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A new species of *Hemipera* Nicoll, 1913 (Digenea: Derogenidae) from fishes of the intertidal rocky zone of Chile

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Abstract

A new species, *Hemipera cribbi* sp. nov., is described. This trematode was found in three intertidal fish species: *Scartichthys viridis* (Valenciennes) (Blenniidae), *Gobiesox marmoratus* Jenyns (Gobiesocidae) and *Myxodes viridis* Valenciennes (Clinidae) from the central and southern coast of Chile. Of 233 individuals of *S. viridis* from the central coast examined, 19 were infected. From the southern coast, nine individuals of *S. viridis* (one infected), five individuals of *G. marmoratus* (four infected), and 16 individuals of *M. viridis* (one fish infected) were examined. *Hemipera cribbi* sp. nov. is distinguished from the five other congeneric species mainly in the body size, being the smallest and narrowest species in the genus, reaching five times longer than wide. This is the first species of the genus described for the South Pacific Ocean off South America. ITS2 rDNA sequences of *Hemipera cribbi* sp. nov. from each host and locality were identified.

Keywords

Hemipera, Derogenidae, *Scartichthys*, intertidal rocky shore, Chile

Introduction

The genus *Hemipera* Nicoll, 1913 comprises five species: *Hemipera ovocaudata* Nicoll, 1913 (the type species), *H. sharpei* Jones, 1933, *H. nicolli* (Manter, 1934) Yamaguti, 1958, *H. manteri* (Crowcroft, 1947) Yamaguti, 1958 and *H. magnaprostatica* Gaevskaya and Aleshkina, 1995. *Hemipera* have been reported from 17 fish species, from the deep ocean (Nicoll 1913, Manter 1934, Dawes 1946, Crowcroft 1947, Gaevskaya and Aleshkina 1995), commercial fisheries (Marques *et al*. 2006a, b) and the intertidal zone (Muñoz and Delorme 2011, Muñoz *et al.* 2013). Although only a few species have been recognised within the genus, they are widely distributed: three species have been found in the North Atlantic Ocean: *H. ovocaudata* from *Lepadogaster lepadogaster* (Bonnaterre) (Gobiesocidae), *Gaidropsarus vulgaris* (Cloquet) (Lotidae), *Ciliata mustela* (Linnaeus) (Lotidae), *Molva molva* (Linnaeus) (Lotidae), *Hippocampus hippocampus* (Linnaeus) (Syngnathidae); *H. nicolli* from *Chaunax nuttingi* German (Chaunacidae), *Diplacanthopoma brachysoma* Günther (Bythitidae) and *Dibranchus atlanticus* Peters (Ogcocephalidae); *H. sharpei* from *Cepola macrophthalma* (Linnaeus) (Cepolidae), *Halobatrachus didactylus* (Bloch and Schneider) (Batrachoididae); one species from the Central-East Atlantic: *H. magnaprostatica* from *Merluccius senegalensis* Cadenat (Merlucciidae) and one species from the South Pacific Ocean: *H. manteri* from *Latridopsis forsteri* (Castelnau) (Latridae) and *Cheilodactylus spectabilis* Hutton (Cheilodactylidae) (Nicoll 1913, Jones 1933, Baylis and Jones 1933, Manter 1934, Sproston 1939, Dawes 1946, Crowcroft 1947, Williams 1960, Rodrigues *et al.* 1975, Gaevskaya and Aleshkina 1995, Olson *et al.* 2003, Pankov *et al.* 2006).

The taxonomic status of species of *Hemipera* has been evolving with the time. The genus *Hemipera* Nicoll, 1913 comprised two species until 1958: *Hemipera ovocaudata* Nicoll, 1913 and *H. sharpei* Jones, 1933 and the genus was part of Hemiuridae. *Hemipera nicolli* (Manter, 1934) and *H. manteri* (Crowcroft, 1947) were originally described in the genus *Hemiperina* as part of the family Hemiuridae, however, Yamaguti in 1958 relocated these two species under *Hemipera* as synonyms of *Hemiperina*, arguing that Manter (1934) was misled by Nicoll's description and figure, misinterpreting the

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male terminalia. The final reorganization of these species occurred in 1979 by Gibson and Bray (1979) in which these species are reorganized in a new family, Derogenidae and in a new subfamily, Gonocercinae, highlighting the position of the testis posteriorly to the ovary and vitelline.

Derogenidae Nicoll, 1910 is characterised by an elongated body, an unarmed tegument, well developed oral and ventral suckers, a short oesophagus, two symmetrical or tandem testes, an oval ovary, numerous eggs with or without filaments, and one or two vitelline masses. Members of the family are parasitic in the gut of freshwater and marine teleosts, but are occasionally recorded from reptiles and fresh water shrimps (Gibson 1996, 2002).

Specimens of *Hemipera* can be recognized for the presence of the ventral sucker in a posterior half of the body, testes symmetrical to oblique at posterior extremity posterior to ovary, seminal vesicle oval to tubular, pars prostatica short which may apparently be enclosed by parenchymatous capsule. Sinus-sac weakly developed. Male and female ducts may open separately on sinus organ or form short hermaphroditic duct. Ovary median, anterior to testis, uterus pre-ovarian. Eggs filamented.

Hemipera have been recorded from several intertidal fish of Chile, since 2002 (Muñoz *et al*. 2002, Muñoz-Muga and Muñoz 2010, Muñoz and Delorme 2011, Muñoz and Castro 2012). However, this material has not been identified to species level. Here we describe a new species, *Hemipera cribbi* sp. nov., from intertidal fishes off the coast of Chile and report the ITS2 rDNA sequence data for this species.

Materials and Methods

Sampling

Specimens of intertidal fishes, *Scartichthys viridis*, *Myxodes viridis* and *Gobiesox marmoratus*, were collected in 2014 from three localities: Las Cruces in Central Chile (33°30´S, 71°38´W); and two localities in the Southern Chile close to Dichato: Burca (36°29´S, 72°55´W) and Merquiche (36°29´S, 72°54´W). Fish were dissected to obtain the trematodes. Parasites were fixed by pipetting them into nearly boiling saline, followed by immediate preservation in 5% formalin for morphological study, and 80% ethanol for molecular analysis (Cribb and Bray 2010).

Morphological analysis

The worms in formalin were stained with Mayer's haematoxylin, destained with 1% HCl and neutralized with NH_3, dehydrated in a graded series of ethanol (50%, 75%, 90%, 95% and 100%), and cleared using methyl salicylate. Specimens were then mounted on slides with Canada balsam. Measurements were made with an Olympus BH-2 microscope and a Spot Insight™ digital camera (Diagnostic Instruments, Inc.) using SPOT™ imaging software. Worms were drawn using an Olympus drawing attachment in an optical microscope, Intuos 3 9 × 12 graphics tablet and Adobe Illustrator and Photoshop CS6 software to digitalise the final drawing.

Specimens were deposited in the Queensland Museum, Australia (QM), and in the Museo de Zoología de Concepción, Chile (MZUC).

Molecular analysis

Total genomic DNA from specimens of *Hemipera* was extracted using universal and rapid salt-extraction procedures (Aljanabi and Martinez 1997). Amplification of the ITS2 nuclear ribosomal DNA region was performed with the forward primers 3S (5'-GGT ACC GGT GGA TCA CGT GGC TAG TG-3' (Bowles *et al*. 1993)), and the reverse primer ITS2.2 (5'CCT GGT TAG TTT CTT TTC CTCCG C-3' (Anderson and Barker 1993)). The ITS2 region location in the sequence was determined using the website Internal Transcribed Spacer 2 Ribosomal RNA Data Base, which gave the exact start and end of the ITS2 region.

PCR amplification of ITS2 rDNA data was developed using a Touchdown PCR (Don *et al*. 1991) with the next protocol: initial denaturing at 95°C for 10 min, followed by touchdown of 10 cycles of 95°C for 15s, 60–50°C for 30s and 72°C for 45s, a second stage of 35 cycles of 95°C for 15s, 50°C for 30s and 72°C for 45s and a final extension phase of 72°C for 30 min (Peña *et al*. 2014). PCR products were visualized in 0.8% agarose gels and the final PCR products were purified and sequenced using the service of Macrogen, South Korea. Sequencher™ version 4.5 (GeneCodes Corp.) was used to assemble and edit contiguous sequences.

Results

A total of 263 intertidal fishes were examined from three localities: 233 specimens of *S. viridis* in Las Cruces (19 infected, 40 parasite specimens collected), 16 specimens of *M. viridis* in Burca (one infected, two parasites collected), 5 specimens of *G. marmoratus* in Merquiche (four infected, 23 parasites collected) and 9 specimens of *S. viridis* in Merquiche (one infection, two parasites collected).

Family Derogenidae Nicoll, 1910
Hemipera Nicoll, 1913
Hemipera cribbi sp. nov. (Fig. 1A-E)

Description. (Measurements based on 12 gravid specimens). Body elongated, with nearly parallel sides 1,056–1,400 (1,211) µm long (Fig. 1A). Body width 214–260 (238) µm. Oral sucker subterminal, more circular than oval, 80–138 (112) µm × 80–133 (110) µm. Prepharynx absent, pharynx small, rounded 39–60 (49) µm long × 45–53 (49) µm wide. Oesophagus short. Intestinal bifurcation in anterior to seminal vesicle. Caeca extend to posterior section of body anterior

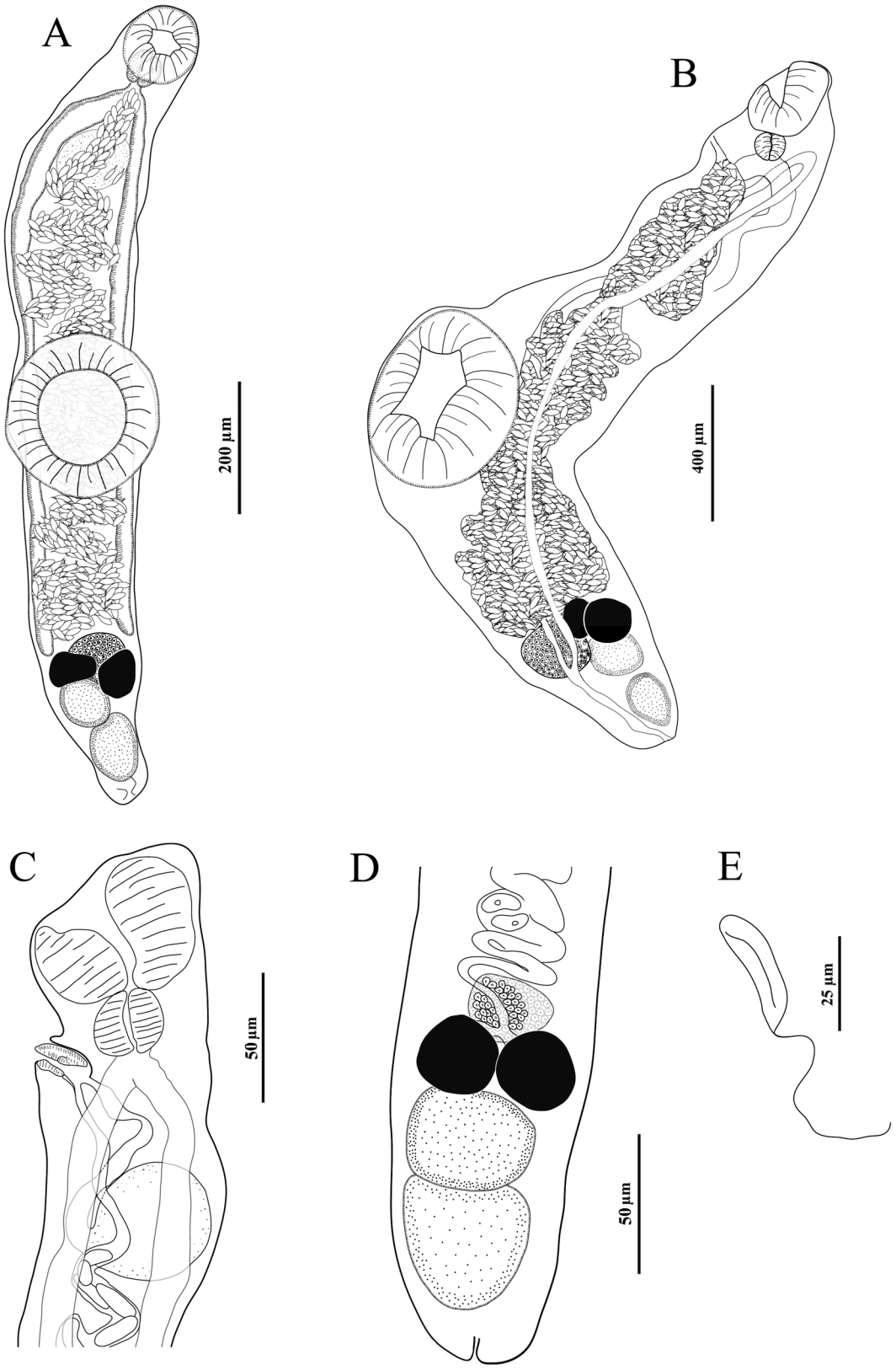

Fig. 1. *Hemipera cribbi* sp. nov. from *Scartichthys viridis*. **A.** Ventral view and **B.** lateral view of an adult specimen. **C.** Dorsal view of the anterior part of the body, with details of oral sucker, pharynx, genital cone and seminal vesicle. **D.** Dorsal view detailing the Mehlis' cells positioned behind the ovary. **E.** Detail of an egg

Table I. Comparisons of morphometric features of *Hemipera cribbi* sp. nov. and five species of the genus. Abbreviations: BL: body length; BW: body width; OS: oral sucker; VS: ventral sucker; PH: pharynx; TP: posterior test; TA: anterior test; OV: ovary; VM: vitelline masses. Measurements of the other species were obtained from the original descriptions

	Hemipera cribbi sp. nov.	***H. ovocaudata***	***H. sharpei***	***H. nicolli***	***H. manteri***	***H. magnaprostatica***
Source	Present study	Nicoll, 1913	Jones, 1933	Manter, 1934	Crowcroft, 1947	Gaevskaya and Aleshkina, 1995
Host	*Scartichthys viridis*	*Lepadogaster lepadogaster*	*Cepola macrophthalma*	*Chaunax nuttingi*	*Latridopsis forsteri*	*Merluccius senegalensis*
				Diplacanthopoma brachysoma	*Cheilodactylus spectabilis*	
				Dibranchus atlanticus		
BL	1,056–1,400 (1,211)	1,540	4,770	2,070–3,130	2,070–3,010	2,830–4,140 (3,630)
BW	214–260 (238)	560	850	720–870	680–830	630–1,270 (1,100)
OS	80–138 (112) × 80–133 (110)	220	370		240–310	220–350 × 260–410
VS	223–284 (248) × 214–241 (231)	400	740		460–620	460–670 × 440–690
PH	39–60 (49) × 45–53 (49)					120–130 × 110–170
TP	68–102 (87) × 64–89 (78)					160–460 × 180–390
TA	55–90 (78) × 71–103 (90)					140–350 × 210–410
OV	62–89 (78) × 66–101 (79)					110–240 × 140–300
VM	60–90 (74) × 55–83 (64)					
EGGS	23–29 (26) × 11–15 (13)	100 × 27	100 × 38	44–52 × 16–20	32–40 × 8–12	122 × 35
BL/BW	4.93–5.38 (5.09)	2.75	5.61	2.80–3.59	3.0–3.6	3.25–4.49
Filaments	Twice eggs length	Short	11 times egg length	20 times egg length	20 times egg length	5 times egg length
VS position	Slightly post equatorial	Centre of body	Posteriorly	Centre of body, slightly posterior	Centre of body, slightly posterior	Posteriorly

to ovary. Ventral sucker rounded 223–284 (248) µm long × 214–241 (231) µm wide. Ventral sucker positioned slightly post-equatorial, protrudes laterally in lateral view. Body bent dorsally at level of ventral sucker, boomerang-shaped in lateral view (Fig. 1B). Excretory pore terminal. Excretory vesicle long, bifurcated at level of ovary, with arms united in forebody, dorsal to pharynx (Fig. 1B).

Testes oval to irregular, tandem reaching close to posterior extremity of body, anterior testis, 64–89 (78) µm long × 68–102 (87) µm wide, posterior testis 71–103 (90) µm long × 55–90 (78) µm wide (Fig. 1A-B). Seminal vesicle oval, large 70–125 (95) µm long × 72–127 (97) µm width, situated posterior to intestinal bifurcation (Fig. 1C). Ejaculatory duct connects in small weakly-developed sinus-sac. Genital pore in anterior part of body, immediately posterior to pharynx. Prostatic gland diffuse, positioned dorsal to genital cone.

Ovary oval 62–89 (78) µm long × 66–101 (79) µm wide, anterior to testes and posterior to vitelline masses. Laurer's canal not observed. Vitellarium composed of two irregular, dense structures anterior to testes and ventral to ovary 60–90 (74) µm long × 55–83 (64) µm wide. Mehlis' cells dorsal to ovary (Fig. 1D). Uterus mostly intercaecal, filling area from just posterior to intestinal bifurcation to anterior edge of ovary. Metraterm simple, opens to hermaphroditic duct. Eggs oval 23–29 (26) µm width × 11–15 (13) µm width, with filaments, slightly narrowing towards filament. Filaments up to twice egg length (Fig. 1E). Egg-mass forming spiral.

Type-host: *Scartichthys viridis* (Valenciennes) (Blenniidae).

Other Hosts: *Myxodes viridis* Valenciennes (Clinidae) and *Gobiesox marmoratus* Jenyns (Gobiesocidae).

Type-locality: Rocky shore of Las Cruces (33°30´S, 71°38´W), Central Chile.

Other locality: Rocky shore of Burca (36°29´S, 72°55´W) and Merquiche (36°29´S, 72°54´W), Southern Chile.

Site in host: intestine.

Specimens deposited: Holotype: QM G235022, Paratypes: QM G235023-G235034; MZUC-UCCC Nº 41746-41747.

Molecular data: Representative sequences: GenBank numbers KU745181, KU745182, KU745183, KU745184. ITS2 rDNA sequences were generated for *H. cribbi* sp. nov. for each host. The ITS2 rDNA fragment was 100% identical for each host/locality combination with a length of 302 bp.

Etymology: The species is named in honour of the first author´s Master and PhD thesis mentor, Dr Thomas H. Cribb from the University of Queensland, and also for his great contribution to the understanding of trematodes for marine fishes.

Remarks

The morphology of *Hemipera cribbi* sp. nov. was compared with the five species described in the genus. Data for the other species were obtained from original descriptions (Manter 1934, Nicoll 1913, Jones 1933, Crowcroft 1947, Gaevskaya and Aleshkina 1995). The new species is the smallest in the genus (Table I), it has the smallest eggs, and the ventral sucker occupies the widest proportion of body width (Fig. 1), this parameter is not reported for the other species. In addition, the body length /body width ratio is greater than for four of the other species (Table 1), meaning that *H. cribbi* sp. nov. is five times longer than wide, similar to *H. sharpei*. However, the latter species is almost three times larger than *H. cribbi* sp. nov., and it has larger eggs with one long filament on the eggs. *Hemipera cribbi* sp. nov. has a prominent seminal vesicle, a feature not observed in the other species.

Discussion

Hemipera cribbi sp. nov. is proposed as a new species on the base of its morphometric dimensions which are distinctive in comparison with the other four species described in the genus; the new species is smaller than all of the other species in the genus. Moreover, the body is very narrow in relation to the body length.

Hemipera cribbi sp. nov. is the first species of *Hemipera* described from the Pacific Ocean off South America. Previous studies have only reported undescribed specimens of *Hemipera* sp. from several fish species, including the type-host of the new taxon described above (*Scartichthys viridis*, *Bovichthys chilensis*, *Helcogrammoides chilensis*, *Auchenionchus microcirrhis* and *Syciases sanguineus*), which share the same habitat in the intertidal rocky zone of the coast of central-south Chile (Muñoz *et al*. 200; Muñoz-Muga and Muñoz 2010, Muñoz and Delorme 2011, Muñoz and Castro 2012). However, *H. cribbi* sp. nov. is not a common species, its prevalence varying between 0.1 and 1.4% and the intensity between 1 and 3 (Muñoz *et al*. 200, Muñoz-Muga and Muñoz 2010, Muñoz and Delorme 2011, Muñoz and Castro 2012). The intensity of this trematode was exceptionally high in two of the host specimens considered in this study; up to 42 trematodes in *S. viridis* and up to 23 in *G. marmoratus*.

Scartichthys viridis (Blenniidae), *Gobiesox marmoratus* (Gobiesocidae) and *Myxodes viridis* (Clinidae) are intertidal fishes distributed in the Southeast Pacific from Peru to central-south of Chile (*S. viridis* and *M. viridis*) (Williams 1990, Stepien 1992) and in the South Atlantic from Uruguay to Argentina (*G. marmoratus*) (Menni *et al*. 1984). Parasites from these fishes have been well studied and most of the trematodes present in these hosts are endemic, and highly host-specific (Díaz and George-Nascimento 2002, Díaz and Muñoz 2010, Muñoz and Randhawa 2011, Muñoz-Muga and Muñoz 2010).

These fishes are ecologically different in terms of their diets (Muñoz and Ojeda 1997, Boyle and Horn 2006). *Scartichthys viridis* is a herbivorous fish, with 92.8% of its diet comprising macroalgae and the other percentage composed of small invertebrate species, molluscs, copepods, crustacean larvae and insect larvae (Muñoz and Ojeda 1997). In contrast, *G. marmoratus* and *M. viridis* consume large numbers of crustaceans (83.9% in *G. marmoratus* and 91.4% in *M. viridis*)

and there is no presence of macroalgae in their diets (Muñoz and Ojeda 1997). Judging from the study by Muñoz and Ojeda (1997), the items common to the diet of all these fish species are amphipods, gastropods and polychaetes and according to Busch *et al*. (2012) amphipods are the most likely intermediate hosts of digeneans. The life-cycle of *Hemipera* is not known, but Køie (1979) summarised the life-cycle of the related species *Derogenes varicus* (Müller, 1784) and found a wide variety of crustaceans served as intermediate host. It seems possible, therefore, that amphipods may be an intermediate host of *H. cribbi* sp. nov.

Finally, in this study we report with the first ITS2 rDNA sequence data (GenBank Numbers to be included) for *Hemipera* for the purposes of future taxonomic and life cycle studies of the species in the genus.

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