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3 High-intensity cardiac infections of *Phthinomita heinigerae* n. sp.

4 (Digenea: Aporocotylidae) in the orangelined cardinalfish, *Taeniamia*

5 *fucata* (Cantor), off Heron Island on the Great Barrier Reef

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30   **ABSTRACT**

31   We report a new species of aporocotylid trematode (Platyhelminthes: Digenea) from the heart of the

32   orangelined cardinalfish, *Taeniamia fucata* (Cantor), from off Heron Island on the southern Great Barrier

33   Reef. We used an integrated approach, analysing host distribution, morphology, and genetic data from the

34   internal transcribed spacer 2 of the ribosomal DNA, to circumscribe *Phthinomita heinigerae* n. sp. This is

35   the first species of *Phthinomita* Nolan & Cribb, 2006 reported from the Apogonidae; existing species and

36   known 'types' are recorded from species of the Labridae, Mullidae, and Siganidae. The new species is

37   distinguished from its 11 congeners in having a body 2977–3539 long and 16.5–22.4 times longer than

38   wide, an anterior testis 6.2–8.2 times longer than wide and 8.3–13.0 times longer than the posterior testis,

39   a posterior testis whose width is 35–56% of the body width, and an ovary positioned 11–13% of the body

40   length from the posterior end. *P. heinigerae* n. sp. differs further in having an ovary that is positioned

41   entirely anterior to the posterior margin of the anterior testis. In addition, 2–34 base differences (0.4–7.0%

42   sequence divergence) were detected among the ITS2 sequence representing *P. heinigerae* n. sp. and the

43   14 representing other *Phthinomita* species/molecular types. Prevalence and intensity of infection with *P.*

44   *heinigerae* n. sp. was relatively high within the heart tissue of *T. fucata*, with 19 of 20 fish examined from

45   off Heron Island infected (95%) with 7–25 adult worms (arithmetic mean 16.6). Infections by these

46   parasites accounted for an occupation of 7–30% of the total estimated heart volume.

47

48   **Keywords**

49   Platyhelminthes

50   Trematoda

51   Apogonidae

52   Internal transcribed spacer 2 (ITS2) of the ribosomal DNA (rDNA)

53   Host-switching

54

1. Introduction

The Aporocotylidae Odhner, 1912 (Platyhelminthes: Trematoda) is a family of parasitic flatworms that has, in recent years, emerged as an increasingly rich, and morphologically diverse, group of digeneans. There are currently 142 accepted species from 37 genera [1-5], which infect a broad range of fishes. Species from seven genera have been recorded from fishes of the Great Barrier Reef (GBR): *Ankistromeces* Nolan & Cribb, 2004 (see [6]); *Braya* Nolan & Cribb, 2006 (see [7]); *Cardicola* Short, 1953 (see [7-9]); *Pearsonellum* Overstreet & Køie, 1989 (see [10, 11]); *Plethorchis* Martin, 1975 (see [12]); *Phthinomita* Nolan & Cribb, 2006 (see [6]); and, *Rhaphidotrema* Yong & Cribb, 2011 (see [13]). *Phthinomita* is the most complex of these, consisting of 11 recognised species and numerous undefined 'types' represented by a unique DNA sequence or single morphological specimen. Unlike most aporocotylids, which are typically characterised by a flat body that may be linear, elliptical, or lanceolate, species of *Phthinomita* are long and thread-like. As adults, they wind through the intertrabecular spaces of the ventricle of their hosts, which to date include species of labrid (wrasses), mullid (goatfishes), and siganid (rabbitfishes or spinefoots). Due to the extreme morphological similarity that exists among species of *Phthinomita*, an effect most likely due to their site of infection, this group is best described as a complex of cryptic species. As such, genetic data are required to enhance traditional methods of species characterisation (i.e. microscopic and morphological examination, host and geographic distribution) and the delineation of species is only possible though this integrated approach (see [14]). Here, we report *Phthinomita heinigerae* n. sp. from the ventricle of the orangelined cardinalfish, *Taeniamia fucata* (Cantor) (Perciformes: Apogonidae), collected during the CReefs project from 2009–2012 (http://www.aims.gov.au/creefs/field-program.html), from off Heron Island on the southern GBR.

2. Materials and methods

2.1. Sample collection

Between 2009 and 2012, 22 species of apogonid from nine genera (Table 1) were collected from five sites off Heron Island on the southern GBR (23.4420° S, 151.9140° E), eight sites off Lizard Island on the northern GBR (14.6680° S, 145.4617° E), and from seven sites on Ningaloo reef, off Western Australia (22.5625, 113.810278). Apogonid fishes were stored in an 80 litre container before being euthanised by an overdose of clove oil, in strict accordance with the Queensland Museum's Animal Ethics Permit 07/01, issued for this research. Immediately upon death the heart, gills, and viscera were excised and processed as described previously [8]. The hearts of infected apogonids were preserved in 10% formalin (room temperature), for histological examination.

91   *2.2. Morphological examination of aporocotylids*

92

93   Fixed worms were washed, stained, and mounted as described by Nolan et al. [8]. Drawings
94   were completed using a drawing tube attached to an Olympus BX53 compound microscope with
95   Nomarski differential interference contrast (DIC) optics. We inferred the dorsal surface by reference to
96   the position of the separate genital pores, which were assumed to be dorsal, as in all *Phthinomita* species.
97   All measurements, in micrometres, were made using an Olympus UC50 digital camera and the software
98   LabSens (Olympus Soft Imaging Solutions), and are presented as a range followed by the arithmetic
99   mean in parentheses. Measurement of morphological characters from the anterior or posterior end of
100   worms reflects the distance from the extremities of each feature. Caecal lengths as a percentage of body
101   length are based on the right caeca only. Type-specimens, hologenophores, and paragenophores were
102   deposited in the Queensland Museum, Australia (QM).

103

104   *2.3. Isolation of genomic DNA, Polymerase chain reaction, and phylogenetic analysis*

105

106   Total genomic DNA (gDNA) was isolated from three separate specimens identified
107   morphologically as putative *P. heinigerae* n. sp. using a DNeasy® Blood and Tissue kit (Qiagen, Hilden,
108   Germany), according to the manufacturer's instructions. PCR amplification of the entire internal
109   transcribed spacer 2 (ITS2) of the ribosomal DNA (rDNA) region was achieved using the primers 3S
110   (forward: 5'-GGTACCGGTGGATCACGTGGCTAGTG-3') and ITS2.2 (reverse: 5'-
111   CCTGGTTAGTTTCTTTTCCTCCGC-3'). PCR was carried out in a 20 µl volume as described by
112   Cutmore et al. [15]. All resultant PCR amplicons were purified and sequenced as described by Nolan et
113   al. [8].
114   Prior to phylogenetic analysis, the sequence representing *P. heinigerae* n. sp. (GenBank
115   accession no. KX168409) was aligned with 30 reference sequences for selected aporocotylid
116   species/genera, presently available in GenBank. Sequences were aligned using the software MUSCLE
117   version 3.7 [16, 17] with ClustalW sequence weighting and UPGMA clustering for iterations 1 and 2. The
118   resultant alignment was adjusted manually using the software BioEdit [18]. Total nucleotide distance
119   matrices were calculated using the pairwise deletion of gaps/missing data option in the software package
120   MEGA v.5 [19].
121   Minimum evolution analysis was conducted on the ITS2 dataset using MEGA v.5. Nodal
122   support for the analysis of this dataset was inferred by bootstrap analysis using a heuristic search of
123   10,000 replicates. The outgroup taxa used were six species/molecular types of *Ankistromeces* (GenBank
124   accession nos. DQ335838–DQ335843, [6]).

125

126   *2.4. Histology*

127

128   Sections (5 μm thick) were cut from 10% formalin fixed tissue samples as described by Heiniger

129 et al. [20]. In brief, tissue sections were stained with haematoxylin and eosin (H & E). Coverslips were

130 applied using DePeX (BDH, England). Digital, light microphotographs of the sections were taken at ×100

131 magnification using an Olympus UC50 digital camera attached to an Olympus BX53 compound

132 microscope.

133

134 *2.5. Estimating volume of heart space occupied by* P. heinigerae *n. sp. in infected* T. fucata

135

136   To estimate the volume of individual worms, additional measurements were taken from each

137 type specimen (n = 9). Because blood flukes vary in diameter over the length of the body, between six

138 and 11 radius and height (i.e. length) measurements were taken and used in the formula for calculating the

139 volume of a cylinder ($V = \pi\, r^2\, h$, where r is the radius and h is the height). The general body morphology

140 of species of *Phthinomita* are more cylindrical than dorso-ventrally flattened, therefore these volume

141 calculations were considered appropriate. These were then combined to obtain the approximate total

142 volume for each type specimen, and then averaged to obtain the arithmetic mean volume of a single

143 worm. To approximate the volume of a *T. fucata* heart, whole formalin fixed hearts (n = 3) were

144 measured and the radius of each used in the formula to calculate the volume of a sphere ($V = 4/3\, \pi\, r^3$).

145 Using the average volume of a worm, the percentage volume of heart 'space' occupied, based on the

146 minimum (n = 7), mean (16.6), and maximum (25) intensities observed, was estimated.

147

148 **3. Results**

149

150 *3.1. Aporocotylid prevalence and specificity*

151

152   The hearts of 19 of the 724 apogonid specimens examined (2.6%) were infected with thread-like

153 aporocotylids. All 19 infected individuals were identified as the orangelined cardinalfish, *T. fucata*, which

154 were all collected from a single site in the Heron Island lagoon (19/20; 95% prevalence); none of the 27

155 *T. fucata* specimens collected from two sites off Lizard Island (Casuarina beach and Turtle beach) were

156 infected.

157

158 *3.2. Morphology*

159

160   Class Trematoda Rudolphi, 1808

161   Subclass Digenea Carus, 1863

162   Order Diplostomida Olson, Cribb, Tkach, Bray & Littlewood, 2003

163   Suborder Diplostomata Olson, Cribb, Tkach, Bray & Littlewood, 2003

164   Superfamily Schistosomatoidea Stiles & Hassall, 1898

165   Family Aporocotylidae Odhner, 1912

166   *Phthinomita* Nolan & Cribb, 2006

167

168 *3.3. P. heinigerae* n. sp.

169

170   Description and measurements (Figs. 1–2): (based on nine whole mature worms). With all

171 features of genus. Body slightly notched at male genital pore, 2977–3539 (3249) × 133–198 (167), 16.5–

172 22.4 times longer than wide. Oral sucker weakly developed, bearing concentric rows of fine spines.

173 Oesophagus straight, 698–887 (783) or 22–25% of body total length. Intestine; right anterior caecum 25–

174 56 (37) or 0.8–1.7% of body total length, left anterior caecum 25–54 (35) long; posterior caeca sinuous,

175 unequal, irregular in outline; right posterior caecum 601–948 (758) or 18.9–31.8% of body total length;

176 left posterior caecum 882–1075 (977) long; 12.4–37.9 times longer than anterior pair.

177   Anterior testis originating posterior to intercaecal field, but antero-dextral to distal termination of

178 left posterior caecum (see Fig. 1), containing dorso-ventral muscle fibres, 844–1206 (1068) or 28–37% of

179 body total length × 130–185 (155) or 83–99% of body total width, 6.2–8.2 times longer than wide, 8.3–

180 13.0 times longer than posterior testis; posterior testis ovoid, rudimentary, 76–114 (99) or 3–4% of body

181 total length × 55–93 (76) or 35–56% of body total width, 1.0–1.6 times longer than wide. Vas deferens

182 seen antero-dextral to posterior margin of anterior testis; duct from posterior testis passing antero-

183 medially. Cirrus-sac tear-shaped, 34–54 (44) × 24–42 (31); 1.3–1.9 times longer than wide; 191–243

184 (219) from posterior extremity, 6–7% of body total length. Internal seminal vesicle ovoid, occupying

185 posterior end of cirrus-sac; ejaculatory duct sinuous; prostatic cells not seen.

186   Ovary spherical to ovoid, entirely anterior to posterior margin of anterior testis (see Fig. 2), 351–

187 435 (394) or 11–13% of body total length from posterior extremity, 68–99 (84) or 2–3% of body total

188 length × 72–94 (82) or 40–60% of body total width. Oviduct originating at posterior dorsal margin of

189 ovary, dorsal to vas deferens, entering oötype postero-dorsally. Vitelline duct forming lateral to ovary,

190 posteriorly dextral to vas deferens and cirrus-sac. Oötype ovoid, 35–47 (42) × 18–24 (21). Mehlis' gland

191 extending anteriorly to posterior margin of cirrus-sac, and posteriorly to anterior margin of posterior

192 testis. Uterus extending from oötype sinuously, sinistral to oviduct. Uterine chamber forming posterior to

193 posterior margin of ovary, sinuous, curving dorsally posteriorly to female pore, 144–185 (171) × 23–39

194 (33). Eggs 14–22 (18) × 8–15 (10) (n = 10). Vitelline follicles extending anteriorly past intestinal

195 bifurcation, sinistral and dextral to oesophagus, posterior caeca and anterior testis, posteriorly extending

196 to anterior margin of ovary.

197

198 *3.4. Taxonomic summary*

199

200   *Type-host*: *Taeniamia fucata* (Cantor), the orangelined cardinalfish (Perciformes: Apogonidae).

201   *Type-locality*: Heron Island lagoon, Heron Island (23.4420° S, 151.9140° E), southern Great

202 Barrier Reef, Queensland, Australia.

203   *Site*: Intertrabecular spaces and lumen of ventricle (heart).

204   *Intensity:* 7−25 (arithmetic mean 16.6).

205   *Prevalence:* 19 of 20 (95%).

206   *Type-material*: Holotype (QM G ~~XXXXXX~~232105), eight paratypes (QM G ~~XXXXXX~~232106-

207 232113).

208   *Molecular sequence data*: ITS2 (complete), three identical replicates.

209   *Molecular voucher data*: Hologenophore (QM G 232114), two paragenophores (QM G 232115-

210 232116).

211   *GenBank accession number*: KX168409~~XXXXXX~~.

212   *Etymology:* Specific name '*heinigerae*' is in reference to our esteemed colleague Dr Holly

213 Heiniger, for whom the initial samples of this species of Apogonidae were collected.

214

215 *3.5. Molecular data*

216

217   Three replicate ITS2 sequences were generated from as many specimens of *P. heinigerae*, all of

218 which were identical. Comparison of the sequence represented by KX168409 ~~XXXXXX~~ with reference

219 data for aporocotylids available on GenBank indicated this sequence type to be new, and two nucleotides

220 different (0.4% sequence divergence over 485 base positions) from the most similar available sequence,

221 represented by DQ335856 [6], which corresponds to *Phthinomita munozae* Nolan & Cribb, 2006 from

222 *Choerodon venustus* (De Vis) (Labriformes: Labridae).

223   Phylogenetic analysis of 31 sequences (including outgroups) aligned over 485 positions

224 (trimmed to match the shortest sequence length) resulted in a phylogram where species of *Phthinomita*

225 formed a monophyletic clade, to the exclusion of the outgroup taxa (i.e. members of the genus

226 *Ankistromeces*). The sequence representing *P. heinigerae* n. sp. grouped with the sequence represented by

227 DQ335856, for *P. munozae*, as expected based on sequence comparisons. These sequences resolved as a

228 strongly supported monophyletic clade together with sequences representing *Phthinomita poulini* Nolan

229 & Cribb, 2006 (DQ335857–DQ335859) from *Parupeneus barberinus* (Lacepède) (Perciformes:

230 Mullidae), *Parupeneus bifasciatus* (Lacepède) [now *Parupeneus trifasciatus* (Lacepède)], and

231 *Parupeneus cyclostomus* (Lacepède), and *Phthinomita* sp. B (DQ335863) from *Mulloidichthys*

232 *vanicolensis* (Valenciennes) (Mullidae) (see Fig. 3). As a result, species of *Phthinomita* that parasitise

233 siganids represent a paraphyletic group. With the exception of sequences representing *Phthinomita*

234 *littlewoodi* Nolan & Cribb, 2006, *Phthinomita hallae* Nolan & Cribb, 2006, and *Phthinomita jonesi* Nolan

235 & Cribb, 2006 (bootstrap value = 69) components of the siganid-infecting species generally formed

236 several well-supported clades (bootstrap values = 82–100). Although sequences did not group based on

237 host or geographic distribution, basal *Phthinomita* species are mainly more 'robust' morphs relative to the

238 smaller, more delicate *P. littlewoodi*, *P. hallae*, and *P. jonesi*.

239

3.6. Pathology

Specimens of *P. heinigerae* n. sp. were found in the lumen and intertrabecular spaces of the ventricle of *T. fucata* during autopsy and in histological sections of heart tissue. Fig. 4 shows a partially dissected heart from a single *T. fucata*. Infections included between 7–25 adult worms, which may each have a volume of between 1.85e+07–3.76e+07 (arithmetic mean 2.74e+07) μm^3 (see Section 2.5.). Based on the minimum and maximum number of worms found in a single infection, this could account for between 7–30% of the total estimated heart volume of *T. fucata*. Fig. 5 shows both transverse and (partial) longitudinal sections of *P. heinigerae* n. sp. within the heart. Cross-sections of *P. heinigerae* n. sp. were identified by the presence of cells within which different parts of the male (i.e. testes) and female (i.e. oötype) genitalia, and the caeca were recognised (see Fig. 5). No direct pathological changes produced by adult worms were detected in the heart tissue of infected fishes.

We did not observe eggs in the gills and/or the heart tissue of infected hosts.

4. Discussion

4.1. Taxonomy

Phthinomita heinigerae n. sp. can be differentiated from all current species of *Phthinomita* by the combined possession of a body 2977–3539 long and 16.5–22.4 times longer than wide, an anterior testis that is 6.2–8.2 times longer than wide and 8.3–13.0 times longer than the posterior testis, a posterior testis whose width is 35–56% of the body width, and having the ovary positioned 11–13% of the body length from the posterior end (see Table 2). In addition, *P. heinigerae* n. sp. differs further from all 11 species in having an ovary that is positioned entirely anterior to the posterior margin of the anterior testis (see Fig. 2); *P. robertsthomsoni* Nolan & Cribb, 2006 and *P. poulini* Nolan & Cribb, 2006 both possess an ovary that is positioned so that the posterior margin of the anterior testis passes adjacent to the ovary's medial line, while the remaining nine species all possess an ovary that is entirely posterior to, abutting, or only slightly overlapping the posterior margin of the anterior testis. *P. heinigerae* n. sp. is different from *Phthinomita brooksi* Nolan & Cribb, 2006 in having vitelline follicles that extend anteriorly past the intestinal bifurcation, and from *P. symplocos* Nolan & Cribb, 2006, *P. brooksi*, *P. hallae*, *P. jonesi*, *P. littlewoodi*, and *P. sasali* Nolan & Cribb, 2006 in having an anterior testis that overlaps the posterior margin of the posterior caeca (see Fig. 1).

Due to the general lack of morphological variation observed among species of *Phthinomita*, preceding work on this genus (and *Ankistromeces*) (see [6]) placed substantial weight on genetic data. To achieve this, a total of 135 sequences, with between one to 17 replicates for 30 host species/parasite species/geographical location combinations, was assembled to provide a robust dataset. Nineteen distinct

276   ITS2 genotypes were separated by 1–41 base differences (0.3–12.7% sequence divergence). These data

277   showed that species of *Phthinomita* could be distinguished by as little as a single base difference (see

278   page 69 in [6]). Here, sequence comparisons again confirmed that the specimens described as *P.*

279   *heinigerae* n. sp. are distinct from the 11 recognised and three putative species of *Phthinomita* (2–34 base

280   differences or 0.4–7.0% sequence divergence over 485 positions). These genetic differences, in

281   combination with the morphological distinctions described above, and the host family, are consistent with

282   *P. heinigerae* n. sp. being a new species.

283

284   *4.2. Host specificity and prevalence of infection*

285

286   This study is the first to report a species of *Phthinomita* from an apogonid fish. Existing species

287   and known 'types' are largely restricted to the Mullidae [seven species - *Mulloidichthys vanicolensis*,

288   *Parupeneus barberinoides* (Bleeker), *P. barberinus*, *P. bifasciatus*, *P. cyclostomus*, *P. indicus* (Shaw),

289   and *P. multifasciatus* (Quoy & Gaimard)] and the Siganidae [nine species - *Siganus argenteus* (Quoy &

290   Gaimard), *S. corallinus* (Valenciennes), *S. doliatus* Guérin-Méneville, *S. fuscescens* (Houttuyn), *S.*

291   *lineatus* (Valenciennes), *S. puellus* (Schlegel), *S. punctatus* (Schneider & Forster), *S. virgatus*

292   (Valenciennes), and *S. vulpinus* (Schlegel & Müller)] in the Indo-West Pacific Ocean [6]. One species, *P.*

293   *munozae*, has been recorded from a labrid fish (*Choerodon venustus*). Although we dissected 724

294   specimens (n = 1–274) of 22 species of apogonid, *P. heinigerae* n. sp. was absent in all species but *T.*

295   *fucata*. These specimens included 52 individuals of five species collected from the same patch reefs in the

296   Heron Island lagoon where infected *T. fucata* were sampled. Similarly strict host specificity has been

297   reported for *Kudoa leptacanthae* Heiniger & Adlard, 2012 (see [21]) (Multivalvulida: Kudoidae) from the

298   apogonids *Zoramia leptacantha* (Bleeker) (74% or 199/269) and *Z. viridiventer* Greenfield, Langston &

299   Randall (82.4% or 61/74) from off Lizard Island. Heiniger and Adlard [21] suggested this high prevalence

300   of infection and the high host specificity might be explained by apogonid developmental biology (i.e. life

301   cycle completion in a single lagoon, recruitment to home reefs, habitat specialists that are site attached

302   and specific; see [22-28]). Furthermore, these authors proposed that the two-host life cycle of *K.*

303   *leptacanthae* could be facilitated by the continual cycling of life stages through an intermediate host

304   (presumably an annelid) in close proximity to home patch reefs. Given the two-host life cycle of marine

305   teleost aporocotylids, which also incorporates an annelid intermediate host (e.g. [29-32]), similar

306   reasoning for the high host and site specificity, and the high prevalence of infection, could be applied

307   here.

308   In our phylogenetic analysis, *P. heinigerae* n. sp. and *P. munozae* (from a labrid) formed a well-

309   supported clade together with the two mullid-infecting species of *Phthinomita*. As such, all the non-

310   siganid infecting species form a clade exclusive to the siganid-infecting species, which form a

311   paraphyletic assemblage. The most parsimonious explanation of this distribution is that the non-siganid

312   clade arose as a host-switch from siganids. Host-switching is presumably difficult within this group as

313   demonstrated by the general fidelity to the Siganidae. The topology of our analysis suggests that,

314 following the initial host-switch out of the Siganidae, the clade has adopted three distinct and only

315 distantly related fish groups – Apogonidae, Labridae, and Mullidae. Although all three families have

316 traditionally been considered members of the Perciformes, the Labridae are now considered by some to

317 belong to a separate order, the Labriformes (see Figs. 9 and 10 in [33]). Comparable evidence of host-

318 switching was reported by Nolan et al. [8], who showed that the lutjanid-infecting aporocotylids

319 *Cardicola beveridgei* Nolan, Miller, Cutmore, Cantacessi, & Cribb, 2014 and *C. milleri* Nolan & Cribb,

320 2006 formed a well-supported clade with the chaetodontid-infecting *C. chaetodontis* Yamaguti, 1970.

321 Similarly, Trieu et al. [34] showed that several apogonids (including *T. fucata*) shared a bivesiculid

322 trematode, the sister species of which occurs in an unrelated pomacentrid. All three cases are evidence of

323 the importance and recent history of host-switching by trematodes of coral reef fishes.

324

325 *4.3. Pathology*

326

327   Histological sections of heart tissue suggests that *P. heinigerae* n. sp. does not elicit

328 immunological responses from *T. fucata* (see Fig. 5). Pathological changes induced by adult

329 aporocotylids are rare, in contrast to the effects stimulated by accumulated eggs and escaping miracidia

330 (see [35-43]). Overstreet and Thulin [44] found *Pearsonellum corventum* Overstreet & Køie, 1989 evoked

331 an increased abundance of melanomacrophage centers in the heart of *Plectropomus leopardus*

332 (Lacepède), while Herbert et al. [45] and Herbert and Shaharom [46] found *Cruoricola lates* Herbert,

333 Shaharom-Harrison & Overstreet, 1994 and *Parasanguinicola vastispina* Herbert & Shaharom, 1995

334 (respectively) do not elicit pathological changes to infected blood vessels in *Lates calcarifer* (Bloch),

335 despite *P. vastispina* possessing large spines that push into the endothelial cell walls (albeit they do not

336 penetrate them). In contrast, Kirk et al. [38] found that attachment of adult *Sanguinicola inermis* Plehn,

337 1905 to vessel walls in the carp, *Cyprinus carpio* Linnaeus, caused hyperplasia of the endothelial lining

338 and the occlusion of blood flow. More recently, Alama-Bermejo et al. [47] reported *Skoulekia meningialis*

339 Alama-Bermejo, Montero, Raga, & Holzer, 2011 induced a *"localised, mild but chronic inflammatory*

340 *response"* (see Fig. 5F, G, and H in [47]) in the ectomeninx of the meninges involving lymphocytes,

341 macrophages, and eosinophilic granulocytes, together with clotted erythrocytes in the meningeal vessels

342 of *Diplodus vulgaris* (Geoffroy Saint-Hilaire). Despite these exceptions, it is conceivable that adult

343 aporocotylids, including *P. heinigerae* n. sp., use a series of strategies similar to those employed by

344 closely related schistosomes (i.e. rapid development, stealth-like host-interfaces, and immunosuppression;

345 {Wilson, 2009 #XXXXXX}), to avoid the immunosurveillance of their hosts {Leow, 2014 #XXXXXX}.

346 This could certainly explain the presence of the tegument and/or mucus observed to cover the spines of *P.*

347 *symplocos* (see Fig. 21, page 37 in {Nolan, 2006 #8092}).

348   Here, although we found fishes infected with between 7–25 adult worms (see Fig. 5), which

349 were calculated to account for between 7–30% of the total estimated heart volume of *T. fucata*, there

350 appears to be little visual impact on host health. This is despite *P. heinigerae* n. sp. also possessing small

351 tegumental spines in incomplete lateral transverse rows along the entire length of the body and the adults

352 being wound extensively throughout the intertrabecular spaces of the ventricle. Despite the absence of
353 evidence of pathogenic effects, we find it unlikely that such dramatic infection of such a key organ could
354 be without significant effect on host health.
355
356 **Conflict of interest**
357
358   The authors declare they have no competing interests.
359
360 **Acknowledgements**
361

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369

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503
504

505 **Table 1**
506 Numbers of specimens examined for 22 apogonid species collected from off Heron
507 Island and Lizard Island on the Great Barrier Reef, and on Ningaloo Reef off Western
508 Australia. The infection of *T. fucata* by *P. heinigerae* n. sp. is presented as number of
509 fish infected/number of fish sampled.
510

Genus Species	Heron Island	Lizard Island	Ningaloo Reef	Totals
Archamia		**1**		**1**
bleekeri (Günther)		1		1
Cercamia		**1**		**1**
eremia (Allen)		1		1
Cheilodipterus	**37**	**65**	**1**	**103**
artus Smith		12		12
intermedius Gon	5*	19	1	25
macrodon (Lacepède)	2*			2
quinquelineatus Cuvier	30*	34		64
Nectamia	**31**	**21**	**1**	**53**
fusca (Quoy & Gaimard)	31*	21		52
savayensis (Günther)			1	1
Ostorhinchus	**52**	**46**	**56**	**154**
angustatus (Smith & Radcliffe)	1		1	2
aureus (Lacepède)			14	14
compressus (Smith & Radcliffe)		5		5
cookii (Macleay)	34	1	1	36
cyanosoma (Bleeker)		4	24	28
doederleini (Jordan & Snyder)	17*	2		19
properuptus (Whitley)		5		5
rubrimacula (Randall & Kulbicki)		29		29
rueppellii (Günther)			16	16
Pristiapogon		**2**		**2**
exostigma (Jordan & Starks)		2		2
Rhabdamia		**10**		**10**
gracilis (Bleeker)		10		10
Taeniamia	**20**	**106**		**126**
fucata (Cantor)	19/20	27		47
zosterophora (Bleeker)		79		79
Zoramia		**274**		**274**
leptacantha (Bleeker)		274		274
Totals	140	526	58	724

511
512 * Species of apogonid sampled from the same Heron Island lagoon patch reefs that
513 infected individuals of *T. fucata* were collected from.
514

Table 2

Morphometric comparison of *Phthinomita heinigerae* n. sp. with the 11 recognised species of *Phthinomita* Nolan & Cribb, 2006. Shading indicates morphometric distinctions between *P. heinigerae* n. sp. and described species. Percentages (%) are based on total body length. Measurement of morphological characters from the anterior or posterior end of worms reflects the distance from the extremities of each feature.

										Character									
Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	References
P. heinigerae n. sp.	2977–3539 (3249)	16.5–22.4	22–25	1–2	19–32	12.4–37.9	6.2–8.2	28–37	83–99	8.3–13.0	1.0–1.6	3–4	35–56	6–7	1.3–1.9	2–3	40–60	11–13	Present study
P. symplocos (type-species)	3536–4858 (4217)	20.6–31.8	20–30	1–2	20–28	12.8–34.8	5.0–9.2	17–30	59–96	3.5–8.1	1.1–3.1	4–7	30–74	7–12	1.0–1.8	1–2	43–65	13–18	[6]
P. adlardi	4353–6294 (5394)	23.8–45.3	13–22	0–3	15–28	7.8–75.0	12.0–21.7	37–59	77–96	12.6–38.2	1.6–3.6	1–4	9–62	6–8	1.0–2.2	2–3	52–76	11–14	[6]
P. brooksi	4078–7843 (5207)	21.4–40.7	23–31	0–2	16–50	12.4–94.0	3.8–14.1	12–24	22–92	2.4–6.8	1.2–3.3	3–6	35–78	5–10	1.3–7.0	1–2	40–71	11–22	[6]
P. hallae	3070–3950 (3507)	24.5–32.8	20–35	?	?	?	10.1–21.7	35–37	48–95	7.6–9.9	1.8–2.5	3–5	37–66	6–10	0.6–1.3	1–2	40–57	9–15	[6]
P. ingramae	2317–2983 (2645)	15.6–27.4	18–32	1–3	21–46	7.8–24.0	5.4–11.3	23–47	44–100	4.9–10.7	1.1–2.4	3–6	46–71	7–10	0.9–2.0	2–3	46–72	13–21	[6]
P. jonesi	2060–5329 (3674)	23.6–75.0	16–40	1–2	15–35	8.1–71.8	5.3–19.8	18–40	71–100	3.5–22.4	1.1–6.3	1–5	29–70	6–12	0.9–1.7	1–2	45–95	9–20	[6]
P. littlewoodi	2993–4133 (3465)	26.3–59.7	24–42	1–2	14–29	8.5–26.7	4.9–15.5	16–31	62–97	4.4–12.4	1.7–3.3	2–4	30–67	6–10	0.9–2.1	1–2	46–80	11–19	[6]
P. robertsthomsoni	3784–5706 (4851)	19.8–34.7	9–36	1–2	13–31	10.0–37.5	9.0–22.9	38–62	65–94	14.8–31.3	1.2–3.3	2–3	15–46	5–10	1.2–1.4	2–4	30–71	10–18	[6]
P. sasali	3765–4017 (3863)	24.7–29.7	25–26	1–2	23–28	12.9–21.0	5.2–10.2	20–29	70–94	3.3–9.6	1.2–2.8	3–4	2	7–10	2.0–2.6	2	46–65	18–20	[6]
P. munozae	2714–6094 (5210)	31.8–41.8	21–28	1–2	30–41	13.1–37.5	11.2–21.2	27–44	67–100	11.7–25.0	1.8–5.0	2–3	11–43	5–7	1.1–2.1	1–2	35–85.0	11–15	[6]
P. poulini	2350–4269 (3451)	21.0–35.6	20–27	1–2	26–34	14.2–39.5	8.6–15.2	35–40	84–94	4.9–13.6	1.6–3.8	3–8	32–64	7–15	1.6–12.8	1–3	50–75	14–26	[6]

Character legend: 1) body length; 2) body length/width; 3) oesophagus %; 4) anterior caeca length %; 5) posterior caeca length %; 6) posterior caeca/anterior caeca; 7) anterior testis length/width; 8) anterior testis length %; 9) anterior testis width %; 10) anterior testis length/posterior testis length; 11) posterior testis length/width; 12) posterior testis length %; 13) posterior testis width %; 14) cirrus-sac position %; 15) cirrus-sac length/width; 16) ovary length %; 17) ovary width %; 18) ovary position (%)

525 **List of Figures**
526
527 **Figs. 1–2.** *Phthinomita heinigerae* n. sp. from *T. fucata* from off Heron Island. 1. Holotype, adult, whole
528 mount, lateral view. 2. Holotype, female terminal genitalia, lateral view. Abbreviations: GP, female
529 genital pore; MG, Mehlis' gland; OD, oviduct; Oö, oötype; Ov, ovary; T, testis; U, uterus; VD, vitelline
530 duct; VF, vitelline follicles. Scale–bars: 1, 500 μm; 2, 250 μm.
531
532 **Fig. 3.** The genetic relationships among species of *Phthinomita* inferred by minimum evolution analysis
533 of the complete ITS2 rDNA dataset. The sequence from the present study is indicated in bold. Bootstrap
534 support is indicated for all major nodes.
535
536 **Figs. 4–5.** The heart of *T. fucata*. 4. Dissected heart illustrating the intensity of a *P. heinigerae* n. sp.
537 infection in a single host fish. 5. Longitudinal section illustrating *P. heinigerae* n. sp. occupying the
538 intertrabecular spaces and lumen of the ventricle. Scale–bar: 4, 200 μm.