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Two new species of digeneans (Lecithasteridae and Haploporidae) of the intertidal blenny *Scartichthys viridis* (Valenciennes) from the central coast of Chile

Gabriela Muñoz[1]*, Mario George-Nascimento[2] and Rodney A. Bray[3]

[1]Laboratorio de Parasitología Marina, Facultad de Ciencias del Mar y de Recursos Naturales, Universidad de Valparaíso, P.O. 5080, Viña del Mar, Chile; [2]Centro de Investigación en Biodiversidad y Ambientes sustentables (CIBAS), Facultad de Ciencias, Universidad Católica de la Santísima Concepción, Alonso de Rivera 2850, Concepción, Chile; [3]Department of Life Sciences, Natural History Museum, London SW7 5BD, United Kingdom

Abstract

Two new digenean species are described from the intertidal blenny *Scartichthys viridis* (Valenciennes) (Blenniidae) collected off the coasts of Chile. The digenean *Monorchimacradena viridis* n. sp. (Lecithasteridae: Macradenininae) differs from the only known species described in the genus, *M. acanthuri* Nahhas and Cable 1964, in the presence of Drüsenmagen in the caeca, the location of the seminal vesicle between the testis and ovary (anterior to the testis in *M. viridis* n. sp.), and the pre-ovarian vitellarium. *Megasolena littoralis* n. sp. (Haploporidae), which is also reported from *Scartichthys gigas* (Steindachner), differs from the five valid species of *Megasolena* in that the post-caecal region (from the posterior edge of the caeca to the end of the body) is larger in *M. viridis* n. sp., meaning that the caeca are shortest in this species. Also, *M. littoralis* n. sp. is distinguished, from the other congeneric species, in a combination of characteristics, e.g., body length, suckers, pharynx, testes, hermaphroditic sac and sucker-length ratio. Sequences of ITS2 rDNA were generated for specimens of both species from various localities, indicating that it was invariant within the species. This study describes species of *Monorchimacradena* and *Megasolena* in Chilean waters, here reported for the first time off the Pacific Coast of South America.

Keywords

Lecithasteridae, Haploporidae, taxonomy, *Scartichthys*, intertidal rocky shore

Introduction

The blenny *Scartichthys viridis* (Valenciennes) is an endemic fish of the South American Pacific coasts, which lives between the intertidal and subtidal zones (Williams, 1990). This fish harbors at least 23 parasite species; 14 of them have been identified to generic level and nine to specific level (Muñoz and Delorme 2011). Of these, four have recently been identified as new species, comprising a copepod, a nematode, a monogenean and a digenean (Muñoz 2010; Castro and Muñoz 2011; Zietara *et al*. 2012; Díaz *et al*. 2016).

Six digenean taxa have been recorded in *S. viridis: Hemipera cribbi* Díaz, Muñoz and George-Nascimento 2016, *Helicometrina* cf. *nimia*, *Monascus filiformis* (Rudolphi, 1819), sp., *Megasolena* sp. Hemiuridae gen. sp. and Lecithasteridae gen. sp. (Díaz and George-Nascimento 2002; Flores and George-Nascimento 2009; Díaz and Muñoz 2010; Muñoz-Muga and Muñoz 2010; Muñoz and Delorme 2011; Muñoz and Randhawa 2011). Two of these, Lecithasteridae gen. sp. and *Megasolena* sp., are the most prevalent and abundant digeneans in *S. viridis* (e.g., Flores and George-Nascimento 2009; Muñoz-Muga and Muñoz 2010), but they still do not have appropriate morphological descriptions. According to its morphological features, the "Lecithasteridae gen. sp." belongs to the subfamily Macradenininae, specifically to the genus *Monorchimacradena*. The other digenean species was initially identified as *Lepidauchen* sp. by Díaz and George-Nascimento (2002) and Flores and George-Nascimento (2009), but subsequently it has been recorded as *Megasolena* sp., belonging to the family Haploporidae (Muñoz and Delorme 2011; Muñoz and Randhawa 2011). Therefore, we aim to describe two species of digeneans (a lecithasterid and a hap-

*Corresponding author: gabriela.munoz@cienciasdelmar.cl

loporid) from the fish *S. viridis* collected from the intertidal rocky zone of central Chile.

Materials and Methods

A sample set of 1,613 blenny *Scartichthys viridis* was collected between 2014 and 2015 from the intertidal rocky zone of central (33°S) and central-south (36°S) coasts of Chile. Some fixed and stained digeneans from past sampling (2009-2011) were also used. The fishes were caught from rocky pools formed in the intertidal zone during low tide, by using hand nets and an anaesthetic solution (0.1%, AQUI-S®, Bayer S.A., Chile). The fishes were dissected and the gut was removed and examined under a Leica M80 stereo microscope (Leica Microsystems, Germany). Digeneans were washed in saline and killed by pipetting them into nearly boiling saline. Some specimens of digeneans were fixed in 5% formalin prepared in a physiological solution for staining procedures, and other specimens were fixed in 100% ethanol for molecular analysis.

The digeneans were stained with haematoxylin, dehydrated in alcohol series from 70% to 100%, cleared in methyl salicylate and mounted in Canada balsam. Digeneans were not fixed under pressure and were just softly pressured when mounting. Measurements and drawings were made with a "camera lucida", attached to an Olympus BH-2 light microscope (Olympus Corporation, USA), using a Digicad Plus digitising tablet and Carl Zeiss KS100 v.3 software (Zeiss Germany), adapted by Imaging Associates. The prevalence and mean intensity of digeneans was calculated according to the method of Bush *et al*. (1997). For the descriptions, all ranges of measurements, followed by the mean in parentheses, are expressed in micrometres (µm).

Two specimens of *Megasolena* sp. were used for scanning electron microscopy (SEM), specifically to observe the tegumental spines. Specimens, initially fixed in 5% formalin, were dehydrated through an ethanol series (70–100%), followed by critical point drying in CO_2 using a Samdri-780A machine (Tousimis Research Corporation, Rockville, MD, USA), sputter-coated with gold using an Ion JFC-1100 Sputter machine and examined using a JEOL T-300 SEM (JEOL, Tokyo, Japan).

Molecular tools were applied to explore if specimens (four of each species) collected from different localities and hosts belong to the same species. Total genomic DNA from specimens of both digenean species 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. The start and end of the ITS2 rDNA region was determined by reference to the ITS2 database (Koetschan *et al*. 2012). The sequences were aligned using CLUSTAL implemented within MEGA v. 5 (Tamura *et al.* 2011).

The sequences of the trematode species of this study were compared with other members of the families, obtained from the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/), such as *Quadrifoliovarium pritchardae* Yamaguti, 1965 and *Quadrifoliovarium maceria* Chambers and Cribb, 2006 (Lecithasteridae) (Chambers and Cribb 2006) and *Saccocoelium tensum* Looss, 1902 and *Haploporus benedenii* (Stossich, 1887) (Haploporidae) (Blasco-Costa *et al.* 2009). The genetic distance matrices were computed with the total number of mutations, and the divergences were calculated among individuals by applying the ML model (Maximum composite likelihood model) on MEGA V5.

Description

Family Lecithasteridae Odhner, 1905
Subfamily Macradenininae Skrjabin and Guschanskaya, 1954
Genus *Monorchimacradena* Nahhas and Cable, 1964
***Monorchimacradena viridis* n. sp.** (Figs 1–6, Table I)

Based on 25 mounted mature specimens: Body elongated, narrower in anterior and posterior regions, 1,115–2,372 (1,846) long and 280–573 (372) wide. Tegument thin and unarmed. Oral sucker almost rounded 85–155 (123) long, 90–158 (129) wide, subterminal at 23–65 (40) from anterior extremity. Prepharynx absent. Pharynx rounded to oval, 84–144 (110) long and 78–130 (104) wide. Oesophagus short, 37–75 (55) long. Intestinal bifurcation with wide lateral chambers ["Drüsenmagen" (Gibson and Bray 1979)] that pass postero-laterally at level of ventral sucker. Drüsenmagen globular, 75–198 (92) in diameter (right side) and 83–103 (93) in diameter (left side). Caeca long, end blindly, with large epithelial cells; longer caecum terminates 114–329 (204) from posterior extremity, shorter caecum terminates 171–527 (279) from posterior extremity. Forebody 297–485 (409) long, representing 25–37% of body length. Ventral sucker oval, 105–183 long and 138–211 wide. Testis single, rounded to oval, in middle of hindbody, 132–303 (204) long, 94–251 (192) wide. Seminal vesicle oval, 84–289 (175) long, 50–179 (90) wide, immediately anterior to testis, may reach 14–162 (69) into hindbody or slightly overlaps ventral sucker when the sinus-organ is everted. Pars prostatica 150–275 (182) long ensheathed by large nucleate prostatic cells. Ejaculatory duct simple 107–230 (174) long, when not contracted. Ovary smooth, rounded to oval, 76–190 (127) long, 94–215 (147) wide, post-testicular. Laurer's canal and seminal receptacle not observed. Vitellarium ventral to ovary, with seven long branched lobes, united centrally. Vitelline field 226–446 (325) long, 214–347 (269) wide. Mehlis' gland distant from ovary and vitellarium. Uterus extending from posterior edge of ventral sucker almost to posterior extremity when full of eggs, 130–1,741 (1,013) in longitudinal extension. Metraterm simple. Eggs oval, 24–37 (30) long, 12–19 (16) wide. Metraterm and ejaculatory duct join within sinus-sac. Sinus-sac elliptical to oval, 83–115 (102) long, 53–82 (65) wide. Genital pore median, between pharynx and ventral sucker, 225–355 (288) from anterior extremity of body. Excretory pore terminal. Excretory vesicle long (observed in three specimens), visible as far as posterior edge of ventral sucker, with three portions, a short simple tube leading from excretory pore, 179 long (measured in one specimen), short glandular portion, 150 long, 16–49 (29) wide; and another long part which is obscured by eggs at level of vitellarium. No bifurcation was observed.

Molecular analysis

ITS2 rDNA sequences of 296 base pairs from *Monorchimacradena* specimens were analyzed. Four digenean specimens (two from the north and two from central and central-south of Chile) were successfully sequenced. Specimens showed 0% of genetic divergence (Table II A), providing convincing evidence that they belong to the same species (*M. viridis* n. sp.).

Taxonomy summary

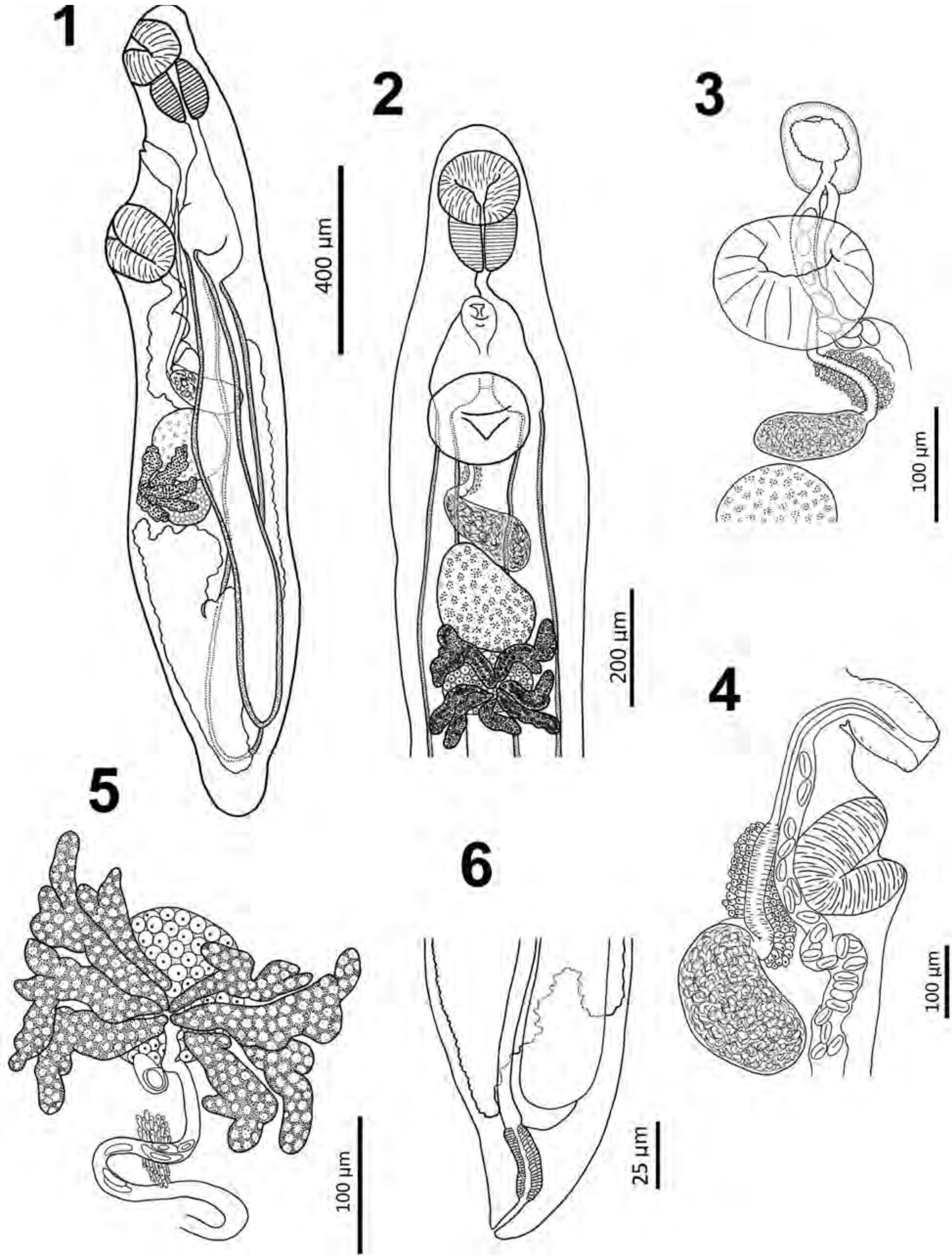
Syns*:* Digenea sp. 1 of Díaz and George-Nascimento (2002); Lecithasteridae gen. sp. of Flores and George-Nascimento (2009), Díaz and Muñoz (2010), Muñoz-Muga and Muñoz (2010), Muñoz and Delorme (2011) and Muñoz and Randhawa (2011).
Type host: *Scartichthys viridis* (Valenciennes) (Blenniidae).
Site of infection: Intestine.
Type locality: Las Cruces, central Chile (33°29´43″S, 71°38´18″W).
Other localities: In central Chile: Isla Negra (33°26´32″S, 71°41´15″W), El Tabo (33°26´43″S, 71°40´57″W), Maitencillo (32°38´41″S; 71°26´14″W), Tunquén (33°16´50″S, 71°39´46″W) and Quintero (33°44´29″S, 71°29´56″W). In central-south of Chile: Burca (36°27´56.22″S, 72°54´24.9″W), Cantera (36°41´09″S, 73°08´17″W) and Merquiche (36°28´59″S, 72°54´35″W).
***Prevalence, abundance (± standard deviation) and intensity range*:** 8.4%, 0.21 ± 1.13 and 1–19, respectively, for *S. viridis* (n = 1,471) from central Chile; 31.7%, 3.2 ± 8.35 and 1–58, respectively, for *S. viridis* (n=142) from the central-south of Chile.
Type-material: Museo Nacional de Historia Natural, Santiago, Chile, MNHNCL PLAT 15001 (Holotype) and MNHNCL PLAT 15003 (Paratype); Natural History Museum, London, England, BMNH 2016.4.14.1–11 (Paratypes).
***GenBank accession numbers*:** KX035011 and KX035012 (specimens from Merquiche), KX035013 and KX035014 (specimens from Las Cruces).
Etymology: The specific name of the host, "*viridis*", was used to name the new trematode species.

Taxonomy remarks

Only one species has been described in the genus *Monorchimacradena* Nahhas and Cable 1964, *M. acanthuri* Nahhas and Cable 1964, found in the Palette Surgeonfish *Paracanthurus* (as *Acanthurus*) *hepatus* (Linnaeus) (Acanthuridae) from Curaçao and Jamaica (Nahhas and Cable 1994) and the Ocean Surgeonfish *Acanthurus bahianus* Castelnau from the Cayman Islands (Nahhas 1993). Some measurements and morphological characteristics of *M. viridis* n. sp. and *M. acanthuri* are similar, such as body length and sucker sizes (Table I). However, there are morphometric features which only slightly overlap; for example, the body width, testis and eggs are larger in *Monorchimacradena viridis* n. sp. than in *M. acanthuri*, while the ventral sucker is wider in *M. acanthuri* (Table I). Three characteristics differ distinctly; the presence of Drüsenmagen in the caeca in *M. viridis* n. sp. but not in *M. acanthuri*; the seminal vesicle is anterior to the testis in *M. viridis* n. sp., while this organ is positioned dorsally, between the testis and ovary in *M. acanthuri*; and the position of the vitellarium is ventral and anterior to the ovary in *M. viridis* n. sp. and is posterior to the ovary in *M. acanthuri*, consequently the position of the posterior vitelline lobes is more anterior in *M. viridis* n. sp. than in *M. acanthuri* (Table I).

Family Haploporidae Nicoll, 1914
Subfamily Megasoleninae Manter, 1935
Genus *Megasolena* Linton, 1910
***Megasolena littoralis* n. sp**. (Figs 7–12, Tables II–IV)

Based on 20 mounted mature specimens: Body elongated, narrower in anterior region, 1,406–3,282 (1,964) long and 394–744 (552) wide. Body width represents 21–40% of body length. Tegument with small spines from anterior extremity up to ventral sucker; spines 3–11 long (Figs. 10–11). Oral sucker almost rounded 184–342 (237) long, 196–344 (258) wide. Muscular ring immediately posterior to oral sucker. Prepharynx 83–272 (151) long. Eye-spot pigments around posterior edge to oral sucker and prepharynx area.



Figures 1–6. *Monorchimacradena viridis* n. sp. **1 –** whole body in lateral view, **2 –** anterior half body in frontal view, **3 –** terminal genitalia showing the junction of the ejaculatory duct and metraterm in the sinus-sac. **4 –** sinus-organ everted and ejaculatory duct everted, **5 –** Vitellarium and first portion of the uterus showing the position of Mehlis' gland, **6 –** posterior part of the excretory vesicle

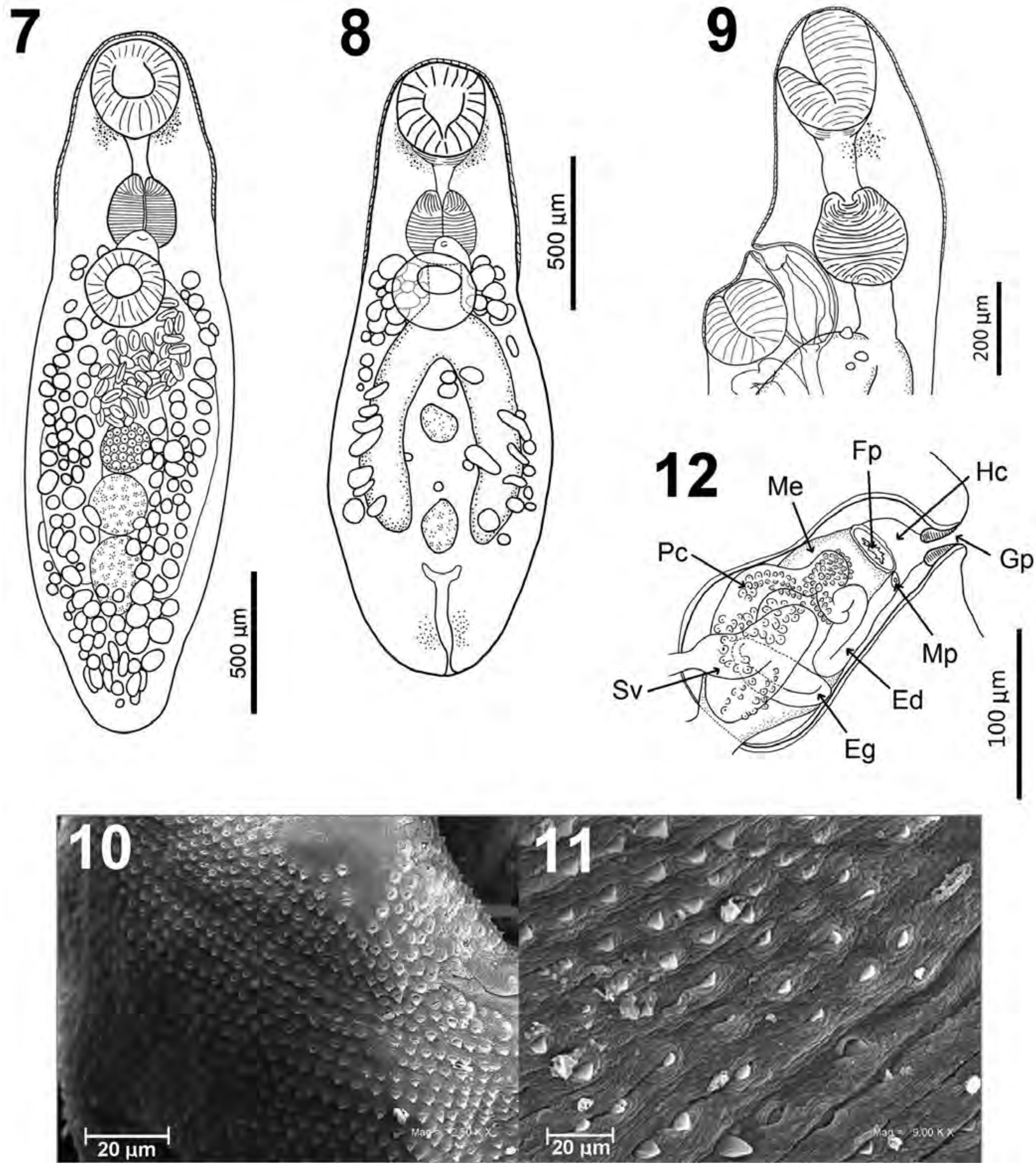
Table I. Comparison between morphometric (μm) and morphological characteristics between *Monorchimacradea viridis* n. sp. and *M. acanthuri.* Abbreviations; BW: body width; BL: body length; FB: forebody; OSL: Oral sucker length; VSL: Ventral sucker length; PVR: distance from the posterior lobule of the vitellarium to the end of the body. *Measurement taken from drawings of the original description

	***M. viridis* n. sp.**	***M. acanthuri***
Hosts	*Scartichthys viridis*	*Acanthurus hepatus*
Locality	Central and central-south of Chile	Curaçao and Jamaica (Caribbean Sea)
Source	This study	Nahhas and Cable (1964)
Body length	1,115–2,373	1,100–2,570
Body width	280–573	240–374
Oral sucker length	85–155	82–145
Oral sucker width	90–159	105–180
Oesophagus	37–85	60–97
Ventral sucker long	105–183	150–266
Ventral sucker width	138–211	250*
Pharynx length	85–145	60–97
Pharynx width	79–130	75*
Testis length	133–304	105–200
Testis width	94–251	68–130
Ovary length	76–190	45–160
Ovary width	94–215	–
Genital pore to anterior end	225–450	450*
Acetabulum to testis distance	133–274	475*
Egg length	24–37	20–28
Egg width	12–19	9–15
BW/BL	0.16–030	0.12*
FB/BL	0.17–0.28	0.21*
OSL/VSL	0.59–0.96	0.68*
PVR/BL	0.31– 0.41	0.24*
Drüsenmagen	Present	Absent
Vitellarium position	Ventral to the ovary	Posterior to the ovary
Seminal vesicle	Anterior to testis	Dorsal (between testis and ovary)

Pharynx robust, 159–289 (206) long, 179–277 (218) wide. Oesophagus 55–318 (133) long. Intestinal bifurcation at level of ventral sucker. Caeca long, end blindly, 175–227 (209) maximum width, with large epithelial cells; longer caecum terminates 244–446 (357) from posterior extremity, shorter caecum terminates 284–522 (429) from posterior extremity. Forebody 468–951 (616) long, representing 25–37% of the body length. Ventral sucker oval, 161–293 (212) long and 188–286 (235) wide. Testes rounded, intercaecal, tandem. Anterior testis 143–376 (229) long, 106–281 (187) wide. Posterior testis 153–388 (236) long, 95–309 (190) wide. Normally no space between testes, except when the testes are small. Sinus sac oval, 158–356 (224) long, 74–149 (112) wide, dorsal to ventral sucker. Genital pore ventral at pharynx level and immediately anterior to ventral sucker, almost in middle line, at 450–850 (568) from anterior extremity. Seminal vesicle oval inside hermaphroditic sac, no part of seminal vesicle was observed outside of hermaphroditic sac because of dense field of vitelline follicles. Prostatic duct convoluted and surrounded by prostatic cells. Metraterm and ejaculatory duct unite to form small hermaphroditic chamber. Ovary rounded, 98–265 (152) long, 90–266 (155) wide, anterior to and contiguous with anterior testis. Mehlis' glands, Laurer's canal and seminal receptacle not observed. Uterus in midbody between ovary and ventral sucker. Eggs oval, 70–106 (87) long, 31–54 (42) wide. Vitelline follicles distributed between ventral sucker and posterior extremity. Excretory pore posteriorly terminal. Excretory vesicle Y-shaped, 225–325 (266) long up to bifurcation, almost reaching posterior margin of posterior testis (observed in one specimen).

Molecular analysis

ITS2 rDNA sequences of 289 base pairs from *Megasolena* specimens were analyzed. Four digenean specimens (one from the north and three from central Chile) were successfully sequenced. They showed 0% of genetic divergence (Table II B), demonstrating that all specimens belong to the same species (*M. littoralis* n. sp.).



Figures 7–12. *Megasolena littoralis* n. sp. **7 –** whole body of a mature and **8 –** an immature specimen in ventral views, **9 –** anterior half body in lateral view, **10–11 –** spines on the tegument, lateral to ventral sucker level, **12 –** hermaphroditic sac. Abbreviations, Fp: female genital pore, Mp: male genital pore, Hc: hermaphroditic chamber, Gp: genital common pore, Me: Metraterm, Pc: prostatic cells, SV: seminal vesicle, Eg: egg, Ed: ejaculatory duct

Table II. Pairwise sequence divergences for the ITS2 region of the rRNA gene among species of the (A) Lecithasteridae and (B) Haploporidae. The divergence distance was calculated using the maximum composite likelihood model, and it is shown as a percentage (below the diagonal). The number of base pairs different between samples is also shown in each clade (above the diagonal); Abbreviations: PSA = Pacific of South-America, IP: Indo-Pacific, MS = Mediterranean Sea

(A) Lecithasteridae clade	**Host**	**Area**	**Locality**	**1**	**2**	**3**	**4**	**5**	**6**
1. *Monorchimacradena viridis* n. sp.	*S. viridis*	PSA	Valparaiso (central Chile)	–	0	0	0	53	52
2. *Monorchimacradena viridis* n. sp.	*S. viridis*	PSA	Valparaiso (central Chile)	0	–	0	0	53	52
3. *Monorchimacradena viridis* n. sp.	*S. viridis*	PSA	Biobio (central-south Chile)	0	0	–	0	53	52
4. *Monorchimacradena viridis* n. sp.	*S. viridis*	PSA	Biobio (central-south Chile)	0	0	0	–	53	52
5. *Quadrifoliovarium pritchardae*	*Naso unicornis*, *N. lituratus*	IP	Geat Barrier Reef and west of Australia, French Polynesia	0.23	0.23	0.23	0.23	–	19
6. *Quadrifoliovarium maceria*	*N. tonganus*, *N. annulatus*, *N. ageniand* *N. revirostris*	IP	Geat Barrier Reef and west of Australia, French Polynesia, and Hawaii	0.22	0.22	0.22	0.22	0.07	–
(B) Haploporidae clade				**1**	**2**	**3**	**4**	**5**	**6**
1. *Megasolena littoralis* n. sp.	*S. gigas*	PSA	Arica (northern Chile)	–	0	0	0	56	62
2. *Megasolena littoralis* n. sp.	*S. viridis*	PSA	Valparaiso (central Chile)	0	–	0	0	56	62
3. *Megasolena littoralis* n. sp.	*S. viridis*	PSA	Valparaiso (central Chile)	0	0	–	0	56	62
4. *Megasolena littoralis* n. sp.	*S. viridis*	PSA	Valparaiso (central Chile)	0	0	0	–	56	62
5. *Saccocoelium tensum*	*Liza aurata*, *L. ramado*	MS	Santa Pola (Spain)	0.23	0.23	0.235	0.23	–	34
6. *Haploporus benedeni*	*L. ramado*	MS	Santa Pola (Spain)	0.26	0.26	0.26	0.26	0.13	–

Table III. Morphometric characteristics (μm) of six species of *Megasolena*. *measurement taken from drawings

	M. littoralis n. sp.	*M. estrix* Linton, 1910		*M. kyphosi* Sogandares-Bernal, 1959	*M. hysterospina* (Manter 1931)				*M. archosargi* Sogandares-Bernal and Hutton (1959)	*M. acanthuri* Machida and Uchida, 1991	*M. dongzhaiensis* Liu, Zhou, Yu and Liu, 2006
Source	**This study**	**Linton (1910)**	**Manter (1935)**	**Sogandares-Bernal (1959)**	**Manter (1931)**	**Overstreet (1969)**	**Amato (1982)**	**Fernandes, Kohn and Pinto (1985)**	**Sogandares-Bernal and Hutton (1959a)**	**Machida and Uchida (1991)**	**Liu, Zhou, Yu and Liu (2006)**
Body length	1,406–3,282	3,000	2,670–3,600	2,790–2,830	2,540	1,900–3,900	1,820–2970	5,830	2,210–2,778*	3,700–5,600	5,270–5,540
Body width	394–744	830	780–980	480–660	870	990–1,560	756–1,080	1,570	990	1,300–2,000	1,620–1,720
Oral sucker length	184–342	380	306–380	320–399	420	330–680	324–540	720	360	320–640	712–720
Oral sucker width	196–344	380	306–380	320–399	420	340–540	324–513	770	330	360–770	640–656
Prepharynx	83–272		45*	57–69	23*	0–130	0–135		49*	60–180	120–184
Pharynx length	159–289	380	300–380	230–275	380	220–460	179–364	500	180	200–260	452–456
Pharynx width	179–277	380	307–380	230–260	350	340	204–432	620	250	280–390	464–544
Oesophagus	55–318		303*	230–275	0*	0*	132*		56*	0–85	80–184
Ventral sucker length	161–293	280	262–280	170–228	240	160–350	148–216	420	170	460–1010	328–352
Ventral sucker width	188–286	280	262–280	170–228	240	200–380	162–351	480	180	600–990	400–416
Ovary length	98–265		121*	130–174	227*	130–210	94–310	330	210	220–360	272–304
Ovary width	90–266			131–152		320–650	297–391	670	260	230–400	512–516
Anterior testis length	143–376		333*	228–320	227*	200–550	337–472	950	210	320–640	520–648
Anterior testis width	106–281			190–220		360–850	243–553	800	260	360–700	608–624
Posterior testis length	153–388		424*	266–322	341*	380–730	432–526	1240	390	400–850	700–836
Posterior testis width	95–309			190–247		370–740	256–499	770	260	380–800	512–568
Intestinal bifurcation to acetabulum	0		0*	0*	0*	51*	59*		139*		455*
Posterior testis region	155–425		924*	684–799	625*	526*	485*		514*	700–1600	1248–1360
Egg length	70–106	60	60–76	60–74	72–75	56–83	54–60		54–60	87–103	62–68
Egg width	31–54	30	30–34	43–47	36–38	35–47	38–46		38–46	58–72	36–39

Table IV. Relative of morphometric characteristics and qualitative feature of six species of *Megasolena*. Abbreviations; BW: body width; BL: body length; FB: forebody; OSL: Oral sucker length; VSL: ventral sucker length; PTR: posterior testis region; PCR: posterior caeca region; PhL: pharynx length; HSL: hermaphroditic sac length. * Measurement taken from drawings

	***M. littoralis* n. sp.**	***M. estrix* Linton, 1910**		***M. kyphosi* Sogandares-Bernal, 1959**	***M. hysterospina* (Manter 1931)**				***M. archosargi* Sogandares-Bernal and Hutton (1959)**	***M. acanthuri* Machida and Uchida, 1991**	***M. dongzhaiensis* Liu, Zhou, Yu and Liu, 2006**
Hosts	*Scarthichthys virdis*	*Kyphosus sectatrix*	*Kyphosus* spp.	*Kyphosus analogus*	*Lagodon rhomboides*	*Archosargus rhomboides*			*Archosargus probato-cephalus*	*Acanthurus* spp.	*Scatophagus argus*
Locality	Central Chile	Florida, USA	Florida, USA	Taboga Island, (Gulf of Panama)	North Carolina, USA	Biscayne Bay (Florida)	Florianopolis, Brazil	Rio de Janeiro State, Brazil	Florida, USA	Japan and Palau	Hainan, China
Source	This study	Linton (1910)	Manter (1935)	Sogandares-Bernal (1959)	Manter (1931)	Overstreet (1969)	Amato (1982)	Fernandes, Kohn and Pinto (1985)	Sogandares-Bernal and Hutton (1959a)	Machida and Uchida (1991)	Liu, Zhou, Yu and Liu (2006)
BW/BL	0.21–0.40	0.27	0.27–0.29	0.17–0.23	0.32*	0.40–0.52	0.36–0.41	0.26	0.44	0.35–0.36	0.3–0.31
FB/BL	0.25–0.37		0.27*	0.32	0.28*	0.29*	0.31*		0.31	0.23*	0.32–0.34
OSL/VSL	0.86–1.39	1.35	1.16–1.35	1.75–1.88	1.75	1.94–2.06	2.18–2.50	1.71	2.11	0.63–0.69	2.04–2.17
PTR/BL	0.09–0.19		0.29*	0.24–0.28	0.24*	0.19*	0.16*		0.18	0.19–0.28	0.23–0.24
PCR/BL	0.15–0.29		0.10*	0.07*	0.06*	0.06*	0.1*		0.07*	0.09*	0.03–0.05
PhL/OSL	0.65–1.12	1.0	0.80–0.98	0.71–0.68	0.90	0.66–0.67	0.55–0.67	0.69	0.5	0.40–0.62	0.63
HSL/BL	0.11–0.15		0.19*	0.09–0.13		0.11*	0.13–0.14	0.11		0.08–0.09	0.10–0.11
Tegumental spines	Forebody	Not mentioned	Body unspined	Body unspined	Around oral sucker and at posterior tip	Forebody	Forebody and around excretory pore	Not mentioned	Whole body. Fore body heavily spined	Body unspined	Whole body
EV bifurcation	Posterior testis level	Not mentioned	Ovary level	Ovary level	Ovary level	Not mentioned	Not observed	Not mentioned	Ovary level	Not mentioned	Not mentioned

Taxonomy summary

Syns: *Lepidauchen* sp. of Díaz and George-Nascimento (2002), Flores and George-Nascimento (2009); *Megasolena* sp. of Díaz and Muñoz (2010), Muñoz-Muga and Muñoz (2010), Muñoz and Delorme (2011) and Muñoz and Randhawa (2011).
Type host: *Scartichthys viridis* (Valenciennes) (Blenniidae).
Other hosts: *Scartichthys gigas* (Steindachner) (Blenniidae)
Site of infection: Intestine.
Type locality: Las Cruces, central Chile (33°29´43″S, 71°38´18″W).
Other localities: In central Chile: Isla Negra (33°26´32″S, 71°41´15″W), El Tabo (33°26´43″S, 71°40´57″W), Maitencillo (32°38´41″S; 71°26´14″W), and Quintero (33°44´29″S, 71°29´56″W). In northern Chile: Arica (18°28´50″S, 70°20´0.5″W).
Prevalence, abundance (± standard deviation) and intensity range**:** 63.7%, 0.71 ± 0.91 and 1–5 respectively for *S. viridis* (n = 80) from the northern Chile; 14.7%, 0.35 ± 1.24 and 1–19, respectively, for *S. viridis* (n = 1,471) from the central-south of Chile; 80%, 1.4 ± 0.89 and 1–2, respectively, for *S. gigas* (n = 5) from the northern Chile.
Type-material: Museo Nacional de Historia Natural, Santiago, Chile, MNHNCL PLAT 15002 (Holotype), and MNHNCL PLAT 15004 (Paratype); Natural History Museum, London, England BMNH 2016.4.14.12–21 (Paratypes).
GenBank accession numbers**:** KX035007 (specimen from Arica); KX035008 and KX035009 (from Las Cruces); KX035010 (from Quintero).
Etymology: "*littoralis*", i.e. "beach zone", refers to the habitat where the definitive hosts are normally found.

Taxonomy remarks

Six species of *Megasolena* have been described: *M. estrix* Linton, 1910, *M. kyphosi* Sogandares-Bernal, 1959, *M. hysterospina* (Manter, 1931), *M. archosargi* Sogandares-Bernal and Hutton, 1959, *M. acanthuri* Machida and Uchida, 1991 and *M. dongzhaiensis* Liu, Zhou, Yu and Liu 2006. Overstreet (1969) suggested that *M. archosargi* is a synonym of *M. hysterospina*. Therefore five species of *Megasolena* are considered valid.

Megasolena littoralis n. sp. differs from these species in a combination of morphometric characteristics, such as sizes of the body, suckers, pharynx, hermaphroditic sac and testes (Table III), and morphological features such as tegumental spination, intestinal bifurcation and excretory vesicle (Tables III and IV). Moreover, the post-caecal region (from the posterior edge of the caeca to the end of the body) is larger in *M. littoralis* n. sp. than any other species, meaning that the caeca are shortest in this species. The testes are relatively more posteriorly situated in relation to the body length than in all species except *H. hysterospina*, as shown by the smaller posterior testis region (PTR/BL) (Table IV).

Megasolena estrix is distinguished from *M. littoralis* n. sp. by its wider body and larger posterior testis, and it has a large excretory vesicle reaching the edge of the anterior testis or ovary.

Megasolena hysterospina also differs from *M. littoralis* n. sp. in the large body size and larger ranges of several morphometric measurements (sucker sizes, pharynx, ovary and testes) and it has a larger sucker-length ratio (OSL/VSL, Table IV). Manter (1931) and Overstreet (1969) did not detect an oesophagus in *M. hysterospina*, although Amato (1982) showed an oesophagus of 132 μm judging from the drawings supplied (Table IV). This contrasts to *M. littoralis* n. sp. because all the specimens exhibited a distinct oesophagus (Figs. 7–9).

M. kyphosi is distinguished from *M. littoralis* n. sp. by its larger sucker-length ratio and it has a large excretory vesicle reaching the anterior testis or ovary.

Megasolena acanthuri is distinguished from *M. littoralis* n. sp. by its larger body, suckers, pharynx, hermaphroditic sac and testes; and smaller sucker-length ratio.

Megasolena dongzhaiensis is also distinguished from *M. littoralis* n. sp. by its larger body, suckers, sucker-length ratio, pharynx, hermaphroditic sac and testes; also it has a spinose tegument reaching to the posterior testis, the caeca reach close to the posterior extremity of the body, the intestinal bifurcation is in the forebody and the uterus is pre-equatorial.

Discussion

The two *Monorchimacradena* species have been recorded in fish hosts with completely different biological characteristics, *Acanthurus hepatus*, the type host for *M. acanthuri* is a coral reef associate, in this case from the Caribbean zone, while *S. viridis*, the type-host for *M. viridis* n. sp., is an endemic fish of the tidal rocky shore of the South American Pacific coast, particularly in Chile and Peru. Moreover, *M. viridis* n. sp. was found in the central to central-south localities of Chile (33 and 36 °S), but not in the north. However, Flores and George-Nascimento (2009) supposedly recorded this species in *Scartichthys* spp. from Iquique, northern Chile (19°S). Therefore, the presence of *M. viridis* n. sp. in the north needs to be convincingly demonstrated.

Species of *Megasolena* have been recorded from tropical and subtropical waters. The type-species, *Megasolena estrix*, was originally reported in the Bermuda Sea Chub, *Kyphosus sectatrix* (Linnaeus) from the Dry Tortugas, Florida (Linton 1910) and later in *K. sectatrix* and the Yellow Sea Chub *Kyphosus incisor* (Cuvier), also from the Dry Tortugas (Manter 1935, 1947, 1954).

Megasolena hysterospina was originally reported as *Lepidauchen hysterospina* from the Pinfish *Lagodon rhomboidalis* (Linneaus) from Beaufort, North Carolina (Manter 1931), and later it was reported in the same host from Bayboro Harbor, Tampa Bay, Florida (Hutton and Sogandares-Bernal 1960). *M. archosargi* was originally reported in the Sheepshead, *Archosargus probatocephalus* (Walbaum) from Bayboro Harbor, Tampa Bay, Florida (Sogandares-Bernal and Hutton 1959), and later from the same host and locality (Hutton and Sogandares-Bernal 1960). Overstreet (1969) redescribed *M. hysterospina* from the Western Atlantic Seabream *Archosargus rhomboidalis* from Biscayne Bay, Florida, realising that the species was not a *Lepidauchen* and moving it to *Megasolena* and suggesting that *M. archosargi* is a synonym. In this connection it is of interest that *M. littoralis* was original identified as a *Lepidauchen* species. The species has also been reported in the West African Spadefish *Chaetodipterus lippei* Steindachner off Sierra Leone (Fischthal and Williams 1971), and from *A. rhomboidalis* off Brazil (Amato 1982; Fernandes *et al.* 1985; Cordeiro and Luque 2005). Using the junior synonym *M. archosargi*, Bunkley-Williams *et al.* (1997) reported the species in *A. rhomboidalis* from Parguera, Puerto Rico.

Megasolena kyphosi has been reported in the Pacific only in the Blue-bronze Sea Chub, *Kyphosus analogus* (Gill), from Taboga Island, off the Pacific coast of Panama (Sogandares-Bernal 1959).

Megasolena acanthuri was found in the Eyestripe Surgeonfish *Acanthurus dussumieri* Cuvier and Valenciennes off Okinawa-jima, Japan, the Orangespot Surgeonfish *A. olivaceus* Bloch and Schneider from Palau, Micronesia, and the Lined surgeonfish *Acanthurus lineatus* (Linneaus) from Amami-oshima, Japan (Machida and Uchida 1991).

M. dongzhaiensis is known only from the Spotted Scat, *Scatophagus argus* (Linnaeus) from Dongzhai Bay, Hainan Province, China (Liu *et al.* 2006).

An unidentified species of *Megasolena* has been recorded in *Ocyurus chrysurus* (Bloch 1791) from Coastal reefs of Veracruz, Mexico (Montoya-Mendoza *et al.* 2014). The species designated *Megasolena* sp. in *Pomadasys* sp. from Charambirá, Pacific Coast of Colombia (Castañeda *et al.* 2003) may not belong to this genus considering the description given by the authors; the description of reproductive systems was not given, however other morphological features do not correspond to *Megasolena*, for example, small size of the pharynx, and narrow caeca. Therefore, previous *Megasolena* species are distributed in tropical waters, mostly in the North, Central and South American coasts and the North Western Pacific Ocean.

In this study, *Megasolena littoralis* n. sp. was found in two species of *Scartichthys*; in *S. viridis* from the central and northern Chile and also in *S. gigas* from the north of Chile. This distribution of the digenean has been previously found in other studies (Díaz and George-Nascimento 2002, Flores and George-Nascimento 2009). However, this digenean species was not found in central-south localities (~36°S). No *Megasolena* species have been recorded in *Scartichthys variolatus* (Valenciennes) from the oceanic islands of central Chile (Díaz and Muñoz 2010). Moreover, there are no records of this parasite in *S. gigas* from the central coast of Perú (Iannacone *et al.* 2012). According to all the parasitic records of digeneans from intertidal fish from Chile, *M. littoralis* n. sp. seems to be specific to the genus *Scartichthys* from the central to northern coasts of Chile (19°S to 33°S) and *S. viridis* appears to be the main host for this digenean species. Thus, this *Megasolena* species shows the most southerly distribution for the genus on the Pacific coast of America.

The abundance and prevalence of *Monorchimacradena viridis* n. sp. and *Megasolena littoralis* n. sp. increase with fish body size, as was previously demonstrated (Muñoz and Randhawa 2011). Larger blenny fish of *S. viridis* eat a greater amount of food and therefore can be infected by digeneans more frequently than small fish. These parasites are transmitted by crustaceans, however, the specific intermediate hosts are still unknown for these two digenean species. Even though *S. viridis* is an herbivorous fish, small crustaceans (e.g. isopods, amphipods) are often found in the diet, showing 59% frequency of occurrence (Ojeda and Muñoz 1999), therefore, small crustaceans may transmit digeneans, and possibly other parasites, to *S. viridis*.

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References

Aljanabi S. M., Martinez I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25, 4692–4693. DOI: 10.1093/nar/25.22.4692

Amato J.F.R. 1982. Digenetic trematodes of percoid fishes of Florianópolis, southern Brasil – Fellodistomidae, Monascidae, Diplangidae, Zoogonidae, and Waretrematidae with description of two new species. *Revista Brasileira de Biologia*, 42, 681–699

Anderson G.R., Barker S.C. 1993. Species differentiation in the Didymozoidae (Digenea): restriction fragment length differences in internal transcribed spacer and 5.8S ribosomal DNA. *International Journal for Parasitology*, 23, 133–136. DOI: 10.1016/0020-7519(93)90106-9

Blasco-Costa I., Balbuena J.A., Kostadinova A., Olson P.D. 2009. Interrrelationships of the Haploporinae (Digenea: Aploporidae): a molecular test of the taxonomic framework based on morphology. *Parasitology International*, 58, 263–269. DOI: 10.1016/j.parint.2009.03.006

Bowles J., Hope M., Tiu W.U., Liu X.S., McManus D.P. 1993. Nuclear and mitochondrial genetic markers highly conserved between Chinese and Philippine *Schistosoma japonicum*. *Acta Tropica*, 55, 217–229. DOI: 10.1016/0001-706X(93)90079-Q

Bunkley-Williams I., Dyer W.G., Williams E.H. 1997. Some aspidogastrid and digenean trematodes of Puerto Rican marine fishes. *Journal of Aquatic Animal Health*, 8, 87–92. DOI: 10.1577/1548-8667(1996)008<0087:SAADTO>2.3.CO;2

Bush A.O., Lafferty K.D., Lotz J.M., Shostak A.W. 1997. Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *Journal of Parasitology*, 83, 575–583. DOI: 10.2307/3284227.

Castañeda L., Carvajal H., Vélez I. 2003. Algunos trematodos digeneos de peces marinos de Charambirá (Chocó, Colombia). *Actualidades Biológicas*, 25, 147–155. (In Spanish)

Castro R., Muñoz G. 2011. Two species of *Colobomatus* (Copepoda: Phylichthydae) parasitic on coastal fishes in Chilean waters. *Crustaceana*, 84, 385–400. DOI: 10.1163/001121611X555417

Cordeiro A., Luque J.L. 2005. Aspectos quantitativos dos metazoários parasitos do sargo-de-dente, *Archosargus rhomboidalis* (Linnaeus, 1758) (Osteichthyes, Sparidae) do litoral do estado do Rio de Janeiro, Brasil. *Revista Brasileira de Zoociências Juiz de Fora*, 7, 7–14. (In Portuguese)

Chambers C.B., Cribb T.H. 2006. Phylogeny, evolution and biogeography of the *Quadrifoliovariinae* Yamaguti, 1965 (Digenea: Lecithasteridae). *Systematic Parasitology*, 63, 61–82. DOI: 10.1007/s11230-005-9007-5

Díaz F., George-Nascimento M. 2002. Estabilidad temporal de las infracomunidades de parásitos en la borrachilla *Scartichthys viridis* (Valenciennes, 1836) (Pisces: Blenniidae) en la costa central de Chile. *Revista Chilena de Historia Natural*, 75, 641–649. DOI: 10.4067/S0716-078X2002000400001. (In Spanish)

Díaz P., Muñoz G. 2010. Diet and parasites of the insular fish *Scartichthys variolatus* (Blenniidae) from Robinson Crusoe Island, Chile: How different is this from two continental congeneric species? *Revista de Biología Marina y Oceanografía*, 45, 293–301. DOI: 10.4067/S0718-19572010000200011

Díaz P. E., Muñoz G., George-Nascimento M. 2016. A new species of *Hemipera* Nicoll, 1913 (Digenea: Derogenidae) from fishes of the intertidal rocky zone of Chile. *Acta Parasitologica*, 61: 516–522. DOI: 10.1515/ap-2016-0068

Don R.H., Cox P.T., Wainwright B.J., Baker K., Mattick J.S. 1991. "Touchdown" PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research*, 19, 4008. DOI: 10.1093/nar/19.14.4008

Fernandes B.M.M., Kohn A., Magalhaes-Pinto R. 1985. Aspidogastrid and digenetic trematodes. Parasites of marine fishes of the coast of Rio de Janeiro State, Brazil. *Revista Brasileira de Biologia*, 45, 109–116

Fischthal J.H., Williams M.O. 1971. Some digenetic trematodes of marine fishes from Sierra Leone. *Journal of Helminthology*, 45, 41–50. DOI: 10.1017/S0022149X0000691X

Flores K., George-Nascimento M. 2009. Las infracomunidades de parásitos de dos especies de *Scartichthys* (Pisces: Blenniidae) en localidades cercanas del norte de Chile. *Revista Chilena de Historia Natural*, 82, 63–71. DOI: 10.4067/S0716-078X2009000100004. (In Spanish)

Gibson D.I., Bray R.A. 1979. The Hemiuroidea: terminology, systematic and evolution. *Bulletin of the British Museum (Natural History) Zoology series*, 36, 35–146. DOI: 10.5962/bhl.part.3604

Hutton R. F., Sogandares-Bernal F. 1960. A list of parasites from marine and coastal animals of Florida. *Transactions of the American Microscopical Society*, 79, 287–292. DOI: 10.2307/3223735

Iannacone J., Vanesa S., Olazabal N., Salvador C., Alvariño L., Molano J. 2012. Índices ecológicos de los parásitos de *Scartichthys gigas* (Steidachner, 1876) (Perciformes, Blenniidae) de las costas de Lima, Perú. *Neotropical Helminthology*, 6, 191–204. (In Spanish)

Koetschan C., Hackl T., Müller T., Wolf M., Förster F., Schultz J. 2012. ITS2 database IV: interactive taxon sampling for internal transcribed spacer 2 based phylogenies. *Molecular Phylogenetic and Evolution*, 63, 585–588. DOI: 10.1016/j.ympev.2012.01.026

Linton E. 1910. Helminth fauna of the dry Tortugas II. Trematodes. *Papers from the Tortugas Laboratory of the Carnegie Institute of Washington*, 4, 11–98

Liu S.F., Zhou L., Yu S.Z., Liu J. 2006. A new species of haploporid (Digenea) from the South China Sea. *Journal of Parasitology*, 92, 620–622. DOI: 10.1645/GE-692R.1

Machida M., Uchida A. 1991. A new species of *Megasolena* (Trematoda, Waretrematidae) from surgeonfishes of Japanese and adjacent waters. *Bulletin of the National Science Museum, Tokyo. Series A. Zoology*, 17, 111–114

Manter H.W. 1931. Some digenetic trematodes of marine fishes of Beaufort, North Carolina. *Parasitology*, 23, 396–411. DOI: 10.1017/S0031182000013755

Manter H.W. 1935. The structure and taxonomic position of *Megasolena estrix* Linton 1910 (Trematoda) with notes on related trematodes. *Parasitology*, 27, 431–439. DOI: 10.1017/S003118200001533X

Manter H.W. 1947. The digenetic trematodes of marine fishes of Tortugas, Florida. *American Midland Naturalist*, 38, 257–416. DOI: 10.2307/2421571

Manter H.W. 1954. Trematoda of the Gulf of Mexico. *Fishery Bulletin of the Fish and Wildlife Service*, 55, 335–350

Montoya-Mendoza J., Jiménez-Badillo M.L., Salgado-Maldonado G. 2014. Helminths of *Ocyurus chrysurus* from coastal reefs in Veracruz, Mexico. *Revista Mexicana de Biodiversidad*, 85, 957–960. DOI: 10.7550/rmb.43343

Muñoz G. 2010. A new species of *Pseudodelphis* (Dracunculoidea: Guyanemidae) in the intertidal fish *Scartichthys viridis* (Blenniidae) from Central Chile. *Journal of Parasitology*, 96, 152–156. DOI: 10.1645/GE-2163.1

Muñoz-Muga P., Muñoz G. 2010. Comunidades de parásitos de *Scartichthys viridis* (Pisces: Blenniidae) de Chile central: localidad vs. longitud del hospedador. *Revista de Biología Marina y Oceanografía*, 45, 165–169. DOI: 10.4067/S0718-19572010000100018. (In Spanish)

Muñoz G., Delorme N. 2011. Variaciones temporales de las comunidades de parásitos de peces intermareales de Chile central: hospedadores residentes vs temporales. *Revista de Biología Marina y Oceanografia*, 43, 313–327. DOI: 10.4067/S0718-19572011000300003. (In Spanish)

Muñoz G., Randhawa H. 2011. Monthly variation in the parasite communities of the intertidal fish *Scartichthys viridis* (Blenniidae) from central Chile: are there seasonal patterns? *Parasitology Research*, 109, 53–62. DOI: 10.1007/s00436-010-2220-4

Nahhas F.M. 1993. Some Acanthocephala and Digenea of marine fish from Grand Cayman, Cayman Islands, British West Indies. *Journal of the Helminthological Society of Washington*, 60, 270–272

Nahhas F.M., Cable R.M. 1964. Digenetic and aspidogastrid trematodes from marine fishes of Curacao and Jamaica. *Tulane Studies in Zoology*, 11, 169–228

Ojeda F.P., Muñoz A.A. 1999. Feeding selectivity of the herbivorous fish *Scartichthys viridis*: effects on macroalgal community structure in a temperate a rocky intertidal coastal zone. *Marine Ecology Progress Series*, 184, 219–229. DOI: 10.3354/meps184219

Overstreet R.M. 1969. Digenetic trematodes of marine teleost fishes from Biscayne Bay, Florida. *Tulane Studies in Zoology and Botany*, 15, 119–176

Peña M.F., Poulin E., Dantas G.P.M., González-Acuña D., Petry M.V., Vianna J.A. 2014. Have Historical Climate Changes Affected Gentoo Penguin (*Pygoscelis papua*) Populations in Antarctica? *PLoS ONE*, 9:e95375. DOI: 10.1371/journal.pone.0095375

Sogandares-Bernal F. 1959. Digenetic trematodes of marine fishes from the Gulf of Panama and Bimini, British West Indies. *Tulane Studies in Zoology*, 7, 69–117

Sogandares-Bernal F., Hutton R.F. 1959. Studies on helminth parasites of the coast of Florida. I. Digenetic trematodes of marine fishes from Tampa and Boca Ciega Bays with descriptions of two new species. *Bulletin of Marine Science of the Gulf and Caribbean*, 9, 53–68

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–2739. DOI: 10.1093/molbev/msr121

Williams J.T. 1990. Phylogenetic relationships and revision of the blenniid fish genus *Scartichthys*. *Smithsonian Contributions to Zoo*logy, 492, 1–30. DOI: 10.5962/bhl.title.48741

Zietara M.S., Lebedeva D., Muñoz G., Lumme J. 2012. A monogenean fish parasite, *Gyrodactylus chileani* n. sp., belonging to a novel marine species lineage found in the South-Eastern Pacific and the Mediterranean and North Seas *Systematic Parasitology*, 83, 159–167. DOI: 10.1007/s11230-012-9379-2

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