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Review of Haploporid (Trematoda) Genera with Ornate Muscularization in the Region of the Oral Sucker, including Four New Species and a New Genus

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Abstract

Species of the Haploporidae Nicoll, 1914 with elaborate muscularization of the oral sucker belong in three trematode genera, including three new species and a new genus from the intestine of fishes in Australian waters. *Spiritestis* Nagaty, 1948 is resurrected and *S. herveyensis* n. sp. is described from the mullet *Moolgarda seheli* (Forsskål) collected in Hervey Bay, Queensland, Australia; the latter differs from *S. arabii* Nagaty, 1948 in that the position of the genital pore is pharyngeal rather than post-pharyngeal and the geographical range is off Australia rather than the Red Sea. A new genus is proposed for two new species, with a uniquely ornamented oral sucker, which infect Australian scatophagids. Members of *Capitimitta* n. g. are distinguished from *Waretrema* Srivastava, 1937, species of which have a simple oral sucker with six radially arranged anterior muscular lobes, in that their oral sucker is V-shaped with six embedded muscular finger-like structures in the anteroventral portion. The relatively small *C. darwinensis* n. sp., collected from *Selenotoca multifasciata* (Richardson) at Darwin, Northern Territory, Australia, is distinguished from *C. costata* n. sp., collected from *Scatophagus argus* (Linnaeus) in the same locality and *S. multifasciata* off Brisbane, Australia, and by having smaller eggs, a vitellarium commencing at a level close to the ventral sucker rather than at greater than one ovarian length posterior to the ventral sucker, and shorter tegumental body spines. Sequence data of a c. 2,500 bp region of the 3' end of 18S, the entire ITS region and the 5' end of the 28S revealed that *Spiritestis* and *Capitimitta* are not as closely related as some morphological features would suggest and are probably not the closest relative of each other. What has been reported as *Waretrema pisciculum* Srivastava, 1937 probably consists of several species, some in different genera, and one,

based on material collected by Dr. Masaaki Machida, is proposed as *Spiritestis machidai* n. sp. from *Crenimugil crenilabis* (Forsskål) off Japan. Phylogenetic hypotheses, based on analysis of an alignment of partial 28S sequences with other haploporids, provide a framework for the evaluation of interrelationships within the Haploporidae. These analyses show that: (1) *Spiritestis* and *Capitimitta* are supported within the Haploporidae; (2) branches to *Forticulcita* Overstreet, 1982, *Saccocoelioides* Szidat, 1954, *Spiritestis* and *Capitimitta* create a clade that is sister to haploporines from the Mediterranean Sea; (3) the branch to *Saccocoelioides*, *Spiritestis*, and *Capitimitta* create a polytomy; and (4) the two new species of *Capitimitta*, plus an immature specimen of an unnamed species, form a monophyletic clade.

Introduction

Haploporid trematodes are cosmopolitan parasites of the alimentary tract of fishes characterized primarily by the presence of a hermaphroditic sac and a single testis (Overstreet & Curran, 2005). The organization of the subfamilies and genera has been called into question by many authors (reviewed by Overstreet & Curran, 2005) and more recently by the proposal of the Forticulcitinae Blasco-Costa, Balbuena, Kostadinova, & Olson, 2009. Haploporids have the greatest diversity in the Mugilidae in both the number of species and genera described, but some also infect members of other fish families. Prior to this study, only two species of haploporids, both placed in *Waretrema* Srivastava, 1937 by Overstreet & Curran (2005), were reported to have an ornamented oral sucker. The type-species for *Waretrema*, the type-genus of Waretrematinae Srivastava, 1937, is *W. piscicolum* Srivastava, 1937. Although this species has been reported six times (Srivastava, 1939; Velasquez, 1961; Gupta & Miglani, 1976; Bilqees, 1980; Machida, 1996; Liu & Yang, 2003), we doubt whether any of the subsequent reports even represent a species in *Waretrema* as diagnosed by Srivastava. *Waretrema piscicolum* was described in detail by Srivastava (1939) from specimens obtained from *Liza vaigiensis* (Quoy & Gaimard) [reported as *Mugil waigiensis* (Quoy & Gaimard)] in the Arabian Sea, off Karachi, Pakistan. Specimens identified as *W. piscicolum*, or *W. piscicola*, have been reported from *Crenimugil crenilabis* (Forsskål) off Okinawa, Japan (Machida 1996) and a marine fish off the Andaman and Nicobar Islands, India (Gupta & Miglani, 1976). It has also been reported from *Scatophagus argus* (Linnaeus) off Karachi, Pakistan (Bilqees, 1980), off the Philippines (Velasquez, 1961) and in the South China Sea (Liu & Yang, 2003). Nagaty (1948) described *W. arabii* (Nagaty, 1948), as *Spiritestis arabii* Nagaty, 1948, from *Mugil* sp. in the Red Sea. *Spiritestis* Nagaty, 1948 was proposed as a junior synonym of *Waretrema* by Overstreet & Curran (2005) because of the superficial similarity of the oral sucker in *S. arabii* and *W. piscicolum*.

In this study, on the basis of old and new specimens, we concluded that haploporids with ornamentation in the region of the oral sucker are best considered as representing three genera. One is *Waretrema* (*stricto sensu*), one is resurrected, and the last is new. We discuss records of *W. piscicolum* and examine several specimens with an ornate anterior end and show that *Waretrema*, as defined by Overstreet & Curran (2005), is polyphyletic, resurrect *Spiritestis*, describe two new species in that genus, propose a new genus for three species infecting *Scatophagus argus* and *Selenotoca multifasciata* (Richardson) in Australian waters, and describe two of these species as new. Molecular data obtained from the ITS1,

ITS2, and 28S gene fragments are used to support decisions to separate *Spiritestis* from the new genus and describe two unique species in the new genus. Also, we place these two new species, and an unnamed one, within a phylogenetic framework of a larger group of haploporids using the 28S gene fragment.

Materials and methods

Specimens of three undescribed species belonging to *Spiritestis* and a new genus, along with other trematodes, were collected from *Moolgarda seheli* (Forsskål), *Selenotoca multifasciata*, and *Scatophagus argus* at several locations in Australian waters during March, 2010 using cast-nets. Fish length was measured as total length (TL), extending from the tip of the snout to the end of the tail. Fish names follow those given by FishBase (Froese & Pauly, 2012).

Trematodes were collected following Cribb & Bray (2010) for gastrointestinal species, often skipping the initial examination under a dissecting scope for mullets due to the volume of the intestinal contents. Live worms were rinsed and cleaned in a container with saline, and examined briefly; then most of the saline was removed from the container, and the worms were killed by pouring hot water (not boiling) over them. This procedure was followed by immediately adding cool water or ethanol to prevent cooking, and then fixing the worms in 70% ethanol. When the number of specimens of a species allowed, a few additional ones were placed at room temperature directly into 95% ethanol for molecular analysis, and a couple were heat-killed while under coverslip pressure for the critical examination of ducts. Worms were stained in aqueous alum carmine, Mayer's haematoxylin, or Van Cleave's haematoxylin; dehydrated in a graded ethanol series; cleared in clove oil (carmine and Van Cleave's) or methyl salicylate (Mayer's); and mounted permanently in Damar gum. Measurements were taken using a differential interference contrast (DIC) equipped Leica compound microscope using a ProgRes CapturePro camera (Version 2.8 Jenoptic, Jena, Germany) and software. All measurements are in micrometers unless noted otherwise. Museum abbreviations are as follows: GCRLM, Gulf Coast Research Laboratory Museum; NTM, Museum and Art Gallery of the Northern Territory, Darwin, Australia; NSMT, National Science Museum Tokyo; QM, Queensland Museum, Brisbane, Australia; and USNPC, US National Parasite Collection, Beltsville Maryland.

With regard to the terminology of structures, we want to clarify one matter involving the terminal genitalia reported differently by various authors. The uterus enters the posterior portion of the hermaphroditic sac, also termed "hermaphroditic pouch" by some, and it continues its path to join the male duct as a hermaphroditic duct or intromittent organ. We do not consider the distal portion of the uterus before entering the hermaphroditic sac a "metraterm" unless there is a distinct sphincter, which is usually considerably more muscular than the uterus-proper. This "metraterm" controls or inhibits the passage of eggs into the hermaphroditic sac. We did not encounter such a structure in specimens reported herein. Once the uterus enters the sac, we consider the tube, which is usually quite muscular, as the "female duct," and this duct can, in rare cases (not observed here), be subdivided into two portions by a sphincter forming an internal metraterm.

For comparisons with previously labeled and deposited specimens, we examined the following from the USNPC: *Spiritestis arabii* (USNPC 038164.00, voucher [Nagaty, 1948]), *Spiritestis arabii* (USNPC 059541.00, paratype [Nagaty, 1948]), and *Waretrema piscicola* (USNPC 039476.00, labeled as topotype [Velasquez, 1961]); and from the NSMT: *W. piscicola* (NSMT PI-3841, 4700 1/2, 4700 2/2, 4705, and 4731 [Machida, 1996]). No type of *W. pisciculum* Srivastava, 1937 was designated at the time of publication.

Genomic DNA was isolated using Qiagen DNAeasy Tissue Kit (Qiagen, Inc., Valencia, California, USA) following the instructions provided. DNA fragments c. 2,500 base pairs (bp) long, comprising the 3' end of the 18S nuclear rDNA gene, internal transcribed spacer region (including ITS1 + 5.8S + ITS2) and the 5' end of the 28S gene (including variable domains D1–D3), were amplified from the extracted DNA by polymerase chain reaction (PCR) on a PTC-200 Peltier Thermal Cycler using forward primers ITSF (5'-CGCCCGTCGCTACTACCGATTG-3') or LSU5 (5'-TAGGTCGACCCGCTGAAAYTTAAGCA-3') and reverse primer 1500R (5'-GCTATCCTGAGGGAAACTTCG-3'). These PCR primers and multiple internal primers were used in sequencing reactions. The internal forward primers were DIGL2 (5'-AAGCATATCACTAAGCGG-30), 300F (5'-CAAGTACCGTGAGGGAAAGTTG-3') and 900F (5'-CCGTCTTGAAACACGGACCAAG-3'), and the internal reverse primers were 300R (5'-CAACTTTCCTCACGGTACTTG-3'), DIGL2R (5'-CCGCTTAGTGATATGCTT-3'), and ECD2 (5'-CTTGGTCCGTGTTTCAAGACGGG-3').

The resulting PCR products were excised from PCR gel using QIAquick Gel Extraction Kit (Qiagen, Inc., Valencia, California, USA) following the kit instructions, cycle-sequenced using ABI BigDye chemistry (Applied Biosystems, Inc., Carlsbad, California, USA), ethanol-precipitated and run on an ABI 3130 Genetic Analyzer. Contiguous sequences were assembled using Sequencher (GeneCodes Corp., Ann Arbor, Michigan, USA, Version 4.10.1) and submitted to GenBank. Previously published 28S ribosomal RNA gene sequences of *Atractotrema sigani* Durio & Manter, 1969, *Dicrogaster contracta* Looss, 1902, *D. perpusilla* Looss, 1902, *Forticulcita gibsoni* Blasco-Costa, Montero, Balbuena, Raga, & Kostadinova, 2009, *Haplodena nasonis* Yamaguti, 1970, *Haploporus benedeni* Looss, 1902, *Lecithobotrys putrescens* Looss, 1902, *Pseudomegasolena ishigakiense* Machida & Kamiya, 1976, *Saccocoelioides* sp. of Overstreet & Curran, (2005), *Saccocoelium brayi* Blasco-Costa, Montero, Balbuena, Raga, Kostadinova, & Olson 2009, *S. cephalis* Blasco-Costa, Montero, Gibson, Balbuena, Raga, & Kostadinova, 2009, *S. obesum* Looss, 1902, *S. tensum* Looss, 1902 and *Paragonimus westermani* (Kerbert, 1878) were used for comparison (see Table 1 for all accession numbers and host information). Sequences were aligned using the ClustalW application in the BioEdit program, Version 7.0.9 (Hall, 1999). The alignment was further refined by eye and trimmed to the shortest sequence on both 5' and 3' ends. The resulting alignment utilized 14 haploporids and the two attractotrematids *Pseudomegasolena ishigakiense* and *Atractotrema sigani*, with *P. westermani* as the outgroup, and it was 1,204 characters long, including gaps, with 790 sites conserved, 411 sites variable, and 276 sites informative. Phylogenetic analysis of the data was performed using Bayesian inference (BI) with MrBayes 3.1.2 software (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The best nucleotide substitution model was estimated with jModeltest Version 0.1.1 (Posada, 2008; Guindon & Gascuel, 2003) as general time reversible with estimates of invariant sites and gamma-distributed among site-rate variation (GTR + I + Γ). The following model parameters were

used in MrBayes: $nst = 6$, $rates = invgamma$, $ngen = 1,000,000$, and $samplefreq = 100$. Burn-in value was 1,780 estimated by plotting the log-probabilities against generation and visualizing plateau in parameter values ($sump\ burnin = 1780$), and nodal support was estimated by posterior probabilities ($sumt$) (Huelsenbeck et al., 2001), with all other settings left as default.

Table 1. List of species, hosts, origins, and GenBank accession numbers of specimens used in this study

Species	Host species	Country	Region, locality	28S
<i>Paragonimus westermani</i>	Experimental host	India	Meghalaya	DQ836244.1
<i>Atractotrema sigani</i>	<i>Siganus lineatus</i>	Australia	Lizard Island	AY222267.1
<i>Hapladena nasonis</i>	<i>Naso unicornis</i>	Australia	Lizard Island	AY222265.1
<i>Pseudomegasolena ishigakiense</i>	<i>Scarus rivulatus</i>	Australia	Heron Island	AY222266.1
<i>Dicrogaster contracta</i>	<i>Liza aurata</i>	Spain	Santa Pola	FJ211261.1
<i>Dicrogaster perpusilla</i>	<i>Liza ramado</i>	Spain	Santa Pola	FJ211238.1
<i>Forticulcita gibsoni</i>	<i>Mugil cephalus</i>	Spain	Santa Pola	FJ211239.1
<i>Haploporus benedeni</i>	<i>Liza ramado</i>	Spain	Santa Pola	FJ211237.1
<i>Lecithobotrys putrescens</i>	<i>Liza saliens</i>	Spain	Ebro Delta	FJ211236.1
<i>Saccocoelium brayi</i>	<i>Liza saliens</i>	Spain	Ebro Delta	FJ211234.1
<i>Saccocoelium cephalic</i>	<i>Mugil cephalus</i>	Spain	Ebro Delta	FJ211233.1
<i>Saccocoelium obesum</i>	<i>Liza ramado</i>	Spain	Ebro Delta	FJ211259.1
<i>Saccocoelium tensum</i>	<i>Liza aurata</i>	Spain	Santa Pola	FJ211258.1
<i>Saccocoeloides</i> sp.	<i>Poecilidae</i>	Nicaragua		EF032696.1
<i>Capitimitta darwinensis</i> n. sp.	<i>Selenotoca multifasciata</i>	Australia	Northern Territory, Darwin	KC206498
<i>Capitimitta costata</i> n. sp.	<i>Selenotoca multifasciata</i> , <i>Scatophagus argus</i>	Australia	Brisbane, Queensland (<i>S. multifasciata</i>); Darwin, Northern Territory (<i>S. argus</i>)	KC206497
<i>Capitimitta</i> sp.	<i>Selenotoca multifasciata</i>	Australia	Causeway Lake, Queensland	KC206499
<i>Spiritestis herveyensis</i> n. sp.	<i>Moolgarda seheli</i>	Australia	Hervey Bay, Queensland	KC206500

Waretrema Srivastava, 1937

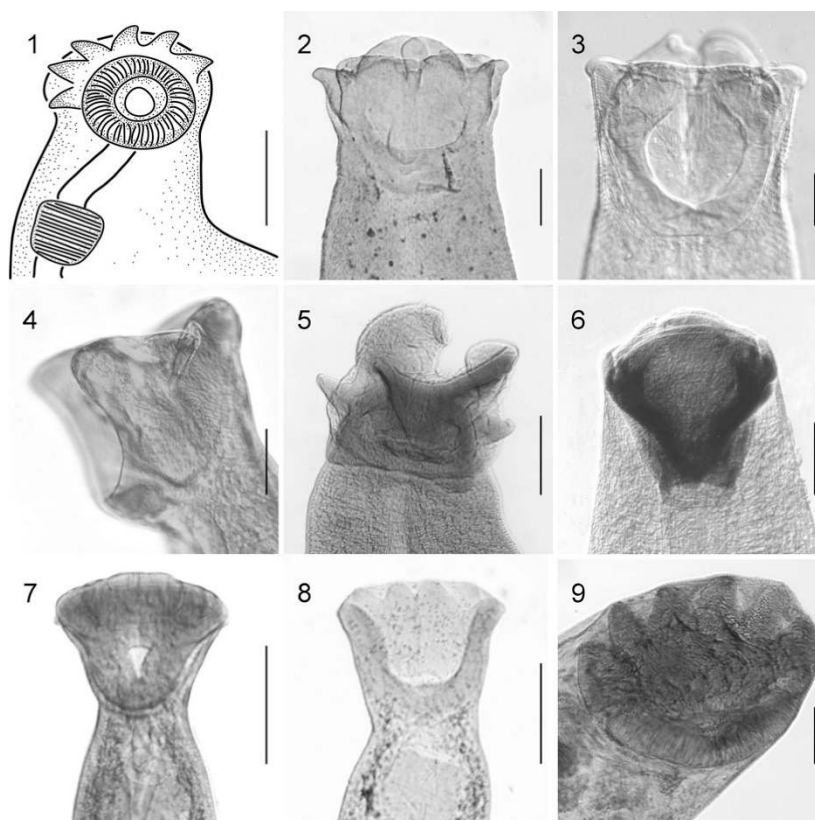
Diagnosis (Fig. 1)

Body fusiform. Eyespot pigment unknown but assumed present. Tegument spinous, with spines on forebody. Oral sucker subspherical, with 6 separate anterodorsal conical lobes (arranged radially in relation to anterior half), ventral, with spines like those of tegument. Ventral sucker larger than oral sucker, in anterior third of body. Prepharynx present. Pharynx well developed. Esophagus relatively long. Intestinal bifurcation posterior to ventral sucker. Caeca elongate, saccular, end blindly anterior to ovary. Testis singular, ovoid, in posterior third of body. External seminal vesicle present. Hermaphroditic sac contains internal seminal vesicle, pars prostatica with surrounding prostatic gland cells, female duct

and hermaphroditic duct; female duct and hermaphroditic duct about equal in length. Ovary slightly dextral, contiguous with testis to pretesticular. Laurer's canal present. Canicular seminal receptacle present. Vitellarium composed of 10 elongate spindle-shaped follicles, extends anteriorly to mid-body and posteriorly close to posterior extremity of body. Uterus pre-ovarian; eggs few, medium-sized, thin-shelled. Excretory vesicle Y-shaped, bifurcates at posterior level of testis; pore subterminal. Parasites of Mugilidae.

Type- and only recognized species: Waretrema piscicolum Srivastava, 1937.

Etymology: The genus was named by Har Dayal Srivastava for Mr. F. Ware, Director, Imperial Veterinary Institute, Mukteshwar-Kumaon, Uttarakhand, India. Because of the Greek neuter "trema" for hole, the genus is treated as neuter in gender.



Figures 1–9. 1. *Waretrema piscicolum*, illustration of the anterior end, redrawn from Srivastava (1939); 2. *Spiritestis arabii*, ventral view of specimen heat-killed while under pressure; 3. *Spiritestis herveyensis* n. sp., ventral view of specimen heat-killed without pressure; 4. *S. herveyensis*, lateral view of specimen heat-killed without pressure; 5. *S. machidai* n. sp., extended oral sucker of specimen killed while under pressure; 6. *S. machidai*, contracted oral sucker of specimen killed while under pressure; 7. *Capitimitta darwinensis* n. sp., oral sucker of specimen heat-killed without pressure; 8. *C. darwinensis*, oral sucker of specimen heat-killed while under pressure; 9. *Capitimitta* sp., oral sucker of worm labelled as "topotype" of *Waretrema piscicolum* collected from *Scatophagus argus* by Velasquez (1961), specimen killed while under pressure. Scale-bars: 1, 250 μm ; 2–9, 100 μm .

***Waretrema piscicolum* Srivastava, 1937**

Syns *Waretrema piscicola* Srivastava, 1937; *W. piscicola* of Srivastava (1939)

Type- and only known host: *Liza vaigiensis* (Quoy & Gaimard, 1825) squaretail mullet (Mugilidae).

Etymology: The Latin adjectival name *piscicolum* refers to “dwelling in” a fish and corresponds with the neuter generic name.

Description: (Fig. 1) With characters of genus.

Remarks

Waretrema piscicolum was first named *W. piscicola* in an abstract presented by Srivastava (1937) that included enough information to separate both species and genus from related haploporid taxa at the time, and, then soon after, Srivastava (1939) provided the description in more detail. In the abstract, Srivastava (1937) recorded the host as *Trichiurus mutieus* Gray, which we assume to be a misspelling of *Trichiurus muticus* Gray, currently considered to be *Eupleurogrammus glossodon* (Gray). With the full description, Srivastava (1939) corrected the identification of the fish from the Arabian Sea to *Liza vaigiensis* (Quoy & Gaimard) [reported as *Mugil waigiensis* Quoy & Gaimard]. Srivastava's (1939) description and illustration are clear and coherent, but subsequent reports (Velasquez, 1961; Gupta & Miglani, 1976; Bilqees, 1980; Machida, 1996; Liu & Yang, 2003) of *W. piscicolum* are suspect and will be discussed under other sections. The two most definitive features of *Waretrema* that separate it from other genera of haploporids are: (1) the possession of six radially arranged lobes located anterior to the oral sucker that are covered with spines resembling those of the body tegument; and (2) the few elongate vitelline follicles.

The most recent diagnosis of *Waretrema* by Overstreet & Curran (2005) included members of both *Spiritestis* and a new genus reported herein, which necessitate the narrowing of their concept to that diagnosed above. The oral area of *W. piscicolum* is very unusual for a haploporid, and this unusual appearance is what we believe lead to a history attributing specimens and species to this genus based on superficial characters. Additionally, when specimens are fixed when under coverslip pressure, the oral sucker area becomes compressed, making the anterior end of different species appear similar. The illustration of, and description by, Srivastava (1939) is clear, concise, and thorough, and there is no evidence to support the possibility that the oral sucker does not have six projections anterior to it. We have provided photomicrographs (Figs. 2–9) of species of *Spiritestis* and the new genus and have redrawn the oral area of *W. piscicolum* (Fig. 1). Based on these comparisons, we believe that variations in the oral sucker in specimens caused authors to treat all as *W. piscicolum*. For example, when we initially examined live specimens of *Spiritestis*, we thought that we were dealing with *Waretrema*, although recognizing others as a new genus of atypical haploporids. The differentiation of *Waretrema* from other genera will be discussed below under *Spiritestis* and the new genus.

Spiritestis* Nagaty, 1948Amended diagnosis*

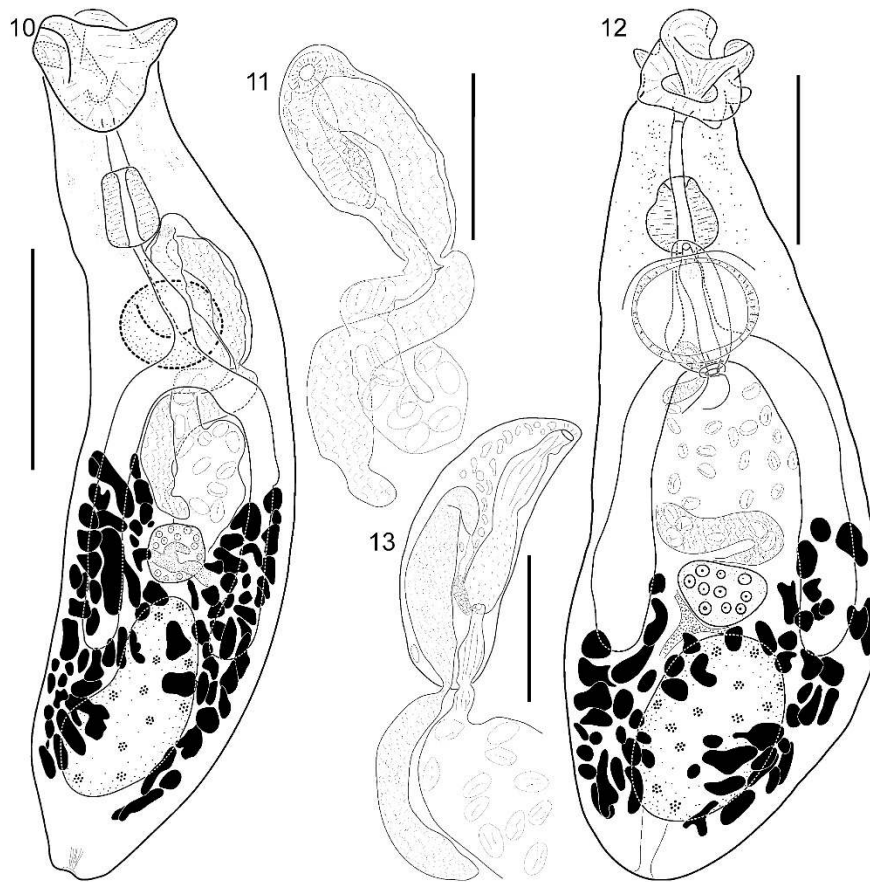
Body elongate. Anterior-most portion of worm deeply cleft, with origin of cleft near level of prepharynx origin. Eyespot pigment dispersed from levels of oral sucker to ventral sucker, densest between pharynx and oral sucker. Tegument spinous. Oral sucker terminal, with 6 muscular lobes arranged in 3 distinct pairs; first pair forms ventral or anterior rim of oral sucker with slight cleft medially; second pair dorsal to first pair and extends laterally; third pair dorsal and extends anteriorly. Mouth subterminal, opens ventrally. Prepharynx relatively long. Pharynx pyriform. Esophagus longer than pharynx. Ventral sucker slightly elevated, without any specialization, located quarter to third of body length from anterior end. Intestinal bifurcation near posterior margin of ventral sucker. Caeca sac-like to relatively long, narrow, end blindly. Testis longer than wide, in hindbody close to posterior end of caeca, with post-testicular field not more than 10% of body length (BL). External seminal vesicle elongate, sinuous, longer than internal seminal vesicle. Hermaphroditic sac elongate, arcuate. Ovary pretesticular. Vitellarium with numerous (> 50) small follicles, located between level slightly anterior to ovary and post-testicular region. Uterus pre-ovarian, posterior to genital pore. Eggs thin-shelled, nonoperculate; miracidium lacks pigmented eyespots. Lymphatic system present in forebody. Excretory vesicle weakly Y-shaped, extends to ovarian region; pore terminal. In Mugilidae; in Indo-Pacific Region. Type-species *Spiritestis arabii* Nagaty, 1948.

Etymology: Nagaty (1948) described *Spiritestis* based on *S. arabii* as having a single, elongate, more-or-less superficially spiraled testis, but he did not provide an etymological origin of the name. We consider the name a combination of the Latin feminine *spira*, meaning coil or twist, and the Latin *testis* and consider the name masculine, since “testis” is clearly masculine.

Remarks

The combination of morphological features, including a spinose tegument, the possession of a hermaphroditic sac, a single testis, and a Y-shaped excretory vesicle, makes members of this genus fully consistent with the diagnosis of the Haploporidae (see Overstreet & Curran, 2005), even though the genus was originally placed in the Lepocreadiidae Odhner, 1905 by Nagaty (1948). We resurrect the available name *Spiritestis*, because it differs significantly from *Waretrema*. Members of *Waretrema* have the oral sucker composed of six conical, muscular, independent, retractable lobes directed anteriorly (Fig. 1). However, in specimens of *Spiritestis*, the oral sucker bears three pairs of lobes (Figs. 2–6, 12, 14), of variable mobility in live material, which, in contracted, fixed specimens, remain distinct and are not retracted into the oral sucker even when the latter is withdrawn into the body. Nagaty (1948) stated that there were four lobes in *S. arabii*; presumably, he was referring to the second and third pairs. Based on our examination of two of his specimens, we found that the first pair was inconspicuous because of the contracted nature of the specimens. In these, there was a noticeable thinning of what we consider to be the first pair, as in our Red

Sea specimens. Other features that distinguish specimens of *Spiritestis* from *Waretrema* include: a pyriform rather than ovoid pharynx; long tubular rather than saccate caeca; numerous small vitelline follicles rather than a few long, relatively large, tubular follicles; and a delicate Y-shaped excretory vesicle extending to the ovary rather than bifurcating at ovarian level and extending at least to the level of the ventral sucker. *Spiritestis* specimens have several features which are odd for a waretrematine, i.e., a long, sinuous external seminal vesicle, a very delicate Y-shaped excretory vesicle, and numerous small vitelline follicles. The testis is not spiraled as the name suggests, but it is usually elongate and may give the appearance of being twisted in specimens under pressure or not heat-killed. Other members of the family Haploporidae that exhibit these characters are in the subfamily Megasoleninae Manter, 1935, suggesting *Spiritestis* may occupy a basal position within the Waretrematinae.



Figures 10–13. 10. *Spiritestis arabii*, dorsal whole-mount, not all eggs or vitelline follicles illustrated, specimens killed while under pressure; 11. *Spiritestis arabii*, hermaphroditic sac of same specimen in Figure 10; 12. *S. machidai* n. sp. ventral whole-mount, not all eggs or vitelline follicles illustrated, specimen killed while pressure; 13. *S. machidai* n. sp. hermaphroditic sac, specimen killed while pressure. *Scale-bars:* 10, 12, 600 μm ; 11, 13, 300 μm .

***Spiritestis arabii* Nagaty, 1948**

Syn. *Waretrema arabii* (Nagaty, 1948) Overstreet & Curran, 2005

Type-host: Unidentified *Mugil* sp. known locally as “Boory or Arabi” (Mugilidae).

Other host: *Crenimugil crenilabis* (Forsskål), fringelip mullet (Mugilidae); *Moolgarda seheli* (Forsskål) bluespot mullet, (Mugilidae).

Type-locality: Red Sea.

Other locality: Off Eilat, Israel, Red Sea.

Material examined: *Spiritestis arabii* Nagaty, 1948 (USNPC 059541.00, paratype; USNPC 038164.00, voucher); 5 voucher specimens from Red Sea collection, 2 from *Crenimugil crenilabis* and 3 from *Moolgarda seheli*, collected from the Gulf of Aqaba, Red Sea, Eilat, Israel, by Ilan Paperna USNPC 106213.00–106215.00.

Description: (Figs. 2, 10–11, Table 2)

[Based on 5 gravid specimens collected by Ilan Paperna killed under varying degrees of coverslip pressure with heat.] Body elongate, ellipsoidal, 2,371–3,249 long, 346–606 wide, with width 14–26% of BL. Forebody 710–926 or 26–31% of BL. Hindbody 1,389–2,155 or 58–66% of BL. Tegument spinous, with spines 8–10 long in forebody. Eyespot pigment dispersed. Oral sucker (Fig. 2) subterminal, with mouth opening ventrally, 229–368 long, 229–397 wide, with 6 muscular lobes; first pair of lobes ventral and anterior to mouth, with weakly M-shaped extension of anterior oral sucker rim projecting ventrally; second pair dorsal to first pair, extending laterally, forming widest part of oral sucker apparatus; third pair dorsal to second and extending anteriorly as anterior-most extension of entire worm, flattened dorsoventrally, with total width about same as first pair, overlapping slightly along median junction, uniting posterior to level of anterior margin of first pair. Ventral sucker slightly elevated, 212–282 long, 210–296 wide. Prepharynx 107–297 long. Pharynx pyriform, 137–209 long, 147–181 wide, widest in posterior half, with length 88–139% of width and 55–195% of prepharynx length. Esophagus 322–482 long, extends to near level of posterior margin of ventral sucker. Intestinal bifurcation 851–1165 from anterior end or 33–41% of BL. Caeca long, tubular, terminate blindly 572–887 from posterior end; postcaecal field 22–28% of BL.

Table 2. Dimensions and ratios of *Capitimitta darwinensis* n. sp., *C. costata* n. sp., *Spiritestis herveyensis* n. sp., and *S. arabii* from our Red Sea collection (the latter had been killed under varying degrees of coverslip pressure)

Species	<i>Capitimitta darwinensis</i>	<i>Capitimitta costata</i>	<i>Spiritestis herveyensis</i>	<i>Spiritestis herveyensis</i> flat	<i>Spiritestis arabii</i> GCRL collection
Host	<i>Selenotoca multifasciata</i>	<i>Selenotoca multifasciata</i> , <i>Scatophagus argus</i>	<i>Moolgarda seheli</i>	<i>Moolgarda seheli</i>	<i>Moolgarda seheli</i> , <i>Crenimugil crenilabis</i>
N	8	2 +1	6	1	3 + 2
Length	785–1,101 (889)	1,142–1,577 (1,375)	2,491–3,140 (2,936)	3,879	2,371–3,249 (2,753)
Width	148–214 (170)	200–287 (252)	447–642 (551)	840	346–606 (517)
Pre-genital pore distance	226–361 (274)	267–339 (310)	643–776 (709)	932	571–785 (660)
Genital pore to ventral sucker	0–75 (31)	8–17 (11)	45–119	185	100–151 (130)
Forebody length	242–388 (307)	372–411 (387)	687–898 (803)	1,172	710–926 (795)
Hindbody length	411–607 (488)	664–1,036 (870)	1563–1,990 (1,778)	2,377	1,389–2,155 (1,710)
Pre-intestinal bifurcation distance	366–526 (425)	573–725 (634)	953–1,220 (1,075)	1,470	851–1,165 (1,001)
Postcaecal distance	248–414 (297)	379–528 (466)	518–694 (589)	948	572–887 (706)
Oral sucker (OS) length	110–157 (124)	155–228 (197)	197–277 (253)	303	229–368 (296)
OS width	126–176 (147)	193–255 (230)	279–342 (311)	386	229–397 (328)
Ventral sucker (VS) length	88–126 (103)	108–140 (128)	219–290 (261)	352	212–282 (246)
VS width	98–122 (106)	127–155 (138)	224–291 (258)	371	210–296 (242)
Prepharyngeal length	30–100 (72)	35–88 (59)	248–396 (342)	443	107–297 (195)
Pharynx length	72–109 (88)	99–123 (113)	157–222 (197)	225	137–209 (172)
Pharynx width	71–102 (85)	75–92 (85)	156–186 (170)	211	147–181 (156)
Esophagus length	116–240 (181)	287–351 (316)	235–365 (309)	518	322–482 (381)
Testis length	144–281 (181)	172–217 (191)	457–669 (594)	782	713–803 (761)
Testis width	101–150 (119)	135–169 (152)	206–320 (268)	250	215–251 (233)
Testis to ventral sucker	64–202 (124)	258–493 (402)	910–1,184 (1053)	1,392	608–1,211 (857)

Table 2. Continued					
Post-testicular field	156–280 (194)	235–334 (285)	86–196 (143)	171	42–266 (135)
Ovary length	101–150 (119)	56–73 (67)	124–199 (153)	199	158–205 (177)
Ovary width	44–85 (57)	45–69 (55)	140–179 (168)	183	110–162 (134)
Ovary to ventral sucker	27–167 (79)	195–425 (344)	416–608 (451)	747	413–609 (519)
Testis to ovary	0	0	153–527 (370)	453	16–412 (152)
Vitellarium to ventral sucker	0–36 (6)	98–235 (185)	400–608 (513)	600	243–578 (404)
Vitellarium to posterior end	43–81 (63)	45–61 (52)	36–128 (78)	96	57–110 (72)
Hermaphroditic sac length	99–177 (137)	161–360 (289)	369–510 (429)	611	415–557 (468)
Hermaphroditic sac width	53–81 (67)	75–99 (89)	116–214 (17)	192	163–222 (193)
Internal seminal vesicle length	65–109 (86)	107–227 (180)	181–286 (229)	353	297–434 (357)
Internal seminal vesicle width	27–40 (33)	47–56 (52)	42–88 (71)	58	82–106 (93)
External seminal vesicle length	44–88 (63)	69–87 (80)	125–272 (203)	636	444–888 (601)
External seminal vesicle width	29–51 (38)	48–64 (58)	41–73 (59)	78	85–137 (117)
Clear egg length	58–64 (61)	67–70 (68)	61–67 (64)	54–58 (55)	61–70 (66)
Clear egg width	29–36 (32)	27–31 (29)	29–36 (32)	35–40 (38)	33–42 (38)
Width %*	18–21% (19%)	18–19% (18%)	17–24% (19%)	22%	14–26% (19%)
Pre-genital pore distance*	25–35% (31%)	22–23% (23%)	22–28% (25%)	24%	22–26% (24%)
Forebody*	31–38% (34%)	26–33% (29%)	25–31% (29%)	30%	26–31% (29%)
Hindbody*	52–58% (55%)	58–66% (63%)	59–66% (63%)	61%	58–66% (62%)
Pre-intestinal bifurcation distance*	43–53% (48%)	43–50% (46%)	34–41% (38%)	38%	33–41% (36%)
Postcaecal distance*	29–38% (33%)	33–35% (34%)	17–23% (21%)	24%	22–28% (26%)
VS length % OS length	80–88% (84%)	60–70% (66%)	94–111% (104%)	86%	70–104% (84%)
VS width % OS width	67–79% (73%)	52–80% (62%)	76–90% (82%)	82%	59–92% (76%)
Pharynx length % prepharynx length	72–377% (150%)	29–75% (53%)	43–90% (59%)	51%	55–195% (101%)

Table 2. *Continued*

Pharyngeal length %	89–108%	132–134%	101–124%	107%	88–139%
pharynx width	(100%)	(133%)	(115%)		(110%)
Testis to ventral sucker*	8–19%	23–32	34–39%	36%	26–37%
	(14)	(29)	(37%)		(30%)
Post-testicular space*	15–28%	20–21	3–7%	4%	2–9%
	(22)	(21)	(5%)		(5%)
Ovary length/ovary to ventral sucker distance	0.5–2.6	2.7–7.4	2.8–4.9	3.8	2.4–3.1
	(1.4)	(5.0)	(3.5)		(2.9)

* Ratio as percentage of body-length

Testis elongate, medial, slightly pointed at posterior end, 713–803 long, 215–251 wide, 608–1,211 from posterior margin of ventral sucker, 42–266 from posterior end of body or post-testicular field 2–9% of BL. External seminal vesicle 444–888 long, 85–137 wide, sinuous, extends posteriorly to near ovary, often obscured by eggs. Hermaphroditic sac thick-walled, arcuate to straight, passes dorsal to ventral sucker, 415–557 long, 163–222 wide, contains internal seminal vesicle measuring 297–434 long by 82–106 wide in posterior region, male duct arising from anterior region of internal seminal vesicle, and pars prostatica which unites with female duct at roughly middle of sac and forms hermaphroditic duct; hermaphroditic duct strongly muscularized, S-shaped, about half length of hermaphroditic sac. Genital pore medial, 100–151 anterior to ventral sucker, 571–785 from anterior extremity or 22–26% of BL.

Ovary medial, 158–205 long, 110–162 wide, 413–609 posterior to ventral sucker, 16–412 anterior to testis. Uterus confined between levels of ovary and slightly posterior to ventral sucker, with proximal portion filled with sperm. Laurer's canal not observed. Vitellarium follicular; follicles numerous, more than 100, ovoid, distinct, appear as extensive dendritic masses when under pressure, commencing near level of ovary, 243–578 dorsal to ventral sucker, densest when surrounding caeca, absent in area between testis and ovary, confined to near tegumental surface, terminate 57–110 from posterior end. Eggs thin-shelled, 61–70 long, 33–42 wide, with those in distal uterus not containing miracidium with pigmented eyespots.

Lymphatic system not observed. Excretory vesicle slightly Y-shaped, bifurcates dorsal to ovary, with arms extending slightly anterior to anterior margin of ovary; excretory pore terminal.

Remarks

Our examination of two specimens of *S. arabii* (USNPC 038164.00 voucher and USNPC 059541.00 paratype) collected by Nagaty revealed that, even when the oral sucker apparatus was contracted, its state was similar to those we described from our material collected from *Crenimugil crenilabis* and *Moolgarda seheli* in the Red Sea. These specimens were fixed a few decades ago, with extreme coverslip pressure being applied to the specimens from *M. seheli* and moderate pressure to those from *C. crenilabis*, making them unsuitable for

comparison with unflattened specimens from recent collections. This species will be discussed under the new species of *Spiritestis*. We encourage the recollection of this species and a redescription.

***Spiritestis herveyensis* n. sp.**

Type- and only host: *Moolgarda seheli* (Forsskål), bluespot mullet (Mugilidae).

Type-locality: Mouth of Beelbi Creek, Hervey Bay, Queensland, Australia (25°14'48"S, 152°40'02"E).

Other locality: Eli Creek, Queensland, Australia (25°15'45"S, 152°48'28"E).

Site of infection: Intestine.

Type-material: Holotype QM G234006; 2 paratypes QM G234007, G234008, USNPC 106216.00–106218.00 (including 1 flattened specimen USNPC 106217.00 and 1 lateral mount USNPC 106218.00); representative DNA sequences partial 18S, entire ITS region, partial (D1–D3) 28S: GenBank accession no. KC206500, two identical sequences from Beelbi Creek, QLD.

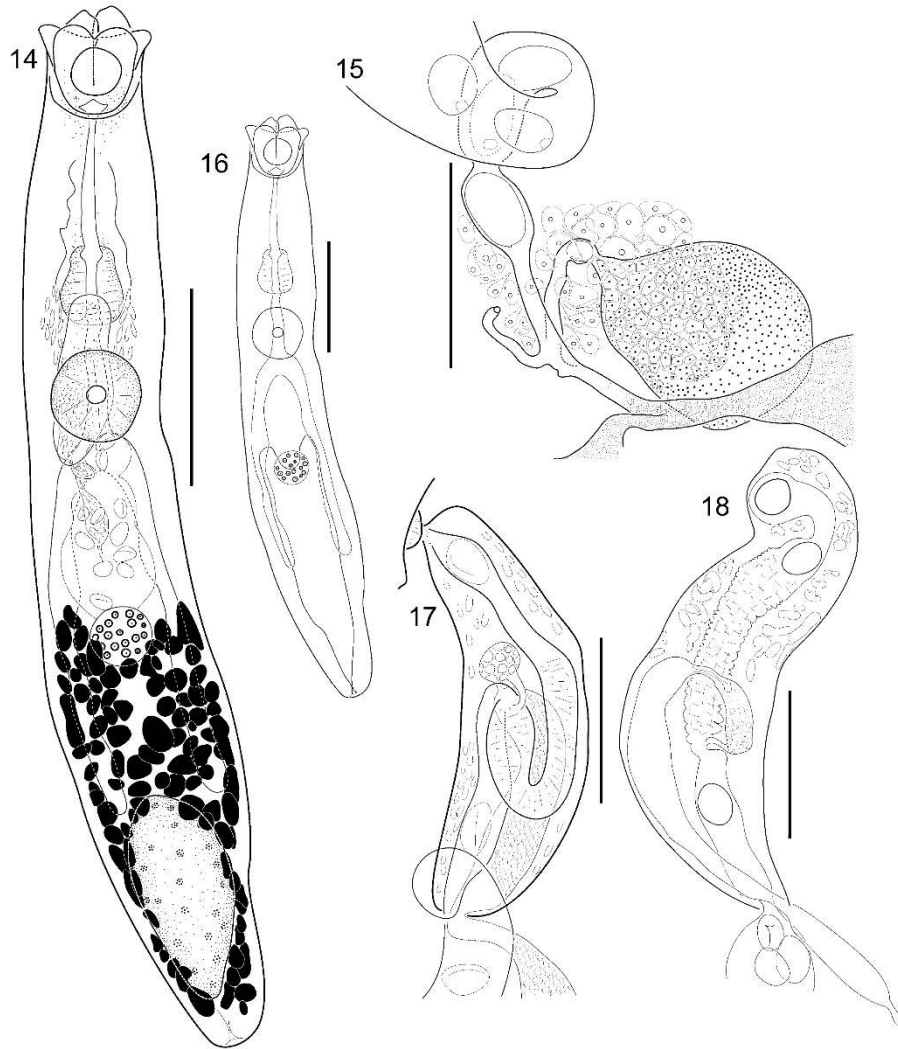
Etymology: The Latinized, adjectival, masculine name refers to Hervey Bay, from which the material was collected.

Description (Figs. 3–4, 14–18; Tables 1, 2, 3, 4)

[Measurements based on 6 gravid, unflattened, whole-mount specimens; measurements of holotype below and of entire series in Table 2.] Body elongate, ellipsoidal, 2,629 long, 447 wide, with width 17% of BL, widest in posterior half of body. Tegument spinose; spines 7–9 long (on forebody of flattened specimen), becoming progressively shorter and more sparse posteriorly. Eyespot pigment dispersed in anterior third of body. Oral sucker (Figs. 3–4, 14) terminal, with 6 muscular lobes (3 pairs), 252 long, 279 wide at widest lateral point of outermost lobe pair (second pair); first pair of lobes less distinctive than other pairs, in form of rounded "m" anterior to mouth, forms dorsoventral rim of oral sucker; second pair extends laterally and slightly anterior to first pair, forming widest part of oral sucker apparatus; third pair dorsal, about as narrow as first but with sharper "m," with deep cleft extending to near mouth; second and third pairs of lobes move independently of other pairs in life. Mouth subterminal, opens ventrally. Ventral sucker slightly elevated, circular in outline, 236 long, 231 wide, with anterior margin 824 from anterior most extremity or 31% of BL. Hindbody 1,563 or 59% of BL. Prepharynx 349 long, with small atrium proximally. Pharynx pyriform, 198 long, 159 wide, widest in posterior half. Esophagus 365 long, extends posteriorly to near level of posterior margin of ventral sucker. Intestinal bifurcation 1,050 from anterior end or 40% of BL. Caeca terminate blindly 606 from posterior end; postcaecal field 23% of BL.

Testis elongate, medial, slightly pointed at posterior end, 524 long, 254 wide, located 913 from posterior margin of ventral sucker, with post-testicular field 127 or 5% of BL. External seminal vesicle 217 long, 62 wide, sinuous, often obscured by eggs. Hermaphroditic sac (Figs. 14, 17–18) thick-walled, arcuate to straight, usually somewhat dextral, passes dorsal to ventral sucker, 425 long, 153 wide, contains internal seminal vesicle in dextroposterior region measuring 209 long by 42 wide, male duct arising from anterior

region of internal seminal vesicle, pars prostatica looping ventral to hermaphroditic duct and uniting with female duct to form hermaphroditic duct roughly in middle of sac; hermaphroditic duct strongly muscularized, U- to S-shaped, with total length about length of hermaphroditic sac. Genital pore medial, anterior to ventral sucker, in pharyngeal region, 732 from anterior extremity or 28% of BL.



Figures 14–18. *Spiritestis herveyensis* n. sp. 14. Ventral whole-mount, not all eggs or vitelline follicles illustrated; 15. ovarian complex of specimen killed while under pressure; 16. ventral whole-mount showing extent of excretory vesicle; 17. hermaphroditic sac of lateral mount; 18. hermaphroditic sac of specimen killed with coverslip pressure. Scale-bars: 14, 16, 400 μm ; 15, 100 μm ; 17, 18, 200 μm .

Table 3. Length and number of variable sites based on pairwise comparison of the ITS1 region (above diagonal) and 5.8S gene (below diagonal) between *Spiritestis herveyensis* n. sp., *Capitimitta darwinensis* n. sp., *C. costata* n. sp., and an undescribed species of *Capitimitta*

		<i>S. herveyensis</i>	<i>C. darwinensis</i>	<i>C. costata</i>	<i>Capitimitta</i> sp.
	Length	626	614	449	583
<i>S. herveyensis</i>	157	—	206	109	194
<i>C. darwinensis</i>	157	4	—	39	68
<i>C. costata</i>	157	6	2	—	33
<i>Capitimitta</i> sp.	157	3	1	3	—

Table 4. Length and number of variable sites based on pairwise comparison of the ITS2 region (above diagonal) and 28S gene (below diagonal) between *Spiritestis herveyensis* n. sp., *Capitimitta darwinensis* n. sp., *C. costata* n. sp., and an undescribed species of *Capitimitta*

		<i>S. herveyensis</i>	<i>C. darwinensis</i>	<i>C. costata</i>	<i>Capitimitta</i> sp.
	Length	310	299	300	301
<i>S. herveyensis</i>	1,383	—	52	58	58
<i>C. darwinensis</i>	1,370	147	—	22	33
<i>C. costata</i>	1,369	145	38	—	24
<i>Capitimitta</i> sp.	1,369	137	37	38	—

Ovary (Fig. 11) medial, circular to triangular in outline, 136 long, 159 wide, 416 from posterior margin of ventral sucker, 359 from testis. Laurer's canal opens dorsally between levels of ovary and testis. Mehlis' gland slightly anterior to ovary. Vitellarium follicular; follicles numerous, more than 100, commence near level of ovary about 400 posterior to ventral sucker, densest where surrounding caeca, absent in area between testis and ovary, confined to near tegumental surface, terminate 63 from posterior end; vitelline reservoir ventral to ovary. Uterus confined between levels of ovary and ventral sucker, with proximal portion filled with sperm. Eggs thin-shelled, 61–65 long, 32–34 wide, with those in distal uterus not containing miracidium with pigmented eye-spots.

Lymphatic system consists of 2 large tubes; canals lateral and parallel to prepharynx, terminate near level of ventral sucker, associated with numerous gland-cells near pharynx. Excretory vesicle slightly Y-shaped, bifurcates dorsal to ovary, terminates slightly anterior to ovary; excretory pore terminal.

Remarks

Based on the combination of features, such as a spinose tegument, hermaphroditic sac, and single testis, the new species is consistent with the Haploporidae. The presence of small and numerous vitelline follicles, a pyriform pharynx, tubular caeca, and a sinuous external seminal vesicle, in addition to the nature of the oral sucker, places the new species in *Spiritestis*.

Spiritestis herveyensis n. sp. can be differentiated from *S. arabii* by its geographical location, with *S. herveyensis* being from Australian waters and *S. arabii* from the Red Sea. In terms of morphology, the position of the genital pore in *S. herveyensis* is anterior to the posterior margin of the pharynx rather than being at the posterior margin of the pharynx

or postpharyngeal; and the third pair of oral lobes (the most anterior and dorsal) in *S. herveyensis* (Fig. 3) are almost conical rather than being dorsoventrally flattened and leaf-like (Fig. 2).

***Spiritestis machidai* n. sp.**

Syn. *Waretrema piscicolum* of Machida (1996); also redrawn in fig. 12.23 from same collection by Overstreet & Curran (2005)

Type- and only known host: *Crenimugil crenilabis* (Forsskål), fringelip mullet (Mugilidae).

Type-locality: Off Nago, Okinawa Prefecture, Japan. Material examined (originally identified as *Waretrema piscicolum*): Holotype herein designated from four specimens as the one circled with a diamond pen on slide NSMT PI-4731; paratypes NSMT PI-3841, 4700 1/2, 4700 2/2, 4705, 4705 2/5, 4731.

Description: Refer to that by Machida (1996, Fig. 1) (Figs. 5–6, 12–13).

Remarks

We examined 21 specimens collected by Machida, including the 10 specimens used for the description of *Waretrema piscicolum* by Machida (1996). The specimens examined possess features, such as the three pairs of oral lobes, pyriform pharynx, and numerous small vitelline follicles, that are consistent with species of *Spiritestis*. We consider these specimens to represent a distinct species. *S. machidai* n. sp. (Figs. 5–6) can be differentiated from both *S. arabii* and *S. herveyensis* n. sp. (Figs. 2–4) by its larger and more elaborate oral sucker; furthermore, in *S. machidai*, the first pair of muscular lobes are directed posteriorly from the oral opening, forming a “W” at the ventroposterior margin of the oral sucker, whereas in *S. arabii* and *S. herveyensis*, the first pair of muscular lobes are directed anteriorly and M-shaped. Even though the specimens of *S. machidai* examined by us were fixed with considerable coverslip pressure, they appear to show that the female duct unites with the male duct to form the hermaphroditic duct more posteriorly in the hermaphroditic sac than in *S. arabii* or *S. herveyensis*. Machida (1996) stated that the tegument of the Japanese specimens was smooth; upon review of the specimens, we determined that many had a few spines in the area around the oral and ventral suckers and assumed that some spines have either been shed or dissolved during fixation or slide preparation. Also, in his figure 1, Machida (1996) shows the vitellarium to be larger with fewer follicles than we observed; this difference probably resulted from the considerable pressure applied to these specimens, skewing some features. *S. machidai* is in need of an amended description based on fresh material that has been killed with hot water without pressure and on accompanying material providing molecular data, preferably including the ITS region and 3' end of the 28S gene.

Capitimitta* n. g.Diagnosis*

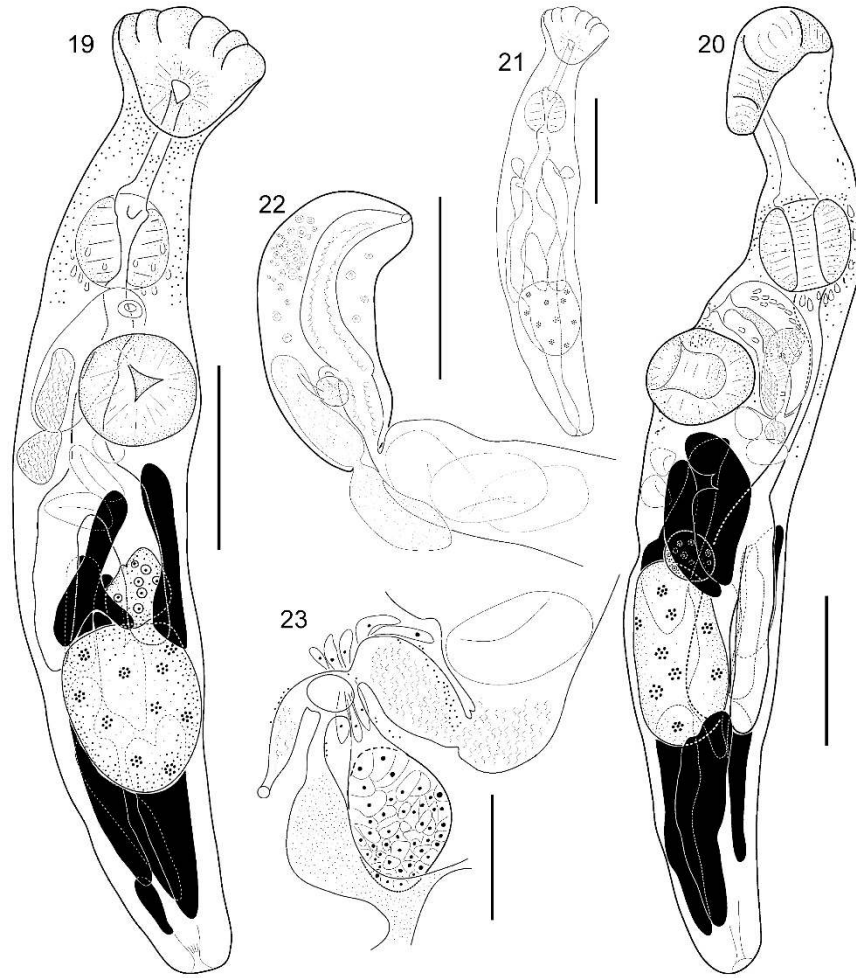
Body fusiform, elongate, with distinct constriction immediately posterior to oral sucker, widest posterior to ventral sucker, tapers posteriorly. Eyespot pigment dispersed in forebody, densest between pharynx and oral sucker. Tegument spinose but spines sparse in area between oral and ventral suckers on ventral surface. Oral sucker specialized, V-shaped, ventroterminal, lies in transverse diagonal plane to body, with anterodorsal margin flat and mouth near posterior margin; anterior region of oral sucker possesses 6 muscular structures in 3 symmetrical pairs; outer pair forms left and right margins of sucker, fanning out to much greater size when extended (like fingers in mitten), with ventral surface of oral sucker muscular without spines. Ventral sucker smaller than oral sucker. Prepharynx distinct but appearance dependent on orientation of oral sucker on "neck" region. Esophagus ranges from indistinct to longer than pharynx. Caeca moderately long, sac-like, terminate near mid-body. Testis slightly longer than wide, located near mid-hindbody. External seminal vesicle sac-like, subspherical. Hermaphroditic sac long, J-shaped, dorsal to ventral sucker, terminates slightly posterior to ventral sucker. Genital pore median, anterior to ventral sucker. Ovary contiguous with anterior margin of testis. Vitellarium follicular; follicles few (< 15), relatively large, elongate, tube-like. Uterus anterior to ovary, posterior to hermaphroditic sac. Eggs relatively few, with miracidium lacking pigmented eyespots. Excretory vesicle Y-shaped; pore terminal. In Scatophagidae; in Indo-West Pacific Region. Type-species *Capitimitta darwinensis* n. sp.

Etymology: The name *Capitimitta* is constructed from the Latin "capitalis," referring to the anterior end of the worm, and the Medieval Latin feminine "mitta," referring to the mitten covering the muscular structures in the oral sucker.

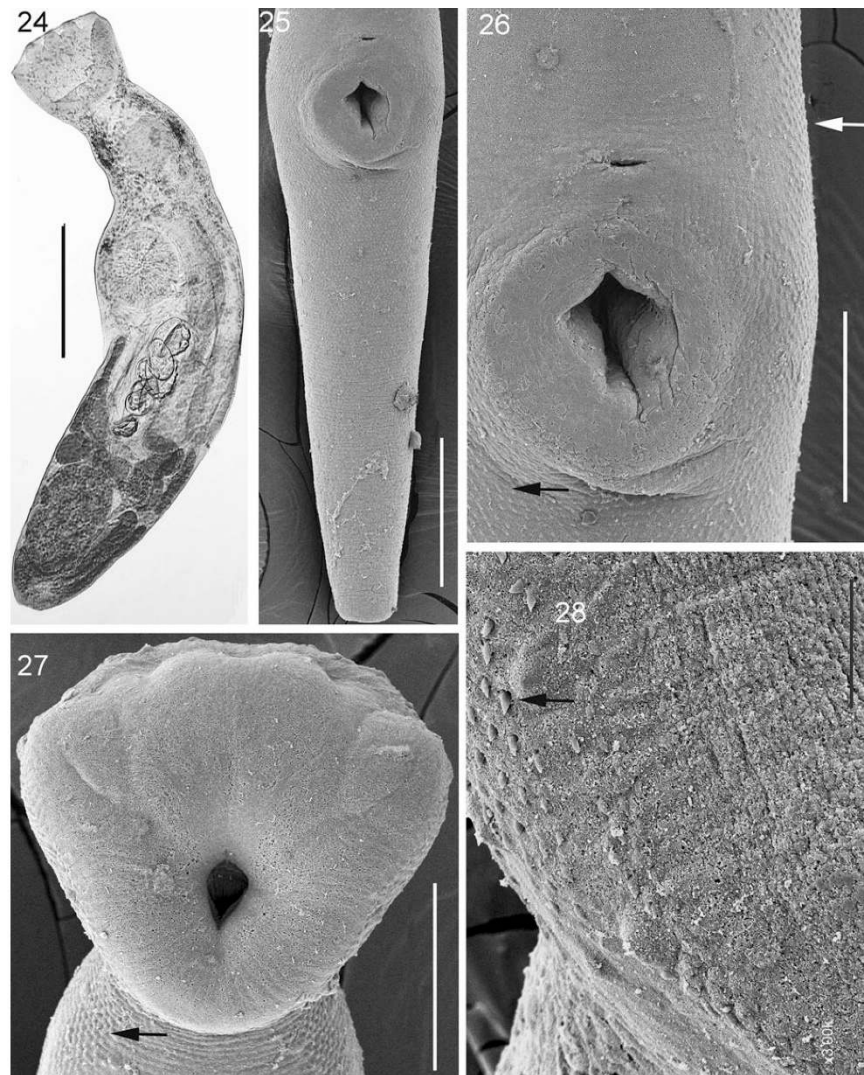
Remarks

Capitimitta n. g. fits within the Haploporidae based on the morphological features listed by Overstreet & Curran (2005), with an emphasis on the possession of a hermaphroditic sac, a single testis, a spinose tegument and a Y-shaped excretory vesicle. The specialized nature of the oral sucker separates it from other haploporid genera, except for *Spiritestis* and *Waretrema*. *Capitimitta* is most similar to *Waretrema* but can be distinguished from it by the nature of the oral sucker. In specimens of *Waretrema*, the oral sucker (Fig. 1) consists of a subspherical oral sucker with six anterodorsal, radially arranged, conical and retractable lobes with a spinose tegument, and the host is a mugilid (Srivastava, 1939). In members of *Capitimitta*, the oral sucker (Figs. 7–9, 19, 20, 24, 27, 29) is basically V-shaped and perched on the "neck" facing ventrally, with the surface of the sucker being smooth. When fixed without pressure, the leading edge of the oral sucker has six humps, which represent the anterior region of the muscular structures. The oral sucker in both extended and contracted live worms resembles the fingers in a mitten, giving a webbed appearance. Even in flattened specimens, there is no indication of a subspherical oral sucker (Figs. 8–9, 24); in such flattened specimens, the oral sucker has the appearance of a large cup with the bottom rim

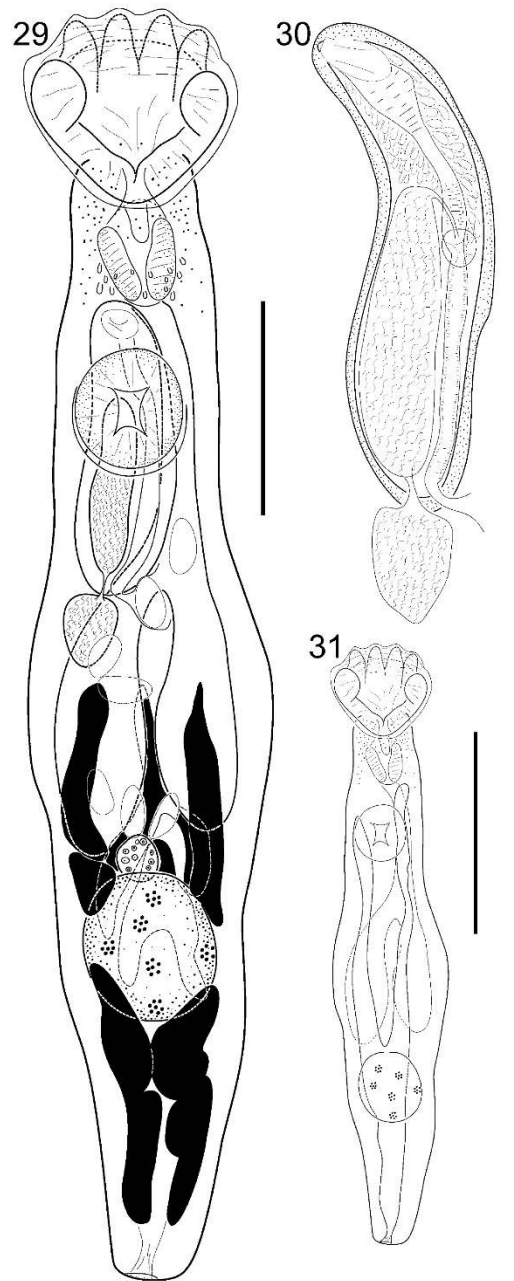
and muscular structures embedded within the anterior region. *Capitimitta* superficially resembles *Spiritestis* in that its species possesses a complex oral sucker. The oral sucker of *Spiritestis* spp. also has six structures, but they are independent of one another. When fixed under pressure, differences in the oral suckers of members of all three genera are not fully apparent because superficially they appear similar.



Figures 19–23. *Capitimitta darwinensis* n. sp. 19. Holotype, ventral aspect of whole-mount, not all eggs illustrated; 20. paratype, lateral aspect mount, not all eggs illustrated; 21. holotype, ventral aspect, whole-mount showing extent of excretory vesicle; 22. hermaphroditic sac of specimen killed while under pressure; 23. ovarian complex of specimen killed while under pressure. *Scale-bars:* 19–21, 200 μm ; 22, 100 μm ; 23, 50 μm .



Figures 24–28. *Capitimitta darwinensis* n. sp. 24. Whole-mount of flattened specimen, note displacement of organs to posterior end and appearance of oral sucker; 25–28. scanning electron micrographs: 25. posterior end of body; 26. ventral sucker with spine free patch anteriorly; 27. oral sucker; 28. transition of muscular oral sucker to spinous tegument. Arrows designate spines. *Scale-bars:* 24, 200 μm ; 25, 100 μm ; 26, 27, 50 μm ; 28, 10 μm .



Figures 29–31. *Capitimitta costata* n. sp. 29. Holotype, ventral aspect mount; 30. holotype, hermaphroditic sac; 31. holotype, ventral aspect, whole-mount showing extent of excretory vesicle. Scale-bars: 29, 250 μm ; 30, 200 μm ; 31, 500 μm .

***Capitimitta darwinensis* n. sp.**

Type- and only known host: *Selenotoca multifasciata* (Richardson), spotbanded scat (Scatophagidae).

Type-locality: Doyles boat ramp, Conacher St., Fannie Bay, in parking lot of NTM, Darwin, Northern Territory (NT), Australia, 12°26'09"S, 130°45'57"E.

Other localities: Buffalo Creek (NT), 12°20'16"S, 130°50'31"E; Sandy Creek (NT), 12°20'37"S, 130°54'05"E.

Site of infection: Intestine.

Type-material: Holotype NTM D001480; paratypes NTM D001481–D001484, USNPC 106219.00–106220.00, and QM G234009, specimens fixed under pressure NTM D001484 and USNPC 106220.00; 2 specimens prepared for SEM; representative DNA sequence of partial 18S, entire ITS region, partial (D1–D3) 28S: GenBank accession no. KC206498, from 6 identical sequences (2 adults from DMANH boat ramp, 1 adult and 1 immature specimen from Buffalo Creek, and 2 immature specimens from Sandy Creek).

Etymology: The Latinized feminine adjectival name *darwinensis* refers to the Darwin Metropolitan Area, where the holotype and all other specimens were collected.

Description (Figs. 7–8, 19–23, 24–28; Tables 1–4)

[Measurements based on 8 gravid, unflattened, whole-mount specimens, with those of holotype given in description and of entire series in Table 2.] Body long, fusiform, widest near mid-body, 817 long, 148 wide, with width 18% of length. Tegument bears minute spines (Figs. 26–28) c. 2 long; spines densest in region between oral sucker and ventral sucker laterally and dorsally, becoming progressively less dense posteriorly; area of tegument between ventral and oral suckers with few irregularly spaced spines (Fig. 26), appearing almost smooth. Eyespot pigment dispersed in anterior quarter of body. Oral sucker (Figs. 7, 19, 20, 27–28) large, V-shaped, terminal, 112 long, 132 wide; 6 papilla-like, muscular structures embedded within sucker, giving scalloped appearance to anterior margin; width of sucker measured at widest point of outer pair of muscular structures considerably wider than body immediately posterior to oral sucker. Mouth opens ventrally. Ventral sucker slightly elevated, 104 long, 98 wide, with anterior margin 283 from anterior-most extremity or 35% of BL; length 88% of oral sucker length; width 79% of oral sucker width. Hindbody 448 or 55% of BL. Prepharynx 96 long, with considerable widening at junction with pharynx; linear length of prepharynx in ventral or dorsal whole-mounts shorter than total length because of oblique angle of neck. Pharynx thick-walled, 81 long, 80 wide. Esophagus 150 long, c. 156% of prepharyngeal length. Glands surrounding prepharynx and pharynx probably associated with digestion. Intestinal bifurcation at level of posterior margin of ventral sucker to slightly further posteriorly, 526 from anterior end of body or 48% of BL. Caeca sac-like, terminate in testicular region, 376 from posterior end of body or 34% of BL.

Testis ovoid, 281 long, 144 wide, 148 from ventral sucker, with post-testicular field 157 long or 19% of body length. External seminal vesicle sac-like, 48 long, 29 wide, with shape

variable, often distorted by eggs. Hermaphroditic sac 138 long, 53 wide, with length appearing shorter because of curvature dorsal to ventral sucker, with majority of posterior region dorsal to ventral sucker, contains internal seminal vesicle measuring 65 long by 27 wide, pars prostatica and hermaphroditic duct, with male and female ducts initing in proximal half. Genital pore medial, anterior to ventral sucker, 262 from anterior end of body or 32% of BL.

Ovary (Figs. 19–20) medial, contiguous with testis, 65 long, 50 wide, 88 from ventral sucker, with oviduct arising from anterior portion. Mehlis' gland anterolateral to ovary. Laurer's canal opens dorsally, anterior to or at level of ovary. Vitellarium tubular, commencing 11 from ventral sucker (extending to ventral sucker in most specimens), terminating 58 from posterior extremity of body. Uterus occupies space between hermaphroditic sac and testis, ventral to caeca, with proximal region containing sperm in most specimens, with no sphincter demarcating uterine seminal receptacle. Mature eggs thin-shelled, operculate, 61–64 long, 29 wide (3 measured from holotype), and 11 eggs from 4 specimens fixed under pressure in permanent mounts measuring 61–64 long by 31–39 wide, with terminal ones not containing miracidium with pigmented eyespots.

Excretory vesicle Y-shaped, bifurcates at anterior region of testis, posterior to ovary, with arms extending to ventral sucker before reflexing and forming small expansion; pore terminal.

Immature specimens generally have same shape as adults. Oral sucker well developed. Caeca relatively wider than in adult, filling predetermined space of uterus and hermaphroditic sac. Testis well developed. Ovary poorly developed or indistinct; vitellarium not developed.

***Capitimitta costata* n. sp.**

Type-host: *Selenotoca multifasciata* (Richardson), spotbanded scat (Scatophagidae).

Other host: *Scatophagus argus* (Linnaeus), spotted scat (Scatophagidae).

Type-locality: Cabbage Tree Creek, Queensland, Australia, 27°19'47"S, 150°03'11"E (*Selenotoca multifasciata*).

Other locality: Buffalo Creek, Northern Territory, Australia, 12°20'16"S, 130°50'31"E (*Scatophagus argus*).

Site: Intestine.

Type-material: Holotype QM G234010; paratype USNPC 106221.00, representative DNA sequence partial 18S, entire ITS region, partial (D1–D3) 28S: GenBank accession no. KC206497, from 3 identical sequences (1 adult and 1 immature specimen from Cabbage Tree Creek, QLD, *Selenotoca multifasciata*, and 1 adult from Buffalo Creek, NT, *Scatophagus argus*).

Etymology: The Latin feminine adjective *costata*, meaning "ribbed," refers to the ribbed appearance of the oral sucker resulting from the protrusion of muscular structures.

Description (Figs. 29–31; Tables 1–4)

[Measurements based on 3 gravid, unflattened, whole-mount specimens, with those of holotype given in description and of entire series in Table 2.] Body elongate, fusiform, 1,405

long, 268 wide in middle third, with width 19% of BL. Tegument armed with spines; spines 2–4 long, dense except for region between oral and ventral suckers where only few irregularly spaced spines occur. Eyespot pigment dispersed in anterior quarter of body. Oral sucker (Fig. 29) large, V- to U-shaped depending on amount of expansion, terminal, 207 long, 243 wide, greatly extensible; aperture almost circular; anterior region with 6 muscular, papilla-like structures embedded within sucker, which appear webbed in between giving scalloped appearance to anterior margin of oral sucker; outer pair curve slightly ventrally in holotype, forming slight cup with mouth at base; width, measured at widest point of outer pair of muscular structures, considerably wider than body immediately posterior to oral sucker. Mouth opens ventrally. Ventral sucker slightly elevated, 140 long, 68% of oral sucker length, 127 wide, with width 52% of oral sucker width. Forebody 372 long or 26% of BL; hindbody 911 long or 65% of BL. Prepharynx 35 long, 28% of pharyngeal length, appearing shorter because of position of oral sucker on peduncle. Pharynx 123 long, 92 wide, length 134% of width; prepharynx and pharynx surrounded by dense dispersed eyespot pigment, surrounded by gland-cells probably associated with digestion. Esophagus 287 long. Intestinal bifurcation posterior to ventral sucker or 43% of BL. Caeca sac-like, terminate 492 from posterior end of body or 35% of BL.

Testis ovoid, 183 long, 151 wide, 454 from ventral sucker or 32% of BL; post-testicular space 285 or 20% of BL. External seminal vesicle sac-like, 87 long, 64 wide, variable in shape. Hermaphroditic sac (Figs. 29–30) 347 long, 94 wide, thick-walled, terminates well posterior to ventral sucker, contains internal seminal vesicle 207 long by 53 wide, pars prostatica surrounded by prostatic cells, female duct, and hermaphroditic duct; male and female ducts unite roughly at mid-point of hermaphroditic sac; female duct thin-walled, about half length of sac; hermaphroditic duct highly muscular. Genital pore medial, anterior to ventral sucker, 325 from anterior end or 23% of BL.

Ovary nearly spherical, smooth, near mid-axis of body, slightly dorsal to testis, 56 long, 45 wide, 412 from the ventral sucker. Laurer's canal not observed. Vitellarium tubular, commences 235 posterior to ventral sucker, terminates 61 from posterior extremity of body. Uterus arises from anterior region of ovary, pretesticular, encroaches into region of ventral sucker, with proximal portion filled with sperm; distal region enters posterior end of hermaphroditic sac, with no evidence of metraterm. Mature eggs thin-shelled, 67–69 long, 27–31 wide (4 measured from holotype), with those in distal uterus not containing miracidium with pigmented eyespots.

Excretory vesicle Y-shaped, bifurcates near ovary, with arms extending to between levels of ventral sucker and pharynx; excretory pore terminal.

Remarks

Capitimitta darwinensis n. sp. and *C. costata* n. sp. can be distinguished by a number of characters; *C. darwinensis* is slightly smaller, and most of the metrical data reflects this difference. The pharynx of *C. darwinensis* is almost equal in length and width, with its length ranging from 89–108% of its width, whereas in *C. costata* the pharynx is noticeably longer than wide, with the length being 132–134% of its width. The vitelline follicles of *C. darwinensis* commence at between half and less of an ovarian length from the ventral sucker, compared with more than one ovarian length in *C. costata*. The hermaphroditic sac and

duct are more muscular and prominent in *C. costata* than in *C. darwinensis*. Finally, the eggs of *C. darwinensis* are shorter and rounder than in *C. costata*, i.e., 58–64 by 29–36 μm rather than 67–70 by 27–31 μm .

Both specimens of *C. costata* described from *Selenotoca multifasciata* in Cabbage Tree Creek, Queensland, were fixed with a near maximum expansion of their oral sucker (Fig. 29). In the mounted specimen of this species from *Scatophagus argus* at Darwin, NT, the oral sucker appeared the same as that of *C. darwinensis* (Figs. 7, 19, 27), with its lobes not extended. Sequences from specimens of *C. costa* from the different hosts in different localities did not differ, even though the worms were separated by a linear distance of more than 2,800 km, or 3,400 km when calculated around the Cape York Peninsula. The mounted specimen from *S. argus* was slightly contracted but exhibited no morphological difference to those from *Selenotoca multifasciata*.

***Capitimitta* sp.**

Host: *Selenotoca multifasciata* (Richardson), spot-banded scat (Scatophagidae).

Locality: Causeway Lake, Queensland, Australia, 23°12'00"S, 150°47'21"E.

Site: Intestine.

Material: No mounted specimen; representative DNA sequence, partial 18S, entire ITS region, partial (D1–D3) 28S: GenBank accession no. KC206499, from single immature specimen from Causeway Lake.

Remarks

A single immature specimen was sequenced from *Selenotoca multifasciata* collected at the outlet of Causeway Lake in Queensland, Australia. The sequence obtained from this specimen matched neither the sequence of *C. darwinensis* n. sp. nor *C. costata* n. sp., and we think it represents an undescribed species. We include it for molecular comparisons and analysis, as well to encourage others to find and describe it.

Species inquirendae

***Waretrema piscicolum* of Velasquez (1961) (Fig. 9)**

This species, reported as *W. piscicolum*, from the scatophagid *Scatophagus argus* in fish ponds at Bulacan and Luzon Island in the Philippines (Velasquez, 1961), appears to belong to *Capitimitta*. We examined three deposited specimens on one slide (USNPC 039476.00), and they conformed with the diagnosis of *Capitimitta*; however, because of the extreme pressure applied to the specimens when fixed, we cannot with confidence assign its specific status. Eggs measured 60–68 by 23–30 μm , or similar in size to eggs of *C. costata*, but other features prevented us from designating them as conspecific. We think these specimens represent an undescribed species, since the arms of the excretory vesicle extend anteriorly to the pharynx, compared with not reaching into the prepharyngeal region as in *C. darwinensis* n. sp. or *C. costata* n. sp. In these Philippine specimens, the oral sucker (Fig. 9)

does not appear as though it would be as large, proportionally to body size, as in *C. darwinensis* (Figs. 8, 19) if not flattened, but the lobe-like structures appear embedded, as in the two species we describe. The caeca of the Philippine specimens appear to be less robust than in *C. darwinensis* and *C. costata*, although the flattening of specimens causes a significant shifting of the position of the organs (Fig. 24).

***Waretrema piscicolum* of Liu & Yang (2003)**

This species, reported as *W. piscicolum* from the scatophagid *Scatophagus argus* off Zhanjiang, China (South China Sea) (Liu & Yang, 2003), also appears to belong to *Capitimitta*. The thorough description of this worm and the accompanying illustration (Liu & Yang, 2003) appear to provide an accurate record. The specimens are very similar to those of Velasquez (1961), and, although unlikely, may represent the same species. Specimens of Velasquez are generally larger (1.25–3.15 vs. 0.96–2.24 mm), the oral sucker reported by Liu & Yang appears to be smaller proportionally in relation to the ventral sucker and body size, and the maximum measurement of the ventral sucker diameter is 296 μm in specimens reported by Liu & Yang (2003) and 240 μm in those measured by Velasquez (1961) despite Velasquez's largest specimen being 1.4 times larger than any measured by Lui & Yang.

***Waretrema piscicolum* of Bilqees (1980)**

This species from *Scatophagus argus* off Karachi, Pakistan, was identified as *W. piscicolum* despite clear differences in its oral sucker (Bilqees, 1980, fig. 7; Bilqees, 1981, same figure with additional measurements). Also, the figured specimen showed the intestinal bifurcation located immediately posterior to the pharynx and an indistinct esophagus. Our attempts to borrow the submitted specimens were unsuccessful. We consider this species to represent an undescribed member of *Capitimitta*.

Species incertae sedis

***Waretrema piscicolum* of Gupta & Miglani (1976)**

Although the record of *W. piscicolum* from an unidentified marine fish off Port Blair (Andaman and Nicobar islands), India, by Gupta & Miglani (1976) provides a scant description, their figure 13 shows a subspherical oral sucker with some type of lobe-like apparatus anteriorly and a pharynx broader than long. These features suggest that this record may represent what we accept as *W. piscicolum*. Although the authors stated that the terminal part of the hermaphroditic duct was protrusible, the duct does not protrude in any healthy fixed specimens of *Waretrema*, *Spiritestis*, or *Capitimitta*; we have seen this condition in other haploporid species when they have been outside the host too long in a nonisotonic solution. Because of the lack of a host identity and questionable state of the described specimens, we consider the material reported by Gupta & Miglani (1976) as *incertae sedis*.

Additional material of species of *Capitimitta* from off China, the Philippines, and Pakistan infecting *Scatophagus argus* and the specimens reported by Gupta & Miglani (1976) from a marine fish in the Andaman and Nicobar islands all require recollection.

Molecular data

We compared DNA sequence data from three species of *Capitimitta* and from *Spiritestis herveyensis*. The fragment sequenced encompassed the 3' end of the 18S gene, the ITS region (ITS1 + 5.8S + ITS2) and c. 1,300 bp of the 5' end of the 28S gene, because this region has been shown to be suitable for species differentiation and phylogenetic analysis (Nolan & Cribb, 2005; Olson & Tkach, 2005; Parker et al., 2010; Tkach et al., 2010). The total length of the region sequenced and used for species discrimination was 2,342 bp in *C. costata*, 2,477 bp in the undescribed *Capitimitta* sp., 2,507 bp in *C. darwinensis* and 2,543 bp in *S. herveyensis*. No intraspecific variation was found in cases when sequences were obtained from multiple individuals of each species. The length differences for different species resulted primarily from indels of various lengths in the ITS1 region (Table 3). For species of *Capitimitta*, the percent variation of the values (Tables 3, 4) were 5.7–11.1% in ITS1, 0.6–1.9% in 5.8S, 7.3–11.0% in ITS2, and 2.7–2.8% in the partial 28S, all of which were consistent with the intrageneric variation found by Blasco-Costa et al. (2009) for species of *Dicrogaster* Looss, 1902 and *Saccocoelium* Looss, 1902. *Capitimitta* spp. differed from *S. herveyensis* (Tables 3, 4) by 17.4–30% in ITS1, 1.3–3.8% in 5.8S, 14.8 to 17.7% in ITS2, and 10.0–10.6% in the 28S. Levels of intrageneric variation reported by Blasco-Costa (2009) are comparable for the ITS2 and higher for the partial 28S regions for species of haploporines but lower than those in species that they considered to be in separate subfamilies. Our values are consistent with our proposal for the separate generic status for *Capitimitta* and *Spiritestis*.

The BI analysis of partial 28S rDNA gene sequences (Fig. 32) included the outgroup *Paragonimus westermani* and two species of the Atractotrematidae, in addition to the 15 species of the Haploporidae. The ingroup of haploporids formed a monophyletic clade. *Hapladena nasonis* appeared to be well supported as basal to the other haploporids. The other 14 haploporid species formed two clades, one composed of the genera *Dicrogaster*, *Lecithobotrys* Looss, 1902, *Haploporus* Looss, 1902 and *Saccocoelium*, and the other formed by *Forticulcita* Overstreet, 1982, *Spiritestis*, *Saccocoelioides*, and *Capitimitta*. *Forticulcita* was basal to a polytomy of the three genera *Spiritestis*, *Saccocoelioides*, and *Capitimitta*. The three species of *Capitimitta* formed a highly supported clade. Because of the high degree of variation among species for available ITS2 sequences, the resulting alignment was highly ambiguous due to indels and left too few informative sites. After the exclusion of ambiguous regions of the alignment, reliable conclusions still could not be made, although the three species of *Capitimitta* formed a clade (data not presented here).

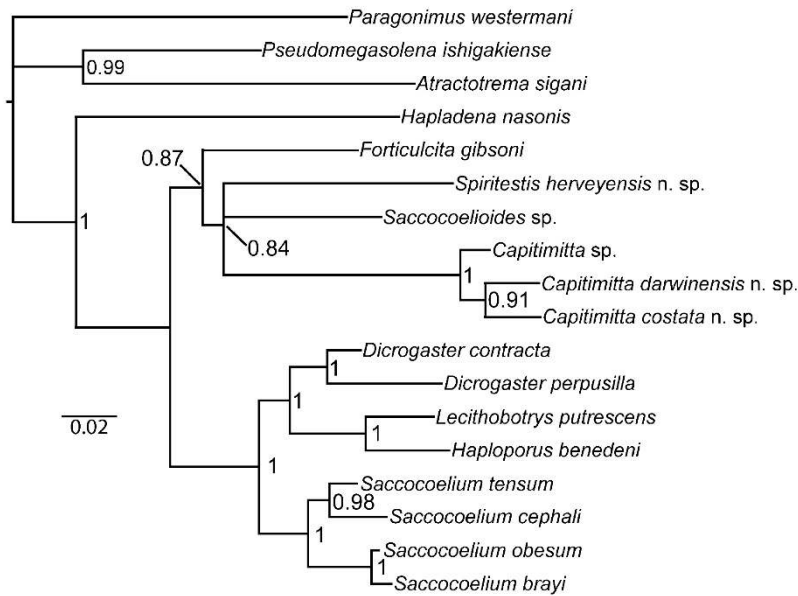


Figure 32. Bayesian Inference tree of phylogenetic relationships of the Haploporidae using partial 28S rDNA sequences with *Paragonimus westermani* as the outgroup. Posterior probability score given at the nodes (see Table 1 for accession numbers).

Key to the species of the Haploporidae with an ornamented oral sucker

- 1a. Vitellarium composed of numerous ([40] small follicles (Figs. 10, 12, 14); in Mugilidae *Spiritestis* Nagaty, 1948 ... (2)
- b. Vitellarium composed of few (c. 12) large tubular rod-like structures (Figs. 19, 20, 24, 29) 4
- 2a. Oral sucker with first pair of oral lobes (ventral pair) directed posteriorly toward oral opening, forming “W” on the posterior margin of oral sucker (Figs. 5, 12); body elongate oval; caeca sac-like *Spiritestis machidai* n. sp.
- b. Oral sucker with ventral pair of lobes directed anteriorly, with lobes forming “M” shape along the anterior rim of the sucker (Figs. 2); body elongate; caeca long, relatively narrow 3
- 3a. Genital pore located at posterior margin of pharynx (Fig. 10); from Red Sea *S. arabii* Nagaty, 1948
- b. Genital pore at level of pharynx (Fig. 14); from Australian waters ... *S. herveyensis* n. sp.
- 4a. Oral sucker with six retractable lobes (Fig. 1); lobes with spines; infecting Mugilidae *Waretrema piscicolum* Srivastava, 1937
- b. Oral sucker with six muscular, nonretractable structures embedded within anterior portion of oral sucker (Figs. 7–9, 19–20, 24, 27, 29), lacking spines on ventral surface of sucker (Figs. 27–28); infecting Scatophagidae *Capitimitta* n. g. (5)

- 5a. Vitelline follicles commencing less than half one ovarian length posterior to ventral sucker (Figs. 19–20); pharyngeal length < 105% of width; eggs 58–64 × 29–36 μm
 *C. darwinensis* n. sp.
- b. Vitelline follicles commencing more than one ovarian length posterior to ventral sucker (Fig. 29); pharyngeal length > 130% of width; eggs 67–70 × 27–31 μm
 *C. costata* n. sp.

Discussion

The status of the type-species of *Waretrema* is uncertain, as discussed above, and the only possible report, other than the original description, is by Gupta & Miglani (1976). They reported the host as a “marine fish” but illustrated the broad pharynx and nature of the oral sucker similar to that originally illustrated for *W. piscicolum* by Srivastava (1939). The specimens of Machida (1996) attributed to *W. piscicolum* clearly represented a species of *Spiritestis* based on the oral sucker, pyriform pharynx, and vitellarium with numerous follicles. Despite the contracted state of the specimens described by Nagaty (1948) as *S. arabii*, and with the addition of our Red Sea specimens also from mullet, the general nature of the oral sucker, pharynx, and vitellarium shows this species to be considerably different from those we which we have placed in *Capitimitta* n. g. The other three records of *W. piscicolum* were reported as hosted by scatophagids. Molecular data from *S. herveyensis* n. sp. support the decision to resurrect *Spiritestis*. The reports from scatophagids by Velasquez (1961), Bilqees (1980), and Liu & Yang (2003) all involve material that has the general characteristics of *Capitimitta*, and most likely include undescribed species. The absence of subsequent records of species of *Waretrema*-like haploporids from other locations and from mullets is perplexing. The illustration and description by Srivastava (1939) show an almost *Capitimitta*-like oral sucker, and the shapes of the vitellarium and pharynx are similar. In subsequent reports, no species referable to *Waretrema* or *Capitimitta* has been reported from a mullet.

Species of *Spiritestis* have been reported from the mullets *Moolgarda seheli* and *Crenimugil crenilabis*. Specimens morphologically identified as *M. seheli*, *C. crenilabis*, unidentified specimens of *Moolgarda* spp. and *Valamugil* spp. from many areas have been found to form a monophyletic clade, probably comprising five species (Durand et al., 2012). Specimens identifiable as *M. seheli* from Australian waters (host to *S. herveyensis* n. sp.) belonged in the same clade but were distinct from the other members identifiable as *M. seheli* from the other areas and identified as *C. crenilabis*. This distinctness in hosts further supports a difference in the species of *Spiritestis*. Based on their hosts, species of *Spiritestis* appear more closely related to the species of *Waretrema* than to species of *Capitimitta*. Moreover, we also collected specimens of the more distantly related *Liza vaigiensis* from the waters of Western Australia, Northern Territory, and Queensland and found no species of haploporid with ornate muscularization in the region of the oral sucker.

Specimens of *Capitimitta* that we collected all occurred in the scatophagids *Selenotoca multifasciata* and *Scatophagus argus* measuring under 12 cm, and each infected fish harbored only a few individuals. Only nine specimens of *Selenotoca multifasciata* and no specimen of *Scatophagus argus* larger than 12 cm were examined, but many specimens of *Selenotoca multifasciata* between 12 and 24 cm, in some cases even caught in the same throw of the castnet

as the small infected individuals, harbored numerous trematodes other than members of *Capitimitta*. Known lifecycles of waretrematines involve the ingestion of cercariae (Sheena & Janardanan, 2007) or metacercariae (Diaz et al., 2009; Shameem & Madhavi, 1991; Tang & Lin, 1979). As the life-cycle for a species of *Capitimitta* is unknown, we only can speculate that the pattern involves diet change by the host related to growth or competition among these and other trematode species; regardless, species of *Capitimitta* seem to be replaced with other trematodes in large fish. The gut contents of small *S. multifaciata* consisted mostly of filamentous algae, and those of large individuals consisted of leaf-like matter (EEP, pers. obs.). No report of material that we consider belongs to *Capitimitta* in scatophagids (Velasquez, 1961; Bilqees, 1980; Liu & Yang, 2003) indicated host sizes but perhaps exhaustive sampling of large fish would produce infections of *Capitimitta*. Future life-cycle work involving the first intermediate host of a species of *Capitimitta* should begin with snails of the Rissoidae and Potamididae, families found to host other haploporids, that inhabit filamentous algae and mangrove habitats in areas where species of *Capitimitta* have been found in scatophagids.

We used a partial 28S alignment of the three species of *Capitimitta*, including the unnamed species represented by juveniles, and *Spiritestis herveyensis*, all waretrematines, along with some available haploporid sequences from GenBank (Table 1). *Hapladena nasonis* appears basal in the haploporid clade, in agreement with Blasco-Costa et al. (2009). Blasco-Costa et al. (2009) proposed the new subfamily Forticulcitinae for Forticulcita because of the paraphyletic classification of the haploporines if Forticulcita is included. We agree that Forticulcita does not belong to the subfamily Haploporinae, but, because of the paucity of genera available for molecular analysis, there remains considerable uncertainty whether the subfamily framework of Overstreet & Curran (2005) is valid or an artificial arrangement.

The result of incorporating sequence data for *S. herveyensis* and *Capitimitta* spp. with previously published sequences and analyses by BI (Fig. 32), shows that *Capitimitta* and *Spiritestis* are no more related to each other than they are to *Saccocoelioides*; consequently, we could consider both *Forticulcita* and *Saccocoelioides* as members of Waretrematinae or we could propose a distinct subfamily for either *Spiritestis* or *Capitimitta*, although none of these proposals seem warranted until more genera of the Haploporidae are analyzed. We view the morphologically based subfamilial placements proposed by Overstreet & Curran (2005) with skepticism, because molecular data are now available for eight of about 29 genera but for only two type-genera, and these do not emphasize geography and convergent evolution. From a geographical and morphological standpoint, the close grouping of *Dicrogaster*, *Lecithobotrys*, *Haploporus*, and *Saccocoelium* seems logical, and all fit well into the Haploporinae. The monophyly of this portion of the clade may result from diversification within the Mediterranean Sea, but other currently accepted haploporines may not fit well with this group. The absence of available species from areas outside the Mediterranean Sea, and of other genera placed within the Haploporinae, may represent a monophyly of portions of the clade, resulting from diversification within the Mediterranean Sea and not accounting for the diversity of species currently regarded as haploporines according to Overstreet & Curran (2005). Until more type-species and representative haploporid and haploporid-like species are sequenced and analyzed, any major revision or

change should proceed with caution. At the current time, five haploporid subfamilies are recognized, but sequence data for the type-genera exist for only *Forticulcita* and *Haploporus*. We emphasize this uncertainty by separating the waretrematine species of *Capitimitta*, *Spiritestis*, and *Waretrema*. Based on published records, descriptions, and systematic treatments, we would have incorrectly expected species attributed to *Spiritestis* and *Capitimitta* to be closely related. The appearance of the superficial similarity of the oral sucker is not the result of shared evolutionary history but rather the ornate muscularization seems to be acquired at least twice. Also, we do not think *W. piscicolum* has been convincingly reported since the original description by Srivastava (1937, 1939), and *W. piscicolum (sensu stricto)* may not be closely related to either *Spiritestis* or *Capitimitta*. Using either *Spiritestis* or *Capitimitta* as a surrogate in phylogenetic treatment of the subfamily is not advisable, as one or both genera may not be members of the Waretrematinae. *Waretrema* shares more characteristics in common with *Capitimitta* than with *Spiritestis*, but the affiliation with the definitive hosts would suggest a closer relationship with *Spiritestis*.

The present study demonstrates the poorly understood nature of the species diversity of haploporids and the importance of proper methods for fixing and preserving trematodes. For reliable morphological differentiation of haploporid genera and species, specimens for comparison should be killed with hot but not boiling water, without pressure. Other hot fixatives also produce good results but are more noxious than hot tap water. With species that possess an ornamented oral sucker, killing with pressure precludes full appreciation of the nature of the oral apparatus (Figs. 8–9, 10, 12, 24) and leads to erroneous interpretations of those features as well as causing shifts in the position of some internal organs (Fig. 24). However, pressure applied to a few additional specimens, not used for measurements or the precise location of their organs, allows the most critical interpretation of the terminal genitalia and the female complex. For example, problems arising from not using hot fixatives and the improper handling of specimens has led to several misidentifications of species as *W. piscicolum*. Molecular data should be collected whenever possible to accompany fixed and mounted specimens and to produce phylogenies.

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