sp. n. in the genus *Krobylos* because of the very weak spiral striae. However, we remark that the distinctness of the genera *Krobylos* and *Tonkinospira* requires further study. *Krobylos sinensis* sp. n. is the only species assigned to *Krobylos* reported outside of Thailand. However, "*Pyramidula*" *laosensis* might also belong to the same genus.

Discussion

Some of the new species reported in this study, especially the member of the genus *Angustopila*, have remarkably tiny shells. Adult individuals of *Angustopila subelevata* sp. n. (shell height = 0.83-0.91 mm, mean = 0.87 mm) and *A. dominikae* sp. n. (shell height of the holotype = 0.86 mm) represent the smallest members of the genus *Angustopila*, since the smallest member of the genus so far was *Angustopila elevata* with 0.92-0.99 mm height (Thompson and Upatham 1997) (Figure 11).

During a non-exhaustive literature survey (Powell 1979, Schileyko 1998a, 1998b, 2002, Panha and Burch 2005 for pulmonates; Boeters et al. 1989, Panha and Burch 2005, Liew et al. 2014 for operculate land snails), we found only very few reports of species smaller than 1 mm. The smallest land snail presented in these literature



Figure 11. Comparison of the sizes of the five smallest *Angustopila* species. **A** *Angustopila fabella* sp. n. **B** *Angustopila szekeresi* sp. n. **C** *Angustopila elevata* **D** *Angustopila subelevata* sp. n. **E** *Angustopila dominikae* sp. n. Dark grey silhouettes represent the smallest, light grey the largest shells. The numbers above the shells indicate the number of shells measured.



Figure 12. The holotype of *Angustopila dominikae* sp. n. in the eye of a sewing needle to picture its extraordinary small size. Photo: B. Páll-Gergely and N. Szpisjak.

is "*Pupisoma* sp." from Thailand, measuring "about 0.9 mm in length" (Panha and Burch 2005). Only a few genera containing species smaller than 1.5 mm according to Schileyko (1998a, 1998b, 2002), for example: *Pupisoma* (H = 1.3–3 mm; Schileyko 1998a), *Salpingoma* Haas 1937 (H = 1.3–1.5 mm; Schileyko 1998a), *Truncatellina* Lowe, 1852 (H = 1.2–2.5; Schileyko 1998b), *Acinolaemus* (H = 0.87–1.61, D = 0.65–1.92; Schileyko 1998b, page 255) and *Punctum* Morse, 1864 (D = 1–2 mm; Schileyko 2002). The height of 0.87 mm in *Acinolaemus* refers to a paratype of *Acinolaemus colpodon* Thompson & Upatham, 1997 measured from the base of the last whorl to the apex, but this is not the largest diameter of that shell. The largest measurement of that paratype is 1.05 mm from the base of the last whorl to the aperture. The diameter of 0.65 mm probably refers to the aperture height of *A. rhamphodon* Thompson & Upatham 1997, which appears as a measurement of the shell width due to the shifting of data in the table presented in the original description (Thompson and Upatham 1997, page 227). *Paralaoma serratocostata* Webster, 1906, which is probably the smallest land snail in New Zealand, is generally less than 1.0 mm



Figure 13. Map showing the distributions of newly described species of Chinese Hypselostomatidae. Filled circle: *Krobylos sinensis* Páll-Gergely & Hunyadi, sp. n. I Type locality of *Angustopila huoyani* 2 new locality of *Angustopila* cf. *huoyani* 3 Type locality of *Angustopila dominikae* Páll-Gergely & Hunyadi, sp. n., *Angustopila subelevata* Páll-Gergely & Hunyadi, sp. n., *Angustopila subelevata* Páll-Gergely & Hunyadi, sp. n. Angustopila sekeresi Páll-Gergely & Hunyadi, sp. n. and *Hypselostoma socialis* Páll-Gergely & Hunyadi, sp. n. 4 Type locality of *Angustopila fabella* Páll-Gergely & Hunyadi, sp. n. and *Hypselostoma lacrima* Páll-Gergely & Hunyadi, sp. n.

maximum shell dimension over a large part of its range (Powell 1979), but in some areas can reach 0.7×1.2 mm (Gary M. Barker, pers. comm.). As for operculated land snails, Liew et al. (2014) mentioned that the genus *Plectostoma* Adams, 1865 has a shell height of 1.0-3.7 mm. *Platyla minutissima* Boeters, Gittenberger & Subai, 1989, which is mentioned as the smallest European land snail, has a shell height of 1.1-1.25 mm. These data suggest that *Angustopila subelevata* sp. n. and *A. dominikae* sp. n. are amongst the smallest land snails ever reported if the largest measurement of the shell is considered. If however, shell volume is calculated according to McCain and Nekola (2008) and Nekola (2014), there are even tinier land snails (e.g. Punctidae spp) occupying the lowest rung of the volume/size scale.

The smallest snails are, however, certainly marine species. The smallest recorded gastropod seems to be *Ammonicera minortalis* Rolán, 1992, ranging in size from 0.32 to 0.46 mm. Although a few marine species less than 1 mm are known, all of them are larger than *A. minortalis*. For example, Europe's smallest gastropod, *Retrotortina*

fuscata Chaster, 1896 measures 0.5–0.75 mm (Gofas and Warén 1998). Extremes in body size of organisms not only attract attention from the public, but also incite interest regarding their adaptation to their environment (Hanken and Wake 1993, Grebennikov 2008, Glaw et al. 2012). Investigating tiny-shelled land snails is important for assessing biodiversity and natural history as well as for establishing the foundation for studying the evolution of dwarfism in invertebrate animals. The present data are insufficient for addressing the evolutionary processes of miniaturization in land snails. However, we hope that these results provide the taxonomic groundwork for future studies concerning the evolution of dwarfism in invertebrates.

Biogeography

The similarity between distantly distributed species (*A. elevata* – *A. subelevata*; *A. tamlod* – *A. huoyani*) and the two populations of *Angustopila huoyani* can be explained by three different hypotheses: (1) These populations may be connected with additional populations (i.e. via contiguous cave systems or interconnected river drainage basins) resulting in a continuous distributional area. The 500–1000 km gap between the known populations is therefore due to lack of additional exploration and thus, additional material; (2) they can be the results of rare long distance dispersal events; or (3) convergent evolution of shell traits. Our present knowledge is insufficient to reject any of these hypotheses.

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RESEARCH ARTICLE



Five new cryptic freshwater gastropod species from New Caledonia (Caenogastropoda, Truncatelloidea, Tateidae)

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Abstract

During the course of a project aiming at the reconstruction of the colonization of the South Pacific islands by tateid gastropods based on molecular data we discovered five new species on New Caledonia belonging to the genera *Hemistomia* and *Leiorhagium*, respectively. We describe these species based on morphological, anatomical and genetic data. All five species are morphologically cryptic as they closely resemble or are even indistinguishable from known species stressing the importance of a comprehensive taxonomic approach integrating several methods. As a consequence of their small and fragmented geographic ranges and the rapidly progressing anthropogenic land cover changes on New Caledonia, all five species qualify as critically endangered according to the criteria of the IUCN.

Keywords

Conservation, cryptic species, endemic, integrative taxonomy, IUCN, New Caledonia, South Pacific, spring snails, Tateidae

Introduction

New Caledonia is famous for being a biodiversity hotspot harboring a high number of endemic species (Myers et al. 2000) including a radiation of small freshwater gastropods belonging to the family Tateidae. This radiation is probably of Oligocene origin and comprises more than 50 species in seven genera (Haase and Bouchet 1998, Zielske and Haase 2015). Many of these species are extreme narrow-range endemics known from only few or single sites (Haase and Bouchet 1998), a pattern typical for Truncatelloidea in freshwaters worldwide (e.g. Giusti and Pezzoli 1980, Radoman 1983, Haase 1996, 2008, Ponder and Colgan 2002, Liu and Hershler 2005, Hershler et al. 2011, Delicado and Ramos 2012). In the frame of a project aiming at the reconstruction of the colonization of the South Pacific islands by tateids based on molecular data (Zielske and Haase 2014a, b, 2015, Zielske, Ponder and Haase in preparation) we visited New Caledonia in May 2012 in order to collect suitable material for sequencing. During this expedition we found five new species of the genera Hemistomia Crosse, 1872 and Leiorhagium Haase & Bouchet, 1998, respectively (Figs 1, 2), which we describe herein based on morphological, anatomical and genetic data. All five species qualify as morphologically cryptic as they closely resemble or are even indistinguishable from known species (see Pfenninger and Schwenk 2007). The discovery of new cryptic species was predicted by Haase and Bouchet (1998), whose revision was based solely on morphology and anatomy. In general, cryptic species are common among different spring snail families of Truncatelloidea (e.g., Liu et al. 2003; Haase et al. 2007; Delicado and Ramos 2012; Collado et al. 2013).

Material and methods

Snails were fixed in 70% ethanol in the field, transferred to propylene glycol for shipping by courier, and returned to ethanol, this time 96%, after arrival in our lab. For measurements, up to 20 snails per sample were photographed under a Zeiss SteREO Discovery.V20 dissecting microscope with a Zeiss Axio Cam MR3. Five dimensions – shell height, shell width, aperture height, aperture width, body whorl width – were measured using the program AxioVision 40 V4.8. (Zeiss) and whorls counted to the nearest eighth (Kerney and Cameron 1979). Up to six shells were dissolved in diluted hydrochloric acid for dissections. Anatomies were photographed as well. These digital images served as template for drawings made on a graphical tablet. For scanning electron microscopy up to three shells, radulae and opercula were cleaned in 5% sodium hypochlorite. The cephalopodia of up to two males were dried using hexamethyldisilazane (Nation 1983). After sputter coating with gold objects were investigated in a Zeiss EVO LS10 Scanning Microscope.

Morphometric analyses of shell measurements including canonical variates analyses (CVA) maximizing the differentiation of groups in multivariate space, multivariate analyses of variance (MANOVA), assignment tests, and Hotelling's T²-tests were conducted



Figure 1. Localities of new species and samples used for morphometric comparisons. Inset shows position of New Caledonia in the Southwest Pacific. Arrows indicate type localities of species represented by more than one sample (see also Table 1).

in PAST 2.12 (Hammer et al. 2001). Sequential Bonferroni-correction was applied to multiple tests. These analyses also included samples of known, similar species the new ones could be mistaken for (Table 1). The selection of species used in comparisons was based on the phylogenetic analysis.

Phylogenetic analyses were based on a selection of sequences generated by Zielske and Haase (2015), who analyzed fragments of the mitochondrial genes cytochrome oxidase subunit I (COI) and 16S rRNA as well as the nuclear internal transcribed spacer 2 (ITS2). For lab protocols see Zielske and Haase (2014a, 2015). We restricted the analysis to 3 specimens per species at most and used *Kanakyella gentilsiana, Crosseana crosseana*, and *C. melanosoma* as outgroups (Table 1). The alignment of 16S rRNA and ITS2 was generated using secondary structure information using RNAsalsa 0.8.1 (Stocsits et al. 2009) (for details see Zielske and Haase 2015) and checked for ambiguous and randomly similar sites in Aliscore 2.0 (Misof and Misof 2009). We defined seven partitions. PartitionFinder 1.1.0 (Lanfear et al. 2012) identified the following scheme and substitution models as optimal among all possible combinations of separate and merged partitions: COI 1st positions (TrNef+I), COI 2nd positions (F81), COI 3rd positions (TVM+I+ Γ), 16S rRNA loops (TrN+ Γ), ITS2 loops (TrNef+I+ Γ), joint stems of 16S rRNA and ITS2 (K80+I). With these settings, tree reconstructions were conducted in a maximum likelihood (ML) frame-

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Species, sample	Locality	Latitutde, longitude	COI	16S	IT2
H. andreae, NeCa 12_1 H. andreae, NeCa 12_2	Bouloupari, Ouaméni valley	21°49'46.9"S; 165°56'42.9"E	KJ490851 KJ490852	KJ490767 	KJ490691
<i>H. cockerelli</i> , paratypes MNHN IM-2012-2694	Bouloupari, Ouaméni, prop. Debels	21°49'12.0"S; 166°56'36.0"E			
H. cockerelli, NeCa 11	Bouloupari, Ouitchambo	21°48'16.8"S; 166°00'00.8"E			
H. cockerelli, NeCa 17	Moindou, road toward barrage	21°39'52.8"S; 165°43"10.3"E	KJ490857	KJ490772	KJ490696
H. cockerelli, NeCa 21A	Farino, Sentier de la Cascade et des Sources	21°38'11.9"S; 165°46'36.6"E	KJ490863		KJ490702
H. cockerelli, NeCa 36	Sarraméa, track to "Trou d'Eau"	21°38'22.1"S; 165°51'37.5"E			
H. cockerelli, NeCa 54	Hienghène, Tendo	20°42'54.7"S; 164°49'20.8"E			
H. eclima, NeCa 19	Moindou, road toward barrage	21°39'58.4"S; 165°43'08.2"E	KJ490858	KJ490773	KJ490697
H. fabrorum, NeCa 1	Dumbéa, Koé, prop. Oesterlin	22°08'59.0"S; 166°29'10.6"E	KJ490829	KJ490749	KJ490670
H. fabrorum, NeCa 25B	Sarraméa, road side of RPN 5	21°34'15.7"S; 165°49'41.2"E	KJ490867	KJ490781	KJ490704
H. minor, NeCa 30	Moindou, road side SW Katrikoin	21°34'21.6"S;165°41'02.5"E	KJ490872	KJ490786	KJ490709
<i>Н. пуо</i> , NeCa 35	Bourail, Oua Oué	21°36'50.3"S; 165°35'31.5"E	KJ490880	KJ490791	KJ490716
H. oxychila, NeCa 43A	Poya, road side between Nétéa and Goipin	21°16'06.0"S; 165°14'32.0"E	KJ490893	KJ490804	KJ490726
H. rusticorum, NeCa 6A	Bouloupari, road side N Nassirah	21°48'08.0"S; 166°04'14.6"E	KJ490836	KJ490755	KJ490677
H. winstonefi, NeCa 3B	Mont Dore, Rue des Roseaux, prop. Solier	22°15'42.4"S; 166°34'08.7"E	KJ490834	KJ490753	KJ490675
L. adioincola, NeCa 43B	Poya, side of road to Goipin	21°16'06.0"S; 165°14'32.0"E	KJ490895	KJ490806	KJ490728
L. adioincola, NeCa 49	Poya, stream into Grotte d'Adio	21°15'24.4"S; 165°14'46.4"E	KJ490901	KJ490812	KJ490734
<i>L. ajie</i> , paratypes MNHN IM-2012-2688	Houailou, Néoua	21°24'00.0"S; 165°38'54.0"E			
L. aremuum, NeCa 33_1 L. aremuum, NeCa 33_2	Moindou, Aremu valley	21°35'04.8"S; 165°39'07.5"E	KJ490878 KJ490879	KJ490789 KJ490790	KJ490714 KJ490715
L. clandestinum, NeCa 30B	Moindou, road side SW Katrikoin	21°34'21.6"S; 165°41'02.5"E	KJ490874	1	KJ490711

Species, sample	Locality	Latitutde, longitude	COI	165	IT2
<i>L. douii</i> , paratypes MNHN IM-2012-2681	Poya, Grotte d'Adio	21°15'30.0"S; 165°14'30.0"E			
L. inplicatum, NeCa 9B	Bouloupari, road side of RP 4	21°44'30.9"S; 166°05'57.9"E	KJ490845	KJ490762	KJ490685
<i>L. kavuneva</i> , paratypes MNHN IM-2012-2690	Sarraméa, prop. Bonnard	21°39'00.0"S; 165°50'48.0"E			
L. kavuneva, NeCa 15B	Bouloupari, Oua Tom	21°47'24.4"S; 165°54'51.2"E	KJ490855	KJ490770	KJ490694
L. kavuneva, NeCa 27	Kouaoua, road side N Koh	21°30'52.2"S; 165°48'05.0"E	KJ490869	KJ490783	KJ490706
L. kavuneva, NeCa 29	Kouaoua, road side N Koh	21°32'02.6"S; 165°49'27.2"E			
L. monachum, paratypes MNHN IM-2012-2679	Poya, Mt. Krapé	21°23'12.0"S; 165°14'30.0"E			
L. montfaouense, paratypes MNHN IM-2012-2684	Poya, Montfaoué	21°16'48.0"S; 165°17'42.0"E			
L. neteae, NeCa 44B	Poya, beginning of road into Vallée d'Adio	21°14'47.9"S; 165°15'45.0"E	KJ490897	KJ490808	KJ490730
L. orokau, NeCa 42	Poya, near Nétéa	21°16'32.2"S; 165°12'17.6"E	KJ490891	KJ490802	KJ490724
L. orokau, NeCa 57	Hienghène, Tendo	20°42'43.9"S; 164°47'47.5"E	KJ490912	KJ490823	KJ490744
C. crosseana, NeCa 51	Koumac, seepage in N of town	20°32'32.2"S; 164°18'33.0"E	KJ490904	KJ490815	KJ490737
C. melanosoma, NeCa 50	Voh, Boyen, overflow of reservoir	20°49'13.6"S; 164°36'56.4"E	KJ490902	KJ490813	KJ490735
K. gentilsiana, NeCa 58	Hienghène, Tendo	20°42'22.4"S; 164°47'20.0"E	KJ490914	KJ490825	KJ490746

work using GARLI 2.01 (Zwickl 2006) with 500 replicates. Robustness was assessed by bootstrapping with 200 replicates.

Type and non-type material is deposited at the Museum National d'Histoire Naturelle in Paris (MNHN) and at the Naturhistorisches Museum Wien (NHMW).

Results

Systematic descriptions

Diagnoses and descriptions of *Hemistomia* and *Leiorhagium* and data used in our comparisons with the new species were provided by Haase and Bouchet (1998). Locality data include site number, district capital, site, coordinates, and date of collection. Shell measurements are given in Table 2 and not repeated in the descriptions.

Genus Hemistomia Crosse, 1872

Type species. Hemistomia caledonica Crosse, 1872

Hemistomia andreae sp. n.

http://zoobank.org/1C80E381-43F7-43EB-9853-425C5C6B925E

Type material. Holotype MNHN IM 2000-27858; paratypes MNHN IM 2000-27859 (> 50), NHMW 110181 (10).

Type locality. NeCa 12, Bouloupari: Ouaméni-valley, small stream on W-side of road in secondary forest, 21°49'46.9"S, 165°56'42.9"E, 22 May 2012.

Etymology. The new species is dedicated to the senior author's daughter on the occasion of her 'quinceañera', the 15th birthday.

Diagnosis. *H. andreae* sp. n. is very similar to *H. cockerelli* and *H. nyo.* It differs from both in a clearer separation of the opercular pegs and a much more delicate penis. The protoconch of the new species has more whorls than *H. nyo* and the palatal denticle is further behind the outer lip.

Shell. Conical, 2.2 times higher than wide, 4.5-5.5 whorls, without colour, transparent; protoconch faintly pitted with 1-1.25 whorls; palatal denticle large, elongate, c. 1/3 whorl behind outer lip; with columellar fold in the body whorl; aperture slightly higher than wide (Figs 2A, 3A,B, 4A,B).

Operculum. Elongate-ellipsoidal, paucisprial, nucleus submarginal, orange, one large and one small non-calcareous white peg, well separated from each other (*N*=5) (Fig. 5A,B).

External features. Epidermis without pigment, eyes black.



Figure 2. Holotypes. **A** *Hemistomia andreae* sp. n. **B** *Leiorhagium adioincola* sp. n. **C** *Leiorhagium aremuum* sp. n. **D** *Leiorhagium clandestinum* sp. n. **E** *Leiorhagium neteae* sp. n. Scale bar = 1 mm.

Mantle cavity. Ctenidium with 24–26 (2 males) or 25–28 (3 females) filaments; osphradium kidney-shaped, behind middle of ctenidium.

Digestive system. Radula formula (N=3) (Fig. 6A): R (rhachis or central tooth): 3 1 3/2 2, L (lateral tooth): 3 1 5, M₁ (inner marginal tooth): 21–25, M₂ (outer marginal tooth): 27–32; stomach without caecum; rectum close to pallial oviduct in females and to prostate in males.

Female genitalia. Ovary without lobes, proximal end c. 1.25 whorls below apex, comprising 0.25-0.5 whorls, eventually reaching stomach; anterior capsule gland yellow-orange, posterior capsule gland opaque-white, albumen gland milky-white; proximal loop of renal oviduct upright comprising 180°, distal loop short; bursa copulatrix pear-shaped, reaching only slightly behind albumen gland; bursal duct long, entering anterior; seminal receptacle on ventral edge of and as long as bursa (N=3) (Fig. 7A).

Male genitalia. Proximal end of lobate testis 1-1.25 whorls below apex, comprising 0.75 whorls, covering proximal end of stomach; vesicula seminalis arising from anterior third of testis; penis fairly delicate with blunt end (N=2) (Fig. 8A,B).

Remarks. This is *Hemistomia* sp. n. 1 of Zielske and Haase (2015). Both *H. andreae* sp. n. and *H. cockerelli* do have the columellar fold in the body whorl assumed to be unique in *H. nyo* by Haase and Bouchet (1998). *H. andreae* sp. n. is only known from the type locality.

Genus Leiorhagium Haase & Bouchet, 1998

Type species. Leiorhagium orokau Haase & Bouchet, 1998

Table 2. Morphometry. Measurements in mm. Shell measures: AH, aperture hight; AW, aperture width; BWW, width of body whorl; SH, shell height; SW, shell width; W, number of whorls; statistics: CV, coefficient of variation corrected for unequal sample sizes; max, maximum; min, minimum; SD, standard deviation. First line of new species contains measurements of holotypes. Note that the holotype was only in case of *L. clandestinum* included in the descriptive statistics. Numbers of whorls were only counted in the new species as this parameter was not used in the statistical analyses.

New species							
	SH	SW	AH	AW	BWW	SH/SW	W
<i>H. andreae</i> sp. n. (<i>N</i> =20)	2.70	1.25	0.90	0.87	1.08	2.17	5.4
min	2.40	1.10	0.80	0.75	0.97	2.00	4.50
max	2.78	1.28	0.93	0.91	1.08	2.35	5.50
mean	2.60	1.18	0.85	0.82	1.02	2.20	5.14
median	2.60	1.17	0.85	0.82	1.01	2.23	5.25
SD	0.11	0.05	0.04	0.04	0.03	0.11	0.28
CV	4.40	3.93	4.23	4.54	2.77	4.94	5.49
L. adioincola sp. n. NeCa 49 (N=20)	2.29	1.24	0.88	0.87	1.09	1.84	4.50
min	2.10	1.16	0.83	0.83	1.04	1.71	4.13
max	2.42	1.31	0.96	0.96	1.15	1.90	4.75
mean	2.25	1.25	0.88	0.89	1.10	1.80	4.36
median	2.24	1.24	0.88	0.89	1.10	1.80	4.25
SD	0.09	0.04	0.04	0.03	0.03	0.05	0.18
CV	4.21	2.96	4.15	3.92	2.94	2.91	4.24
L. aremuum sp. n. (N=20)	2.19	1.35	0.97	0.91	1.16	1.62	4.25
min	2.03	1.29	0.87	0.86	1.10	1.53	3.75
max	2.43	1.46	1.03	1.00	1.25	1.69	4.25
mean	2.19	1.35	0.94	0.92	1.16	1.62	4.03
median	2.15	1.35	0.93	0.92	1.17	1.62	4.00
SD	0.11	0.05	0.04	0.04	0.04	0.04	0.15
CV	4.92	4.06	4.76	4.54	3.77	2.71	3.78
L. clandestinum sp. n. (N=4)	2.49	1.32	0.94	0.95	1.16	1.91	4.50
min	2.23	1.26	0.89	0.88	1.07	1.77	4.25
max	2.49	1.32	0.94	0.95	1.16	1.91	4.50
mean	2.38	1.28	0.91	0.92	1.10	1.86	4.41
median	2.41	1.27	0.90	0.93	1.09	1.89	4.44
SD	0.11	0.03	0.02	0.03	0.04	0.06	0.12
CV	4.83	2.30	2.68	3.44	4.00	3.52	2.89
<i>L. neteae</i> n. sp. (<i>N</i> =18)	2.07	1.12	0.75	0.77	0.91	1.84	4.50
min	1.85	0.97	0.65	0.70	0.82	1.76	4.25
max	2.23	1.17	0.79	0.80	0.95	2.01	5.00
mean	2.05	1.09	0.73	0.75	0.88	1.88	4.46
median	2.04	1.10	0.73	0.75	0.87	1.88	4.38
SD	0.12	0.05	0.03	0.03	0.03	0.07	0.19
CV	6.05	4.82	4.71	4.69	3.99	3.72	4.25
Material for comparisons							
	SH	SW	AH	AW	BWW	SH/SW	
H. cockerelli Types (N=20)							
min	2.58	1.18	0.88	0.83	1.03	2.05	
max	3.21	1.39	1.03	0.97	1.16	2.40	
mean	2.79	1.27	0.94	0.91	1.09	2.19	

median	2.74	1.25	0.93	0.90	1.09	2.18	
SD	0.17	0.06	0.04	0.04	0.05	0.09	
CV	6.20	4.91	4.31	4.52	4.23	4.36	
H. cockerelli NeCa11 (N=20)							
min	2.20	1.06	0.77	0.73	0.94	1.93	
max	2.48	1.25	0.87	0.91	1.04	2.28	
mean	2.33	1.13	0.81	0.81	0.97	2.06	
median	2.32	1.12	0.80	0.80	0.96	2.03	
SD	0.08	0.04	0.03	0.04	0.02	0.10	
CV	3.49	3.70	3.36	4.60	2.36	4.75	
H. cockerelli NeCa17 (N=20)							
min	2.35	1.16	0.83	0.83	1.04	1.96	
max	2.62	1.28	0.92	0.93	1.14	2.19	
mean	2.50	1.21	0.87	0.87	1.08	2.07	
median	2.51	1.21	0.87	0.88	1.08	2.07	
SD	0.07	0.04	0.03	0.03	0.02	0.07	
CV	2.90	3.20	3.16	3.25	2.32	3.43	
H. cockerelli NeCa21A (N=8)							
min	2.26	1.09	0.74	0.77	0.96	2.03	
max	2.74	1.23	0.89	0.87	1.08	2.38	
mean	2.49	1.17	0.84	0.83	1.03	2.12	
median	2.49	1.17	0.85	0.83	1.05	2.09	
SD	0.14	0.05	0.04	0.03	0.04	0.11	
CV	5.87	4.33	5.41	3.89	3.66	5.50	
H. cockerelli NeCa36 (N=13)							
min	2.32	1.14	0.79	0.82	1.03	1.97	
max	2.64	1.23	0.91	0.91	1.12	2.14	
mean	2.43	1.18	0.85	0.85	1.06	2.05	
median	2.42	1.19	0.86	0.85	1.06	2.04	
SD	0.10	0.03	0.03	0.03	0.03	0.05	
CV	4.25	2.42	3.79	3.22	2.64	2.64	
H. cockerelli NeCa54 (N=20)							
min	2.28	1.16	0.78	0.82	1.04	1.86	
max	2.63	1.31	0.96	0.93	1.14	2.14	
mean	2.47	1.23	0.87	0.88	1.09	2.00	
median	2.47	1.23	0.86	0.87	1.10	2.02	
SD	0.10	0.04	0.04	0.03	0.03	0.07	
CV	4.18	3.08	4.18	3.28	2.62	3.62	
H. nyo NeCa35 (N=7)							
min	2.43	1.25	0.88	0.89	1.09	1.93	
max	2.75	1.34	0.96	0.96	1.15	2.08	
mean	2.62	1.30	0.92	0.92	1.12	2.01	
median	2.69	1.30	0.91	0.91	1.11	2.03	
SD	0.12	0.04	0.03	0.03	0.02	0.06	
CV	4.80	2.84	3.30	3.10	2.00	3.00	
L. ajie Types (N=6)							
min	2.35	1.31	0.93	0.94	1.12	1.61	
max	2.74	1.62	1.10	1.06	1.34	1.80	
mean	2.50	1.46	1.01	1.00	1.25	1.72	
							1

median	2.43	1.46	1.01	1.00	1.27	1.70	
SD	0.16	0.12	0.07	0.05	0.08	0.07	
CV	6.50	8.31	6.95	4.88	6.32	4.12	
L. douii Types (N=20)							
min	1.87	0.98	0.68	0.68	0.86	1.84	
max	2.50	1.16	0.84	0.79	0.97	2.16	
mean	2.06	1.05	0.73	0.71	0.91	1.96	
median	2.02	1.06	0.72	0.71	0.91	1.95	
SD	0.14	0.04	0.04	0.02	0.03	0.08	
CV	7.04	4.02	5.11	3.51	3.21	4.23	
<i>L. kavuneva</i> Types (<i>N</i> =20)							
min	2.17	1.17	0.78	0.82	1.02	1.77	
max	2.42	1.32	0.94	0.93	1.13	1.93	
mean	2.31	1.26	0.88	0.88	1.07	1.84	
median	2.33	1.25	0.89	0.88	1.07	1.85	
SD	0.07	0.04	0.04	0.03	0.03	0.05	
CV	3.24	3.16	4.52	3.15	2.87	2.58	
L. kavuneva NeCa15B (N=20)							
min	2.20	1.21	0.84	0.88	1.07	1.76	
max	2.46	1.31	0.94	0.98	1.20	1.97	
mean	2.34	1.27	0.90	0.92	1.12	1.84	
median	2.35	1.28	0.91	0.92	1.12	1.83	
SD	0.07	0.03	0.03	0.03	0.03	0.06	
CV	3.14	2.30	3.31	3.00	2.54	3.14	
L. kavuneva NeCa29 (N=20)							
min	2.17	1.20	0.85	0.85	1.06	1.76	
max	2.54	1.36	1.00	0.99	1.17	1.97	
mean	2.35	1.28	0.91	0.93	1.12	1.83	
median	2.34	1.27	0.90	0.93	1.13	1.82	
SD	0.10	0.04	0.04	0.04	0.03	0.05	
CV	4.27	3.12	4.50	3.93	2.91	2.62	
<i>L. monachum</i> Types (<i>N</i> =3)							
min	2.07	1.04	0.72	0.69	0.88	1.88	
max	2.18	1.10	0.82	0.78	0.97	2.00	
mean	2.11	1.08	0.76	0.74	0.92	1.96	
median	2.07	1.09	0.76	0.75	0.92	1.99	
SD	0.07	0.03	0.05	0.05	0.05	0.07	
CV	3.36	3.19	7.25	6.89	5.51	3.67	
L. montfaouense Types (N=10)							
min	1.80	1.03	0.68	0.64	0.83	1.76	
max	2.30	1.16	0.81	0.77	0.99	1.99	
mean	2.01	1.08	0.73	0.70	0.90	1.87	
median	2.02	1.05	0.72	0.68	0.89	1.86	
SD	0.15	0.05	0.05	0.04	0.06	0.09	
CV	7.73	4.89	6.40	6.29	6.87	4.79	


Figure 3. Shells (all paratypes). **A, B** *Hemistomia andreae* sp. n. **C, D** *Leiorhagium adioincola* sp. n. **E, F** *Leiorhagium aremuum* sp. n. **G** *Leiorhagium clandestinum* sp. n. **H, I** *Leiorhagium neteae* sp. n.

Leiorhagium adioincola sp. n.

http://zoobank.org/CCC4F863-76C3-44C2-A4AA-CE9DE0B726AB

Type material. Holotype MNHN IM 2000-27860; paratypes MNHN IM 2000-27861 (29), NHMW 110182 (5).

Type locality. NeCa 49, Poya: Massif d'Adio, stream flowing into Grotte d'Adio, open secondary forest, 21°15'24.4"S, 165°14'46.4"E, 29 May 2012.

Other material. NeCa 43, Poya: small stream on W-side of road between Nétéa and Goipin, on forest edge, 21°16'06.0"S, 165°14'32.0"E, 28 May 2012, MNHN-IM-2012-36075 (23), NHMW 110183 (10).

Etymology. Adioincola is composed of the name of the area of Adio and the Latin noun incola meaning inhabitant, and thus refers to the type locality of the new species.

Diagnosis. *L. adioincola* sp. n. is very similar to *L. kavuneva* and *L. clandestinum* sp. n. The former pair differs in penial shape, slender vs. basally broad with long terminal filament. *L. adioincola* sp. n. tends to have fewer radular denticles than *L. kavuneva*. Genetically, these species differed on average at 9.65% of the positions of COI. Due

to the lack of anatomical data, both new species can only be distinguished genetically. Their COI sequences differed on average by 9.5% (p-distance).

Shell. Pupiform, 1.8 times higher than wide, 4.125-4.75 whorls, without colour, transparent; protoconch faintly pitted with c. 1 whorl; palatal denticle a small droplet 1/8 whorl behind outer lip; aperture as high as wide (Figs 2B, 3C, D, 4C, D).

Operculum. Elongate-ellipsoidal, paucisprial, nucleus submarginal, orange, usually two non-calcareous white pegs, eventually accompanied by a small third one (N=3) (Fig. 5C,D).

External features. Epidermis without pigment, eyes black.

Mantle cavity. Ctenidium with 18-19 (3 males) or 21–24 (2 females) filaments; osphradium kidney-shaped, behind middle of ctenidium.

Digestive system. Radula formula (N=3) (Fig. 6B): R: 4 1 4/2 2, L: 4-5 1 6, M₁: 22-27, M₂: 21-29; stomach without caecum; rectum close to pallial oviduct in females and to prostate in males.

Female genitalia. Ovary without lobes, proximal end 1.25 whorls below apex, comprising 0.25-0.5 whorls, eventually reaching stomach; anterior capsule gland yellow-orange, posterior capsule gland opaque-white, albumen gland milky-white; proximal loop of renal oviduct bent forward, distal loop short; bursa copulatrix almost cubical, reaching behind albumen gland; bursal duct long, entering anterior; no seminal receptacle (N=2) (Fig. 7B).

Male genitalia. Proximal end of lobate testis 1.25-1.5 whorls below apex, comprising 0.5-0.75 whorls, covering proximal end of stomach; vesicula seminalis arising from anterior half of testis; penis slender, terminal end occasionally forming short filament (N=3) (Fig. 8C).

Remarks. This is *Leiorhagium* sp. n. 4 of Zielske and Haase (2015). *L. adioincola* sp. n. occurs in the area between the villages of Nétéa and Goipin including the Massif d'Adio.

Leiorhagium aremuum sp. n.

http://zoobank.org/3B015791-A03B-48BB-8C1D-1A829588B5E2

Type material. Holotype MNHN IM 2000-27862; paratypes MNHN IM 2000-27863 (28), NHMW 110184 (10).

Type locality. NeCa 33, Moindou: spring-fed stream close to road in Aremu valley, under shrub, 21°35'04.8"S, 165°39'07.5"E, 26 May 2012.

Etymology. The new species is named after the Aremu valley, where it has been discovered.

Diagnosis. *L. aremuum* sp. n. is most similar to *L. ajie*, which is, however, larger and slightly more slender, lacks the palatal denticle, and has a more massive penis. The prolonged capsule gland is unique among New Caledonian tateids. The COI sequences had a p-distance of 9.4%.

Shell. Broadly pupiform, 1.62 times higher than wide, 3.75-4.25 whorls, without colour, transparent; protoconch faintly pitted with 0.75-0.9 whorls; palatal denticle a



Figure 4. Protoconchs (left) and close-up views of apical microstructure (right). **A**, **B** *Hemistomia andreae* sp. n. **C**, **D** *Leiorhagium adioincola* sp. n. **E**, **F** *Leiorhagium aremuum* sp. n. **G**, **H** *Leiorhagium clandestinum* **I**, **J** *Leiorhagium neteae* sp. n. Scale bars 50 μm (**A**, **C**, **E**, **G**, **I**), 10 μm (**B**, **D**, **F**, **H**, **J**).



Figure 5. Operculum. A, B Hemistomia andreae sp. n. C, D Leiorhagium adioincola sp. n. E, F Leiorhagium aremuum sp. n. G, H Leiorhagium neteae sp. n.

small droplet 1/8 whorl behind outer lip; aperture practically as high as wide (Figs 2C, 3E,F, 4E,F).

Operculum. Elongate-ellipsoidal, paucisprial, nucleus submarginal, orange, two noncalcareous white pegs, eventually accompanied by a small third one (N=4) (Fig. 5E, F).

External features. Epidermis without pigment, eyes black.

Mantle cavity. Ctenidium with 15-16 (2 males) or 19-20 (2 females) filaments; osphradium elongate, slightly behind middle of ctenidium.

Digestive system. Radula formula (N=3) (Fig. 6C): R: 4-5 1 4-5/2-3 2-3, L: 4-5 1 4-6, M₁: 26-31, M₂: 20-32; stomach without caecum; rectum close to pallial oviduct in females, with short loop left of prostate in males.

Female genitalia. Ovary without lobes, proximal end 1.25-1.75 whorls below apex, comprising 0.25-0.5 whorls, reaching stomach; capsule gland with long and slender, opaque-white vestibulum, anterior capsule gland yellow-orange, toward posterior capsule gland covered with brown spots, posterior capsule gland opaque-white with a central milky section, albumen gland milky-white; proximal loop of renal oviduct bent forward, distal loop long; bursa copulatrix higher than long, reaching behind albumen gland; bursal duct long, entering anterior; no seminal receptacle (N=3) (Fig. 7C).

Male genitalia. Proximal end of lobate testis 1 whorl below apex, comprising c. 0.75 whorls, covering proximal end of stomach; vesicula seminalis arising from distal third of testis; penis very long and slender (N=2) (Fig. 8D).



Figure 6. Radula. **A** *Hemistomia andreae* sp. n. **B** *Leiorhagium adioincola* sp. n. **C** *Leiorhagium aremuum* sp. n. **D** *Leiorhagium neteae* sp. n. Arrows indicate membranous junction of flank and face of lateral teeth typical for most Pacific tateid genera (partly dissolved in **A** and **D**).

Remarks. This is *Leiorhagium* sp. n. 3 of Zielske and Haase (2015). *L. aremuum* sp. n. is only known from the type locality.

Leiorhagium clandestinum sp. n.

http://zoobank.org/723A9EA1-CBFC-486A-AA37-69728E99AC3A

Type material. Holotype MNHN IM 2000-27865; paratypes MNHN IM 2000-27866 (3).

Type locality. NeCa 30, Moindou: spring along road SW of Katrikoin, under shrub, 21°34'21.6"S, 165°41'02.5"E, 26 May 2012.

Etymology. The Latin adjective clandestinus means clandestine and refers to the new species' external identity with *L. kavuneva*.

Diagnosis. L. clandestinum sp. n. is most similar to L. adioincola sp. n. and L. kavuneva. For the distinction from L. adioincola sp. n. see above. Due to the lack of anatomical data, L. clandestinum sp. n. and L. kavuneva can only be distinguished based on 7.6% average sequence divergence of COI (p-distance).

Shell. Pupiform, 1.86 times higher than wide, 4.25-5 whorls, without colour, transparent; protoconch very faintly pitted with c. 1 whorl; palatal denticle a small droplet 1/8 whorl behind outer lip; aperture as high as wide (Figs 2D, 3G, 4G, H).



Figure 7. Female genitalia. **A** *Hemistomia andreae* sp. n. **B** *Leiorhagium adioincola* sp. n. **C** *Leiorhagium aremuum* sp. n. **D** *Leiorhagium neteae* sp. n. ac anterior capsule gland, ag albumen gland, bc bursa copulatrix, bd bursal duct, go genital opening, od oviduct, pc posterior capsule gland, rs receptaculum seminis, vc vestibular capsule gland, ve ventral channel.

External features. Epidermis without pigment, eyes black.

Remarks. This is *Leiorhagium* sp. n. 2 of Zielske and Haase (2015). *L. clandesti-num* sp. n. is only known from the type locality.

Leiorhagium neteae sp. n.

http://zoobank.org/7B81AF32-3FDA-49C7-A316-D84B1A5ED324

Type material. Holotype MNHN IM 2000-27867; paratypes MNHN IM 2000-27868 (20).

Type locality. NeCa 44, Poya: stream at side of small road branching off road between Nétéa and Goipin toward the Vallée d'Adio, under shrub close to overgrown garden, 21°14'47.9"S, 165°15'45.0"E, 28 May 2012.

Etymology. The new species is named after the village of Nétéa, which is closely proximal to our collecting locality.

Diagnosis. *L. neteae* sp. n. is very similar to *L. douii* and *L. montfaouense*. In *L. neteae* sp. n. the palatal denticle is slightly larger and 1/8 whorl further behind the outher lip. The operculum has only a single denticle compared to 2-3 in *L. douii* and *L. montfaouense*. The distal loop of the renal oviduct of the new species forms a 270° loop counter-clockwise, whereas in the other two species this part of the oviduct is bent



Figure 8. Penis. **A, B** *Hemistomia andreae* sp. n. **C** *Leiorhagium adioincola* sp. n. **D** *Leiorhagium aremuum* sp. n. **E** *Leiorhagium neteae* sp. n. Scale bars = 100 μm.

180° clockwise. The penis of *L. neteae* sp. n. is long and slender in contrast to the other species, where it has a broad base and a very long filament.

Shell. Elongate-pupiform, 1.88 times higher than wide, 4.25–5 whorls, without colour, transparent; protoconch faintly pitted with c. 1 whorl; palatal denticle an elongate droplet c. 1/4 whorl behind outer lip; aperture slightly wider than high (Figs 2E, 3H, I, 4I, J).

Operculum. Elongate-ellipsoidal, paucisprial, nucleus submarginal, orange, one non-calcareous white peg (N=4) (Fig. 5G, H).

External features. Epidermis without pigment, eyes black.

Mantle cavity. Ctenidium with 15 (1 male) or 19-22 (5 females) filaments; osphradium short-elongate, behind middle of ctenidium.

Digestive system. Radula formula (N=4) (Fig. 6D): R: 4 1 4/2-3 2-3, L: 4-5 1 5, M₁: 20-25, M₂: 24-27; stomach without caecum; rectum close to pallial oviduct in females, with short loop left of prostate in male.

Female genitalia. Ovary without lobes, proximal end 1.25-1.5 whorls below apex, comprising 0.25-0.5 whorls, not reaching stomach; anterior capsule gland yellow-orange, posterior capsule gland opaque-white, albumen gland milky-white; proximal loop of renal oviduct bent forward, distal loop short; bursa copulatrix globular, reaching slightly behind albumen gland; bursal duct long, entering anterior; no seminal receptacle (Fig. 7D).

Male genitalia. Proximal end of lobate testis 1 whorl below apex, comprising slightly more than 0.5 whorls, covering proximal end of stomach; vesicula seminalis arising approximately in middle of testis; penis very long and slender (N=1) (Fig. 8E).

Remarks. This is *Leiorhagium* sp. n. 5 of Zielske and Haase (2015). *L. neteae* sp. n. is only known from the type locality.

Morphometry

The CVA plot (Fig. 9) comparing species of *Hemistomia* shows the high variability of *H. cockerelli*. The associated MANOVA was highly significant (Wilk's $\lambda = 0.062$, DF₁ = 35, DF₂ = 490.4, *F* = 13.16, *p* = < 0.001). Many pairwise comparisons of populations were significant as well (Table 3). *H. nyo* and *H. andreae* sp. n. fell within the variation of *H. cockerelli*. According to the CVA, they were not more different from each other than from populations of *H. cockerelli*. Assignment and jacknifed assignment tests allocated 80 (62.5%) and 67 (52.3%) of a total of 128 shells to their original sample indicating the considerable overlap of shapes.

The CVA (Fig. 10) for *Leiorhagium* revealed species clusters with *L. adioincola* sp. n. and *L. clandestinum* sp. n. overlapping with *L. kavuneva* and *L. neteae* sp. n. largely grouping with *L. douii* and *L. monachum*. The MANOVA was again highly significant (Wilk's $\lambda = 0.009$, DF₁ = 50, DF₂ = 669.2, F = 23.56, p = < 0.001), as were most pairwise comparisons (Table 4). Note that comparisons involving *L. clandestinum* sp. n. or *L. monachum* were less meaningful because of the small sample sizes. Assignment and jacknifed assignment tests performed similar as for *Hemistomia* with only 103 (64.0%) and 88 (54.7%) correctly allocated shells of a total of 161.



Figure 9. CVA plot for *Hemistomia*. Samples without numbers are paratypes.

Table 3. Pairwise morphometric comparisons of *Hemistomia* samples. Hotelling's T^2 tests, based on five shell measures; significance assessed after sequential Bonferroni correction; sample sizes are given in Table 2. * p < 0.05; NS, not significant.

	1	2	3	4	5	6	7
1 H. andreae							
2 H. cockerelli Types	*						
3 H. cockerelli NeCa11	*	*					
4 <i>H. cockerelli</i> NeCa17	*	*	*				
5 <i>H. cockerelli</i> NeCa21	NS	*	*	NS			
6 H. cockerelli NeCa36	*	*	*	NS	NS		
7 <i>H. cockerelli</i> NeCa54	*	*	*	NS	*	NS	
8 <i>H. nyo</i> NeCa35	*	*	*	NS	NS	*	NS

Table 4. Pairwise morphometric comparisons of *Leiorhagium* samples. Hotelling's T² tests, based on five shell measures; significance assessed after sequential Bonferroni correction; sample sizes are given in Table 2. * p < 0.05; NS, not significant.

	1	2	3	4	5	6	7	8	9	10
1 L. adioincola NeCa49										
2 L. aremuum	*									
3 L. clandestinum	NS	*								
4 L. neteae	*	*	*							
5 <i>L. ajie</i> Types	*	*	NS	*						
6 L. douii Types	*	*	*	*	*					
7 L. kavuneva Types	*	*	NS	*	*	*				
8 <i>L. kavuneva</i> NeCa15B	NS	*	NS	*	*	*	*			
9 <i>L. kavuneva</i> NeCa29	NS	*	NS	*	*	*	*	NS		
10 L. monachum Types	*	*	NS	*	NS	NS	*	*	*	
11 L. montfaouense Types	*	*	*	*	*	NS	*	*	*	NS



Figure 10. CVA plot for *Leiorhagium*. Samples without numbers are paratypes.

Phylogenetic analysis

In the phylogenetic analysis (Fig. 11), *Hemistomia* and *Leiorhagium* were sister groups, both with 100% bootstrap support. Within *Leiorhagium*, the elongate-pupiform species *L. orokau*, *L. inplicatum* and *L. neteae* sp. n. were paraphyletic with respect to the more conical-pupiform species, which received a bootstrap support of 91%. Otherwise, relationships among species of *Leiorhagium* were not well supported. All four new species were (phylo)genetically well distinct as indicated by the branch lengths expressing genetic distances. Within *Hemistomia*, the picture was very similar with well differentiated species but otherwise little resolution. Average pairwise uncorrected genetic distances based on the COI-fragment were $\geq 7.4\%$ and are summarized in Table 5.



Figure 11. Maximum likelihood phylogram showing bootstrap support when > 50%. Outgroup pruned from tree; new species highlighted by bold face type.

	1	2		
1 H. andreae				
2 H. cockerelli	8.6			
3 H. nyo	8.8	9.5		
	1	2	3	4
1 L. adioincola				
2 L. ajie	9.3			
3 L. aremuum	10.6	9.4		
4 L. clandestinum	9.5	7.8	7.4	
5 L. kavuneva	9.7	8.1	8.5	7.6

Table 5. Average pairwise uncorrected (p) distances between selected species based on the COI-fragment (in %).

Our phylogenetic analyses based on DNA sequence data confirmed the suspicion of Haase and Bouchet (1998) that additional cryptic species in this snail fauna will be identified once molecular methods are applied emphasizing the huge morphological variability of certain nominal species. Recent accounts on tateid gastropods from Vanuatu and Fiji (Zielske and Haase 2014a, b) have revealed extensive radiations of morphologically very similar species. However, in contrast to the New Caledonian taxa, the radiations on those archipelagos are comparatively young (Zielske and Haase 2015). Four of the five species described here are hardly distinguishable from known taxa based on measurements despite being genetically well differentiated with even uncorrected distances (see Fregin et al. 2012) of at least 7.4% to their next similar congeners. Whether this means that morphologically similar species occupy similar niches is impossible to tell at this stage because the relationship of shell morphology to habitat has not been investigated among truncatelloidean gastropods except for a few accounts on Potamopyrgus antipodarum (Haase 2003, Holomuzki and Biggs 2006, Kistner and Dybdahl 2013). Although ranges overlap or are contiguous, sibling species have not (yet) been encountered in sympatry, i.e. in the same spring or stream.

The new species provide an additional truncatelloid example stressing the importance of an integrative taxonomic approach combining morphological, anatomical and genetic methods (e.g. Haase et al. 2007, Delicado and Ramos 2012). Given the mosaic nature of evolution of these small gastropods with morphologically as well as genetically cryptic species (e.g., Haase et al. 2007, Haase 2008, Zielske et al. 2011, Delicado and Ramos 2012, Liu et al. 2013), we do not adhere to a fairly strict scheme of species identification as advocated elaborately e.g. by Schlick-Steiner et al. (2010). Instead we advocate the approach of Padial and de la Riva (2010) who have a more natural vision of the evolutionary processes potentially involved in speciation. For instance, they acknowledge that the congruence of different character sets, pivotal for taxonomic decisions for Schlick-Steiner et al. (2010), may be plesiomorphic.

Genetic differentiation was an important indicator of species status. Pairwise pdistances > 7.4% are far above any threshold suggested by advocates of barcoding (e.g., Hebert et al. 2003, 2004; Ratnasingham and Hebert 2007). However, again we do not adhere to a strict scheme as there may be no mitochondrial differentiation between good species as well as considerable variation within species of spring snails (e.g. Haase 2008; Zielske et al. 2014a; see also Fregin et al. 2012). That genetic differentiation does reflect species status for the new taxa is also indicated by the comparison of their branch lengths to branch lengths among morphologically well defined species in our phylogenetic analysis.

While conducting our morphometric analyses we appreciated that the measuring methods applied for the material described previously (Haase and Bouchet 1998) and for this account are incompatible. Obviously, using an ocular micrometer fitted to a dissecting microscope produced inaccurate data, although the measurements were quite consistent judging from the fairly low coefficients of variation, which were of a similar order of magnitude as those computed for the present data. Therefore, we had to re-measure the old samples used in our comparisons.

Another methodological problem almost expectedly occurred in the field. All collections made for our previous monograph (Haase and Bouchet 1998) were georeferenced from maps. This proved to be fairly inaccurate when we tried to relocate sites in 2012 guided by GPS. Additional difficulties arose from recent road development and land-use changes. Many villages are now accessible on much broader roads than 20 years ago. Construction has obviously destroyed small road-side springs and seepages and changed the course of streams. Other sites were destroyed by extensive fires affecting entire valleys or hills. *Crosseana melanosoma*, in our analysis part of the outgroup, used to be common when first collected in 1992. Now we found only a few specimens. It remains to be seen whether there are other (extant) populations in the unexplored hinterland of Boyen. In contrast, *H. yalayu*, collected in a few seepages on Col d'Amoss in the far Northeast in 1989, is now probably extinct. The entire area has lost its primary vegetation. Today, the fire resistant niaouli (*Melaleuca quinquenervia*) and shrubland are dominating and streams harbor a very depauperate fauna.

Four of the five new species were found in single sites and the fifth was found at only two sites. Considering the vulnerability of small habitats like springs and the rapid anthropogenic development and changes on New Caledonia just outlined immediately raises concern regarding the chances of long-term survival of these species (see also Haase et al. 2010). Most sites we surveyed were rather easily accessible, close to roads, so that one can assume that there are other populations deeper in the forests or forest remnants. Nevertheless, given that the area of occupancy of each species is certainly less than 10 km², that ranges of spring snails are almost naturally severely fragmented, and the rapidly progressing change of land cover, areas of occupancy as well as habitat, hence the numbers of populations will decline. Therefore, all five species and probably the majority of New Caledonian tateids qualify as critically endangered according to the criteria (CE, B2,a,II-IV) of the International Union for Conservation of Nature (IUCN 2012).

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RESEARCH ARTICLE



On Afromantispa and Mantispa (Insecta, Neuroptera, Mantispidae): elucidating generic boundaries

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Abstract

The genus *Afromantispa* Snyman & Ohl, 2012 was recently synonymised with *Mantispa* Illiger, 1798 by Monserrat (2014). Here morphological evidence is presented in support of restoring the genus *Afromantispa* **stat. rev.** to its previous status as a valid and morphologically distinct genus. Twelve new combinations (**comb. n.**) are proposed as species of *Afromantispa* including three new synonyms.

Keywords

Mantispidae, Afromantispa, Mantispa, Afrotropics, Palearctic

Introduction

Mantispidae (Leach, 1815) is a small cosmopolitan family in the very diverse order Neuroptera. The former is characterised by an elongated prothorax, elongated procoxa protruding from the anterior pronotal margin and conspicuous raptorial forelegs. Recently, one of the genera, *Mantispa* Illiger, 1798 has been the focus of taxonomic studies (Snyman et al. 2012; Monserrat 2014). *Mantispa* was originally described by Illiger (1978) and quickly became the most speciose genus with a cosmopolitan distribution. Studies by Lambkin (1986a; b), Hoffman (2002), Machado and Rafael (2010) and Snyman et al. (2012) excluded *Mantispa*'s distribution from much of the world and consequently *Mantispa* is no longer thought to occur in the Neotropics, Nearctic, Afro-

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tropics or Australasia. *Mantispa*, according to the morphology of the type species *M. pagana* (Fabricius, 1775) (synonymised with *M. styriaca* (Poda, 1761)), is thus probably a small genus from the Palearctic. As the previously mentioned studies focused on the fauna elsewhere the generic boundaries between *Mantispa* and other similar groups have remained poorly understood.

In their study on the Afrotropical mantispid genera, Snyman et al. (2012) proposed that the majority of the *Mantispa*-like species from the Afrotropics and south western parts of Europe can be defined as a separate genus and consequently described *Afromantispa*. The authors unfortunately did not provide a list of species belonging to the newly erected genus claiming it would be best left until a full revision of the genus could be launched. It appears that this might have caused some additional confusion.

Monserrat (2014) synonymised *Afromantispa* with *Mantispa* in a study only focussing on the local fauna of the Iberian Peninsula and Balearic Islands. A new species *Mantispa incorrupta* was also described. Additionally, the author synonymised *Sagittalata* Handschin, 1959 with *Mantispa*. The status of *Sagittalata* is currently still in dispute and not well understood.

Afromantispa, Mantispa and Sagittalata are quite difficult to distinguish, but several morphological characters do support separation of the genera. Adding to the difficulty is that there is a distribution overlap between species from both genera in southern and western Europe. The antennae, prothorax, mesothorax, pterostigma, and fifth tergite are morphologically different between members of the genera (Table 1). Mantispa are represented by only two species that can confidently be placed in the genus (supplementary files: Appendix II). The status of Afromantispa (Snyman & Ohl, 2012) is hereby restored as a genus morphologically distinct from Mantispa, and a list of the species that belong to Afromantispa is provided. This study thus aims to elucidate the boundaries between these two genera.

Material and methods

The specimens used in this study are housed at the following institutions:

AMG	Albany Museum, Grahamstown, South Africa
BMNH	The Natural History Museum, London, Great Britain
HUAC	Personal collection H. and U Aspöck, Vienna, Austria
MNHN	Museum National d'Histoire Naturelle, Paris, France
MRAC	Musee Royal de l'Afrique Centrale, Tervuren, Belgium
MZBS	Museo Zoologia, Barcelona, Spain
NHMB	Naturhistorisches Museum, Basel, Switzerland
OUM	University Museum, Oxford, Great Britain
SANC	South Arfican National Collection, Roodeplaat, South Africa
VMC	Personal collection V. Monserrat, Madrid, Spain
ZMB	Museum für Naturkunde, Berlin, Germany

Photos were taken with either a Canon 500D equipped with a 100mm Canon macro lens or with a Leica Z16 APOA camera setup.

All type specimens that are not housed at MRAC and ZMB were studied using high resolution photographs provided by ZMB (supplementary files: Appendix IV). Adult morphological terminology follows that of Lambkin (1986a; b).

Taxonomic amendments

Morphological overview

Head: The flagella of the genera are quite similar in appearance but all species of Afromantispa have a pale band in the distal third of the flagella, this character is not shared by the species of Mantispa. The band is even distinct in Afromantispa species with light yellowish flagella. In the latter, the band is then presented by a few dark antennules prior to the band so it remains visible (Fig. 1b; d) (Monserrat 2014: fig. 43). In Mantispa, the occiput is covered by short stout setae dorsolaterally (Fig. 1f); this feature is not present in the Afromantispa species studied. The rest of the head capsule is very similar between the taxa. The prothorax dorsum of Afromantispa is always covered in granules and setae, where the Mantispa species lack granules, even if small pigmentation "dots" are visible at the origin of the setae on *M. styriaca* (Fig. 1a-f) (Monserrat 2014: fig. 23-24). Peculiarly, a region in the lateral mid-zone of the prothorax of Afromantispa always lacks granules (Fig. 2e; f) (Monserrat 2014: fig: 45). Mantispa in turn have short stout setae on the dorsum of the mesothorax, which is lacking in *Afromantispa* (Fig. 1f). The wing venation of both genera is very similar in structure except for features pertaining to the pterostigma (Fig. 2a-d). The costal space in *Mantispa* seems slightly larger than in Afromantispa, but it can vary. The pterostigma in Mantispa however, is different. The subcosta and radius of Afromantispa is always pale/yellowish in colouration up to or just distal to midway of radial cell 2. Thereafter, the pterostigma commences. The proximal end of the pterostigma is the same pale colouration of the subcosta and radius veins. The centre of the large distal half is always reddish or dark in colouration flanked by a thin yellowish margin until meeting the veins. The pterostigma of some species might be slightly truncated and anteriorly rounded (Fig. 2b). Mantispa in turn always have a reddish monocoloured pterostigma (Fig. 2c-d). The terminalia of both genera are similar (Fig. 3a-b) in structure where variation on ectoproct size and length is common in Afromantispa. Both genera have an extrusible gland present between tergite V and VI (Eltringham 1932). Tergite V of Afromantispa is conspicuously enlarged, especially in fresh specimens (Fig. 3c). From various photos of live M. styriaca and M. aphaveselte Aspöck & Aspöck, 1994, including those of Monserrat (2014), it was determined that this tergite is not as prominent in Mantispa.

The elongated line on the procoxa of *Sagittalata* suggested by Snyman et al. (2012) was considered as a weak character by the authors. *Sagittalata* lacks the greatly enlarged gland present between the V and VI tergites. In addition, species from *Sagittalata* have



Figure I. Prothorax in dorsal view of **a** *Afromantispa capeneri* (Handschin, 1959) **b** *Afromantispa moucheti* (Navás, 1925) **c** *Afromantispa nana* (Erichson, 1839) **d** *Afromantispa tenella* (Erichson, 1839) **e** *Mantispa styriaca* **f** *Mantispa aphavexelte* (photo credits: **a–d** Johan Saayman).



Figure 2. Right wings of **a** *Afromantispa tenella* **b** *Afromantispa moucheti* **c** *Mantispa styriaca* **d** *Mantispa aphavexelte*. Prothorax in lateral view of **e** *Afromantispa moucheti* **f** *Mantispa styriaca* (photo credits **a–b; e–f** Johan Saayman).

a dorsally enlarged inner flange on the caudal apex of the gonocoxites as illustrated by Poivre (1981a (fig. 2 J; 4E); 1981b (fig. 1T; 3X; 6R; 7S); 1983 (fig. 3 H, L)). The cusp on the anterior margin of the pronotum lacks short stout setae which are present in *Mantispa*. The pronotal dorsum lacks short stout setae that are present in *Mantispa*, but may have a few sparsely distributed setae. The mesothorax completely lacks prominent setae and is either glaborous or pubescent (velvet appearance) which is also different in *Mantispa*. *Sagittalata* might be forming part of the previously synonymised genus *Mantispilla*, thus, changing the taxonomic status of the genus in this paper only



Figure 3. Terminalia of **a** *Afromantispa zonaria* (Navás, 1925) – type specimen from MRAC **b** *Mantispa aphavexelte*. Freshly killed *Afromantispa tenella* indicating enlarged fifth male tergite (photo credits: **a** Ludwig Eksteen, **c** Morgan Trimble).

	Afromantispa	Mantispa
Short stout setae on occiput (Fig. 1f. (i))		•
Pale band in distal third of the antennae (Fig. 1b. (i))	•	
Granulated prothorax (Fig. 1a–d; 2e–f)	•	
Short stout setae on mesothorax (Fig. 1f. (ii))		•
Bicoloured pterostigma (Fig. 2a. (i); b. (i))	•	
Enlarged fifth male tergite (Fig. 3c. (i))	•	

Table I. Morphological characters separating Mantispa and Afromantispa.

to be considered moot in subsequent publications seems illogical (Snyman et al. in prep). The current synonymy suggested by Monserrat (2014) are considered valid but are excluded from the species list in the Suppl. material 1.

Discussion

These two genera are possibly quite closely related and therefore present several confusing morphological characteristics. The suggestion by Monserrat (2014) to synonymise the genera has cascading effects on the taxonomy of Mantispinae. Even though western Europe is not specifically rich in mantispid species, these genera are not confined to that area. The suggested synonymy by Monserrat (2014) means that *Mantispa* will again include 144 species spanning Africa, Europe, Asia and some Australasian islands (numbers from Ohl 2004). This might be possible, but should be approached with caution and include a substantially larger number of species than what was included by the author. Monserrat (2014) further only considered species formally recorded from Spain, where a much larger scope should have been included.

The following species all conform to the characters proposed in this study and are consequently regarded as belonging to Afromantispa: capeneri (Handschin), comb. n., dispersa (Navás), comb. n., incorrupta (Monserrat), comb. n., meadewaldina (Navás), comb. n., moucheti (Navás), comb. n., nana (Erichson), comb. n., nanyukina (Navás), comb. n. natalensis (Navás), comb. n., navasi (Handschin), comb. n., verruculata (Navás), comb. n., zonaria (Navás), comb. n., zonata (Navás), comb. n. Afromantispa arabica (Navás, 1914f), syn. n. is a new synonym of Afromantispa nana (Erichson, 1839). Afromantispa variolosa (Navás, 1914d), syn. n. is a new synonym of Afromantispa tenella. Afromantispa schoutedeni (Navás, 1929), syn. n. is a new synonym of Afromantispa moucheti (Navás) (supplementary files: Suppl. material 1 I). Several other species have been described with a distribution in the Afrotropics (supplementary files: Suppl. material 1 III). The type specimens of these species have not yet been studied and the placement of the species remains uncertain. The distribution of the genus suggests that these might belong to Afromantispa or another less likely, another Afrotropical genus. Their current placement in Mantispa is most likely a historical one and possibly erroneous. Until the type specimens are studied, the names should remain in Mantispa.

This study confidently presents enough data for the separation of *Afromantispa* and *Mantispa*. Current integrative studies including the authors of this study are ongoing focussing on the elucidation of the world genera of the mantispines.

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Supplementary material I

List and table of species names, updated from Ohl (2004)

Authors: Louwtjie P. Snyman, Catherine L. Sole, Michael Ohl Data type: species data

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RESEARCH ARTICLE



Croatian mayflies (Insecta, Ephemeroptera): species diversity and distribution patterns

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Abstract

Knowledge of the mayfly biodiversity in the Balkan Peninsula is still far from complete. Compared to the neighbouring countries, the mayfly fauna in Croatia is very poorly known. Situated at the crossroads of central and Mediterranean Europe and the Balkan Peninsula, Croatia is divided into two ecoregions: Dinaric western Balkan and Pannonian lowland. Mayflies were sampled between 2003 and 2013 at 171 sites, and a total of 66 species was recorded. Combined with the literature data, the Croatian mayfly fauna reached a total of 79 taxa. Of these, 29 species were recorded for the first time in Croatia while 15 species were not previously recorded in Dinaric western Balkan ecoregion. Based on the mayfly assemblage, sampling sites were first structured by ecoregion and then by habitat type. In comparison with the surrounding countries, the Croatian mayfly fauna is the most similar to the Hungarian and Bosnian fauna. Some morphologically interesting taxa such as *Baetis* cf. *nubecularis* Eaton, 1898 and *Rhithrogena* from the *diaphana* group were recorded. *Ephemera* cf. *parnassiana* Demoulin, 1958, the species previously recorded only from Greece, was also recorded.

Keywords

Ephemeroptera, species list, biodiversity, Balkan Peninsula

Introduction

Mayflies (Ephemeroptera) have a worldwide distribution, being absent only from Arctic region, Antarctica and some remote oceanic islands (Barber-James et al. 2008). According to the literature (Bauernfeind and Soldán 2012), 369 species are recorded for Europe and North Africa. Mayflies are a merolimnic insect order (i.e. with aquatic larval stages and terrestrial adults) that plays a critical role in running and standing waters where they hold an important position in secondary production, as an important food source for diverse freshwater and terrestrial predators. In recent decades, human impacts on the distribution and abundance of many aquatic insects, including mayflies, are becoming more and more evident. During the 20th century, increasing industrialisation, population growth, overexploitation of natural resources and different types of pollutions have greatly impacted many European freshwater ecosystems, and also endangering the species inhabiting them (Brittain and Sartori 2009). Highly sensitive, confronted with habitat alteration, mayfly species are among the first to disappear. Therefore they are important indicators of freshwater health and widely used in bio-monitoring programmes over the world (Elliott et al. 1988, Sartori and Brittain 2015). The knowledge of the mayfly biodiversity in the Balkan Peninsula is still far from complete. Moreover, many taxa lack appropriate morphological descriptions for the larval and/or adult stages. The mayfly fauna in Croatia is no exception. Published data on Croatian mayflies are generally part of diverse limnological studies (e.g. Matoničkin 1959, 1987, Matoničkin and Pavletić 1961, 1967, Filipović 1976, Habdija and Primc 1987, Habdija et al. 1994, 2004) in which mayflies were investigated only as part of the overall macroinvertebrate fauna. In most studies, identification tools are generally not cited, thus the accuracy of mayfly species identification is questionable. In summary, 50 mayfly species were recorded from Croatia (Bauernfeind and Soldán 2012, Kovács and Murányi 2013, Ćuk et al 2015). In comparison with the number of species recorded in the neighbouring countries, i.e. 68 in Slovenia, 106 in Italy, and 93 in Hungary (Bauernfeind and Soldán 2012), it can be assumed that the Croatian mayfly fauna has been underestimated to date.

Studies on distribution and biodiversity are of crucial importance in determining the conservation status of certain species and in investigating factors that influence that diversity (de Silva and Medellín 2001). Therefore, knowledge of the mayfly faunal composition, seasonal dynamics, distribution, ecology, biogeography and especially their sensitivity as bio-indicators can enable high-quality classification and protection of Croatian freshwater habitats.

Materials and methods

This research is based on recent mayfly studies conducted in the last decade (2003–2013). The results of field studies were then combined with the literature data given in Bauernfeind and Soldán (2012), Kovács and Murányi (2013) and Ćuk et al. (2015), for the purpose of obtaining a comprehensive checklist of the Croatian mayfly fauna.

Sampling and laboratory methods

Croatia is a relatively small country situated at the crossroads of Central and Mediterranean Europe and Balkan Peninsula, and is divided into two ecoregions: Dinaric western Balkan (ER5) and Pannonian lowland (ER11) (Illies 1978). Specimens were collected in lotic and lentic freshwater habitats throughout the Croatian territory (Fig. 1). Additionally, specimens housed in the collection of the Slovene National History Museum were identified.

The list of the 171 sampling site names with number codes (site ID), altitude, latitude and longitude is presented in Table 1 as well as on the map (Fig. 1). Larvae were sampled using a Surber sampler and hand net, adults using hand nets and pyramidal emergence traps.



Figure 1. Map of the mayfly fauna sampling sites, Croatia (See Table 1 for codes).

Site ID	Sampling site	Altitude	Longitude	Latitude	Ecoregion	Basin
1	Karašica River, Valpovo	85	N45°37'44"	E18°27'28"	11	BS
2	Vučica River, Valpovo	85	N45°38'14"	E18°25'09"	11	BS
3	Čarna channel, Tikveš, near Bilje	85	N45°40'23"	E18°50'46"	11	BS
4	Veličanka River, Mihaljevci	155	N45°21'36"	E17°40'54"	11	BS
5	Sava River, Slavonski Brod	85	N45°07'35"	E18°02'18"	11	BS
6	Sava River, Štitar	80	N45°05'47"	E18°37'38"	11	BS
7	Sutla River, Klanjec	160	N46°02'46"	E15°43'49"	11	BS
8*	Drava River, Varaždin	170	N46°19'50"	E16°20'22"	11	BS
9	Drava River, Čakovec, left drainage ditch	165	N46°18'49"	E16°27'49"	11	BS
10	Drava River, Dubrava, right drainage ditch	145	N46°18'54"	E16°42'15"	11	BS
11	Stream, Trakošćan	275	N46°15'44"	E15°56'30"	11	BS
12	Stiper stream, Ljubešćica, Kalnik Mountain	185	N46°09'04"	E16°22'18"	11	BS
13	Bliznec stream, Medvednica Mountain	380	N45°52'38"	E15°58'33"	11	BS
14	Veliki potok stream, Medvednica Mountain, Mikulići	300	N45°51'29"	E15°56'08"	11	BS
15	Kraljevec stream, Medvednica Mountain	565	N45°52'48"	E15°56'28"	11	BS
16	Sitnik spring, Žumberak- Samoborsko Gorje Mountain	745	N45°44'40"	E15°32'39"	11	BS
17	Slapnica stream, Žumberak- Samoborsko Gorje Mountain	290	N45°44'12"	E15°29'29"	11	BS
18*	Kupa River, Sisak	90	N45°28'32"	E16°22'37"	11	BS
19	Sava River, Rugvica	100	N45°44'01"	E16°13'11"	11	BS
20	Sava River, Mlaka	90	N45°14'14"	E17°01'11"	11	BS
21	Sava River, Zagreb, bridge	110	N45°47'03"	E16°00'10"	11	BS
22	Bregana River, Jarušje	560	N45°46'21"	E15°34'36"	11	BS
23	Stream, Mečenčani	180	N45°17'07"	E16°25'53"	11	BS
24	Stream Zeleni dol, Hrastovica/ Hrvatski Čuntić	160	N45°21'51"	E16°16'15"	11	BS
25	Pond Zeleni dol, Hrastovica/ Hrvatski Čuntić	160	N45°21'51"	E16°16'18"	11	
26	Petrinjčica River, Prnjavor Čuntićki	150	N45°21'05"	E16°16'57"	11	BS
27	Petrinjčica River, Tješnjak, bridge	150	N45°22'52"	E16°17'11"	11	BS
28	Utinja River, Križ Hrastovački	140	N45°25'15"	E16°14'32"	11	BS
29	Žirovnica River, Donja Ljubina	135	N45°05'39"	E16°17'39"	11	BS
30	Moštanica stream, Moštanica	155	N45°21'55"	E16°21'06"	11	BS
31	Sunja River, Rakovac	120	N45°18'40"	E16°32'33"	11	BS
32	Sunja River, Donji Kukuruzari	150	N45°16'01"	E16°29'14"	11	BS
33	Kupa River, Brest	90	N45°26'56"	E16°15'38"	11	BS

Table 1. The list of the sampling sites in Croatia. Ecoregions are taken from Illies (1978); Dinaric western Balkan (5) and Pannonian lowland (11). BS = Black Sea Basin; AS = Adriatic Sea Basin.

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34	Kupa River, Bubnjarci	135	N45°38'42"	E15°21'24"	5	BS
35	Una River, Hrvatska Kostajnica	105	N45°13'37"	E16°32'22"	11	BS
36	Glina River, Marinbrod	100	N45°23'19"	E16°08'20"	11	BS
37	Glina River, Cerjak	110	N45°21'27"	E16°04'58"	11	BS
38	Cemernica stream, Topusko	125	N45°19'08"	E15°57'30"	11	BS
39	Sava River oxbow, Mužilovčica	90	N45°23'23"	E16°40'37"	11	BS
40*	Sava River, Martinska Ves	95	N45°35'09"	E16°22'14"	11	BS
41*	Sava River, Desno Trebarjevo	95	N45°35'56"	E16°20'43"	11	BS
42*	Sava River, Krapje	90	N45°18'10"	E16°49'23"	11	BS
43	Sava River, Lukavec Posavski	90	N45°24'36"	E16°31'03"	11	BS
44	Sava River, Drenov bok	90	N45°15'58"	E16°50'04"	11	BS
45	Mire Plavnica, Šatornja	125	N45°19'58	E16°00'26"	11	
46	Javošnica stream, Donji Javoranj	140	N45°07'14"	E16°21'44"	11	BS
47	Odra River, Sisak	95	N45°29'54"	E16°21'04"	11	BS
48	Zrinčica River, Zrin	240	N45°11'41"	E16°22'13"	11	BS
49	Čatlan River, Gornja Oraovica	170	N45°09'26"	E16°25'03"	11	BS
50	Spring Izvor bijele stijene Križ, Župić	135	N45°25'44"	E16°13'52"	11	BS
51	Šanja River, Gora	140	N45°25'08"	E16°11'42"	11	BS
52	Radonja River, Vojnić	140	N45°19'26"	E15°41'55"	11	BS
53	Lonja River, Brežnički Hum	200	N46°07'34"	E16°17'18"	11	BS
54	Lonia River, Breznica	180	N46°04'11"	E16°18'07"	11	BS
55	Mrežnica River. Generalski stol	140	N45°22'05"	E15°24'55"	5	BS
56	Mrežnica River Duga Resa	120	N45°27'31"	E15°29'38"	5	BS
57	Dretulia River Plaški spring	390	N45°04'31"	E15°20'32"	5	BS
58	Dretulja River, Plaški, middle reach	375	N45°05'06"	E15°21'56"	5	BS
59	Trupinjska rijeka River, Keserov potok	150	N45°17'04"	E15°37'28'	5	BS
60*	Gojačka Dobra River, Gorinci, downstream from the waterfall	160	N45°21'10"	E15°20'44"	5	BS
61*	Gojačka Dobra River, Gorinci, waterfall above the dam	160	N45°20'60"	E15°20'45"	5	BS
62*	Gojačka Dobra River, Tomašići	145	N45°22'33"	E15°21'18"	5	BS
63	Bukovska Dobra River, Turkovići	340	N45°16'59"	E15°10'49"	5	BS
64	Ribnjak stream, Trošmarija	195	N45°19'43"	E15°16'25"	5	BS
65	Vitunjčica stream, Vitunj	340	N45°17'01"	E15°09'48"	5	BS
66	Bistrica stream, Bistrac	230	N45°16'27"	E15°17'28"	5	BS
67	Sušik stream, Drežnica	465	N45°08'44"	E15°04'41"	5	BS
68	Bračana stream, Škuljari	45	N45°24'57"	E13°55'36"	5	AS
69	Rečica stream, Pengari	90	N45°23'21"	E13°59'13"	5	AS
70	Draga River, Selca	160	N45°23'36"	E13°59'46"	5	AS
71	Račićki potok stream, Juradi	50	N45°20'17"	E13°57'20"	5	AS
72	Mirna River, Kotli	155	N45°22'06"	E14°01'	5	AS
73	Jadova River, Gornia Ploča	610	N44°27'03"	E15°38'58"	5	AS
74	Obsenica stream, near Lovinac	560	N44°21'09"	E15°40'36"	5	AS
75	Ričica stream, Ričice	560	N44°20'23"	E15°45'08"	5	AS

76	Lika River, Lički Ribnik	565	N44°29'13"	E15°27'38"	5	AS
77	Gacka River, Ličko Lešće	450	N44°48'46"	E15°19'18"	5	AS
78	Gacka River,Prozor	450	N44°50'23"	E15°15'21"	5	AS
79*	Bijela rijeka River, NP Plitvice Lakes, upper reach	715	N44°50'04"	E15°33'33"	5	BS
80*	Bijela rijeka River, NP Plitvice Lakes, spring	760	N44°49'56"	E15°33'22"	5	BS
81*	Crna rijeka River, NP Plitvice Lakes, spring	710	N44°49'43"	E15°36'49"	5	BS
82*	Crna rijeka River, NP Plitvice Lakes, upper reach	680	N44°50'10"	E15°36'30"	5	BS
83*	Crna rijeka River, NP Plitvice Lakes, lower reach	670	N44°50'22"	E15°35'59"	5	BS
84*	Korana River, NP Plitvice Lakes	390	N44°55'33"	E15°37'09"	5	BS
85*	Plitvica stream, NP Plitvice Lakes	555	N44°54'08"	E15°36'27"	5	BS
86*	Tufa barrier Novakovića Brod, NP Plitvice Lakes	510	N44°54'07"	E15°36'38"	5	BS
87*	Tufa barrier Labudovac, NP Plitvice Lakes	630	N44°52'17"	E15°35'59"	5	BS
88*	Tufa barrier Kozjak-Milanovac, NP Plitvice Lakes	545	N44°53'39"	E15°36'32"	5	BS
89*	Kozjak Lake, NP Plitvice Lakes	555	N44°53'18"	E15°36'38"	5	BS
90*	Prošće Lake, NP Plitvice Lakes	665	N44°51'51"	E15°36'06"	5	BS
91*	Ciginovac Lake, NP Plitvice Lakes	640	N44°52'22"	E15°35'51"	5	BS
92*	Kaluđerovac Lake, NP Plitvice Lakes	540	N44°54'05"	E15°36'41"	5	BS
93	Suha Ričina stream, Jurandvor, Krk island	20	N44°58'38"	E14°43'52"	5	AS
94	Zeleni vir, Skrad	540	N45°25'25"	E14°53'53"	5	BS
95	Curak stream, Zeleni vir	330	N45°25'37"	E14°53'33"	5	BS
96	Veli potok stream, Dobrinj, Krk island	35	N45°08'06"	E14°35'43"	5	AS
97	Kupica River spring, Mala Lešnica, NP Risnjak	270	N45°25'48"	E14°51'07"	5	BS
98	Mijića vrelo stream, Mijići	60	N44°09'37"	E15°52'38"	5	AS
99	Krupa River, Krupa	130	N44°11'34"	E15°54'34"	5	AS
100	Krupa River, Kudin bridge	90	N44°11'16"	E15°50'44"	5	AS
101	Pond, Zvjerinac	245	N43°56'45"	E16°12'56"	5	
102	Jaruga stream, Jelavića bridge, Zmijavci	260	N43°24'46"	E17°15'09"	5	AS
103	Otuča River, Deringaj, Kijani	615	N44°21'02"	E15°52'34"	5	AS
104	Vransko Lake, main channel, Biograd	0	N43°56'20"	E15°30'59"	5	AS
105	Vransko Lake, Biograd, Drage	5	N43°53'44"	E15°33'07"	5	AS
106	Krka River, Roški slap waterfall, NP Krka	75	N43°54'23"	E15°58'30"	5	AS
107	Visovac Lake, NP Krka	50	N43°51'38"	E15°58'55"	5	AS

108	Brljan Lake, NP Krka	205	N44°00'30"	E16°02'41"	5	AS
109*	Kosovčica River, upper reach, Vučenovići	230	N43°58'30"	E16°12'45"	5	AS
110*	Kosovčica River, lower reach, Biskupija	220	N44°00'26"	E16°12'52"	5	AS
111	Krka River, Knin	220	N44°01'56"	E16°11'26"	5	AS
112	Krka River, upstream of Kosovčica river mouth, Knin	220	N44°02'24"	E16°13'42"	5	AS
113	Krka River, downstream of Kosovčica river mouth, Knin	215	N44°01'41"	E16°12'48"	5	AS
114	Orašnica River, Knin	225	N44°01'56"	E16°12'04"	5	AS
115	Zrmanja River, Mokro polje, Prkos	200	N44°05'31"	E16°02'00"	5	AS
116	Zrmanja River, Vekići	130	N44°06'06"	E15°56'41"	5	AS
117	Zrmanja River, Palanka	270	N44°08'23"	E16°04'25"	5	AS
118	Zrmanja River, Muškovci, Berberi buk	20	N44°11'50"	E15°46'07"	5	AS
119	Zrmanja River, Kravlja Draga, bridge	240	N44°05'50"	E16°04'30"	5	AS
120	Zrmanja River, Žegar, bridge	60	N44°09'10"	E15°53'08"	5	AS
121	Zrmanja River, Draga	55	N44°09'50"	E15°50'43"	5	AS
122	Lopuško vrelo stream, Lake	220	N44°01'11"	E16°13'21"	5	AS
123	Krčić River, Kovačić	315	N44°02'19"	E16°16'42"	5	AS
124	Krčić River, Mlinica	380	N44°01'38"	E16°19'25"	5	AS
125	Šarena jezera lake, Biskupija	220	N44°01'36"	E16°13'22"	5	
126	Čikola River, near Rakići	100	N43°50'13"	E16°04'25"	5	AS
127	Čikola River, Otavice	270	N43°50'36"	E16°15'25"	5	AS
128	Vrba River, Vrba	425	N43°43'21"	E16°23'58"	5	AS
129	Vrba River, Čavoglave	290	N43°47'28"	E16°18'52"	5	AS
130	Butižnica River, Knin	220	N44°02'44"	E16°11'39"	5	AS
131	Brodic stream, Markovac, Biskupija	250	N43°57'03"	E16°15'00"	5	AS
132	Karakašica, Karakašica	320	N43°43'04"	E16°38'19"	5	AS
133	Boggy seepages, Bruvno, Gračac	690	N44°23'15"	E15°53'08"	5	
134	Ričina stream, Proložac	400	N43°29'20"	E17°09'11"	5	AS
135*	Cetina River, Spring Glavaš	385	N43°58'36"	E16°25'48"	5	AS
136	Grab River, Spring	330	N43°38'24"	E16°46'20"	5	AS
137*	Cetina River, Preočki most bridge	370	N43°57'59"	E16°25'53"	5	AS
138*	Cetina River, Crveni most bridge	365	N43°57'35"	E16°25'46"	5	AS
139*	Cetina River, Obrovac Sinjski	300	N43°43'58"	E16°41'11"	5	AS
140*	Cetina River, Trilj1	295	N43°36'54"	E16°43'42"	5	AS
141*	Cetina River, Čikotina lađa	250	N43°31'58"	E16°44'42"	5	AS
142*	Cetina River, Radmanove mlinice	15	N43°26'19"	E16°45'06"	5	AS
143*	Cetina River, Trilj2	295	N43°36'19"	E16°43'28"	5	AS
144	Cetina River, Peruča Reservoir	360	N43°47'45"	E16°35'32"	5	AS
145	Cetina River, Zadvarje	205	N43°26'02"	E16°53'18"	5	AS
146*	Ruda River, spring	295	N43°40'07"	E16°47'39"	5	AS

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147*	Ruda River, upper reach	320	N43°40'06"	E16°47'28"	5	AS
148	Cetina River tributary stream, Vukovići, Paško polje	370	N43°58'06"	E16°25'07"	5	AS
149	Cetina River tributary stream, Kotluša, Paško polje	375	N43°56'54"	E16°24'06"	5	AS
150	Jadro River 1, Solin	10	N43°32'23"	E16°29'45"	5	AS
151	Matica River, Vrgorac	60	N43°12'21"	E17°23'46"	5	AS
152	Matica River, Umčani	40	N43°10'28"	E17°22'32"	5	AS
153	Stinjevac spring, Dusina	30	N43°10'29"	E17°25'02"	5	AS
154	Cetina River, Čitluk	300	N43°44'48"	E16°39'49"	5	AS
155	Vukovića vrilo spring, Bitelići, Hrvace	505	N43°49'12"	E16°37'28"	5	AS
156	Ljuta River, spring	90	N42°32'20"	E18°22'46"	5	AS
157	Ljuta River, upper reach, Donja Ljuta	60	N42°32'05"	E18°22'39"	5	AS
158	Vodovađa stream, Palje Brdo	110	N42°30'29"	E18°24'34"	5	AS
159	Konavočica River, near Karasovići	110	N42°30'19"	E18°24'37"	5	AS
160	Stream, near Zastolje	75	N42°31'17"	E18°23'31"	5	AS
161	Stream, near Brajkovići	90	N42°31'49"	E18°23'14"	5	AS
162	Vrljika River, Kamenmost	265	N43°25'52"	E17°11'42"	5	AS
163	Vrljika River, Kapuše	270	N43°26'33"	E17°10'32"	5	AS
164	Jarun Lake, Zagreb	110	N45°46'47"	E15°55'17"	11	BS
1650	Stream under the village Beram	290	N45°15'10"	E13°54'18"	5	AS
1660	Spring by the church, Stajnica, Porkulabi	500	N45°02'31"	E15°14'18"	5	AS
1670	Danube River, Ilok	75	N45°13'49"	E19°23'26"	11	BS
1680	Ljubica stream, Baške Oštarije, Linići, Velebit Mountain	910	N44°31'37"	E15°09'41"	5	AS
1690	Spring by the church, Slano	15	N42°47'01"	E17°53'26"	5	AS
1700	Spring by the sea, Dubrovnik, Mali Zaton	5	N42°42'06"	E18°02'40"	5	AS
1710	Tounjčica stream, Tounj	220	N45°14'56"	E15°20'04"	5	BS

* Sampling sites used in calculating Shannon-Weaver and Simpson indices and in Cluster analysis.

 Samples stored in Slovene Natural History Museum. The remaining samples are stored at the University of Zagreb, Faculty of Science, Department of Biology, Division of Zoology, Zagreb.

Mayflies were sampled in every season at 34 sites, while at the remainder of sites, sampling was usually performed only once between April and September. Specimens were stored in 80% ethanol and identified in the lab using a stereomicroscope and microscope. A reference collection was made by preparing permanent slide mounts of identified species. Larvae were treated with 10% KOH and 99% acetic acid to remove all muscle parts. Mouth parts, legs, gills, thorax, abdomen, paraproct plate in Baetidae and cerci, necessary for the species identification, were fixed in Euparal and examined under a microscope. Adult specimens were mostly identified by the imaginal male genitalia. The collected material (larvae and adult specimens) was identified using Müller-Liebenau

(1969), Elliott and Humpesch (1983), Malzacher (1984), Elliott et al. (1988), Studemann et al. (1992), Haybach (1999), Bauernfeind and Humpesch (2001), combined with numerous publications with species descriptions (e.g. Tomka and Rasch 1993).

Data analysis

All recorded specimens were included in the Croatian mayfly species list. Data for the sites with the same sampling effort were statistically analysed using the PRIMER 6 software package (Clarke and Warwick 2001). As such, only 34 sampling sites were compared out of the total 171 (Table 1). These sites were sampled in all seasons, at the available microhabitats and they represent habitats in each ecoregion and each sea basin. Species diversity, evenness, and similarity between sites with respect to the mayfly composition and abundance were determined by the Shannon-Weaver and Simpson indices. For estimation of similarity among sites was determined using the Bray-Curtis similarity index. SIMPER (Similarity Percentage) was used to assess which taxa are primarily responsible for the similarities between the sites of the same habitat type. The Croatian mayfly species richness was compared with the surrounding countries (Bosnia & Herzegovina, Hungary, Slovenia, Italy) by compiling species list for these countries taken from Bauernfeind and Soldán (2012) and the Sørensen Index of Similarity was calculated.

Results

Species richness

In total, 79 mayfly taxa (Table 2) were recorded for Croatia. Of the 171 sites (55 in ER11, 116 in ER5) investigated during this study (Table 1), 66 taxa were sampled, of which 29 were recorded for the first time (Table 2). The presence of 13 (16%) previously recorded species could not be confirmed (Table 2). The most diverse genera were *Baetis* Leach, 1815 and *Ecdyonurus* Eaton, 1868 both with 11 species. *Baetis rhodani* (Pictet, 1843) and *Serratella ignita* (Poda, 1761) were the most widely distributed species, present in 83 and 76 sampling sites, respectively. Fourteen species were recorded at only one sampling site: *Cloeon simile* Eaton, 1870, *Procloeon nana* (Bogoescu, 1951), *Caenis pusilla* Navàs, 1913, *Ephemera* cf. *parnassiana* Demoulin, 1958, *Leptophlebia vespertina* (Linnaeus, 1758), *Ecdyonurus vitoshensis* Jacob & Braasch, 1984, *Ecdyonurus zelleri* (Eaton, 1885), *Electrogena mazedonica* (Ikonomov, 1954), *Heptagenia coerulans* Rostock, 1878, *H. flava* Rostock, 1878, *H. longicauda* (Stephens, 1835), *Rhithrogena iridina* (Kolenati, 1839), *Rh.* gr. *diaphana* and *Rh. semicolorata* (Curtis, 1834).

Approximately half of the species (30) were present in both ecoregions. A total of 50 species was recorded as present only in the Dinaric western Balkan ecoregion (ER5) and 48 only in the Pannonian lowland ecoregion (ER11) (Table 2). Nearly half the species (32)

 Table 2. Croatian mayfly fauna.

Mayfly taxa	Ecoregion	Habitat type	Basin
Ametropodidae			
■ <i>Ametropus fragilis</i> Albarda, 1878	11	3	BS
Ameletidae			
▲ Ameletus inopinatus Eaton, 1887	-	-	-
▲ Metreletus balcanicus (Ulmer, 1920)	-	-	-
Siphlonuridae			
▲ Siphlonurus armatus (Eaton, 1870)	-	-	-
Siphlonurus croaticus Ulmer, 1920	11	2,3,4	AS
Siphlonurus lacustris (Eaton, 1870)	5, 11	2,3	BS, AS
Baetidae			
Alainites muticus (Linnaeus, 1758)	5	2,3,4	BS, AS
Baetis alpinus (Pictet, 1843)	5, 11	1,2,3	BS
• Baetis buceratus Eaton, 1870	11	3	BS
Baetis fuscatus (Linnaeus, 1761)	5, 11	3	BS
• Baetis liebenauae Keffermüller, 1974	5, 11	1,2,3	BS, AS
Baetis lutheri Müller-Liebenau, 1967	5, 11	1,3	BS, AS
• Baetis melanonyx (Pictet, 1843)	5	1,2,3	AS
• Baetis cf. nubecularis (Eaton, 1898)	5	1,2,3,4	BS
Baetis rhodani (Pictet, 1843)	5, 11	1,2,3,4	BS, AS
Baetis scambus Eaton, 1870	11	3	BS
• Baetis tricolor Tshernova, 1928	11	3	BS
• Baetis vernus Curtis, 1834	5, 11	3	BS, AS
• Baetopus tenellus (Albarda, 1878)	5, 11	2,3	BS
•Nigrobaetis niger (Linnaeus, 1761)	5, 11	2,3	BS, AS
Centroptilum luteolum (Müller, 1776)	5, 11	2,3,4,5	BS, AS
Cloeon dipterum (Linnaeus, 1761)	5, 11	2,3,5	BS, AS
Cloeon simile Eaton, 1870	5	5	AS
Procloeon bifidum (Bengtsson, 1912)	5, 11	2,3	BS, AS
• Procloeon nana (Bogoescu, 1951)	5	2	AS
Procloeon pennulatum (Eaton, 1870)	5, 11	3,4	BS, AS
Caenidae			
▼ Brachycercus harrisellus Curtis, 1834	11	3	BS
• Caenis beskidensis Sowa, 1973	5	3	AS
Caenis horaria (Linnaeus, 1758)	5, 11	3,4,5	BS, AS
Caenis macrura Stephens, 1835	5, 11	3	BS, AS
• <i>Caenis pusilla</i> Navàs, 1913	5	3	BS
• Caenis rivulorum Eaton, 1884	11	3	BS
• Caenis robusta Eaton, 1884	11	2,3,5	BS
Ephemerellidae			
• Ephemerella mucronata (Bengtsson, 1909)	5, 11	2,3	BS, AS
Serratella ignita (Poda, 1761)	5, 11	1,2,3,4	BS, AS
Torleya major (Klapalek, 1905)	5, 11	2,3,4	BS, AS
Ephemeridae		/ -	
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Ephemera danica Müller, 1764	5, 11	2,3,4,5	BS, AS
▲ <i>Ephemera glaucops</i> Pictet, 1843	-	-	-
<i>Ephemera lineata</i> Eaton, 1870	5	2,3,5	AS
• Ephemera cf. parnassiana Demoulin, 1958	5	2	AS
Ephemera vulgata Linnaeus, 1758	5, 11	2,3,5	BS, AS
•Ephemera zettana Kimmins, 1937	5	2,3	AS
Palingeniidae			
▲ Palingenia longicauda (Olivier, 1791)	-	-	-
Polymitarcyidae			
▲ <i>Ephoron virgo</i> (Olivier, 1791)	-	-	-
Leptophlebiidae			
▲ Choroterpes picteti (Eaton, 1871)	-	-	-
Habroleptoides confusa Sartori and Jacob, 1986	5, 11	2,3	BS, AS
Habrophlebia fusca (Curtis, 1834)	5, 11	1,2,3	BS, AS
Habrophlebia lauta Eaton, 1884	5, 11	2,3,5	BS, AS
•Leptophlebia vespertina (Linnaeus, 1758)	5	2,5	BS, AS
Paraleptophlebia submarginata (Stephens, 1835)	5, 11	2,3,4	BS, AS
• Paraleptophlebia werneri Ulmer, 1920	5	2,5	BS
Oligoneuriidae			
Oligoneuriella rhenana (Imhoff, 1852)	11	3	BS
Potamanthidae			
Potamanthus luteus (Linnaeus, 1767)	11	3	BS
Heptageniidae			
▲ Ecdvonurus aurantiacus (Burmeister, 1839)	-	_	_
Ecdvonurus dispar (Curtis, 1834)	5	2,3	BS, AS
Ecdyonurus insignis (Eaton, 1870)	5, 11	3	BS, AS
• Ecdvonurus macani Thomas & Sowa, 1970	5, 11	3	BS, AS
▲ Ecdvonurus siveci Hefti, Tomka & Zurwerra, 1986	-	-	-
• Ecdvonurus starmachi Sowa, 1971	5, 11	2.3	BS, AS
• Ecdvonurus submontanus Landa, 1969	5	3	BS
Ecdyonurus torrentis Kimmins, 1942	5	2.3	BS, AS
Ecdyonurus venosus (Fabricius, 1775)	5	2.3	AS
Ecdyonurus vitoshensis Jacob & Braasch, 1984	11	2	BS
• Ecdvonurus zelleri (Eaton 1885)	11	2	BS
• Electrogena affinis (Eaton, 1883)	5	23	AS
Flectrogena lateralis (Curtis 1834)	5 11	234	BS AS
• Flectrogena mazedonica (Ikonomov 1954)	5	3	AS
• Electrogena vihelvii (Sowa 1981)	5 11	1.2	BS AS
Eteanus asimilis Eston 1885	5,11	1.2.3	BS AS
Heptagenia coerulans Rostock 1878	11	3	BS
Heptagenia flava Rostock 1878	11	3	BS
Hentagenia langicanda (Stenhans, 1835)	5	3	BS
Haptagenia culphunga (Müller, 1776)) 11	2	BC DO
i iepiagenia saipisarea (iviunei, 1770)	11	5	50

▲ Kageronia fuscogrisea (Retzius, 1783)	-	-	-
• <i>Rhithrogena braaschi</i> Jacob, 1974	5	1,2,3	BS, AS
•Rhithrogena gr. diaphana	11	3	BS
▲ Rhithrogena germanica Eaton, 1885	-	-	-
• Rhithrogena iridina (Kolenati, 1839)	11	2	BS
Rhithrogena semicolorata (Curtis, 1834)	11	2	BS

- ▲ Only literature data: Bauernfeind and Soldán (2012) presence in Croatia noted without referent to exact localities.
- ▼ Only literature data: Kovács and Murányi (2013).
- Only literature data: Ćuk et al. (2015).
- New records for the Croatian mayfly fauna.
- Ecoregion: 5 = Dinaric western Balkan, 11 = Pannonian lowland.

Habitat type: 1 = spring, 2 = stream, 3 = river, 4 = tufa barrier, 5 = lake, - = unknown/missing data.

Basin: BS = Black Sea Basin; AS = Adriatic Sea Basin.

Table 3. Sørensen Index of Similarity between mayfly assemblages for surrounding countries in relation to Croatia. CRO = Croatia, B&H = Bosnia and Herzegovina, I = Italy, SLO = Slovenia, HUN = Hungary.

	CRO	B&H	Ι	SLO
CRO				
B&H	64.62			
Ι	55.44	51.89		
SLO	61.64	56.67	51.72	
HUN	74.85	60.69	54.27	52.17

were recorded in both the Black and Adriatic Sea Basins, while 25 species were recorded only for Black Sea basin and 11 species only for Adriatic Sea basin (Table 2).

The Sørensen Index of Similarity indicated the Croatian mayfly fauna had the greatest similarity with the Hungarian assemblage (Table 3).

Mayflies (Insecta, Ephemeroptera) of Croatia

For the distribution data, the following format was used: "Literature data" were mainly taken from Bauernfeind and Soldán (2012), which listed the presence of each species in Croatia but without reference to their exact localities. Two and one species and localities where they were recorded were mentioned in Kovács and Murányi (2013) and Ćuk et al., respectively. "Literature data with new records" corresponds to data obtained as a part of this study but were already published. "New records" are data obtained in this study but were not yet published. For every species, the site ID is listed. All sampling sites and their ID numbers are listed in Table 1.

- New records for the Croatian mayfly fauna
- Only adults recorded

I. Ametropodidae Bengtsson, 1913

1. *Ametropus fragilis* Albarda, 1878 **Literature data:** Drava River, Donji Miholjac (Ćuk et al. 2015)

II. Ameletidae McCafferty, 1991

2. *Ameletus inopinatus* Eaton, 1887 **Literature data:** Bauernfeind and Soldán (2012)

3. *Metreletus balcanicus* (Ulmer, 1920) Literature data: Bauernfeind and Soldán (2012)

III. Baetidae Leach, 1815
4. Alainites muticus (Linnaeus, 1758)
Literature data: Bauernfeind and Soldán (2012)
Literature data with new records: 79, 80■, 82, 84, 85, 86 (Vilenica et al. 2014)
New records: 68, 70, 115, 150, 158, 160, 161, 162, 163,165, 168

5. *Baetis alpinus* (Pictet, 1843) **Literature data:** Bauernfeind and Soldán (2012) **New records:** 13, 15, 57, 63

6. *Baetis buceratus* Eaton, 1870 • New records: 2, 36

7. Baetis fuscatus (Linnaeus, 1761)
Literature data: Bauernfeind and Soldán (2012)
New records: 5, 7, 8, 10, 18, 19, 26, 29, 31, 32, 35, 36, 40, 56, 60, 61, 62

8. Baetis liebenauae Keffermüller, 1974 •
New records: 1, 2, 9, 10, 35, 36, 37, 62, 98, 109, 110, 111, 112, 113, 122, 128, 131, 134, 139, 140, 141, 143, 151, 152, 153, 162, 171

9. Baetis lutheri Müller-Liebenau, 1967
Literature data: Bauernfeind and Soldán (2012)
New records: 7, 18, 19, 35, 61, 62, 103, 116, 141, 142, 146, 147, 150, 157

10. *Baetis melanonyx* (Pictet, 1843) ● **New records:** 115, 117, 120, 146, 147, 156, 157, 158, 159, 160, 161, 162, 163

11. Baetis cf. nubecularis Eaton, 1898 •

Literature data with new records: 79, 80, 81, 82, 83, 84, 85, 86, 87 (Vilenica et al. 2014)

12. Baetis rhodani (Pictet, 1843)

Literature data: Bauernfeind and Soldán (2012)

Literature data with new records: 79, 80, 81, 82, 83, 84, 85, 87, 88 (Vilenica et al. 2014) New records: 9, 10, 13, 15, 16, 23, 24, 26, 28, 29, 30, 31, 32, 34, 35, 48, 50, 51, 53, 59, 61, 62, 63, 64, 65, 66, 68, 70, 77, 78, 98, 99, 100, 103, 109, 110, 112, 113, 114, 115, 116, 117, 118, 120, 122, 123, 124, 128, 131, 132, 134, 135, 137, 138, 139, 140, 141, 142, 146, 147, 148, 149, 153, 157, 158, 159, 160, 161, 162, 163, 166, 169, 170, 171

13. Baetis scambus Eaton, 1870Literature data: Bauernfeind and Soldán (2012)New records: 7, 26

14. *Baetis tricolor* Tshernova, 1928 • New records: 20, 43, 44

15. *Baetis vernus* Curtis, 1834 ● **New records:** 7, 9, 10, 36, 38, 53, 54, 76

16. *Baetopus tenellus* (Albarda, 1878) ● **New records:** 19, 64, 94

17. *Nigrobaetis niger* (Linnaeus, 1761) ● Literature data with new records: 138 New records: 15, 36, 38, 93, 103, 109, 110, 128, 131

18. *Centroptilum luteolum* (Müller, 1776)
Literature data: Bauernfeind and Soldán (2012)
Literature data with new records: 84, 85, 86, 87, 88, 89, 90, 91, 92 (Vilenica et al. 2014)
New records: 1, 12, 23, 27, 28, 31, 32, 35, 61, 62, 69, 74, 77, 78, 103, 107, 109, 110, 121, 127, 128, 141, 142, 143, 144, 159

19. Cloeon dipterum (Linnaeus, 1761)
Literature data: Bauernfeind and Soldán (2012)
New records: 1, 5, 20, 24, 35, 37, 39, 41, 43, 44, 45, 46, 47, 60, 67, 78, 101, 103, 104, 105, 121, 125, 127, 128, 129, 152

20. *Cloeon simile* Eaton, 1870Literature data: Bauernfeind and Soldán (2012)New records: 125

21. Procloeon bifidum (Bengtsson, 1912)
Literature data: Bauernfeind and Soldán (2012)
New records: 6, 19, 20, 28, 29, 31, 32, 40, 41, 42, 44, 47, 62, 68, 69, 71, 115, 121, 141

22. *Procloeon nana* (Bogoescu, 1951) • New records: 68

23. Procloeon pennulatum (Eaton, 1870)
Literature data: Bauernfeind and Soldán (2012)
Literature data with new records: 84, 85, 86 (Vilenica et al. 2014)
New records: 26, 27, 61, 127, 129

IV. Caenidae Newman, 1853

 Brachycercus harrisellus Curtis, 1834
 Literature data: Vojlovica River at the bridge of road No. 2, Vojlovica (Kovács and Murányi 2013)

25. *Caenis beskidensis* Sowa, 1973 • New records: 139, 140, 141, 143, 142

26. *Caenis horaria* (Linnaeus, 1758)
Literature data: Bauernfeind and Soldán (2012)
Literature data with new records: 86, 87, 89, 90, 91, 92 (Vilenica et al. 2014)
New records: 39, 73, 78, 101, 106, 107

27. *Caenis macrura* Stephens, 1835
Literature data: Bauernfeind and Soldán (2012)
New records: 8, 9, 10, 18, 26, 27, 28, 31, 32, 35, 40, 41, 54, 61, 68, 71, 115, 140, 141, 142, 143

28. *Caenis pusilla* Navàs, 1913 •New records: 62

29. *Caenis rivulorum* Eaton, 1884 ● New records: 40, 41

30. *Caenis robusta* Eaton, 1884 • New records: 1, 24, 39, 47

V. Ephemerellidae Klapálek, 1909 31. Ephemerella mucronata (Bengtsson, 1909) • New records: 14, 134, 139, 163

32. Serratella ignita (Poda, 1761)
Literature data: Bauernfeind and Soldán (2012)
Literature data with new records: 83, 84, 85, 86, 88 (Vilenica et al. 2014))

New records: 1, 7, 8, 9, 10, 12, 17, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 48, 49, 53, 46, 58, 59, 60, 61, 62, 64, 65, 66, 68, 69, 73, 76, 98, 99, 100, 103, 108, 109, 110, 113, 114, 115, 116, 117, 118, 119, 121, 122, 129, 134, 137, 138, 139, 140, 141, 142, 143, 144, 146, 147, 148, 150, 153, 157, 158, 159, 162, 163, 171

33. Torleya major (Klapalek, 1905)
Literature data: Bauernfeind and Soldán (2012)
Literature data with new records: 84, 86 (Vilenica et al. 2014)
New records: 53, 66, 117, 118, 139, 141

VI. Ephemeridae Latreille, 1810

34. Ephemera danica Müller, 1764

- Literature data: Bauernfeind and Soldán (2012)
- Literature data with new records: 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92 (Vilenica et al. 2014)
- New records: 8, 14, 17, 23, 27, 28, 30, 33, 48, 49, 53, 59, 60, 61, 63, 64, 66, 68, 95, 100, 115,141, 142

35. *Ephemera glaucops* Pictet, 1843 Literature data: Bauernfeind and Soldán (2012)

36. Ephemera lineata Eaton, 1870

Literature data: Bauernfeind and Soldán (2012)

New records: 106, 107, 108, 109, 110, 118, 119, 122, 137, 138, 139, 140, 141, 142, 143, 147

37. *Ephemera* cf. *parnassiana* Demoulin, 1958 • New records: 98

38. *Ephemera vulgata* Linnaeus, 1758
Literature data: Bauernfeind and Soldán (2012)
New records: 11, 54, 55, 59, 100, 125, 128, 154, 164

39. *Ephemera zettana* Kimmins, 1937 ● ■ **New records:** 102, 118, 134, 136, 138, 141, 142, 154, 155

VII. Heptageniidae Needham, 1901

40. *Ecdyonurus aurantiacus* (Burmeister, 1839) **Literature data:** Bauernfeind and Soldán (2012)

41. *Ecdyonurus dispar* (Curtis, 1834) Literature data: Bauernfeind and Soldán (2012) New records: 61, 63, 66, 68, 69 42. *Ecdyonurus insignis* (Eaton, 1870) Literature data: Cetina River, between Podgrade and Slime (Kovács and Murányi 2013) New records: 26, 27, 32, 116, 141, 145

43. *Ecdyonurus macani* Thomas & Sowa, 1970 • New records: 7, 26, 27, 137, 138, 139, 141, 147

44. *Ecdyonurus siveci* Hefti, Tomka & Zurwerra, 1986 **Literature data:** Bauernfeind and Soldán (2012)

45. *Ecdyonurus starmachi* Sowa, 1971 • New records: 13, 14, 26, 53, 103, 120

46. *Ecdyonurus submontanus* Landa, 1969 • Literature data with new records: 82, 83 (Vilenica et al. 2014)

47. *Ecdyonurus torrentis* Kimmins, 1942 Literature data: Bauernfeind and Soldán (2012) New records: 95, 99, 118, 119, 120

48. Ecdyonurus venosus (Fabricius, 1775)
Literature data: Bauernfeind and Soldán (2012)
New records: 97■, 99, 100, 109, 110, 112, 118, 119, 120, 137, 138, 139, 141, 148, 150, 162

49. *Ecdyonurus vitoshensis* Jacob & Braasch, 1984 Literature data: Bauernfeind and Soldán (2012) New records: 12

50. *Ecdyonurus zelleri* (Eaton, 1885) • New records: 53

51. *Electrogena affinis* (Eaton, 1883) • New records: 68, 69, 70

52. *Electrogena lateralis* (Curtis, 1834)
Literature data: Bauernfeind and Soldán (2012)
Literature data with new records: 86 (Vilenica et al. 2014)
New records: 12, 27, 61, 96, 165

53. *Electrogena mazedonica* (Ikonomov, 1954) ● New records: 128

54. *Electrogena ujhelyii* (Sowa, 1981) ● **New records:** 11, 13, 16, 24, 50, 93

55. Epeorus assimilis Eaton, 1885
Literature data: Bauernfeind and Soldán (2012)
New records: 4, 13, 94, 97∎, 98, 99, 115, 116, 117, 120, 135∎, 137, 138, 141, 142, 146, 147, 156

56. *Heptagenia coerulans* Rostock, 1878Literature data: Bauernfeind and Soldán (2012)New records: 18

57. *Heptagenia flava* Rostock, 1878Literature data: Bauernfeind and Soldán (2012)New records: 167

58. *Heptagenia longicauda* (Stephens, 1835) •New records: 63

59. *Heptagenia sulphurea* (Müller, 1776) **Literature data:** Bauernfeind and Soldán (2012) **New records:** 7, 8, 18, 21, 40, 42

60. *Kageronia fuscogrisea* (Retzius, 1783) Literature data: Bauernfeind and Soldán (2012)

61. *Rhithrogena braaschi* Jacob, 1974 ●
Literature data with new records: 79, 80, 81, 82, 83, 85 (Vilenica et al. 2014)
New records: 57, 58, 109, 110, 112, 117, 120, 122, 124, 135, 137, 138, 139, 141, 142, 143, 146, 147, 162, 163

62. *Rhithrogena* gr. *diaphana* ● New records: 32

63. *Rhithrogena germanica* Eaton, 1885 Literature data: Bauernfeind and Soldán (2012)

64. *Rhithrogena iridina* (Kolenati, 1839) ● New records: 27

65. *Rhithrogena semicolorata* (Curtis, 1834) **Literature data:** Bauernfeind and Soldán (2012) **New records:** 53

VIII. Leptophlebiidae Banks, 1900

66. *Choroterpes picteti* (Eaton, 1871) **Literature data:** Bauernfeind and Soldán (2012)

67. *Habroleptoides confusa* Sartori and Jacob, 1986 Literature data: Bauernfeind and Soldán (2012) New records: 22, 120, 158

68. *Habrophlebia fusca* (Curtis, 1834)
Literature data: Bauernfeind and Soldán (2012)
New records: 27, 28, 30, 35, 38, 48, 59, 69, 70, 131, 168, 169

69. *Habrophlebia lauta* Eaton, 1884
Literature data: Bauernfeind and Soldán (2012)
Literature data with new records: 82, 83, 85, 90 (Vilenica et al. 2014)
New records: 25, 26, 27, 29, 48, 49, 61, 65, 66, 68, 70, 109, 110

70. *Leptophlebia vespertina* (Linnaeus, 1758) ● Literature data with new records: 90, 91 (Vilenica et al. 2014) New records: 134

71. Paraleptophlebia submarginata (Stephens, 1835)
Literature data: Bauernfeind and Soldán (2012)
Literature data with new records: 79, 83, 84, 85, 86, 87, 88 (Vilenica et al. 2014)
New records: 8, 14, 26, 53, 60, 61, 74, 77, 98, 109, 110, 118, 119, 120, 128, 134, 137, 138, 139, 141, 142, 162

72. *Paraleptophlebia werneri* Ulmer, 1920 ● **Literature data with new records:** 85, 90 (Vilenica et al. 2014)

IX. Oligoneuriidae Ulmer, 1914

73. Oligoneuriella rhenana (Imhoff, 1852)
Literature data: Bauernfeind and Soldán (2012)
New records: 26, 27, 32

X. Palingeniidae Albarda, 1888 74. *Palingenia longicauda* (Olivier, 1791) Literature data: Bauernfeind and Soldán (2012)

XI. Polymitarcyidae Banks, 1900 75. *Ephoron virgo* (Olivier, 1791) Literature data: Bauernfeind and Soldán (2012)

XII. Potamanthidae Albarda, 1888

76. *Potamanthus luteus* (Linnaeus, 1767) Literature data: Bauernfeind and Soldán (2012) New records: 7, 8, 9, 10, 18, 35, 36, 37, 40

XIII. Siphlonuridae Ulmer, 1920 (1888)

77. Siphlonurus armatus (Eaton, 1870) Literature data: Bauernfeind and Soldán (2012)

78. Siphlonurus croaticus Ulmer, 1920
Literature data: Bauernfeind and Soldán (2012)
Literature data with new records: 82, 83, 85, 87 (Vilenica et al. 2014)
New records: 55, 66, 111, 123, 128, 130, 135∎, 137

79. *Siphlonurus lacustris* (Eaton, 1870) Literature data: Bauernfeind and Soldán (2012) New records: 26, 27, 30, 73, 76

Community composition

The majority of the Croatian mayfly species were found to be associated with rivers and streams (Table 2). Among these, larvae of ten species also occurred within the spring areas (Table 2). Eleven species recorded in lakes and/or ponds were also found to inhabit flowing-water habitats. Cluster analysis (Fig. 2) showed that based on the



Figure 2. Cluster analysis of mayfly community composition, based on Bray-Curtis Similarity (See Table 1 for codes).

Sampling site	S	H'	1-λ
8	7	1.38	0.65
18	6	1.05	0.54
40	6	1.19	0.62
41	5	1.09	0.55
42	2	0.56	0.4
60	5	0.31	0.12
61	11	0.76	0.31
62	8	0.85	0.44
79	5	0.95	0.56
80	4	1.01	0.61
81	3	0.98	0.59
82	7	0.77	0.39
83	10	1.70	0.75
84	9	1.43	0.69
85	12	1.67	0.75
86	10	1.51	0.71
	7	1.41	0.67
88	5	1.06	0.59
89	3	0.86	0.56
90	6	0.52	0.24
91	4	0.86	0.43
92	3	1.06	0.66
109	10	1.77	0.75
110	9	1.42	0.69
135	2	0.21	0.11
137	9	1.30	0.66
138	9	1.26	0.65
139	11	1.35	0.61
140	6	1.31	0.65
141	18	1.96	0.81
142	11	1.83	0.82
143	7	1.09	0.52
146	4	1.09	0.59
147	8	1.08	0.56

Table 4. Species richness (S), Shannon-Weaver (H') and Simpson $(1-\lambda)$ indices of diversity, calculated for 34 sites. Sites with the highest H' and $1-\lambda$ are in bold.

mayfly assemblage, sampling sites were mainly structured first by ecoregion and then by habitat type. Species richness at the sampling sites and diversity indices are presented in Table 4. Species richness ranged from 2 and 18 species, Shannon-Weaver index between 0.21 and 1.96 and Simpson index between 0.11 and 0.82. All sampling sites with the highest species richness and diversity indices were situated in the Dinaric western Balkan ecoregion (ER5).

The SIMPER analysis between sites within the same habitat type showed an average similarity ranging from 35.1% for the Pannonian lowland rivers to 57.3% for the springs (Table 5).

Habitat type	Average similarity	Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
	35.10	C. macrura	3.56	10.09	1.12	28.76	28.76
D		H. sulphurea	2.64	9.02	0.95	25.69	54.45
Pannonian		P. luteus	2.72	7.64	0.98	21.77	76.22
lowiand river		P.bifidum	0.98	3.74	0.58	10.66	86.88
		C. rivulorum	1.46	2.32	0.32	6.61	93.49
	37.92	S.ignita	4.64	11.97	1.47	31.57	31.57
		B. rhodani	4.46	10.05	1.49	26.49	58.06
		Rh.braaschi	3.16	5.18	0.73	13.67	71.73
		P. submarginata	1.85	2.41	0.69	6.35	78.08
Dinaric river		E. lineata	1.62	1.68	0.59	4.43	82.51
		B. liebenauae	1.1	0.99	0.4	2.6	85.11
		B. lutheri	1.45	0.89	0.27	2.36	87.47
		C. luteolum	1.04	0.64	0.45	1.7	89.16
		E. danica	0.94	0.6	0.31	1.58	90.74
S	57.32	Rh. braaschi	5.21	33.1	3.43	57.75	57.75
Spring		B. rhodani	4.44	20.02	3.11	34.93	92.67
	53.92	E. danica	4.66	18.86	12.75	34.98	34.98
		P. submarginata	2.99	11.45	9.54	21.24	56.21
Tufa barrier		C. luteolum	2.85	8.47	2.05	15.7	71.92
		B. rhodani	2.31	6.07	0.58	11.26	83.18
		B. cf. nubecularis	2.94	5.71	0.58	10.59	93.77
	54.64	C. horaria	4.44	21.65	2.46	39.63	39.63
Lake		E. danica	2.42	16.91	2.67	30.96	70.59
		C. luteolum	3.08	13.41	1.9	24.55	95.14

Table 5. SIMPER analysis for similarities in mayfly community composition in different habitat types (Pannonian lowland river, Dinaric river, Spring, Tufa barrier, Lake). Average similarity reflects the percentage between samples within one habitat type.

Av. abund. = average abundance, av. sim. = average similarity, Sim/SD = standard deviation of similarity, Contrib% = contribution to similarity, cum.(%) = cumulative percentage of similarity.

Discussion

Due to the paucity of systematic studies, mayfly fauna and their habitat preferences in Croatia were very poorly known, with records of only 50 species (Bauernfeind and Soldán 2012, Kovács and Murányi 2013, Ćuk et al. 2015). As expected, this study showed a higher diversity: 66 taxa were recorded, of which 29 for the first time in Croatia (Table 2). Combined with the literature, the species list consists of 79 taxa. Croatia is a relatively small Balkan country divided into two Ecoregions: Dinaric western Balkan (ER5) and Pannonian lowland (ER11) (Illies 1978) due to its position on the crossroads of Central and Mediterranean Europe, which is why its mayfly fauna shows transitive characteristics.

As a result, species with wide (e.g. *Baetis rhodani*, *Cloeon dipterum*, *Caenis horaria*, *Serratella ignita*), patchy (e.g. *Procloeon nana*, *Leptophlebia vespertina*, *Caenis beskiden*-

sis) central European (e.g. Baetis cf. nubecularis, Ecdyonurus zelleri, Electrogena ujhelyii) as well as southern (e.g. Ephemera zettana) and Balkan (e. g. Electrogena mazedonica, Rhithrogena braaschi, Ephemera cf. parnassiana) distribution were recorded in Croatia. Additionally, 15 taxa were found that were not previously recorded in the Dinaric western Balkan ecoregion: Baetis cf. nubecularis, Procloeon nana, Caenis beskidensis, Ephemera cf. parnassiana, Ecdyonurus macani, E. submontanus, E. torrentis, Electrogena affinis, E. mazedonica, E. ujhelyii, Heptagenia longicauda, Rhithrogena braaschi, Habroleptiodes confusa, Leptophlebia vespertina and Paraleptophlebia werneri (Buffagni et al. 2007, 2009, Bauernfeind and Soldán 2012).

The new records include several morphologically interesting taxa: Rhithrogena from the diaphana group, Baetis cf. nubecularis and Ephemera cf. parnassiana. The Rhithrogena species from the diaphana group is morphologically similar to Rhithrogena savoiensis Alba-Tercedor & Sowa, 1987. However, DNA analysis based on mitochondrial COI gene shows it to be more closely related to Rhithrogena beskidensis Alba-Tercedor & Sowa, 1987 (Vuataz unpubl. results). Thus, reliable identification cannot be distinguished at this time. Comparison with other Balkan Rhithrogena diaphana group species and further detailed studied are required. A similar case is recorded for the Baetis alpinus group (sensu Müller-Liebenau, 1969), which presents the morphological characteristics that are intermediate between Baetis alpinus and B. nubecularis. Interestingly, the species is only recorded in high numbers (Vilenica et al. 2014) in the mountain Dinaric karst streams and tufa barriers in the area of Plitvice Lakes National Park (Table 1, Fig. 1). One male imago of the genus Ephemera Linnaeus, 1758, was caught in the Lopoško vrelo stream in southern Croatia. Its morphological features correspond to Ephemera parnassiana, a species that has currently only been recorded from Greece; however due to the small sample size, additional specimens are necessary for accurate identification of the species.

As most sites were in running waters and often with a stony substrate, the most diverse genera were Baetis and Ecdyonurus, which are known to be very common in running waters of the Northern Hemisphere (Bauernfeind and Soldán 2012). The most widely distributed species were two eurytopic and eurythermic species: Baetis rhodani and Serratella ignita. Further study is required at new sampling sites to determine the distribution of eleven species recorded only at only a single sampling site (Cloeon simile, Procloeon nana, Caenis pusilla, Ephemera cf. parnassiana, Leptophlebia vespertina, Ecdyonurus vitoshensis, E. zelleri, Electrogena mazedonica, Heptagenia coerulans, H. flava, H. longicauda, Rhithrogena iridina, Rh. gr. diaphana and Rh. semicolorata), as well as to determine the presence of the thirteen species listed in the literature which were not confirmed in this study (Ametropus fragilis, Ameletus inopinatus, Metreletus balcanicus, Siphlonurus armatus, Brachycercus harrisellus, Ephemera glaucops, Palingenia longicauda, Ephoron virgo, Choroterpes picteti, Ecdyonurus aurantiacus, E. siveci, Kageronia fuscogrisea and Rhithrogena germanica). The rare or unconfirmed presence of most of these species is likely due to the lack of seasonal sampling. It is possible that they were present at some sampling sites included in this study, but at a very young instar or even egg stage, and as such were overlooked. Additionally, some species might have

become extinct from the Croatian rivers, such as *Palingenia longicauda*, which at present likely only inhabits the Danube River and Tisza River in Hungary, Slovakia and Ukraine (Bauernfeind and Soldán 2012).

The Black Sea basin includes 62% of Croatian rivers (Jelić et al. 2008), which likely explains the higher number of mayfly species recorded in this basin than in the Adriatic Sea basin.

The Dinaric region is considered to be a biodiversity hotspot (Bánárescu 2004, Griffiths et al. 2004, Ivković and Plant 2015). Despite a similar number of taxa recorded in each ecoregion, the highest species diversity was recorded for the fast flowing streams and rivers in the Dinaric western Balkan ecoregion. Similar results were obtained in the study of aquatic dance flies in Croatia (Ivković et al. 2013). The lowest number of mayfly species was found in springs and lakes (Table 4). Various studies have shown that mayfly species diversity is generally low in spring areas (Berner and Pescador 1988, Bauernfeind and Moog 2000, Maiolini et al. 2011). The only spring with four species was the spring of the Ruda River (146) in southern Croatia (Fig. 1), which is largely fed with water from the Buško Blato reservoir (Štambuk-Giljanović 2001, Bonacci and Roje-Bonacci 2003) that is relatively rich in nutrients and organic matter (Štambuk-Giljanović 2001). Thus, mayfly communities in the Ruda River spring are more species diverse and have a high proportion of detritivores (Vilenica unpubl. results). Most mayfly species prefer lotic habitats with a larger array of microhabitats, and these are less diverse in spring areas and lentic habitats. The present study confirmed the results of many previous studies (Berner and Pescador 1988, Elliott et al. 1988, Bauernfeind and Humpesch 2001, Bauernfeind and Soldán 2012).

Mayfly larvae inhabit flowing and standing freshwater ecosystems where they occupy a range of microhabitats in correlation with different biotic and abiotic factors. Additionally, in running water habitats, due to the longitudinal gradient of the physico-chemical characteristics of the water, different parts of the watercourse are inhabited by different mayfly species (Elliott et al. 1988, Bauernfeind and Humpesch 2001). Cluster analysis (Fig. 2) based on mayfly assemblage generally showed that sampling sites are structured first by ecoregion and then by habitat type. For this reason, due to their morphology and water properties (Lucić et al. 2015), the large, slow Pannonian lowland rivers (Sava, Drava, Kupa) are separated from the other sampling sites situated in the Dinaric western Balkan ecoregion . SIMPER analysis (Table 5) showed that the Pannonian mayfly community consisted of species that prefer epipotamalic sections of rivers, such as Caenis macrura, Procloeon bifidum, Heptagenia sulphurea and Potamanthus luteus (Buffagni et al. 2007, 2009, Bauernfeind and Soldán 2012). Due to the two common mayfly species present in high numbers, Baetis rhodani and Rhithrogena braaschi (Vilenica et al. 2014, Vilenica unpubl. results), the investigated springs clustered together with the small mountain karst rivers. Larger karst rivers together due to the presence of species with a wide ecological range as *Baetis rhodani*, Centroptilum luteolum, Serratella ignita and Paraleptophlebia submarginata, and species with a southern European distribution such as Rhithrogena braaschi. Another common species was Baetis liebenauae, previously recorded in smaller streams with a sandy

or stony bottom as well as in large lowland rivers, where it can be found as a habitat specialist on macrophytes (Buffagni et al. 2007, 2009, Bauernfeind and Soldán 2012). The presence of a stony bottom and submerged vegetation may be a suitable habitat combination for the species. Further research is required to determine the more specific preferences at the microhabitat scale and physico-chemical properties of the water. The mayfly species diversity is generally quite poor in lentic habitats, though certain taxa can be very abundant. The main reason why lakes clustered together and apart from other sites was due to their species composition consisting of taxa from lentic (e.g. *Caenis horaria*) or a wide range of habitat type preferences (e.g. *Centroptilum luteolum, Ephemera danica*; Bauernfeind and Soldán 2012). Due to the presence and abundance of the species *Baetis rhodani*, *B. cf. nubecularis*, *Centroptilum luteolum, Serratella ignita*, *Ephemera danica* and *Paraleptophlebia submarginata*, the lower streams in the Plitvice Lakes National Park (sites 84 and 85) grouped together with the tufa-barriers (see also in Vilenica et al. 2014).

In comparison with the neighbouring countries and with consideration of their surface areas, the Ephemeroptera diversity in Croatia could be characterised as relatively high. Together with Croatia, Bosnia and Herzegovina is also situated in Dinaric western Balkan ecoregion (ER5) (Illies 1978). However, as its mayfly fauna is currently poorly known, with only 52 species recorded, and as a large part of Croatian territory belongs to the Pannonian lowland ecoregion, to which most of the Hungarian territory also belongs, the Croatian mayfly fauna was found to be most similar to the Hungarian fauna (75%, Table 3). This is due to the presence of widely distributed species and of the species inhabiting the larger rivers. Even though the mayfly fauna of Bosnia and Herzegovina is currently poorly known, 65% of the species were similar to the Croatian fauna. Thus, it is possible that a much greater similarity between these countries can be expected in the future. Italy is divided into two completely different ecoregions than Croatia: Italy (ER3) and Alps (ER4) (Illies 1978). It had a much higher mayfly diversity and the lowest similarity with the Croatian mayfly assemblage (55%, Table 3). This is possibly due to its geographical position and large surface area that includes a great variety of geographical features and diverse habitats. For example, the Alps, which are not present in Croatia, are well-known for their mayfly diversity and endemism, especially in the genus Rhithrogena Eaton, 1881 (Vuataz et al. 2011).

Conclusions

As expected, this study revealed a higher number of mayfly taxa inhabiting Croatian freshwater habitats than known from the previous literature. As two of the most similar mayfly assemblages of the neighbouring countries have several taxa that could also inhabit Croatian habitats (e. g. *Baetis vardarensis* Ikonomov, 1962, *Rhithrogena picteti* Sowa, 1971, *Leptophlebia marginata* (Linnaeus, 1767), *Ephemerella notata* Eaton, 1887, *Caenis luctuosa* (Burmeister, 1839)) but were not yet recorded, due to the lack of systematic sampling in all seasons, future studies should include seasonal sampling

of a higher number of sites and habitat types. Additionally, the main focus should be on the eastern lowland part of the country, where a lower number of sites was visited during this study.

In the present study, some interesting taxa with restricted European and local distributions were recorded (e.g. *Rhithrogena* gr. *diaphana*, *Baetis* cf. *nubecularis* and *Ephemera* cf. *parnassiana*). Considering these species were recorded from a small number of sites in this study, they could be considered rare. Future studies on the taxonomic status, ecological features and detailed distribution of these species is necessary.

Additionally, as *Baetis liebenauae* was recorded on larger karstic rivers, a different habitat type than previously known, more detailed information on its preferences at the microhabitat scale and water physico-chemical properties should be investigated.

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RESEARCH ARTICLE



Redescription of Temnothorax antigoni (Forel, 1911) and description of its new social parasite Temnothorax curtisetosus sp. n. from Turkey (Hymenoptera, Formicidae)

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Abstract

Temnothorax antigoni (Forel, 1911) is redescribed basing on a new material from southwestern Turkey (Antalya province), Lesbos and Rhodes (Greece, Aegean and Dodecanese islands). The gyne of this species is described for the first time. *Temnothorax curtisetosus*, a new species of social parasite collected in a nest of *T. antigoni*, is described. Colour photos of both taxa are given. A key to the worker caste of the eastern Mediterranean species belonging to both *T. recedens* and *T. muellerianus* groups are provided.

Keywords

Mediterranean subregion, Crematogastrini, taxonomy, Turkey, Greece, Temnothorax

Introduction

The genus *Temnothorax* Mayr, 1861 is one of the most speciose in the Myrmicinae subfamily. The most recent catalogue lists 380 valid species and 47 valid subspecies (Bolton 2015). Most species are distributed in northern hemisphere, mostly in temperate and warm temperate habitats, including taxa occurring in mountain habitats. More than a half of the described taxa are known from Europe and the Mediterranean basin (Borowiec 2014).

However, museum collections suggest that many species remain undescribed. Originally, the genus *Temnothorax* included only taxa related to *Temnothorax recedens* (Nylander), which were characterized by an extremely deep mesonotal groove. Subsequently, Bolton (2003) synonymized several genera with *Temnothorax* and moved most of the species, placed originally in the genus *Leptothorax*, to this taxon. Social parasitism is often encountered in this group of ants and parasitic species were usually described in the separate genera. A recent phylogeny of the subfamily Myrmicinae, based on molecular data, showed that the parasitic taxa are nested within *Temnothorax* and cause non-monophyly of the genus. As a consequence, they were also synonymized with *Temnothorax* (Ward et al. 2015).

Temnothorax antigoni (Forel, 1911), a member of *Temnothorax recedens* group, was described from Western Turkey and has been known only from the type specimen until the present study. Heinze (1988) listed Turkish members of the tribe Leptothoracini and cited *T. antigoni* with a comment: [good species ?]. The junior author collected recently a nest samples of this rare species in Lesbos, Rhodes and in SW Turkey. The nest from Turkey contained specimens of a new socially parasitic ant belonging to the former *Chalepoxenus*. Below we redescribe *Temnothorax antigoni* (Forel), described gyne of this species for the first time, and describe the new socially parasitic species.

Material and methods

Specimens were compared using standard methods of comparative morphology. Photos were taken using a Nikon SMZ 1500 stereomicroscope, Nikon D5200 photo camera and Helicon Focus software.

All given label data are in their original spelling; a vertical bar (|) separates data on different rows and double vertical bar (||) separates labels.

Abbreviations of repositories

DBET	Department of Biodiversity and Evolutionary Taxonomy, University of
	Wrocław, Poland;
MNHW	Museum of Natural History, University of Wrocław, Poland;
NHMC	Natural History Museum of Crete, Heraklion, Greece;
SSC	Sebastian Salata collection.

Measurand indices

- **EL** eye length; measured along the maximum diameter of eye;
- **EW** eye width; measured along the maximum width of eye (diameter perpendicularly to EL);
- **HL** head length; measured in straight line from mid-point of anterior clypeal margin to mid-point of occipital margin in full-face view;

- **HW** head width; measured above the eyes in full-face view;
- **MH** mesosoma height; measured from the upper edge of mesonotum to the lowest point of the mesopleural margin, in lateral view;
- **ML** mesosoma length; measured as diagonal length from the anterior end of the neck shield to the posterior margin of the propodeal lobe;
- **PH** petiole height; maximum height of petiole in lateral view;
- **PL** petiole length; maximum length of petiole in lateral view;
- **PPH** postpetiole height; maximum height of postpetiole in lateral view;
- **PPL** postpetiole length; maximum length of postpetiole in lateral view;
- **PPW** postpetiole width; maximum width of postpetiole in dorsal view;
- **PW** petiole width; maximum width of petiole in dorsal view;
- **SDL** spiracle to declivity length; minimum distance from the center of the propodeal spiracle to the propodeal declivity;
- **SL** maximum straight-line length of the scape;
- SPBA maximum distance between outer margins of spines measured at the base;
- **SPT** maximum distance between outer margins of spines measured at the top;
- **PSL** propodeal spine length; measured from the center of the propodeal spiracle to the top of the propodeal spine.

Example of measurements: $1.617 \pm 0.135 (1.073 - 1.717) =$ The average measurement ± standard deviation (range of variation).

Indices

EI	eye index; EL/HL × 100;
HI	head index: HW/HL × 100;
SI	scape index: SL/HL × 100;
SPI	propodeal spines index; PSL/HW × 100.

All lengths are in millimeters.

Descriptions

Temnothorax antigoni (Forel, 1911)

Leptothorax (Temnothorax) antigoni Forel, 1911: 333; Heinze 1988: 87; Kiran and Karaman 2012: 25.

Material examined. Syntype worker photograph examined: T. antigoni | ♀ type Forel | Coccarinali | p. Smyrne (Forel) || Typus || Sp. T. antigoni | Forel || Coll. Forel. || ANTWEB | CASENT | 0909060 (Available from: https://www.antweb.org/specimen/CASENT0909060, accessed 21 June 2015).

Other examined material. Turkey, Antalya Prov.: 5 gynes, 6 workers from the single locality; Greece, Rhodes: 12 gynes, 177 workers from 5 localities; Greece, Lesbos: 3 gynes, 70 workers, 5 males from 5 localities (for detailed data of examined material see Suppl. material 1).

Redescription. Worker (n=20). Measurements and indices: HL: 0.659 ± 0.04 (0.581-0.721); HW: 0.521 ± 0.032 (0.458-0.581); EL: 0.125 ± 0.09 (0.112-0.142); EW: 0.094 ± 0.005 (0.089-0.106); SL: 0.641 ± 0.039 (0.578-0.704); ML: 0.814 ± 0.062 (0.715-0.927); PSL: 0.139 ± 0.026 (0.078-0.179); SDL: 0.113 ± 0.024 (0.044-0.145); PL: 0.306 ± 0.028 (0.257-0.358); PPL: 0.188 ± 0.017 (0.156-0.218); PH: 0.186± 0.014 (0.162-0.212); PPH: 0.191± 0.016 (0.165-0.223); SPBA: 0.143 ± 0.02 (0.112-0.179); SPT: 0.149 ± 0.022 (0.112-0.19); PW: 0.146 ± 0.015 (0.123-0.168); PPW: 0.221 ± 0.025 (0.179-0.268); HI: 79.0 ± 1.6 (76.4-81.6); EI: 18.8 ± 0.8 (17.3-20.3); SPI: 27.2 ± 3.4 (19.9-32.7); SI: 97.2 ± 1.6 (93.8-99.7).

Whole body pale yellow, including antennae and legs, only first gastral tergite with pale, brown, regular transverse band apically (Figs 1, 2).

Head 1.2-1.3 times as long as wide, posterior margin of the head straight and laterally rounded in full-face view, gena almost parallel-sided (Fig. 7). Eyes small, 1.3 times as long as wide, gena 1.5 times as long as eye length, distance between line connecting hind margins of eyes to posterior margin of head 1.8 times as long as eye length. Anterior margin of clypeus regularly rounded, clypeal lines distinct, slightly divergent, reaching to line connecting anterior margin of eyes. Almost entire surface of head smooth and shiny, only gena with indistinct microreticulation. Clypeus, frons and top of head with numerous, long, erect hairs, the longest hair to 1.2 times longer than eye width, ventral surface of head with numerous long hairs, on the top of head hairs only slightly shorter. Antennal scape approximately as long as head, thin, in widest part only 1.8 times as wide as antennal base. Surface of scape smooth and shiny, covered with long, moderately dense, erect hairs. Funiculus 1.2 times as long as scape with three-segmented thin club, first segment twice longer than wide, second segment 1.3 times as long as wide, segments 3-5 approximately as long as wide, club very long, 0.75 times as long as segments 1-9 combined. Mesosoma elongate, 2.8 times as long as wide, with deep metanotal groove. Pronotum rounded on sides, regularly convex in profile, smooth and shiny, with 8-20 long, erect hairs. Promesonotal suture very fine but visible, mesonotum forms with pronotum regular arch, surface smooth and shiny with 4-8 long hairs. Mesopleura with regular granulate sculpture, metapleural suture distinct. Propodeum slightly convex in profile, surface with granulate sculpture but shiny, propodeal spines very short, triangular (Fig. 2), metapleura with granulate sculpture. Petiole elongate, 1.6 times as long as high, dorsal surface shallowly concave, petiolar lobe regularly rounded, ventral margin of petiole straight, carinate, with small, sharp denticle at the base. Petiolar lobe almost parallel-sided in dorsal view, then slightly converging to base. Petiolar lobe smooth and shiny with 4 long erect hairs, sides of petiole with granulate sculpture. Postpetiole globular in profile, from dorsal view slightly transverse with subangulate sides (Fig. 1), top of postpetiole smooth and shiny with 4-6 long, erect hair, sides with granulate sculpture. Gaster as long as meso-



Figures 1-2. Temnothorax antigoni (Forel), worker 1 dorsal 2 lateral. Scale bar: 1 mm.

soma, surface smooth and shiny covered with numerous long, erect hairs (Fig. 2). Legs elongate, smooth and shiny, with sparse, semierect hairs, femora along underside with row of 3-4 long erect hairs. Hind tarsus 1.6 times as long as hind tibia.

Description. Gyne (n=5). Measurements and indices: HL: 0.741 ± 0.012 (0.726-0.754); HW: 0.642 ± 0.015 (0.615-0.659); EL: 0.201 ± 0.08 (0.190-0.212); EW: 0.155 ± 0.06 (0.145-0.162); SL: 0.664 ± 0.023 (0.637-0.693); ML: 1.258 ± 0.025 (1.219-1.284); MH: 0.680 ± 0.044 (0.598-0.723); PSL: 0.199 ± 0.012 (0.184-0.218); SDL:

0.141 ± 0.013 (0.123-0.156); PL: 0.401 ± 0.025 (0.369-0.441); PPL: 0.237 ± 0.018 (0.212-0.257); PH: 0.277 ± 0.015 (0.257-0.302); PPH: 0.283 ± 0.007 (0.274-0.291); SPBA: 0.311 ± 0.006 (0.301-0.318); SPT: 0.281 ± 0.019 (0.251-0.302); PW: 0.200 ± 0.011 (0.190-0.223); PPW: 0.300 ± 0.017 (0.268-0.313); HI: 86.7 ± 1.5 (84.7-89.3); EI: 27.2 ± 1 (26.2-28.5); SPI: 31 ± 1.8 (28.4-34); SI: 89.6 ± 1.7 (87.7-91.9).

Whole body pale yellow, including antennae and legs, only first gastral tergite with pale brown, regular transverse band apically and subsequent tergites with brownish posterior margin (Figs 3, 4).

Head 1.1 times as long as wide, posterior margin of head rounded in full-face view, gena almost parallel-sided (Fig. 8). Eyes large, 1.4 times as long as wide, gena 0.7 times as long as eye length, distance between line connecting hind margins of eyes to posterior margin of head 1.3 times as long as eye length. Anterior margin of clypeus regularly rounded, clypeal lines distinct, slightly divergent, reaching slightly behind line connecting anterior margin of eyes. Upper half of head smooth and shiny, frons on sides microreticulate but shiny, gena with rugose sculpture and along inner margin of eye run 2-3 thin carinae. Clypeus, frons and top of head with numerous, moderately long, erect hairs, the longest hairs slightly shorter than eye width, ventral surface of head with numerous moderately long hairs. Antennal scape 1.1 times as long as head, thin, in widest part only 1.6 times as wide as antennal base. Surface of scape smooth and shiny, covered with moderately long, moderately dense, more or less erect hairs. Funiculus 1.2 times as long as scape with three-segmented thin club, first segment twice as long as wide, second segment as long as wide, segments 3-5 elongate 1.3-1.4 times as long as wide, club long, approximately as long as segments 1-9 combined. Mesosoma 1.8 times as long as wide. Pronotum narrow, not visible from above, smooth and shiny. Scutum of mesonotum convex, smooth and shiny, covered with numerous moderately long, erect setae. Scutellum convex, smooth and shiny with view erect setae. Anepisternite with indistinct microreticularion, shiny, mesopleuron smooth and shiny. Propodeum short, surface with few transverse carinae, propodeal spines short, 1.1 times as long, acute, near apex with one long seta (Fig. 4), metapleura with distinct carinae. Petiole elongate, 1.5 times as long as high, dorsal side almost flat, petiolar lobe subangulate in profile, with short carina on sides, distinctly microreticulate, ventral margin of petiole straight, carinate, with small, sharp denticle at base. Petiolar lobe feebly rounded on sides in dorsal view, then distinctly converging to base. Petiolar lobe behind top microreticulate with two long setae. Postpetiole globular in profile, from dorsal view distinctly transverse, 1.3 times as wide as long, with carinate sides (Fig. 3), top of postpetiole microreticulate with several thin, longitudinal carinae and 7-9 long, erect setae, sides microreticulate with few short carinae. Gastral tergites smooth and shiny covered with numerous long, erect hairs (Fig. 4). Legs elongate, smooth and shiny, with moderately dense, semierect to erect hairs, femora along underside with row of 4-5 long erect hairs. Hind tarsus 1.7 times as long as hind tibia.

Differential diagnosis. *Temnothorax antigoni* is a species belonging to the former subgenus *Temnothorax* sensu stricto. The following related species occur in the eastern



Figures 3-4. Temnothorax antigoni (Forel), gyne 3 dorsal 4 lateral. Scale bar: 1 mm.

part of the Mediterranean: *Temnothorax finzii* (Menozzi) known from Italy, Macedonia and Turkey, *Temnothorax recedens* (Nylander) widespread in the Mediterranean area, *T. rogeri* Emery noted from Croatia, Montenegro, and Greece, and *T. solerii* (Menozzi) known from Greece (endemic to Karpathos island).



Figures 5-6. Temnothorax antigoni (Forel), head and antennae 5 worker 6 gyne. Scale bars: 0.5 mm.

Workers of *Temnothorax finzii* distinctly differ by a very large eyes (EI > 24.8 in *T. finzii* vs EI < 20.3 in *T. antigoni*) and a longitudinal striation with rugosity covering entire lateral surface of the head while the head in *T. antigoni* is smooth and shiny.



Figures 7-8. Temnothorax antigoni (Forel), head 7 worker 8 gyne. Scale bars: 0.25 mm.

Another four species are very similar: T. rogeri differs in very long propodeal spine, at least twice as long as its width at base (in T. antigoni the spine is short, forms a denticle, not or only slightly longer than its width at base), T. solerii differs in entire body uniformly yellowish-brown to brown (in T. antigoni the body is uniformly pale yellow with darker transverse apical band on the first gastral tergite). At the first glance T. antigoni can be mistakenly determined as a pale variation of T. recedens. Workers of *T. recedens* are always bicoloured with head and gaster mostly dark and mesosoma usually with a darker spots on meso- and metapleura. Even pale workers of this species have always head and gaster gently darker than mesosoma with a pale basal spot on the first gastral tergite. In our collection we possess 17 gynes and 262 workers from 67 localities in Spain, Italy, Greece and Cyprus (see Suppl. material 1) and we have never found a specimens with colouration typical for *T. antigoni* (more than 230 examined specimens). In T. antigoni head and mesosoma are uniformly yellow, devoid of any darker discolourations and the gaster is mostly yellow with a darker transverse apical band on the first gastral tergite. This colouration is constant in all examined samples. The only observed variability was a degree of saturation of dark apical band on the first gastral tergite. Moreover, T. antigoni has average smaller eyes than T. recedens (EI: 18.8 ± 0.8 in *T. antigoni* vs 22.0 ± 1.6 in *T. recedens*).

Gynes are known only for *Temnothorax recedens* and *T. rogeri*. The gyne of *T. rogeri* distinctly differs in long propodeal spine, distinctly longer than width at base, head partly infuscate and gaster mostly brown with yellow spot at base of first tergite (in *T. antigoni* propodeal spine is triangular, as long as wide, body mostly uniformly yellow with darker transverse apical band on first gastral tergite and narrowly infuscate apical margin of subsequent tergites). The gyne of *T. recedens* differs in head and mesosoma usually bicoloured, with at least infuscate spot on meso- and metapleura, and mostly dark gaster (in *T. antigoni* the body is mostly uniformly yellow with darker transverse apical band on the first gastral tergite and narrowly infuscate apical margin of subsequent tergites).

Biological data. In Turkey a nest of *Temnothorax antigoni* was found under a stone on a rocky side of a sandy path which runs through a pine forest. The locality is placed inside archeological site of the ancient Greek city Phaselis, close to the sea, only 6 m a.s.l. In the three Rhodes localities nests were found in rocks in mountain pine forest habitats at altitudes 522-598 m. Nests were located between schists of the volcanic rocks placed in the shade. In the five Lesbos localities nests were found in pine forest, oak forest and river valleys with platanus trees at altitudes 74-485 m. Nests were located under a moss overgrowing a large stones and between a schists of the volcanic rocks placed in the shade. A single workers were collected also on the surface of large stones or rocks. The following ant species were recorded in the same areas as *T. antigoni*:

- Turkey, Antalya province, ancient Phaselis: Aphaenogaster festae Emery, Aphaenogaster sporadis Santschi, Camponotus aegaeus Emery, Camponotus lateralis (Olivier), Camponotus rebeccae Forel, Camponotus samius Forel, Cardiocondyla bulgarica Forel, Crematogaster ionia Forel, Lasius neglectus Van Loon, Boomsma & Andrasfalvy, Lepisiota caucasica (Santschi), Lepisiota dolabellae (Forel), Lepisiota sp., Messor cf. structor, Pheidole koshewnikovi Ruzsky, Plagiolepis pallescens sensu Radchenko, Tapinoma sp., and Tetramorium cf. semilaeve;
- Greece, Rhodes, Attavyros loc. 2: Aphaenogaster sporadis Santschi, Camponotus aegaeus Emery, Camponotus boghossiani Forel, Camponotus truncatus (Spinola), Crematogaster ionia Forel, Lepisiota melas (Emery), Pheidole koshewnikovi Ruzsky, and Temnothorax dessyi (Menozzi);
- Greece, Rhodes, Attavyros location 3: Aphaenogaster festae Emery, Aphaenogaster sporadis Santschi, Camponotus boghossiani Forel, Camponotus kiesenwetteri (Roger), Camponotus lateralis (Olivier), Camponotus samius Forel, Crematogaster ionia Forel, Lepisiota melas (Emery), Temnothorax dessyi (Menozzi), and Plagiolepis taurica Santschi;
- Greece, Rhodes, road to Prof. Ilias location 2: *Aphaenogaster sporadis* Santschi, *Camponotus aegaeus* Emery, *Camponotus oertzeni* Forel, *Crematogaster ionia* Forel, and *Plagiolepis taurica* Santschi;
- Greece, Lesbos, Ligona Valley: Aphaenogaster balcanica (Emery), Aphaenogaster epirotes (Emery), Aphaenogaster lesbica Forel, Camponotus aegaeus Emery, Camponotus boghossiani Forel, Camponotus gestroi Emery, Camponotus lateralis (Olivier), Camponotus samius Forel, Camponotus truncatus (Spinola), Cataglyphis nodus (Brullé), Cataglyphis viaticoides (André), Crematogaster ionia Forel, Dolichoderes quadripunctatus (Linnaeus), Lasius alienus (Förster), Messor oertzeni Forel, Messor orientalis (Emery), Messor wasmanni Krausse, Monomorium monomorium Bolton, Pheidole pallidula (Nylander), Plagiolepis pallescens sensu Radchenko, Prenolepis nitens (Mayr), Temnothorax bulgaricus (Forel), Temnothorax cf. parvulus, Tetramorium cf. caespitum, Tetrarmorium diomedeum Emery, and Tetramorium punctatum Santschi;
- Greece, Lesbos, Antissa: Aphaenogaster festae Emery, Camponotus lateralis (Olivier), Cataglyphis nodus (Brullé), Crematogaster ionia Forel, Crematogaster schmidti

(Mayr), Dolichoderes quadripunctatus (Linnaeus), Lepisiota frauenfeldi (Mayr), Messor orientalis (Emery), Pheidole pallidula (Nylander), Temnothorax bulgaricus (Forel), and Trichomyrmex perplexus (Radchenko);

- Greece, Lesbos, 3 km N of Kalloni: Camponotus lateralis (Olivier), Camponotus sanctus Forel, Crematogaster ionia Forel, Messor orientalis (Emery), Plagiolepis pallescens sensu Radchenko, Temnothorax bulgaricus (Forel), Temnothorax cf. exilis, and Temnothorax semiruber (André);
- Greece, Lesbos, M. Pythariou: Aphaenogaster festae Emery, Camponotus lateralis (Olivier), Cataglyphis nodus (Brullé), Crematogaster ionia Forel, Lepisiota frauenfeldi (Mayr), Liometopum microcephalum (Panzer), Pheidole pallidula (Nylander), Temnothorax bulgaricus (Forel), Tetramorium cf. chefketi, Tetramorium cf. semilaeve, Tetramorium rhodium Emery;
- Greece, Lesbos, Ipsilometopo: Aphaenogaster balcanica (Emery), Aphaenogaster epirotes (Emery), Aphaenogaster festae Emery, Camponotus boghossiani Forel, Camponotus kiesenwetteri (Roger), Camponotus lateralis (Olivier), Camponotus samius Forel, Camponotus sanctus Forel, Cataglyphis nodus (Brullé), Cataglyphis viaticoides (André), Crematogaster ionia Forel, Crematogaster lorteti Forel, Lasius alienus (Förster) Lepisiota frauenfeldi (Mayr), Messor oertzeni Forel, Messor orientalis (Emery), Monomorium monomorium Bolton, Pheidole cf. pallidula, Plagiolepis taurica Santschi, Prenolepis nitens (Mayr), Temnothorax cf. affinis, Temnothorax cf. luteus, Temnothorax cf. tristis, Temnothorax bulgaricus Forel, Tetramorium cf. punctatum.

Distribution. Described from Turkey: "Coccarinali près Smyrne" [now Izmir, Izmir province]. New locality in Turkey (ancient Phaselis) is placed in Antalya province approximately 370 km southeast from the type locality, three localities on Rhodes (Greece, Dodecanese) are placed 231-139 km southwest from the second locality in Turkey, and localities on Lesbos are placed 100-120 km northwest from the type locality (Fig. 16). Species new to Greek fauna.

A key to the worker caste of the East Mediterranean species belonging to the *T. recedens* group.

1	Head rectangular, rugulose with longitudinal striation
_	Head oval, smooth and shiny2
2	Whole body uniformly brown to pale brown, Greece: Karpathos IsT. solerii
_	Body bicoloured, at least gaster with darker transverse apical band on the first
	gastral tergite
3	Propodeal spines very long, claw-shaped, more or less curved apically T. rogeri
_	Propodeal spines short, never claw-shaped, pointed more or less upward4
4	Head and masosoma uniformly pale yellow, gaster pale yellow with darker
	transverse apical band on the first gastral tergite, EI < 20.3 T. antigoni
_	Head and mesosoma usually bicoloured, with at least infuscate spot on meso-
	and metapleura, gaster dark with pale basal spot of first tergite, EI > 20.3
	T. recedens

Temnothorax curtisetosus Salata & Borowiec, sp. n. http://zoobank.org/CF1D977F-E8B0-45AE-B747-21F5590949C2

Etymology. Named after the very short setae on mesosoma dorsum and gastral tergites.

Material examined. Holotype worker (MNHW no. 1226): TURKEY, Antalya Prov. | ancient Phaselis | c. 6 m, 36.5262N/30.5455E | 29 VI 2010, L. Borowiec || Collection L. Borowiec | Formicidae | LBC-TR00059 || Temnothorax | curtisetosus sp. n. | in nest of T. antigoni | det. Salata & Borowiec; paratype worker: the same data as holotype (DBET).

Description. Measurements: Workers (n = 2). HL: 0.715-0.737 (0.726); HW: 0.536-0.570 (0.553); EL: 0.178-0.184 (0.181); EW: 0.145-0.151 (0.148); SL: 0.575-0.603 (0.589); ML: 0.899-0.905 (0.902); PSL: 0.162-0.170 (0.166); PL: 0.296-0.330 (0.313); PPL: 0.212-0.235 (0.2235); PH: 0.279-0.279 (0.279); PPH: 0.223-0.246 (0.2345); SPBA: 0.201-0.190 (0.1955); SPT: 0.229-0.223 (0.226); PW: 0.212-0.235 (0.2235); PPW: 0.313-0.313 (0.313); HI: 75-77.3 (76.2); EI: 24.9-25 (24.95); SPI 28.4-31.7 (30.1); SI: 80.4-81.8 (81.1).

Head yellowish, in dorsal half slightly darker than in frontal parts and below eyes. Mesosoma, petiole, postpetiole, antennae and legs uniformly yellowish, first gastral tergite yellowish-brown with paler large patch at base, subsequent tergites yellowish-brown, sternites yellow (Figs 9, 10).

Head 1.4 times as long as wide, posterior margin of head straight and laterally rounded in full-face view, gena almost parallel-sided (Fig. 11). Eyes moderately large, 1.2 times as long as wide, gena 1.2 times as long as eye length, distance between line connecting hind margins of eyes to posterior margin of head 1.3 times as long as eye length. Anterior margin of clypeus regularly rounded, clypeal lines distinct, slightly divergent, reaching to the line connecting posterior margin of eyes. Almost whole surface of head smooth and shiny, only gena with rugose sculpture and along inner and outer margin of eye run 2-3 thin carinae. Clypeus, frons and top of the head with numerous, moderately long, erect hair, the longest hairs slightly shorter than eye width, ventral surface of head with numerous moderately long hairs. Antennal scape 0.8 times as long as head, thin, in widest part only 1.5 times as wide as antennal base. Surface of scape smooth and shiny, covered with moderately long, moderately dense, more or less erect hairs. Funiculus 1.3 times as long as scape with threesegmented thin club, first segment 1.8 times as long as wide, second segment as long as wide, segments 3-5 slightly transverse, club very long, only slightly shorter than segments 1-9 combined. Mesosoma elongate, 2.5 times as long as wide, with deep metanotal groove. Pronotum rounded on sides, regularly convex in profile, smooth and shiny, with 5-6 moderately long and few short, erect hairs. Promesonotal suture very fine but visible, mesonotum forms with pronotum regular arch, dorsal surface smooth and shiny with 4-8 moderately long hairs, sides with few longitudinal carinae. Mesopleura with indistinct microreticularion and few carinae. Propodeum distinctly convex in profile, surface with indistinct microreticulation but shiny, with 5-6 moderately long erect setae, propodeal spines short, 1.1 times as long as width at



Figures 9-10. Temnothorax curtisetosus sp. n., worker 9 dorsal 10 lateral. Scale bar: 1 mm.

base, acute, near apex with one long seta (Fig. 10), metapleura with indistinct microreticularion and few carinae. Petiole short, 1.1 times as long as high, dorsal surface shallowly concave, petiolar lobe rounded, on sides with short carina, ventral margin of petiole straight, carinate, at base with moderately large, sharp denticle. In dorsal view petiolar lobe almost round, then distinctly converging to base. Petiolar lobe smooth and shiny with 4 long and two short erect hairs, sides of petiole microreticulate but shiny. Postpetiole globular in profile, from dorsal view distinctly transverse,

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				WORKER			
			T. recedens group			T. muelleria	inus group
	T. antigoni	T. finzii	T. recedens	T. rogeri	T. solerii	T. muellerianus	T. curtisetosus
	n=20	n=2	n=25	n=1 7	n=20	n=20	n=2
111	0.659 ± 0.04	7 U CL3 U	0.624 ± 0.05	0.675 ± 0.02	0.702 ± 0.034	0.816 ± 0.044	0.715-0.737
IJL	(0.581 - 0.721)	0.0-2/C.U	(0.503 - 0.715)	(0.636 - 0.726)	(0.648 - 0.76)	(0.715 - 0.888)	(0.726)
	0.521 ± 0.032	201 0 211 0	0.501 ± 0.05	0.552 ± 0.02	0.564 ± 0.032	0.615 ± 0.031	0.536-0.570
M LI	(0.458 - 0.581)	0.440-0.40)	(0.408 - 0.581)	(0.518 - 0.598)	(0.503 - 0.615)	(0.536 - 0.668)	(0.553)
ET	0.125 ± 0.09	0110 2710	0.135 ± 0.016	0.152 ± 0.008	0.15 ± 0.009	0.209 ± 0.014	0.178-0.184
EL	(0.112 - 0.142)	0.14/-0.149	(0.106 - 0.156)	(0.14 - 0.168)	(0.134 - 0.168)	(0.179 - 0.235)	(0.181)
EWV	0.094 ± 0.005	012 0110	0.092 ± 0.015	0.105 ± 0.008	0.104 ± 0.006	0.168 ± 0.009	0.145-0.151
EW	(0.089 - 0.106)	0.12-0.117	(0.067 - 0.112)	(0.095 - 0.123)	(0.089 - 0.112)	(0.156 - 0.179)	(0.148)
CI	0.641 ± 0.039	777 0 157 0	0.605 ± 0.06	0.677 ± 0.018	0.674 ± 0.035	0.637 ± 0.028	0.575-0.603
31	(0.578 - 0.704)	0.401-0.404	(0.491 - 0.698)	(0.648 - 0.709)	(0.626 - 0.726)	(0.57 - 0.693)	(0.589)
1) I	0.814 ± 0.062	JOO U C22 U	0.759 ± 0.095	0.831 ± 0.037	0.889 ± 0.053	0.992 ± 0.059	0.899-0.905
INIT	(0.715-0.927)	000.0-26/.0	(0.609 - 0.911)	(0.782 - 0.893)	(0.804 - 0.983)	(0.893 - 1.106)	(0.902)
DCI	0.139 ± 0.026	0 100 0 1100	0.138 ± 0.027	0.216 ± 0.014	0.172 ± 0.016	0.237 ± 0.039	0.162 - 0.170
тел	(0.078 - 0.179)	0.100-0.1170	(0.084 - 0.179)	(0.19 - 0.243)	(0.154 - 0.221)	(0.179 - 0.363)	(0.166)
IUS	0.113 ± 0.024	7010 7010	0.126 ± 0.024	0.131 ± 0.009	0.131 ± 0.009	0.175 ± 0.01	0 172 0 172
OUL	(0.044 - 0.145)	0.100-0.104	(0.087 - 0.162)	(0.111 - 0.145)	(0.123 - 0.151)	(0.156 - 0.196)	0.120-0.120
DI	0.306 ± 0.028	1000/	0.284 ± 0.039	0.32 ± 0.026	0.337 ± 0.025	0.39 ± 0.03	0.296-0.330
I.L	(0.257 - 0.358)	167.0-1	(0.212 - 0.346)	(0.24 - 0.363)	(0.296-0.374)	(0.363 - 0.447)	(0.313)
Idd	0.188 ± 0.017	0 100 /	0.206 ± 0.035	0.232 ± 0.015	0.199 ± 0.016	0.303 ± 0.028	0.212-0.235
LTL	(0.156 - 0.218)	0.100-/	(0.156 - 0.318)	(0.201-0.257)	(0.173 - 0.226)	(0.279 - 0.346)	(0.224)
рн	0.186 ± 0.014	00007	0.194 ± 0.026	0.213 ± 0.012	0.201 ± 0.016	0.283 ± 0.021	0.279-0.279
	(0.162 - 0.212)	(07.0-1	(0.156 - 0.243)	(0.201 - 0.24)	(0.173 - 0.221)	(0.268 - 0.335)	(0.279)
наа	0.191 ± 0.016	0 205 /	0.19 ± 0.027	0.213 ± 0.014	0.205 ± 0.018	0.281 ± 0.023	0.223-0.246
1111	(0.165 - 0.223)	1-607.0	(0.145 - 0.243)	(0.179 - 0.234)	(0.173 - 0.235)	(0.251 - 0.318)	(0.235)
V B B V	0.143 ± 0.02	0 1 / 3 0 1 / 0	0.137 ± 0.022	0.155 ± 0.011	0.163 ± 0.018	0.220 ± 0.019	0.201 - 0.190
VIIIO	(0.112-0.179)	U.147-U.147	(0.089 - 0.19)	(0.134 - 0.173)	(0.134 - 0.19)	(0.19-0.257)	(0.196)

				WORKER			
			T. recedens group			T. muelleri	anus group
	T. antigoni	T. finzii	T. recedens	T. rogeri	T. solerii	T. muellerianus	T. curtisetosus
	n=20	n=2	n=25	n=1 7	n=20	n=20	n=2
TuS	0.149 ± 0.022	0 1 0 0 0 1 0	0.144 ± 0.023	0.154 ± 0.016	0.181 ± 0.02	0.265 ± 0.021	0.229-0.223
0F 1	(0.112 - 0.19)	0.102-0.210	(0.106 - 0.201)	(0.19 - 0.246)	(0.145-0.217)	(0.223 - 0.302)	(0.226)
	0.146 ± 0.015	2710 0710	0.139 ± 0.018	0.154 ± 0.008	0.16 ± 0.013	0.244 ± 0.017	0.212-0.235
I W	(0.123 - 0.168)	0.147-0.10/	(0.109 - 0.168)	(0.145 - 0.168)	(0.134 - 0.184)	(0.212 - 0.279)	(0.224)
	0.221 ± 0.025		0.209 ± 0.03	0.241 ± 0.012	0.246 ± 0.024	0.371 ± 0.037	0.313-0.313
FT W	(0.179 - 0.268)	667.0-77.0	(0.156-0.257)	(0.223–0.265)	(0.212 - 0.279)	(0.269 - 0.430)	(0.313)
111	79.0 ± 1.6	0 00 0 02	80.3 ± 3.0	81.9 ± 4.2	80.3 ± 1.6	75.4 ± 1.8	
111	(76.4 - 81.6)	/ 0.0-00.0	(71.9–85.9)	(72.5–87.9)	(76.3–82.8)	(72.8–80.7)	(7.0/) C.//- C/
	18.8 ± 0.8	0 % 1 20	22.0 ± 1.6	22.5 ± 1.4	21.2 ± 1.3	25.6 ± 1	(30 % 0 / 20 0 % 0
E	(17.3 - 20.3)	0.1-24.0	(20.3.7 - 26.5)	(20.0-24.8)	(19.2 - 23.7)	(22.9–27.7)	(((,+2))) ()-(,+2)
cnr	27.2 ± 3.4	00% 60%	27.2 ± 3.5	39.2 ± 3.0	30.4 ± 2.2	37.1 ± 2.0	JO & 21 7 (30 1)
110	(19.9–32.7)	42.2-40.0	(20.6 - 34.4)	(34.9 - 46.9)	(27.3 - 37.3)	(33.4 - 40.4)	(1.00) /.10-4.07
CI	97.2 ± 1.6		96.9 ± 3.2	100.3 ± 2.8	96.0 ± 2.2	77.7 ± 2.2	00 % 01 0 /01 1)
10	(93.8–99.7)	C.//_0.0/	(92.3 - 108.1)	(92.0 - 103.6)	(92.2 - 101.6)	(73.4 - 81.5)	(1.10) 0.10-+.00



Figure 11. Temnothorax curtisetosus sp. n., worker head. Scale bar: 0.5 mm.

1.4 times as wide as long, with regularly rounded sides (Fig. 9), top of postpetiole smooth and shiny with 8–10 moderately long, erect hair, sides microreticulate with few short carinae. Gaster slightly shorter than mesosoma, surface smooth and shiny covered with numerous moderately long, erect hairs (Fig. 10). Legs elongate, smooth and shiny, with sparse, semierect to erect hairs, femora along underside with row of 3–4 long erect hairs. Hind tarsus 1.4 times as long as hind tibia.

Differential diagnosis. Temnothorax curtisetosus sp. n. belongs to a monophyletic group of social parasites formerly classified as a separate genus Chalepoxenus Menozzi and recently synonymized with Temnothorax Mayr (Ward et al. 2015). The group comprises five species in Europe and the Mediterranean subregion, all are parasites of various Temnothorax species: Temnothorax brunneus (Cagniat) from Morocco, Temnothorax kutteri (Cagniat) from France: mainland, and Spain: mainland, Temnothorax muellerianus (Finzi) from Bulgaria, Croatia, Cyprus, France: mainland, Germany, Greece: Crete, Ionian Is., mainland, Sicily, Portugal, Serbia, Slovenia, Spain: mainland, Switzerland, Turkey and Ukraine, Temnothorax inquilinus Ward, Brady, Fisher & Schultz from Ukraine and Temnothorax tramieri (Cagniat) from Morocco.


Figure 12–13. Worker mesosoma lateral 12 *Temnothorax curtisetosus* sp. n. 13 *Temnothorax muellerianus* (Finzi). Scale bars: 0.5 mm.

Temnothorax curtisetosus and T. muellerianus (Finzi) differ significantly from other members of this group in having tibiae covered with long, erect setae. Temnothorax muellerianus is the most widely distributed and the most variable species of this group (Buschinger et al. 1988). Temnothorax curtisetosus distinctly differs from T. muellerianus in very short setae on the mesosoma, petiole and postpetiole (Fig. 12 versus Fig. 13), and especially on gastral tergites (the total length of 10 setae combined on the first tergite is 741 µm in T. curtisetosus vs. 1111-1325 µm in T. muellerianus Figs 14-15). Temnothorax curtisetosus is smaller than most specimens of T. muellerianus and has shorter antennal scapes and higher SI index. At the first glance T. curtisetosus reminds a workers of T. finzii. Besides a clear differences in the biology of these species, T. finzii is a non-parasitic species inhabiting dry open habitats and nesting deep in the soil, usually under stone (Bračko et al. 2014), these species can be distinguished also in morphological features. T. curtisetosus differs from T. finzii by a weaker longitudinal striation covering only sides of the frons and its head is devoid of rugosity (in T. finzii whole head is covered by longitudinal striation with rugosity between it) and in presence of a dentiform plate on the ventral margin of the petiole, a character associated



Figure 14-15. Worker gaster lateral 14 *Temnothorax curtisetosus* sp. n. 15 *Temnothorax muellerianus* (Finzi). Scale bar: 0.5 mm.

with a *Chalepoxenus* line. Moreover *T. curtisetosus* has also smaller propodeal spines (SPI < 31.7 vs SPI>40.8 in *T. finzii*).

Distribution. SW Turkey, Antalya Province.

Comments. We found only two workers of *Temnothorax curtisetosus* in a nest of *Temnothorax antigoni*. The large number of gynes in relation to number of workers of the host species (5 gynes/6 workers) suggests that the nest was in the initial stage. Both specimens of the parasite have constant characters, especially very short dorsal setae. Very small specimens of *T. muellerianus* have dorsal setae proportionally 1.5



Figure 16. Distribution of *Temnothorax antigoni* (Forel), blue circle – locus typicus, red circle – new locality in Turkey (also locus typicus for *Temnothorax curtisetosus* sp. n.), yellow circles – new localities in Rhodes, black circle – new localities in Lesbos.

times longer than both specimens of *T. curtisetosus*. Kutter (1973) and Buschinger et al. (1988), basing on a large material from the entire Mediterranean basin, discussed variability and status of several taxa closely related to *T. muellerianus* but none of the samples studied by them were characterized by short dorsal setae. Although we have only two specimens of *T. curtisetosus*, the clear gap in the length of dorsal setae between these specimens and all examined samples of *T. muellerianus* (37 workers from 12 localities of 4 countries, see Suppl. material 1), a shorter propodeal spines and the analysis of variability within various populations of *T. muellerianus* discussed by Buschinger et al. (1988) convinced us to describe these two specimens as a species new for science.

A key to a worker caste of east Mediterranean species belonging to the *T. muellerianus* group is provided below.

1	Tibiae bearing long, erect setae	2
_	Tibiae never with long, erect setae	. T. kutteri group
2	Setae on mesosoma and gaster short, the total length of 10 s	setae combined on
	the first gastral tergite less than 741 µm; propodeal spines	short; SPI<31.7
	<i>T</i> .	<i>curtisetosus</i> sp. n.
_	Setae on mesosoma and gaster long, the total length of 10 s	setae combined on
	the first gastral tergite more than 1100 µm; propodeal spine	s long; SPI>33.4
		<i>uellerianus</i> (Finzi)

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Supplementary material I

Table with specimens data

Authors: Sebastian Salata, Lech Borowiec

Data type: specimens data

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