

1 RH: Andres et al. – Systematics of marine Haploporoidea

2 **On the systematics of some marine haploporids (Trematoda) with the description of a new**
3 **species of *Megasolena* Linton, 1910**

4 Michael J. Andres^{1*}, Eric E. Pulis^{1,2}, Stephen S. Curran¹, and Robin M. Overstreet¹

5 ¹ Division of Coastal Sciences, The University of Southern Mississippi, 703 East Beach Drive,
6 Ocean Springs, MS 39564

7 ² Current address: Department of Science and Mathematics, Northern State University, 1200
8 South Jay Street, Aberdeen, SD 57401

9 * Corresponding author, michael.andres@usm.edu

10

11

12

13

14 **Highlights**

15 • A new species of *Megasolena* is described and molecular data is provided for the first
16 time for five other marine haploporids

17 • Molecular phylogenetics suggest the current framework of haploporid subfamilies
18 required revision and a new subfamily is proposed

19 • Haploporoids had a common marine ancestor with two testes and radiated with a shift to
20 euryhaline hosts that can act as ecological bridges

21

22 **Abstract**

23 *Megasolena mikra* sp. nov. is described from the queen angelfish, *Holacanthus ciliaris*
24 (Linnaeus), off Florida, USA. The new species can be differentiated from all other species of
25 *Megasolena* Linton, 1910 except *Megasolena littoralis* Muñoz, George-Nascimento, and Bray,
26 2017 in possessing testes that are smaller in diameter than the ovary. The new species can be
27 differentiated from *M. littoralis* in lacking tegumental spines and possessing oral sucker papillae.
28 Molecular data are provided for two species each of *Cadenatella* Dollfus, 1946, *Hapladena*
29 Linton, 1910, and *Megasolena* Linton, 1910. Bayesian inference analysis of concatenated
30 internal transcribed spacer region-2 (ITS2) and partial 28S rDNA sequences of 50 haploporoids
31 revealed 1) a monophyletic Atractotrematidae Yamaguti, 1939 sister to the rest of the
32 haploporoids tested; 2) a paraphyletic Megasoleninae Manter, 1935 – if *Hapladena* is included;
33 and 3) a monophyletic Cadenatellinae Gibson and Bray, 1982 sister to the 'mugilid' haploporids.
34 The 'mugilid' haploporids formed a monophyletic clade consisting of the subfamilies
35 Chalcinotrematinae Overstreet and Curran, 2005, Forticulcitinae Blasco-Costa, Balbuena,
36 Kostadinova, and Olson, 2009, Haploporinae Nicoll, 1914, and Waretrematinae Srivastava,
37 1937. Based on our analysis we restrict the Megasoleninae to include *Megasolena*,
38 *Vitellibaculum* Montgomery, 1957, and *Metamegasolena* Yamaguti, 1970, all of which have
39 species with two testes. To accommodate the former megasolenine taxa with a single testis, we
40 erect the Hapladeninae subf. nov. for species in *Hapladena* and tentatively, *Myodera*
41 Montgomery, 1957. Our results further support that haploporoids had a common marine ancestor
42 with two testes, and that members of the superfamily underwent diversification following a shift
43 from a primarily marine life history with eupericarian hosts to a more euryhaline one with
44 diadromous hosts (namely mullet).

45 **Keywords:** Haploporidae; phylogeny; *Cadenatella*; *Hapladena*; ITS region; 28S rRNA

46

47 **1. Introduction**

48 Members of the Haploporoidea Nicoll, 1914 parasitize marine, estuarine, and freshwater
49 teleosts, particularly species that are herbivorous or omnivorous. They are generally thought to
50 be united in possessing a hermaphroditic sac that encloses the terminal portion of the male and
51 female genitalia, and most members possess a single testis. The superfamily currently comprises
52 the Atractotrematidae Yamaguti, 1939 and the Haploporidae Nicoll, 1914 [1] as well as one
53 subfamily of uncertain placement, the Cadenatellinae Gibson and Bray, 1982 [2]. Overstreet and
54 Curran [3] reviewed the Haploporidae and accepted four subfamilies: the Haploporinae Nicoll,
55 1914; the Megasoleninae Manter, 1935 (syn. Scorpodicolinae Yamaguti, 1971); the
56 Waretrematinae Srivastava, 1937; and erected the Chalcinotrematinae Overstreet and Curran,
57 2005. Blasco-Costa et al. [4] erected a fifth subfamily, the Forticulcitinae Blasco-Costa,
58 Montero, Balbuena, Raga, and Kostadinova, 2009, based on morphological and molecular data, a
59 consideration that was further supported by Andres et al.[5] with the addition of molecular data
60 for three other taxa.

61 Recent phylogenetic hypotheses for members of Haploporoidea (e.g., [(5,6,7,8)]) have
62 focused on “typical” haploporids members of the Haploporinae, Chalcinotrematinae,
63 Forticulcitinae, and Waretrematinae that primarily parasitize mugilids, possess a single testis,
64 and are relatively small bodied - whereas the Megasoleninae only has a single representative,
65 *Hapladena nasonis* Yamaguti, 1970, with molecular data. The revelation that cadenatellids are
66 haploporoids was surprising, given that species of *Cadenatella* Dollfus, 1946 lack a
67 hermaphroditic sac, and demonstrated the need for better molecular representation of basal

68 haploporoids [2]. In subsequent phylogenetic hypotheses that have included species of
69 *Cadenatella*, the subfamily has not been clearly resolved within the superfamily but has
70 generally been closer to *H. nasonis* [8; 9].

71 Overstreet and Curran [3] accepted five genera within the Megasoleninae: *Megasolena*
72 Linton, 1910; *Hapladena* Linton, 1910 (syns. *Deredena* Linton, 1910; *Hairana* Nagaty, 1948);
73 *Vitellibaculum* Montgomery, 1957 (syn. *Allomegasolena* Siddiqi and Cable, 1960); *Myodera*
74 Montgomery, 1957 (syn. *Scorpidicola* Montgomery, 1957); and *Metamegasolena* Yamaguti,
75 1970. However, in the same review, they also suggested that the genera containing members
76 with two testes (*Megasolena*, *Metamegasolena*, and *Vitellibaculum*) may not belong within the
77 Haploporidae because all other haploporids described to date had a single testis. Presumably,
78 they believed that megasolenine genera possessing two testes may have a closer affiliation with
79 the Atractotrematidae. Atractotrematids also possess two testes, but they have a smaller and
80 more circular to fusiform body and have oblique testes rather than tandem to semi-oblique testes
81 [10]. Andres et al. [9] recently provided sequence data for three species of *Isorchis* Durio and
82 Manter, 1969, and Huston et al. [11] added data for another, bringing the total number of
83 attractotrematids with molecular data up to six species spanning three genera. The convoluted
84 taxonomic history of the Megasoleninae, the revelation of the Cadenatellinae within the
85 Haploporoidea, and the depauperate molecular representation of cadenatellines and
86 megasolenines compared with the typical or ‘mugilid’ haploporids (subfamilies with largely-
87 mugilid dwelling species) illustrate the need for increased molecular representation of those two
88 groups. Therefore, the purpose of this study is to 1) describe a species of *Megasolena* and
89 provide morphological data for additional basal haploporoids; 2) help clarify some of the deeper

90 phylogenetic relationships of the Haploporoidea; and 3) determine the validity of the
91 Megasoleninae (sensu Overstreet and Curran [3]).

92 **2. Methods**

93 *2.1 Sampling*

94 Specimens of *Cadenatella americana* Manter, 1949 and *Cadenatella floridae* Overstreet,
95 1969 were obtained from specimens of the Bermuda sea chub, *Kyphosus sectatrix* (Linnaeus),
96 off Long Key, Florida, USA, by baited hook-and-line in July 2012. During April 2009 dead and
97 moribund specimens of *Hapladena* were recovered from the doctorfish, *Acanthurus chirurgus*
98 (Bloch), from a fish market in Christiansted, St. Croix, U.S. Virgin Islands. Specimens of
99 *Megasolena hysterospina* (Manter, 1931) Overstreet, 1969 were obtained from the Western
100 Atlantic seabream, *Archosargus rhomboidalis* (Linnaeus), off Missouri Key and Marathon,
101 Florida, in November 2011 and July 2012 as well as from the Bermuda porgy, *Diplodus*
102 *bermudensis* Caldwell, from Harrington Sound, Bermuda. Finally, specimens we attribute to a
103 new species of *Megasolena* were collected from the intestine of the queen angelfish,
104 *Holacanthus ciliaris* (Linnaeus), from the Gulf of Mexico approximately 130 km south of
105 Apalachicola, Florida. Specific fish names follow those given by FishBase [12].

106 *2.2 Morphological material preparation*

107 Haploporoids were isolated in a similar manner to the method of Cribb and Bray [13],
108 fixed as outlined in Andres et al. [9], and preserved in 70% ethanol. Staining and mounting also
109 follow the methods outlined in Andres et al. [9]. Measurements were made using a compound
110 microscope equipped with a differential interference contrast and calibrated digital software
111 (iSolutions Lite Version 8.2 ©). All measurements are in micrometres. For the description of the
112 new species, data for the type-specimen are followed by the range of data for the other

113 specimens in parenthesis. We followed the terminology of Pulis and Overstreet [14] pertaining to
114 the hermaphroditic sac and its structures and use the same terminology for cadematellids despite
115 their lack of a hermaphroditic sac. Museum abbreviations are as follows: GCRLM, Gulf Coast
116 Research Laboratory Museum, Ocean Springs, MS, USA, USNM, Smithsonian National
117 Museum of Natural History, Washington, DC, USA, and USNPC United States National Parasite
118 Collection now housed at the USNM.

119 *2.3 molecular techniques and phylogenetic analyses*

120 Genomic DNA was extracted from one hologenophore sensu Pleijel et al. [15] of the new
121 species and *C. americana*, two hologenophores of *C. floridae*, one entire specimen each of
122 *Hapladena* cf. *varia* Linton, 1910 and *Hapladena acanthuri* Siddiqi and Cable, 1960, and three
123 entire specimens of *M. hysterospina* that were either fixed in cool 95% ethanol or heat killed
124 worms fixed in 70% ethanol. When an entire worm was used for DNA extraction, the individual
125 was first partially cleared in nuclease free water, photographed with the same scope mentioned
126 above, and then placed back into ethanol. Methods used for DNA extraction, PCR, and
127 sequencing as well as the primers used for PCR and sequencing follow those of Andres et al.
128 [5,9,16]. Amplification and sequencing targeted the 3' end of 18S rDNA gene, internal
129 transcribed spacer region (ITS)-1, 5.8S rDNA gene, ITS-2, and 5' end of the 28S rDNA gene
130 (including domains D1–D3). Contiguous sequences from the species were assembled using
131 Sequencher™ (GeneCodes Corp., Ann Arbor, Michigan, USA, Version 4.10.1) and submitted to
132 GenBank. Sequences of other haploporoids and outgroup species obtained from GenBank and
133 used in our phylogenetic analysis are presented in Table 1. Sequences for phylogenetic
134 estimation and pairwise comparison were aligned with MAFFT version 6 [26] with 1,000 cycles
135 of iterative refinement and the *genafpair* algorithm. For phylogenetic estimation, the alignment

136 was masked with ZORRO [27] using default settings and positions with confidence scores <0.4
137 were excluded. The monorchid *Hurleytrematoides chaetodoni* (Manter, 1942) Yamaguti, 1954
138 (GenBank number XXNNNNNN; voucher USNM TBD) was used as the outgroup based on its
139 position relative to the Haploporoidea [18] and because we know the ITS2 and 28S rDNA
140 sequence was obtained from the same specimen. Bayesian Inference analysis (BI) was performed
141 using MrBayes 3.1.2 software [28], and the best nucleotide substitution model was estimated
142 with jModeltest-2 [29] as general time reversible with estimates of invariant sites and gamma-
143 distributed among site-rate variation (GTR + I + Γ) for both the ITS2 and 28S region. The two
144 regions were partitioned and the analysis was run with the following model parameters: nst = 6,
145 rates = invgamma, ngen = 1,000,000 and samplefreq = 100. The first 25% of trees were
146 discarded by setting the “burnin” parameter at 2,500 generations, and nodal support was
147 estimated by posterior probabilities over 2,500 generations.

148 All pairwise comparisons of sequence data exclude gaps. All sequences used for pairwise
149 comparisons were trimmed to the shortest sequence.

150 **3. Results**

151 *3.1 Description*

152 *Megasolena mikra* sp. nov. Andres and Pulis (Fig. 1A–B).

153 Description (measurements based on 4 gravid wholemounts). Body elongate, cylindrical,
154 tapering posteriorly, 2,744 (2,558–3,038) long, 871 (792–986) wide at level of midbody
155 representing 32 (30–34)% of body length (BL). Tegument unspined. Forebody 755 (752–937)
156 long representing 28 (27–31)% of BL. Hindbody 1,730 (1,555–1,820) long representing 63 (60–
157 63)% of BL. Eyespot pigment diffuse, in anterior ½ of forebody. Oral sucker subglobular,
158 subterminal, 492 (419–528) long, 489 (454–545) wide, with inner rim surrounded by ring of 12

159 small papillae, with muscular ring at posterior margin. Ventral sucker 259 (235–289) long, 248
160 (231–291) wide. Ratio of oral sucker to ventral sucker widths 1:0.51 (0.51–0.53). Ratio of oral
161 sucker to ventral sucker lengths 1:1.90 (1.78–1.90). Prepharynx 34 (34–73) long. Pharynx
162 subglobular, contiguous with oral sucker, 408 (360–463) long, 388 (334–404) wide. Ratio of oral
163 sucker width to pharynx width 1:0.8 (1:0.7–0.8). Oesophagus 57 (48–73) long. Intestinal
164 bifurcation dorsal to level of ventral sucker, 799 (799–968) from anterior margin representing 29
165 (29–33)% of BL. Caeca long, approximately 8–12 times longer than wide, terminating blindly
166 237 (216–248) from posterior end, with postcaecal space representing 9 (7–9)% of BL.

167 Testes contiguous, slightly oblique, irregular, near midbody, intercaecal; anterior testis
168 231 (186–231) long, 198 (195–215) wide; posterior testis 228 (205–241) long, 189 (177–206)
169 wide. Post-testicular space 966 (759–979) long representing 35 (30–35)% of BL. External
170 seminal vesicle elongate, 73 (57–98) long, 15 (15–32) wide, posterior to ventral sucker.
171 Hermaphroditic sac thin-walled, anterodorsal to dorsal of ventral sucker, ovoid, 242 (234–330)
172 long, 291 (275–357) wide representing 93 (93–114)% of ventral sucker length; containing
173 terminal genitalia; internal seminal vesicle 129 (101–145) long, 81 (67–83) wide; prostatic bulb
174 elongate to subglobular, 56 (52–71) long, 40 (31–45) wide; male duct short, uniting with female
175 duct at midlevel of hermaphroditic sac; hermaphroditic duct eversible, approximately 1/2 length
176 of hermaphroditic sac. Genital pore medial, 31 (31–66) anterior to anterior margin of ventral
177 sucker.

178 Ovary irregular to longitudinally elongate, medial, intercaecal, contiguous with anterior
179 testis 247 (201–253) long, 262 (221–334) wide, 174 (174–201) from posterior margin of ventral
180 sucker. Mehlis' gland anterodorsal to ovary, 131 long (103–135), 204 (125–217) wide. Uterus
181 confined between level of anterior margin of ovary and hermaphroditic sac, mostly intercaecal.

182 Laurer's canal containing sperm, posterosinistral to Mehlis' gland, opening dorsally. Vitellarium
183 follicular, extending from near posterior margin of body to about midlevel of ventral sucker,
184 surrounding caeca, dorsal to reproductive organs, ventral to testes; vitelline reservoir dorsal to
185 ovary. Eggs thin-shelled, numerous, 72–76 (71–76) long, 29–32 (29–34) wide.

186 Excretory vesicle Y-shaped, branching near midlevel of ovary to posterior margin of
187 ovary; pore terminal.

188 Type-and only known host: *Holacanthus ciliaris* (Linnaeus), queen angelfish,
189 Pomacanthidae.

190 Type-Locality: West Florida Middle Grounds, Gulf of Mexico (28°32'37"N,
191 84°46'43"W), approximately 130 km south of Apalachicola, Florida, USA; from 59 m depth.

192 Site: Intestine.

193 Specimens deposited: Holotype USNM TBD; hologenophore USNM TBD; paratypes
194 USNM TBD (n=2).

195 Molecular Representative: Partial 18S, entire ITS region (ITS1, 5.8S, ITS2), partial (D1–
196 D3) 28S: GenBank accession no. MH244122 from 1 hologenophore and 1 entire immature
197 specimen (identical sequence to hologenophore).

198 Etymology: The Greek feminine “*mikra*” refers to the small size of the testes relative to
199 the ovary.

200 Remarks. *Megasolena mikra* sp. nov. can be differentiated from congeners in possessing
201 an ovary that is larger in diameter than the testes. It can be further differentiated from *M.*
202 *hysterospina*, *Megasolena dongzhaiensis* Liu, Zhou, Yu, and Liu, 2006, and *Megasolena*
203 *littoralis* Muñoz, George-Nascimento, and Bray, 2017 in having an aspinous tegument. The new
204 species can be further separated from *M. hysterospina* and *M. littoralis* in having testes located

205 further anterior (posttesticular space representing approximately 1/3 of the body length rather
206 than 1/4 or less of the body length). *Megasolena mikra* sp. nov. can also be differentiated from
207 *Megasolena acanthuri* Machida and Uchida, 1991 in possessing a ventral sucker that is smaller
208 than the oral sucker (oral sucker width to ventral sucker width ratio 1: 0.5 rather than 1: 1.3-1.7).
209 *Megasolena mikra* sp. nov. possesses testes that are contiguous rather than separated as in
210 *Megasolena kyphosi* Sogandares-Bernal, 1959. *Megasolena mikra* sp. nov. is differentiated from
211 *Megasolena estrix* Linton, 1910 in possessing a hermaphroditic sac that is approximately half as
212 large (in both length and width). No measurement of the hermaphroditic sac of *M. estrix* was
213 given by Linton [30], Manter [31], or Sogandares-Bernal [32]; thus, comparisons were made
214 based on the illustrations provided by those authors.

215 We also examined three other adult specimens of *H. ciliaris* from shallow water (<2 m)
216 off Grassy Key, Florida, but did not collect any haploporoids. Perhaps, this suggests that the new
217 species' life cycle occurs further off-shore, but additional hosts from the different habitats are
218 required to test this.

219 3.2 Molecular analyses

220 3.2.1 Sequence comparisons

221 The DNA sequence fragment lengths for the newly provided specimens are in Table 2.
222 No intraspecific variation was found in any of the replicates, when multiple specimens were
223 sequenced. Sequences of *M. hysterospina* and *M. mikra* sp. nov. did not differ in either the 3' end
224 of the 18S nor in the 5.8S, but they differed by 6 bp (1.1%) in the ITS1 and 9 bp (0.7%) in the
225 28S. Sequences of *C. americana* and *C. floridae* did not differ in the 3' end of 18S, differed by
226 49 bp (9.6%) in the ITS1, differed by 1 bp (0.6%) in the 5.8S, and 25 bp (9.8%) in the ITS2.
227 Sequences of *Hapladena* cf. *varia* and *H. acanthuri* differed by 2 bp (2.2%) in the 3' end of the

228 18S, by 23 bp (3.8%) in the ITS1, and 16 bp (5.7%) in the ITS2, but they did not differ in the
229 5.8S. Pairwise comparison of ITS2 sequence data for species of *Megasolena* are in Table 3, and
230 pairwise comparison of 28S sequence data for species of *Cadenatella* and species of *Hapladena*
231 are in Table 4 and Table 5, respectively.

232 3.2.2 Phylogenetic relationships among the Haploporoidea

233 The BI analysis of the concatenated rDNA sequences (ITS2 and partial 28S) of 50 taxa
234 resolved the Atractotrematidae as the strongly supported sister to the rest of the Haploporoidea
235 (Fig. 2). As observed in previous studies [8,9], the representative cadenatellids formed the sister
236 group to the mugilid haploporids (members of the subfamilies Haploporinae,
237 Chalcinotrematinae, Forticulcitinae, and Waretrematinae). Representatives of *Megasolena* and
238 *Hapladena* did not form a monophyletic clade separate from the rest of the haploporoids.
239 Therefore, the present estimation suggests that the Megasoleninae (if *Hapladena* is included) is
240 paraphyletic unless all other haploporids are considered megasolenines. As such, we advocate
241 that the Megasoleninae be restricted to haploporid species with two testes; the Cadenatellinae be
242 considered a subfamily of the Haploporidae; and that a new subfamily be established for species
243 possessing a single testis and formerly considered megasolenines.

244 Based on our phylogenetic analysis we recognize the Atractotrematidae, with the single
245 subfamily Atractotrematinae Yamaguti, 1939, and the Haploporidae containing the following
246 subfamilies: Haploporinae, Chalcinotrematinae, Forticulcitinae, Waretrematinae, Cadenatellinae,
247 and Megasoleninae, and establish a new subfamily to accommodate *Hapladena* and, tentatively,
248 *Myodera*.

249 3.3 Morphological review of treated subfamilies and additional data for studied basal 250 haploporids

251 Cadenatellinae Gibson and Bray, 1982

252 Diagnosis. Body elongate. Tegument spined. Oral sucker complex, with 8–10 lobes on
253 dorsal and dorsolateral margins. Ventral sucker approximately in anterior third to eighth of body
254 length. Accessory sucker absent or present, if present 1 or more. Prepharynx present, shorter to
255 longer than pharynx. Pharynx well developed. Oesophagus variable. Intestinal bifurcation
256 anterior to ventral sucker. Uroproct present. Testis single, elongate, ellipsoidal to irregular, in
257 anterior to mid-hindbody. Hermaphroditic-sac absent. Seminal vesicle sinuous, extending into
258 hindbody. Pars prostatica vesicular, short. Hermaphroditic duct short. Genital pore median,
259 anterior to ventral sucker, with one or more accessory suckers. Ovary entire, rounded to oval,
260 pretesticular. Uterus confined between ovary and hermaphroditic duct. Uterine seminal
261 receptacle present. Eggs thin-shelled. Vitellarium with numerous small follicles, restricted to
262 hindbody. Excretory vesicle reaches ovary or ventral sucker. In marine teleosts (*Kyphosus*).

263 Type-genus: *Cadenatella* Dollfus, 1946.

264 Remarks. *Cadenatella* was originally allocated to the Enenteridae Yamaguti, 1958 but
265 was resolved as a haploporoid using BI analysis of partial 28S rDNA sequences [2]. Our analysis
266 revealed *Cadenatella* as monophyletic and occupying a position on the tree that has been
267 consistent with recent molecular analyses [8,9]. Bray et al. [2] remarked that the Cadenatellinae
268 will likely be recognized at the family level once additional molecular data for more members of
269 the Haploporoidea were available; however, we believe that such a move at this time would be
270 premature as additional data for basal haploporids are needed.

271 In the diagnosis, we use the term 'hermaphroditic duct' rather than genital atrium as the
272 structure has been previously called. Species of *Cadenatella* are the only currently known
273 haploporoids that lack a hermaphroditic sac surrounding the terminal genitalia. Thus, their

274 inclusion in the Haploporoidea strongly suggests a secondary loss of the hermaphroditic sac wall
275 [2]; therefore, at least a portion of this structure is a hermaphroditic duct. The Cadenatellinae is
276 distinguished from all other Haploporoidea by its members lacking a hermaphroditic sac and
277 possessing an 8–10 lobed oral sucker (waretrematines with a lobed oral sucker have 6 lobes
278 [14]).

279

280 *Cadenatella americana* Manter, 1949

281 Type-host: *Kyphosus incisor* (Cuvier), yellow sea chub, Kyphosidae.

282 Other host: *Kyphosus sectatrix* (Linnaeus), Bermuda sea chub, Kyphosidae.

283 Type-locality: off Tortugas, Florida, USA.

284 Other localities: Biscayne Bay, Florida [34]; off Quintana Roo, Mexico [unpublished
285 report in 35]; off Long Key, Florida (24°50'22"N, 80°46'48"W).

286 Site: Intestine.

287 Holotype: USNM 1345544 (USNPC 46365).

288 Vouchers: Hologenophore USNM TBD, vouchers USNM TBD; GCRLM TBD:

289 Description based on 8 mature specimens (Fig. 3A). Body elongate, 1,999–3,965 long,
290 299–687 wide at level of final third of BL representing 13–19% of BL. Forebody 492–962 long
291 representing 17–26% of BL. Hindbody 1,301–2,801 long representing 65–75% of BL. Oral
292 sucker not retracted into forebody, 205–265 long, 170–237 wide. Ventral sucker 189–281 long,
293 178–306 wide. Ratio of oral sucker to ventral sucker widths 1: 0.96–1.33. Accessory sucker 62–
294 111 long, 62–118 wide. Prepharynx 100–336. Pharynx 119–325 long, 147–206 wide.

295 Oesophagus 11–26. Intestinal bifurcation 515–935 from anterior margin. Postcaecal space 38–99
296 representing 1–3% of BL. Uroproct present. Testis single, ellipsoidal, 389–830 long, 153–349

297 wide. Posttesticular space 611–1,299, representing 31–36% of BL. Ovary 93–153 long, 77–183
298 wide; 229–487 from posterior margin of ventral sucker, contiguous with to 57 from testis. Eggs
299 57–64 long, 28–36 wide.

300 Molecular Representative: Partial 18S, entire ITS region, partial (D1–D3) 28S: GenBank
301 accession no. MH244117 from 1 hologenophore.

302 Remarks. This species was described by Manter [33] as having a retracted oral sucker,
303 contracted forebody, and lateral outfoldings of the proximal portion of the prepharynx all of
304 which likely resulted from contraction of the body. Our specimens all have the oral sucker
305 protruded and a distinct prepharynx. Manter [33] also reported a small, inconspicuous, thin-
306 walled cirrus sac, and Overstreet [34] stated 'a thin membrane appears to surround the vesicle in
307 some wholemounds', a feature we did not observe in our specimens nor in any of the photographs
308 of the type-specimen. Perhaps a careful examination of living material or counter staining may
309 help determine if the hermaphroditic sac wall remains in some specimens of *C. americana*.

310

311 *Cadenatella floridae* Overstreet, 1969

312 Type-host: *Kyphosus sectatrix* (Linnaeus), Bermuda sea chub, Kyphosidae.

313 Type-locality: Biscayne Bay, Florida, USA.

314 Other localities: off Long Key, Florida, USA (24°50'22"N, 80°46'48"W).

315 Site: Pyloric caeca and intestine.

316 Holotype: USNM 1366878 (USNPC 71301).

317 Molecular Representative: Partial 18S, entire ITS region, partial (D1–D3) 28S: GenBank
318 accession no. MH244118 from 2 hologenophores (identical sequences).

319 Vouchers: ; Hologenophore USNM TBD, vouchers USNM TBD; GCRLM TBD

320 Description based on 7 mature lateral mounts (Fig. 3B). Body very elongate, 3,716–7,226
321 long, 217–397 deep at level of midbody representing 4–11% of BL. Hindbody 2,632–5,320
322 representing 71–74% of BL. Oral sucker 156–187 long, 104–132 deep, with 8 preoral lobes.
323 Ventral sucker 135–178 long, 108–158 deep, with ring of small papillae. Ratio of oral sucker to
324 ventral sucker depths 1: 1.00–1.25. Accessory suckers 8–10, extending midventrally from level
325 of posterior margin of pharynx to anterior margin of ventral sucker; smallest sucker first (most
326 anterior), 30–54 long, 26–47 deep; largest sucker second to last (7th–9th), 56–86 long, 57–76
327 deep. Prepharynx 268–536. Pharynx 166–259 long, 94–132 deep. Oesophagus 17–34 long.
328 Intestinal bifurcation 766–1,212 from anterior margin. Postcaecal space 61–99 representing 1–
329 2% of BL. Uroproct present. Testis single, ellipsoidal, 416–855 long, 94–167 deep. Posttesticular
330 space 1,363–2,562 representing 31–38% of BL. Ovary 105–177 long, 88–136 deep; 662–1,623
331 from posterior margin of ventral sucker, contiguous with to 91 from anterior margin testis. Eggs
332 46–62 long, 28–34 wide.

333 Remarks: Our specimens are slightly larger than those described by Overstreet [34] but
334 otherwise agree well with his description.

335

336 Megasoleninae Manter, 1937

337 Diagnosis. Body fusiform to elongate. Tegument thick, spined or unspined. Eyespot
338 pigmentation absent to diffuse. Oral sucker subterminal to terminal. Ventral sucker near anterior
339 third of body. Prepharynx variable. Pharynx large, well developed. Oesophagus variable. Caeca
340 saclike to cylindrical, wide, terminating blindly at level of posterior third of hindbody to near
341 posterior margin of body. Testes two, tandem to slightly oblique, contiguous to separated.
342 Hermaphroditic sac subglobular to elongate. External seminal vesicle elongate, narrow. Ovary

343 immediately pretesticular. Uterus confined between ovary and hermaphroditic sac. Eggs non-
344 filamented. Vitellarium with numerous small, large, or dendritic follicles, filling available space
345 or lateral fields of hindbody. Lymphatic system present. Excretory vesicle I- or Y-shaped. In
346 marine fishes.

347 Type-genus: *Megasolena* Linton, 1910.

348 Remarks. In addition to *Megasolena*, we consider species of *Metamegasolena* and
349 *Vitellibaculum* to belong in Megasoleninae. Overstreet and Curran [3] suggested that those three
350 genera may not belong in the Haploporidae, as they possess two testes rather than a single testis.
351 The subfamily can be differentiated from the other haploporoids, except the Atractotrematidae in
352 possessing two testes. Megasoleninae can be differentiated from the attractotrematids in
353 possessing tandem to slightly oblique testes rather than oblique testes as well as having a larger
354 body and a more robust tegument.

355 *Megasolena hysterospina* (Manter, 1931) Overstreet, 1969

356 syns. *Lepidauchen hysterospina* Manter, 1931; *Megasolena archosargi* Sogandares-
357 Bernal and Hutton, 1959.

358 Type-host: *Lagodon rhomboides* (Linnaeus), pinfish, Sparidae.

359 Other Hosts: *Archosargus probatocephalus* (Walbaum), sheepshead; *Archosargus*
360 *rhomboidalis* (Linnaeus), Western Atlantic seabream; *Diplodus bermudensis* Caldwell, Bermuda
361 porgy, all Sparidae.

362 Type Locality: off Beaufort, North Carolina, USA.

363 Other Locality: Bayboro Harbor, Tampa Bay, Florida, USA [36]; Biscayne Bay, Florida
364 [34]; Jamaica [37]; Florianópolis, Santa Catarina, Brazil [38]; Rio de Janeiro State, Brazil [39];
365 coastal zone of Rio de Janeiro [40]; Little Duck Key, Florida (24°40'47"N, 81°14'5"W);

366 Marathon, Florida (24°46'31"N, 80°55'46"W); Harrington Sound, Bermuda (32°19'23"N,
367 64°44'12"W).

368 Site: Intestine.

369 Holotype: USNM 1321180 (USNPC 8432).

370 Material Examined: 6 specimens from off Marathon, Florida; 5 specimens from
371 Harrington Sound, Bermuda.

372 Vouchers: USNM TBD; GCRLM TBD.

373 Molecular Representative: Partial 18S, entire ITS region, partial (D1–D3) 28S: GenBank
374 accession no. MH244121 from 1 entire specimen from Harrington Sound, Bermuda; 2 immature
375 specimens from off Marathon, Florida (all identical sequences).

376 Remarks. The specimens we collected off Florida and Bermuda morphologically and
377 morphometrically conform to the descriptions of Manter [41], Overstreet [34], and Amato [38].
378 One of our mature specimens (USNPC TBD) is shorter than all other accounts (1,031µm) and
379 the absolute measurements of all structures reflect the smaller body size as well. All specimens
380 are attenuated in the forebody at the level of the pharynx and possess an external seminal vesicle
381 that is 2–3 times longer than the internal seminal vesicle. We agree with Overstreet [34] in
382 considering *M. archosargi* as a junior synonym of *M. hysterospina*, based on morphological
383 features, geography, and host similarity.

384 Fischthal and Williams [42] reported one adult and three immature specimens of *M.*
385 *hysterospina* from the West African spadefish, *Chaetodipterus lippei* Steindachner (Ephippidae),
386 from the Sierra Leone River estuary, Sierra Leone. We have examined their specimens (USNM
387 1363273 and 1366373) and believe that these specimens likely represent an undescribed species
388 of *Megasolena* based on the narrower body of the adult, presence of tegumental spines around

389 the excretory pore, and differences in the final host and geographic location. We refrain from
390 describing this species on the basis of a single individual because of the morphological variation
391 observed for *M. hysterospina* throughout its range.

392

393 Hapladeninae subf. nov.

394 Diagnosis. Body variable in shape, generally elongate. Eyespot pigment generally absent.
395 Tegument thick, with or without spines. Oral sucker subterminal to terminal. Ventral sucker in
396 anterior third of body, pedunculate or not. Prepharynx variable, generally less than to equal
397 pharynx length. Pharynx well developed. Oesophagus equal to or longer than pharynx. Caeca
398 cylindrical, terminating blindly near posterior end of body, forming cyclocoel, or uroproct. Testis
399 single, spherical to elongate, smooth to irregular, located in midhindbody or more posteriorly.
400 Hermaphroditic sac elongate to saccular. External seminal vesicle cylindrical, elongate, generally
401 longer than hermaphroditic sac. Ovary pretesticular in hindbody. Uterus confined to region
402 between ovary and hermaphroditic sac. Eggs operculate or not, filamented or not. Vitellarium
403 with numerous small follicles; follicles can be elongate, coalesced as tubules, or in rosette
404 pattern, usually fill entire available space in hindbody, generally restricted to hindbody.
405 Lymphatic system present or not. Excretory vesicle I-shaped. In marine fishes.

406 Type-genus: *Hapladena* Linton, 1910 (syns. *Deredena* Linton, 1910; *Hairana* Nagaty,
407 1948).

408 Type-species: *Hapladena varia* Linton, 1910.

409 Remarks. We consider Hapladeninae subf. nov. to include *Hapladena* and, tentatively,
410 *Myodera*, the only genera with members containing a single testis considered in the
411 Megasoleninae by Overstreet and Curran [3]. The name Scorpodicolidae Yamaguti, 1971 is

412 available; however, we have chosen to use a derivative of *Hapladena* because we only
413 tentatively consider *Myodera* to have a close affinity with *Hapladena*. Species of *Myodera* have
414 features that may suggest they are closer aligned to species of *Cadenatella*; namely the
415 possession of an uroproct and kyphosid final hosts. Because cadenatellines were only recently
416 resolved within the Haploporidae, it is difficult to discern how important the lack of a
417 hermaphroditic sac is as a character. Overstreet and Curran [3] considered the presence of an
418 uroproct to be a generic character, and for the sake of parsimony, we have elected to tentatively
419 include the two species of *Myodera* within the new subfamily. Depending on the true position of
420 *Myodera*, Scorpodicolinae has priority over both Cadenatellinae and Hapladeninae subf. nov.;
421 therefore, future workers will have to correct the subfamilial classification for the marine, single
422 testis subfamilies once molecular data for members of the genus are available (unless species of
423 *Myodera* represent a distinct clade).

424 The Hapladeninae subf. nov. can be differentiated from the Megasoleninae in having a
425 single testis. It can be differentiated from Cadenatellinae in possessing a hermaphroditic sac
426 enclosing the terminal genitalia, lacking oral lobes, and lacking accessory suckers (in part). The
427 Hapladeninae subf. nov. is differentiated from the rest of the haploporids with a single testis (the
428 ‘mugilid’ haploporids) in having a larger body size (in part), a robust tegument (in part), lacking
429 oral lobes (in part), and being in marine eupercarians (in part), primarily acanthurids, scarids,
430 pomacanthids, and kyphosids.

431

432 *Hapladena acanthuri* Siddiqi and Cable, 1960.

433 Type-Host: *Acanthurus coeruleus* Bloch and Schneider, blue tang surgeonfish,

434 Acanthuridae.

435 Other host: *Acanthurus chirurgus* (Bloch), doctorfish, Acanthuridae.
436 Type Locality: off Parguera, Puerto Rico.
437 Other Locality: off Desecheo Island, Puerto Rico [43]; off St. Croix, U.S.V.I. (host
438 purchased at a fish market).
439 Site: Intestine.
440 Holotype: USNM 1339799 (USNPC 39346).
441 Voucher: USNM TBD.
442 Molecular Representative: Partial 18S, entire ITS region, partial (D1–D3) 28S: GenBank
443 accession no. MH244119 from 1 entire dead specimen from St. Croix, U.S.V.I.
444 Remarks. Our specimens match the description of *H. acanthuri* by Siddiqi and Cable
445 [44], but have slightly smaller ventral and oral suckers in terms of absolute values but not in
446 relative values (ratio). *Hapladena acanthuri* is differentiated from all other species of *Hapladena*
447 except *Hapladena tanyorchis* Manter and Pritchard, 1961 in possessing dendritic vitellaria.
448 *Hapladena acanthuri* can be separated from *H. tanyorchis* based on a shorter, smooth testis
449 rather than an irregular and elongate testis. Additionally, *H. tanyorchis* was described off
450 Hawaii, USA, whereas *H. acanthuri* was described from the Caribbean Sea.
451
452 *Hapladena varia* Linton, 1910
453 Type-Host: *Acanthurus chirurgus* (Bloch), doctorfish, Acanthuridae.
454 Other hosts: *Acanthurus bahianus* Castelnau, ocean surgeonfish; *Acanthurus coeruleus*
455 Bloch and Schneider, blue tang surgeonfish, both Acanthuridae.
456 Type Locality: off Dry Tortugas, Florida, USA.

457 Other Locality: off Tortugas [45]; Drowned Cays, Belize [46]; off Jamaica [47]; off St.
458 Croix, U.S.V.I.

459 Site: Intestine.

460 Syntypes: USNM 1321261, 1321262 (USNPC 8513, 8514).

461 Molecular Representative: Partial 18S, entire ITS region, partial (D1–D3) 28S: GenBank
462 accession no. MH244120 from 1 entire specimen from St. Croix, U.S.V.I.

463 Remarks: Unfortunately, we do not possess a voucher specimen for *H. cf. varia* as only
464 one dead specimen of a large morphotype of *Hapladena* was obtained from the intestinal tract of
465 *A. chirurgus*. Based on initial examination, a photograph of the specimen prior to sequencing, its
466 final host, and comparison with the type material, this species is close to *H. varia*. Randall [48]
467 (and subsequently reported by Palm and Bray [49]) reported *H. varia* from the Convict
468 Surgeonfish, *Acanthurus triostegus* (Linnaeus), off the Hawaiian Islands. We believe it is
469 unlikely that these specimens represent *H. varia* based on the disparate geographical locations.
470 Interestingly, Manter and Pritchard [50] described *Hapladena spinosa* Manter and Pritchard,
471 1961 from the same host and same general locality (Hawaii), but did not mention the specimens
472 collected by Randall [48]. Although we cannot say for certain, it is likely that the specimens
473 collected by Randall [48] are *H. spinosa*.

474 *3.4 Other taxonomic considerations*

475 *Hapladena invaginata* Caballero, 1987 *nom. nud.*

476 Specimen Deposited: Institute of Biology, National Autonomous University of Mexico,
477 National Collection of Helminths, Mexico City, Mexico no. 2539.

478 Remarks. This species was never described (pers. comm. Gerardo Pérez-Ponce de León).
479 It was reported by Caballero et al. [51] and cited by Overstreet et al. [52]. Additionally, based on
480 pictures of the specimen sent by Gerardo Pérez-Ponce de León it resembles *H. varia*.

481

482 Species transferred to other genera

483 *Parasaccocoelium gymnocephali* (Sheena and Janardanan, 2007) comb. n.

484 syn. *Hapladena gymnocephali* Sheena and Janardanan, 2007.

485 Remarks. Sheena and Janardanan [53] described and elucidated the life cycle of this
486 species from the Chaliyar and Kadalundi rivers in Kozhikode, India. The first intermediate host
487 is a freshwater gastropod, *Gabbia travancorica* (Benson), and the final host is an estuarine
488 perciform, *Ambassis gymnocephalus* (Lacepède). The inclusion of fresh and estuarine hosts in
489 the life cycle, combined with a relatively small body length, restricted uterus, extensive vitelline
490 field, and paired caeca strongly suggest that this species belongs in the Waretrematinae.

491 *Hapladena gymnocephali* has morphological characters in common with *Parasaccocoelium*
492 Zhukov, 1971 that Overstreet and Curran [3] considered a junior synonym of *Pseudohapladena*
493 Yamaguti, 1952, but was recently restored by Besprozvannykh et al. [6]. Morphologically, *H.*
494 *gymnocephali* possesses characters in line with the diagnosis of *Pseudohapladena* by
495 Besprozvannykh et al. [6], namely vitelline fields along the lateral margin that do not unite
496 posttesticularly, a uterus that is short and contains few eggs, and a testis that is longitudinally
497 elongate. Besprozvannykh et al. [6] consider members of the genus to only infect mugilids, but
498 host switching within the Waretrematinae seems to be common across genera [3,14]. Thus, we
499 do not consider a perciform rather than mugilid host to be of significance for its generic

500 affiliation, whereas this species, if maintained in *Hapladena* would represent the only species of
501 fresh water or estuarine fish.

502

503 **4. Discussion**

504 Based on the results of our phylogenetic analysis, the interrelationships of basal
505 haploporids are more complex than the morphological subfamilial considerations *sensu*
506 Overstreet and Curran [3]. Overstreet and Curran [3] stated their own reservations about
507 including species with either one or two testes in their concept of the Megasoleninae. Our
508 molecular analysis supported that reservation, and we felt confident that the Megasoleninae
509 required revision despite the limited molecular representation of basal haploporids in our
510 analysis. However, the limited sample size of this study precludes us from making more
511 substantial revisions. Future researchers may prefer to restrict the Haploporidae to only those
512 subfamilies comprising the ‘mugilid’ haploporids and elevate the Megasoleninae, the
513 Hapladeninae subf. nov., and the Cadenatellinae to family level, or perhaps they may choose to
514 restrict the Haploporidae to include members possessing a single testis and elevate the
515 Megasoleninae. We believe that either action would be justified if our proposed subfamilial
516 concepts hold up to scrutiny by molecular analyses that include additional basal taxa (especially
517 species of *Myodera* and *Vitellibaculum*). In any event the haploporoids are a natural group.

518 The present phylogeny suggests that two testes and, perhaps, marine eupercharian fishes as
519 hosts represent the basal state for the superfamily. Regarding two testes, this seems likely given
520 the relative position of the Haploporoidea in general (e.g., [18]) and the basal position of
521 *Megasolena* relative to the rest of the haploporids. Most attractotrematids and megasolenines and
522 all haplidenines and cadenatellines parasitize marine eupercharians. Within the ‘mugilid’

523 haploporid clade, all four subfamilies include species that parasitize mugilids, and most genera
524 contain species that parasitize the flathead grey mullet, *Mugil cephalus* (Linnaeus). Ecologically,
525 this could be significant because *M. cephalus* is a cosmopolitan species throughout coastal
526 waters in tropical and temperate regions and many populations (or species [54, 55]) are
527 diadromous. Manter [56] was the first to suggest that mugilids were responsible for the diversity
528 of haploporids in estuarine and freshwater habitats because they are widespread and capable of
529 traveling between marine, estuarine, and freshwater habitats and thereby acting as “ecological
530 bridges.” Our phylogeny certainly seems to support the ecological bridge hypothesis and likely
531 explains why the basal lineages are depauperate relative to the mugilid haploporids.

532 Finally, our results may indicate that the Indo-Pacific Region is the center of origin for
533 the Haploporoidea. All known attractotrematids are from this region as are many of the basal
534 haploporids (Megasoleninae, Hapladeninae subf. nov., and Cadenatellinae). Although only a few
535 examples are included in our analysis, we observe that representatives from the Indo-Pacific are
536 resolved as sister to representatives from Atlantic seas in the Megasoleninae, Hapladeninae subf.
537 nov., Cadenatellinae, and Haploporinae. As more haploporoids have molecular data made
538 available, these and other hypothesis can be tested.

539 **5. Conclusion**

540 Over the past decade, beginning in earnest with Blasco-Costa et al. [4], molecular
541 techniques have helped to improve the systematics of the Haploporoidea, and our study further
542 helps frame the interrelationships of the superfamily. Within each of the haploporid subfamilies
543 we recognize, additional clarification is still needed. In addition to needing a better
544 understanding of the basal groups as discussed above, the Waretrematinae and
545 Chalcinotrematinae are in need of rigorous molecular investigation. For the Waretrematinae,

546 *Spiritestis herveyensis* Pulis and Overstreet, 2013 continues to be one of the most labile taxa;
547 *Unisaccus tonkini* Besprozvannykh, Atopkin, Ngo, Ha, Tang, and Beloded, 2017 was resolved as
548 a waretrematine in this study as it was in [25], despite *Unisaccus* Martin, 1973 morphologically
549 belonging in Haploporinae; and no molecular data are yet available for the type-species of the
550 subfamily, *Waretrema piscicolum* Srivastava, 1937. Similar to most of the subfamilies, the
551 Chalcinotrematinae also lacks molecular material for the type-species, *Chalcinotrema salobrense*
552 Freitas, 1947, but the Chalcinotrematinae is also of particular interest because it contains more
553 freshwater species and a broader host range (including mugilids) than the other subfamilies [3,8].

554 **Acknowledgements**

555 We thank the National Marine Fisheries Service Laboratory in Pascagoula, Mississippi, USA for
556 making sampling possible and continuing a productive collaboration. We are especially grateful
557 to William Driggers III, Christopher Gledhill, Marc Grace, Alonzo Hamilton, Michael Hendon,
558 Nick Hopkins, Walter Ingram, Lisa Jones, Adam Pollack, Kevin Rademacher, and the crew of
559 the NOAA ships *Gordon Gunter*, *Oregon II*, and *Pisces*. We thank Pat Pilitt, formally USNPC,
560 and Gerardo Pérez-Ponce de León (Universidad Nacional Autónoma de México) for providing
561 photographs of voucher specimens and Anna Phillips (USNM) for loaning specimens. We thank
562 the staff at the Keys Marine Laboratory for providing laboratory space for MJA and EEP and
563 Wolfgang Sterrer, Bermuda Biological Station, for providing laboratory space for RMO. From
564 the University of Southern Mississippi, we thank Jean Jovonovich and Janet Wright for their
565 assistance with DNA sequencing reactions. The material treated here is based on work supported
566 by the National Science Foundation under grant no. 0529684, Ocean and Human Health
567 Initiative grant no. NA08NOS4730322, USM University Research Council's Research, Scholarly,

568 and Creative Activity Award, and US Fish and Wildlife Service/Mississippi Department of Marine

569 Resources MSCIAF MS.R.798 Award M10AF20151.

570

571

572 **References**

- 573 [1] A. Jones, Superfamily Haploporoidea Nicoll, 1914, in: A. Jones, R.A. Bray, and D.I. Gibson
574 (Eds.), Keys to the Trematoda, Volume 2, CABI Publishing and the Natural History
575 Museum, London, 2005, pp. 127–128.
- 576 [2] R.A. Bray, T.H. Cribb, A. Waeschenbach, D.T.J. Littlewood, Molecular evidence that the
577 genus *Cadenatella* Dollfus, 1946 (Digenea: Plagiorchiida) belongs in the superfamily
578 Haploporoidea Nicoll, 1914, Syst. Parasitol. 89 (2014) 15–21.
- 579 [3] R.M. Overstreet, S.S. Curran, Family Haploporidae Nicoll, 1914, in: A. Jones, R.A. Bray,
580 and D.I. Gibson (Eds.), Keys to the Trematoda, Volume 2, CABI Publishing and the Natural
581 History Museum, London, 2005, pp. 129–165.
- 582 [4] I. Blasco-Costa, J.A. Balbuena, A. Kostadinova, P.D. Olson, Interrelationships of the
583 Haploporinae (Digenea: Haploporidae): A molecular test of the taxonomic framework based
584 on morphology, Parasitol. Int. 58 (2009) 263–269.
- 585 [5] M.J. Andres, S.S. Curran, T.J. Fayton, and R.M. Overstreet, An additional genus and two
586 additional species of Forticulcitinae (Digenea: Haploporidae), Folia Parasitol. 62 (2015) 025.
- 587 [6] V.V. Besprozyannykh, D.M. Atopkin, A.V. Ermolenko, A. Nikitenko, Restoration of the
588 genus *Parasaccocoelium* Zhukov, 1971 (Digenea: Haploporidae) and a description of two
589 new species from mugilid fish in the Far East of Russia, J. Helminthol. 89 2014 565–576.
- 590 [7] L. Andrade-Gómez, C.D. Pinacho-Pinacho, M. García-Varela, Molecular, morphological and
591 ecological data of *Saccocoelioides* Szidat, 1954 (Digenea: Haploporidae) from Middle
592 America supported the reallocation from *Culuwiya cichlidorum* to *Saccocoelioides*, J.
593 Parasitol. 103 (2017) 257–267.

- 594 [8] S.S. Curran, E.E. Pulis, M.J. Andres, R.M. Overstreet, Two new species of *Saccocoelioides*
595 (Digenea: Haploporidae) with phylogenetic analysis of the family, including species of
596 *Saccocoelioides* from North, Middle, and South America, *J. Parasitol.* 104 (2018).
- 597 [9] M.J. Andres, E.E. Pulis, R.M. Overstreet, Description of three species of *Isorchis* (Digenea:
598 Atractotrematidae) from Australia. *Acta Parasitol.* 61 (2016) 590–601.
- 599 [10] R.M. Overstreet, S.S. Curran, Family Atractotrematidae Yamaguti, 1939, in: A. Jones, R.A.
600 Bray, and D.I. Gibson (Eds.), *Keys to the Trematoda, Volume 2*, CABI Publishing and the
601 Natural History Museum, London, 2005, pp. 167–174.
- 602 [11] D.C. Huston, S.C. Cutmore, T.H. Cribb, *Isorchis cannoni* n. sp. (Digenea:
603 Atractotrematidae) from Great Barrier Reef rabbitfishes and the molecular elucidation of its
604 life cycle, *J. Helminthol.* (2017) <https://doi.org/10.1017/S0022149X17000906>.
- 605 [12] R. Froese, D Pauly, FishBase, <http://www.fishbase.org>, 2017 (accessed December 2017).
- 606 [13] T.H. Cribb, R.A. Bray, Gut wash, body soak, blender and heat-fixation: approaches to
607 effective collection, fixation and preservation of trematodes of fishes, *Syst. Parasitol.* 76
608 (2010) 1–7.
- 609 [14] E.E. Pulis, R.M. Overstreet, Review of haploporid (Trematoda) genera with ornate
610 muscularisation in the region of the oral sucker, including four new species and a new genus,
611 *Syst. Parasitol.* 84 (2013) 167–191.
- 612 [15] F. Pleijel, U. Jondelius, E. Norlinder, A. Nygren, B. Oxelman, C. Schander, P. Sundberg, M.
613 Thollessen, Phylogenies without roots? A plea for the use of vouchers in molecular
614 phylogenetic studies, *Mol. Phylogenet. Evol.* 48 (2008) 369–371.

- 615 [16] M.J. Andres, E.E. Pulis, T.H. Cribb, R.M. Overstreet, Erection of the haploporid genus
616 *Litosaccus* n. g. and its phylogenetic relationship within the Haploporidae Nicoll, 1914, Syst.
617 Parasitol. 89 (2014) 185–194.
- 618 [17] K.R. Devi, K. Narain, J. Mahanta, T. Nirmolia, D. Blair, S.P. Saikia, T. Agatsuma, Presence
619 of three distinct genotypes within the *Paragonimus westermani* complex in northeastern
620 India, Parasitol. 140 (2013) 76–86.
- 621 [18] P.D. Olson, T.H. Cribb, V.V. Tkach, R.A. Bray, D.T.J. Littlewood, Phylogeny and
622 classification of the Digenea (Platyhelminthes: Trematoda), Int. J. Parasitol. 33 (2003) 733–
623 755.
- 624 [19] R.A. Bray, A. Waeschenbach, T.H. Cribb, G.D. Weedall, P. Dyal, D.T.J. Littlewood, The
625 phylogeny of the Lepocreadiidae (Platyhelminthes: Digenea) inferred from nuclear and
626 mitochondrial genes: implications for their systematics and evolution, Acta Parasitol. 54
627 (2009) 310–329.
- 628 [20] E.E. Pulis, T.J. Fayton, S.S. Curran, R.M. Overstreet, A new species of *Intromugil*
629 (Digenea: Haploporidae) and redescription of *Intromugil mugilicolus*. J. Parasitol. 99 (2013)
630 501–508.
- 631 [21] G. Muñoz, M. George-Nascimento, R.A. Bray, Two new species of digeneans
632 (Lecithasteridae and Haploporidae) of the intertidal blenny *Scartichthys viridis*
633 (Valenciennes) from the central coast of Chile, Acta Parasitol. 62 (2017) 50–62.
- 634 [22] L. Andrade-Gómez, C.D. Pinacho-Pinacho, J.S. Hernández-Orts, A.L. Sereno-Urbe, M.
635 García-Varela, Morphological and molecular analyses of a new species of *Saccocoelioides*
636 Szidat, 1954 (Haploporidae Nicoll, 1914) in the fat sleeper *Dormitator maculatus* (Bloch)
637 (Perciformes: Eleotridae) from the Gulf of Mexico, J. Helminthol. 91 (2016) 504–516.

- 638 [23] V.V. Besprozvannykh, D.M. Atopkin, A.V. Ermolenko, A.Y. Beloded, Morphometric and
639 molecular analyses of *Skrjabinolecithum pyriforme* n. sp. (Digenea: Haploporidae) in mullet
640 fish from the Primorsky Region, Russia, *J. Helminthol.* 91 (2016) 625–632.
- 641 [24] D.M. Atopkin, A.Y. Beloded, H.D. Ngo, N.V. Ha, N.V. Tang, Molecular genetic
642 characterization of the far eastern trematode *Skrjabinolecithum spasskii*, Belous, 1954
643 (Digenea: Haploporidae), a parasite of mullets, *Mol. Phylogenet.* 49 (2015) 373–379.
- 644 [25] V.V. Besprozvannykh, D.M. Atopkin, H.D. Ngo, N.V. Tang, A.Y. Beloded, Morphometric
645 and molecular analyses of two digenean species from the mullet: *Skrjabinolecithum*
646 *spinosum* n. sp. from the Russian southern Far East and *Unisaccus tonkini* n. sp. from
647 Vietnam, *J. Helminthol.* (2017) [https://doi:10.1017/S0022149X17000943](https://doi.org/10.1017/S0022149X17000943)
- 648 [26] K. Katoh, K.-I. Kuma, H. Toh, T. Miyata, MAFFT version 5: improvement in accuracy of
649 multiple sequence alignment. *Nucleic Acids Res.* 33 (2005) 511–518.
- 650 [27] M. Wu, S. Chatterji, J.A. Eisen, Accounting for alignment uncertainty in phylogenomics,
651 *PLOS ONE* 7 (2012) e30288.
- 652 [28] J.P. Huelsenbeck, F. Ronquist, MRBAYES: Bayesian inference of phylogeny,
653 *Bioinformatics* 17 (2001) 754–755.
- 654 [29] D. Darriba, G.L. Taboada, R. Doallo, D. Posada, jModelTest 2: more models, new heuristics
655 and parallel computing. *Nat. Methods* 9 (2012) 772.
- 656 [30] E. Linton, Helminth fauna of the Dry Tortugas. II. Trematodes, Papers from the Tortugas
657 Laboratory of the Carnegie Institute of Washington, 4 (1910) 11–98.
- 658 [31] H.W. Manter, The structure and taxonomic position of *Megasolena estrix* Linton, 1910
659 (Trematoda) with notes on related trematodes, *Parasitol.* 27 (1935) 431–439.

- 660 [32] F. Sogandares-Bernal, Digenetic trematodes of marine fishes from the Gulf of Panama and
661 Bimini, British West Indies, Tulane Stud. Zool. 7 (1959) 71–117.
- 662 [33] H.W. Manter, An additional trematode from Tortugas, Florida, and a new name for
663 *Opisthoporus* Manter, 1947, preoccupied, Am. Midl. Nat. 41 (1949) 432–435.
- 664 [34] R.M. Overstreet, Digenetic trematodes of marine teleost fishes from Biscayne Bay, Florida,
665 Tulane Stud. Zool. 15 (1969) 119–176.
- 666 [35] G. Pérez-Ponce de León, L. Garcia-Prieto, B. Mendoza-Garfias, Trematode parasites
667 (Platyhelminthes) of wildlife vertebrates in Mexico. Zootaxa. 1534 (2007) 1–247.
- 668 [36] F. Sogandares-Bernal, R.F. Hutton, Studies on helminth parasites of the coast of Florida. I.
669 Digenetic trematodes of marine fishes from Tampa and Boca Ciega Bays with descriptions of
670 two new species, Bul. Mar. Sci. Gulf Caribbean, 9 (1959) 53–68.
- 671 [37] F.M. Nahhas, R.M. Cable, Digenetic and aspidogastrid trematodes from marine fishes of
672 Curaçao and Jamaica, Tulane Stud. Zool. 11 (1964) 169–228.
- 673 [38] J.F.R. Amato, Digenetic trematodes of percoid fishes of Florianópolis, southern Brasil –
674 Fellodistomidae, Monascidae, Diplangidae, Zoogonidae, and Waretrematidae with
675 description of two new species, Rev. Bras. Biol. 42 (1982) 681–699.
- 676 [39] B.M.M. Fernandes, A. Kohn, R. Magalhaes-Pinto, Aspidogastrid and digenetic trematodes.
677 Parasites of marine fishes of the coast of Rio de Janeiro State, Brazil, Rev. Bras. Biol. 45
678 (1985) 109–116.
- 679 [40] A.d.S. Cordeiro, J.L. Luque, Quantitative aspects of the metazoan parasites of sea bream,
680 *Archosargus rhomboidalis* (Linnaeus, 1758) (Osteichthyes, Sparidae) from the coastal zone
681 of the Rio de Janeiro, Brazil. Rev. Bras. Zoociencias, 7 (2005) 7–15.

- 682 [41] H.W. Manter, Some digenetic trematodes of marine fishes of Beaufort, North Carolina,
683 Parasitol. 22 (1931) 369–411.
- 684 [42] J.H. Fischthal, M.O. Williams, Some digenetic trematodes of marine fishes from Sierra
685 Leone, J. Helminthol. 45 (1971) 41–50.
- 686 [43] W.G. Dyer, E.H. Williams, Jr., L. Bunkley Williams, Digenetic trematodes of marine fishes
687 of the western and southwestern coasts of Puerto Rico. Proc. Helminthol. Soc. Wash. 52
688 (1985) 85–94.
- 689 [44] A.H. Siddiqi, R.M. Cable, Digenetic trematodes of marine fishes of Puerto Rico, Sci. Sur.
690 Porto Rico and the Virgin Is. 17 (1960) 257–369.
- 691 [45] H.W. Manter, The digenetic trematodes of marine fishes of Tortugas, Florida, Am. Midl.
692 Nat. 38 (1947) 257–416.
- 693 [46] J.H. Fischthal, Some digenetic trematodes of marine fishes from the Barrier Reef and Reef
694 Lagoon of Belize, Zool. Scripta 6 (1977) 81–88.
- 695 [47] F.M. Nahhas, K. Carlson, Digenetic trematodes of marine fishes of Jamaica, West Indies,
696 Ecological Survey of Jamaica 2 (1994) 1–60.
- 697 [48] J.E. Randall, A contribution to the biology of the convict surgeonfish of the Hawaiian
698 Islands, Pac. Sci. 15 (1961) 215–272.
- 699 [49] H.W. Palm, R.A. Bray, Marine Fish Parasitology in Hawaii, Westarp and Partner
700 Digitaldruck, Hohenwarsleben, Germany, 2014, pp. 302.
- 701 [50] H.W. Manter, M.H. Pritchard, Studies on digenetic trematodes of Hawaiian fishes: Families
702 Monorchiidae and Haploporidae, J. Parasitol. 47 (1961) 483–492.
- 703 [51] G. Caballero, C. Río-Estrada, L. Rodríguez-Álvarez, G.C. Tello-Sandoval, Trematodes as
704 pollution levels indicators by hydrogen sulfide in the Gulf of Mexico, and this acid's impact

705 on public health, An. Inst. Cienc. de Mar y Limnol. Univ. Nal. Autón. México 19 (1992)
706 151–161.

707 [52] R.M. Overstreet, J.O. Cook, R.W. Heard, Trematoda (Platyhelminthes) of the Gulf of
708 Mexico, in: D.L. Felder, D.K. Camp (Eds.), Gulf of Mexico: Origin, waters, and biota.
709 Volume 1: Biodiversity, Texas A&M University Press, College Station, Texas, USA, 2009,
710 pp. 419–486.

711 [53] P. Sheena, K.P. Janardanan, The life cycle of *Hapladena gymnocephali* sp. nov. (Digenea:
712 Haploporidae) from the bald glassy perchlet *Ambassis gymnocephalus* in Kerala, India. J.
713 Helminthol. 81 (2007) 301–306.

714 [54] J.-D. Durand, K.N. Shen, W.J. Chen, B.W. Jamandre, H. Blel, K. Diop, M Nirchio, F.J.
715 Garcia de Leon, A.K. Whitfield, C.W. Chang, P. Borsa, Systematics of the grey mullet
716 (Teleostei: Mugiliformes: Mugilidae): molecular phylogenetic evidence challenges two
717 centuries of morphology-based taxonomy, Mol. Phylogenet. Evol. 64 (2012) 73–92.

718 [55] A.K. Whitfield, J. Panfili, J.-D. Durand, A global review of the cosmopolitan flathead
719 mullet *Mugil cephalus* Linnaeus 1758 (Teleostei: Mugilidae), with emphasis on the biology,
720 genetics, ecology and fisheries aspects of this apparent species complex, Rev. Fish Biol.
721 Fisheries 22 (2012) 641–681.

722 [56] H.W. Manter, Host specificity and other host relationships among the digenetic trematodes
723 of marine fishes, in: First Symposium on host specificity among parasites of vertebrates,
724 Institut de Zoologie, Universite de Neuchâtel, Neuchatel, Switzerland, 1957, pp. 185–198.
725
726

Table 1 Species, hosts, and GenBank numbers for sequences used for phylogenetic analysis in this study.

Family	Species	Host	GenBank	Reference
Monorchiidae	<i>Hurleytrematoides chaetodoni</i> (Manter, 1942) Yamaguti, 1954	<i>Chaetodon striatus</i> Linnaeus	MH244116	This study
Paragonimidae	<i>Paragonimus siamensis</i> Miyazaki and Wykoff, 1965	<i>Sartoriana spinigera</i> (Wood-Mason)	JQ322634 (ITS2) JQ322628 (28S)	[17]
	<i>Paragonimus westermani</i> (Kerber, 1878) Braun, 1899	<i>Maydelliathelphusa lugubris</i> (Wood-Mason)	JN656190 (ITS2) JN656176 (28S)	[17]
Atractotrematidae	<i>Atractotrema sigani</i> Durio and Manter, 1969	<i>Siganus lineatus</i> (Valenciennes)	AY222267	[18]
	<i>Isorchis anomalus</i> Andres, Pulis, and Overstreet, 2016	<i>Chanos chanos</i> (Forsskål)	KU873018	[9]
	<i>Isorchis cannoni</i> Huston, Cutmore, and Cribb, 2017	<i>Siganus lineatus</i>	MF803156 (ITS2) MF803154 (28S)	[11]
	<i>Isorchis currani</i> Andres, Pulis, and Overstreet, 2016	<i>Selenotoca multifasciata</i> (Richardson)	KU873016	[9]
	<i>Isorchis megas</i> Andres, Pulis, and Overstreet, 2016	<i>Selenotoca multifasciata</i>	KU873015	[9]
	<i>Pseudomegasolena ishigakiense</i> Machida and Kamiya, 1976	<i>Scarus rivulatus</i> Valenciennes	AY222266	[18]
Cadenatellinae	<i>Cadenatella americana</i> Manter, 1949	<i>Kyphosus sectatrix</i> (Linnaeus)	MH244117	This study
	<i>Cadenatella floridae</i> Overstreet, 1969	<i>Kyphosus incisor</i> (Cuvier)	MH244118	This study
	<i>Cadenatella isuzumi</i> Machida, 1993	<i>Kyphosus vaigiensis</i> Quoy and Gaimard	FJ788497	[19]
	<i>Cadenatella pacifica</i> (Yamaguti, 1970) Bray and Cribb, 2001	<i>Kyphosus vaigiensis</i>	FJ788498	[19]
Haploporidae	<i>Capitimitta costata</i> Pulis and Overstreet, 2013	<i>Selenotoca multifasciata</i>	KC206497	[14]
	<i>Capitimitta darwinensis</i> Pulis and Overstreet, 2013	<i>Selenotoca multifasciata</i>	KC206498	[14]
	<i>Dicrogaster contracta</i> Looss, 1902	<i>Chelon auratus</i> (Risso)	FJ211267 (ITS2) FJ211261 (28S)	[4]
	<i>Dicrogaster perpusilla</i> Looss, 1902	<i>Chelon ramada</i> (Risso)	FJ211248 (ITS2) FJ211238 (28S)	[4]
	<i>Forticulcita apiensis</i> Andres, Curran, Fayton, Pulis, and Overstreet, 2015	<i>Mugil cephalus</i> Linnaeus	KP761087	[5]
	<i>Forticulcita platana</i> Andres, Curran, Fayton, Pulis, and Overstreet, 2015	<i>Mugil liza</i> Valenciennes	KP761086	[5]
	<i>Hapladena acanthuri</i> Siddiqi and Cable, 1960	<i>Acanthurus chirurgus</i> (Bloch)	MH244119	This study
	<i>Hapladena nasonis</i> Yamaguti, 1970	<i>Naso unicornis</i> (Forsskål)	AY222265	[18]
	<i>Hapladena</i> cf. <i>varia</i> Linton, 1910	<i>Acanthurus chirurgus</i>	MH244120	This study
	<i>Haploporus benedeni</i> (Stossich, 1887) Looss, 1902	<i>Chelon ramada</i>	FJ211247 (ITS2) FJ211237 (28S)	[4]
	<i>Intromugilalachuaensis</i> Pulis, Fayton, Curran, and Overstreet, 2013	<i>Mugil cephalus</i>	KC430095	[20]
	<i>Intromugil mugilicolus</i> (Shireman, 1964) Overstreet and Curran, 2005	<i>Mugil cephalus</i>	KC430096	[20]
	<i>Lecithobotrys putrescens</i> Looss, 1902	<i>Chelon saliens</i> (Risso)	FJ211246 (ITS2) FJ211236 (28S)	[4]

<i>Litosaccus brisbanensis</i> (Martin, 1974) Andres, Pulis, Cribb, and Overstreet, 2014	<i>Mugil cephalus</i>	KM253765	[16]
<i>Megasolena hysterospina</i> (Manter, 1931) Overstreet, 1969	<i>Archosargus rhomboidalis</i> (Linnaeus)	MH244121	This study
<i>Megasolena littoralis</i> Muñoz, George-Nascimento, and Bray, 2017	<i>Scartichthys gigas</i> (Steindachner)	KX035007 (ITS2)	[21]
<i>Megasolena mikra</i> sp. nov.	<i>Holacanthus ciliaris</i> (Linnaeus)	MH244122	This study
<i>Parasaccocoelium haematocheilum</i> Besprozvannykh, Atopkin, Ermolenko, and Nikitenko, 2014	<i>Planiliza haematocheila</i> (Temminck and Schlegel)	HF548461	[6]
<i>Parasaccocoelium mugili</i> Zhukov, 1971	<i>Planiliza haematocheila</i>	HF548468	[6]
<i>Parasaccocoelium polyovum</i> Besprozvannykh, Atopkin, Ermolenko, and Nikitenko, 2014	<i>Planiliza haematocheila</i>	HF548474	[6]
<i>Ragaia lizae</i> Blasco-Costa, Montero, Gibson, Balbuena, and Kostadinova, 2009	<i>Chelon auratus</i>	FJ211245 (ITS2) FJ211235 (28S)	[4]
<i>Saccocoelioides beauforti</i> (Hunter and Thomas, 1961) Overstreet, 1971	<i>Mugil cephalus</i>	MG925103 (ITS2) MG925104 (28S)	[8]
<i>Saccocoelioides chauhani</i> Lamothe-Argumedo, 1974	<i>Astyanax aeneus</i> (Günther)	KU061105 (ITS2) KU061119 (28S)	[22]
<i>Saccocoelioides cichlidorum</i> (Aguirre-Macedo and Scholz, 2005) Andrade-Gómez, Pinacho-Pinacho, and García-Varela, 2017	<i>Amatitlania septemfasciata</i> (Regan)	MG925105 (ITS2) MG925106 (28S)	[8]
<i>Saccocoelioides elongatus</i> Szidat, 1954	<i>Prochilodus lineatus</i> (Valenciennes)	MG925107 (ITS2) MG925108 (28S)	[8]
<i>Saccocoelioides lamothei</i> Aguirre-Macedo and Violante-González, 2008	<i>Dormitator latifrons</i> Richardson	KU061099 (ITS2) KU061120 (28S)	[22]
<i>Saccocoelioides magnus</i> Szidat, 1954	<i>Cyphocarynx voga</i> (Hensel)	MG925111 (ITS2) MG925112 (28S)	[8]
<i>Saccocoelioides nanii</i> Szidat, 1954	<i>Prochilodus lineatus</i>	MG925113 (ITS2) MG925114 (28S)	[8]
<i>Saccocoelioides olmecae</i> Andrade-Gómez, Pinacho-Pinacho1, Hernández-Orts, Sereno-Urbe, and García-Varela, 2016	<i>Dormitator maculatus</i> (Bloch)	KU061114 (ITS2) KU061134 (28S)	[22]
<i>Saccocoelioides orosiensis</i> Curran, Pulis, Andres, and Overstreet, 2018	<i>Poecilia gillii</i> (Kner)	MG925115 (ITS2) MG925116 (28S)	[8]
<i>Saccocoelioides sogandaresi</i> Lumsden, 1963	<i>Poecilia latipina</i> (Lesueur)	MG925119 (ITS2) MG925120 (28S)	[8]
<i>Saccocoelioides tkachi</i> Curran, Pulis, Andres, and Overstreet, 2018	<i>Astyanax aeneus</i> (Günther)	MG925121 (ITS2) MG925122 (28S)	[8]
<i>Saccocoelium obesum</i> Looss, 1902	<i>Chelon ramada</i>	FJ211266 (ITS2) FJ211260 (28S)	[4]
<i>Saccocoelium tensum</i> Looss, 1902	<i>Chelon auratus</i>	FJ211264 (ITS2) FJ211258 (28S)	[4]

<i>Skrjabinolecithum pyriforme</i> Besprozvannykh, Atopkin, Ermolenko, and Beloded, 2016	<i>Planiliza haematocheila</i>	LN864990 (ITS2)	[23]
<i>Skrjabinolecithum spasskii</i> Belous, 1954	<i>Planiliza haematocheila</i>	HE806359 (28S)	
		HG530223 (ITS2)	[24]
		HE806363 (28S)	
<i>Skrjabinolecithum spinosum</i> Besprozvannykh, Atopkin, Ngo, Ha, Tang, and Beloded, 2017	<i>Mugil cephalus</i>	MF176832 (ITS2)	[25]
		MF176829 (28S)	
<i>Spiritestis herveyensis</i> Pulis and Overstreet, 2013	<i>Moolgarda seheli</i> (Forsskål)	KC206500	[14]
<i>Unisaccus tonkini</i> Besprozvannykh, Atopkin, Ngo, Ha, Tang, and Beloded, 2017	<i>Osteomugil cunnesius</i> (Valenciennes)	MF176838 (ITS2)	[25]
		MF176843 (28S)	
<i>Xiha fastigata</i> (Thatcher and Sparks, 1958) Andres, Curran, Fayton, Pulis, and Overstreet, 2015	<i>Mugil cephalus</i>	KP761088	[5]

Table 2 Base pair lengths of sequences for the partial 18S rRNA gene, internal transcribed spacer (ITS) region 1, 5.8S rRNA gene, ITS2, and partial 28S rRNA gene of *Cadenatella americana*, *C. floridae*, *Megasolena mikra* sp. nov., *M. hysterospina*, *Hapladena acanthuri*, and *H. cf. varia*.

	18S	ITS1	5.8S	ITS2	28S
<i>Cadenatella americana</i>	107	511	157	258	1,384
<i>Cadenatella floridae</i>	107	517	157	258	1,384
<i>Megasolena hysterospina</i>	101	551	157	294	1,392
<i>Megasolena mikra</i> sp. nov.	101	552	157	294	1,392
<i>Hapladena acanthuri</i>	89	602	157	279	1,407
<i>Hapladena cf. varia</i>	89	603	157	279	1,407

Table 3 Pairwise comparisons (excluding gaps) of percent nucleotide similarity and number of base pair differences (in parentheses) of the internal transcribed spacer region 2 rDNA sequences of the three species of *Megasolena*.

	<i>Megasolena littoralis</i> KX035007	<i>Megasolena hysterospina</i>
<i>Megasolena hysterospina</i>	84.1 (46)	-
<i>Megasolena mikra</i> sp. nov.	84.4 (45)	99.7 (1)

Table 4 Pairwise comparisons (trimmed to GenBank sequences; excluding gaps) of percent nucleotide similarity and number of base pair differences (in parentheses) of the partial 28S rDNA sequences of the four species of *Cadenatella*.

	<i>Cadenatella pacifica</i> FJ788498	<i>Cadenatella americana</i>	<i>Cadenatella floridae</i>
<i>Cadenatella americana</i>	92.6 (98)	-	-
<i>Cadenatella floridae</i>	92.1 (105)	96.7 (44)	-
<i>Cadenatella isuzumi</i>	88.7 (150)	89.4 (141)	88.7 (151)

Table 5 Pairwise comparisons (trimmed to GenBank sequence AY222265; excluding gaps) of percent nucleotide similarity and number of base pair differences (in parentheses) of the partial 28S rDNA sequences of the three species of *Hapladena*.

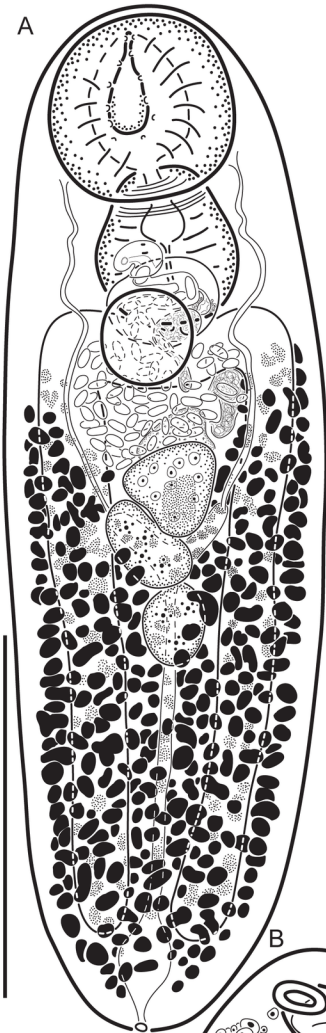
	<i>Hapladena nasonis</i> AY222265	<i>Hapladena acanthuri</i>
<i>Hapladena acanthuri</i>	92.2 (101)	-
<i>Hapladena cf. varia</i>	91.5 (110)	97.5 (32)

Fig. 1. *Megasolena mikra* sp. nov. from *Holacanthus ciliaris* (Linnaeus) A: Ventral view of holotype with everted hermaphroditic duct. B: Ventral view of hermaphroditic sac of paratype USNM TBD. Scale bars: A = 1,000µm; B = 100µm.

Fig. 2. Phylogenetic relationships among members of the Haploporoidea Nicoll, 1914 resulting from Bayesian Inference analysis of concatenated partial 28S rDNA and internal transcribed spacer region 2 (ITS2) sequences. Bolded taxa represent sequences derived for this study; gray box denotes Haploporidae Nicoll, 1914; vertical bars denote family or subfamily groups; black circles denote number and orientation of the testes possessed by members of the clade; dark gray mullet denotes subfamilies with at least one member that parasitizes mullet (Mugilidae) whereas all other major groups include taxa that parasitize marine eupercarians. Waret. = Waretrematinae.

Fig. 3. Terminal genitalia of *Cadenatella americana* (A - Ventral) and *Cadenatella floridae* (B - lateral). AS = accessory sucker; GP = genital pore; HD = hermaphroditic duct; VS = ventral sucker. Scale bars = 100µm.

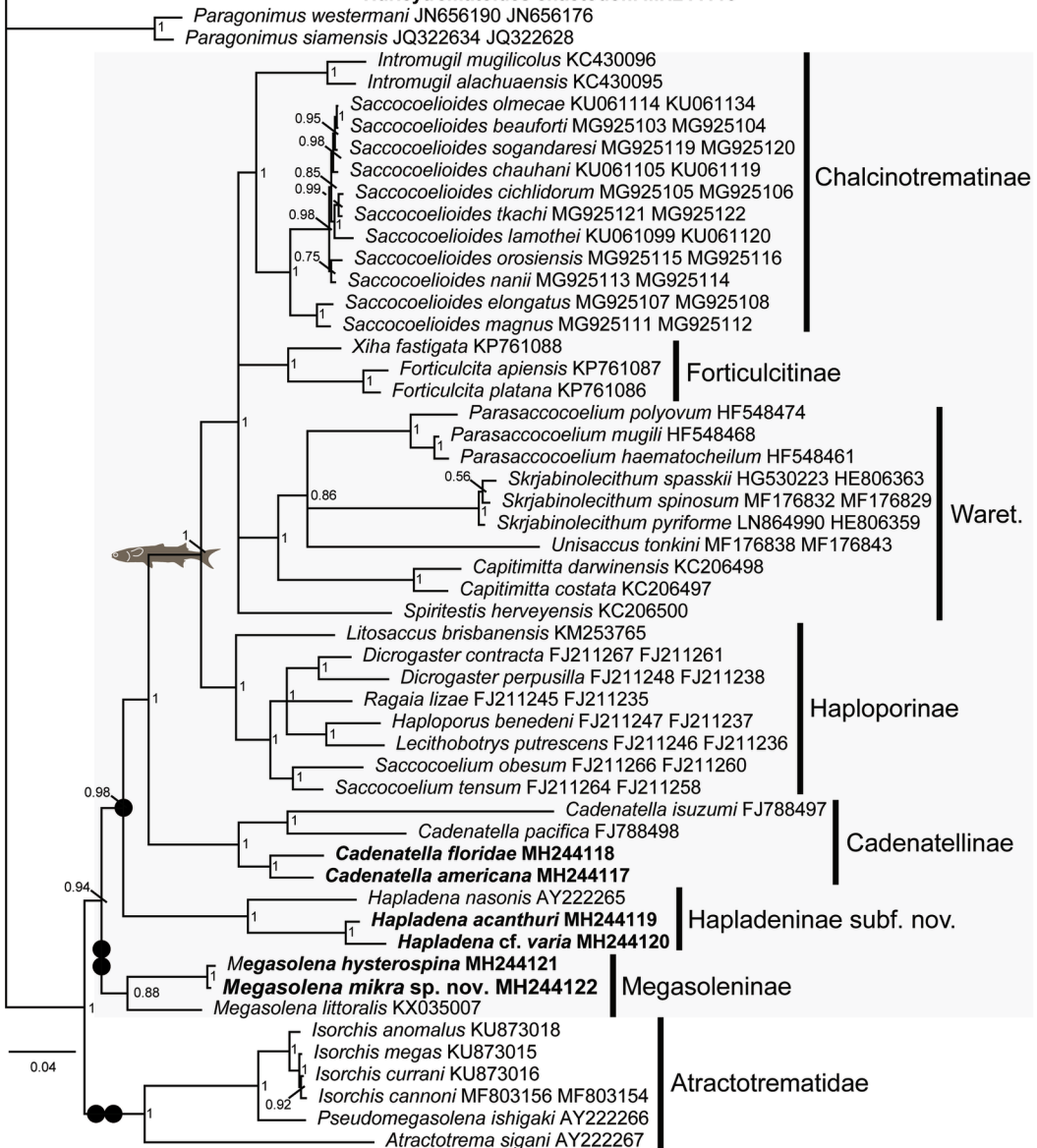
A



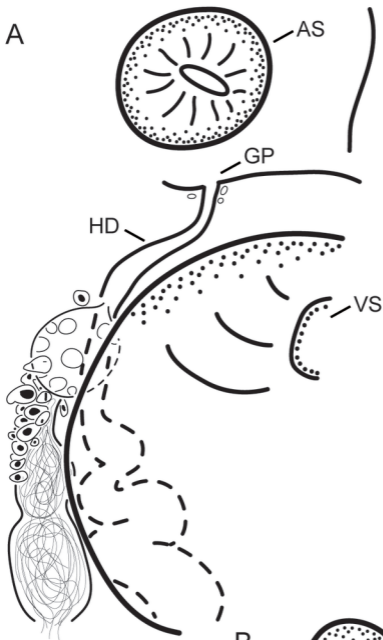
B



Hurleytrematoides chaetodonti MH244116



A



B

