1 **Molecular phylogeny and systematics of the Echinostomatoidea Looss, 1899 (Platyhelminthes:**
2 **Digenea) [☆]**

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17 [☆]Note: New nucleotide sequence data reported in this paper are available in GenBank under
18 accession numbers **KT956912** – **KT956960**.

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20 Note: Supplementary data associated with this article.

21

22 ABSTRACT

23 The Echinostomatoidea is a large, cosmopolitan group of digeneans currently including nine
24 families and 101 genera, the vast majority parasitic, as adults, in birds with relatively few taxa
25 parasitising mammals, reptiles and, exceptionally, fish. Despite the complex structure, diverse
26 content and substantial species richness of the group, due to the lack of data almost no attempt has
27 been made to elucidate its phylogenetic relationships at the suprageneric level based on molecules.
28 Herein, we evaluate the consistency of the present morphology-based classification system of the
29 Echinostomatoidea with the phylogenetic relationships of its members based on partial sequences of
30 the nuclear *lsrRNA* gene for a broad diversity of taxa (80 species, representing eight families and 40
31 genera), including representatives of five subfamilies of the Echinostomatidae, which currently
32 exhibits the most complex taxonomic structure within the superfamily. This first comprehensive
33 phylogeny for the Echinostomatoidea challenged the current systematic framework based on
34 comparative morphology. A morphology-based evaluation of this new molecular framework
35 resulted in a number of systematic and nomenclatural changes consistent with the phylogenetic
36 estimates of the generic and suprageneric boundaries and a new phylogeny-based classification of
37 the Echinostomatoidea. In the current systematic treatment: (i) the rank of two family level lineages,
38 the former Himasthlinae and Echinochasminae, is elevated to full family status; (ii) *Caballerotrema*
39 is distinguished at the family level; (iii) the content and diagnosis of the Echinostomatidae (*sensu*
40 *stricto*) (*s. str.*) are revised to reflect its phylogeny, resulting in the abolition of the Nephrostominae
41 and Chaunocephalinae as synonyms of the Echinostomatidae (*s. str.*); (iv) *Artyfechinostomum*,
42 *Cathaemasia*, *Rhopalias* and *Ribeiroia* are re-allocated within the Echinostomatidae (*s. str.*),
43 resulting in the abolition of the Cathaemasiidae, Rhopaliidae and Ribeiroiinae, which become
44 synonyms of the Echinostomatidae (*s. str.*); and (v) refinements of the generic boundaries within the
45 Echinostomatidae (*s. str.*), Psilostomidae and Fasciolidae are made.

46
47 **Keywords:** Echinostomatoidea; Molecular phylogeny; Systematics; Echinostomatidae (*sensu*
48 *stricto*); Caballerotrematidae n. fam.; Himasthliidae, Echinochasmiidae; Host associations

49 **1. Introduction**

50 The Echinostomatoidea Looss, 1899 is a large, globally distributed and heterogeneous group
51 of cosmopolitan, hermaphroditic digeneans that parasitise, as adults, vertebrate hosts of all classes
52 but exhibit a particularly high diversity in birds (Kostadinova and Jones, 2005). Some of the
53 members of Echinostomatoidea cause diseases in wildlife and humans, and some are used as
54 laboratory model species. Species of this superfamily, most notably its central family, the
55 Echinostomatidae Looss, 1899, have traditionally received significant attention from researchers,
56 resulting in extensive literature on various subjects of their systematics, morphology, biology and
57 ecology (see e.g. Fried and Graczyk, 2000; Kostadinova and Gibson, 2000; Fried, 2001; Fried and
58 Toledo, 2009; and references therein).

59 First defined by Faust (1929), the superfamily was first recognised (at the subordinal rank,
60 Echinostomata) by Szidat (1939). Subsequent conceptions of the Echinostomatoidea (see La Rue,
61 1957; Odening, 1963) have largely preserved the classification of Szidat (1939) with a few
62 exceptions. According to the most recent classification, the Echinostomatoidea is subdivided into
63 eight families comprising a total of 82 genera: Echinostomatidae Looss, 1899 (with 10 subfamilies
64 and 44 genera), Cathaemasiidae Fuhrmann, 1928 (with two subfamilies and five genera),
65 Fasciolidae Railliet, 1895 (with three subfamilies and six genera), Philophthalmidae Looss, 1899
66 (with five subfamilies and 11 genera), Psilostomidae Looss, 1900 (with six subfamilies and 13
67 genera), Rhytidodidae Odhner, 1926 (with two genera) plus the monotypic Calycodidae Dollfus,
68 1929 and Rhopaliidae Looss, 1899 (see Kostadinova, 2005a, b, c; Kostadinova and Jones, 2005).
69 Kanev et al. (2002) placed the Cyclocoelidae Stossich, 1902 within the superfamily Cyclocoeloidea
70 Stossich, 1902, following the concept of La Rue (1957). However, both molecular phylogenies of
71 the Digenea (Cribb et al., 2001; Olson et al., 2003) recovered the Cyclocoelidae within the
72 Echinostomatoidea. This is accepted here, thus increasing the number of subfamilies and genera
73 within the superfamily by six and 18, respectively (i.e. 32 subfamilies and 101 genera, see
74 Supplementary Table S1 for detailed classification to the generic level). The Cotylotretidae

75 Travassos, 1922 was not recognised by Kostadinova and Jones (2005), since its type genus,
76 *Cotylotretus* Odhner, 1902, was treated as a *genus inquirendum* by Kostadinova (2005a); this is
77 accepted here.

78 Although the Echinostomatoidea is characterised by a complex structure, diverse content
79 and substantial species richness, almost no attempt has been made to elucidate its phylogenetic
80 relationships at the suprageneric level based on molecular data due to the very limited sampling,
81 e.g. the most comprehensive phylogeny of the Digenea of Olson et al. (2003) included molecular
82 data for eight of 101 echinostomatoid genera and four families have not been sampled to date
83 (Kostadinova and Pérez-del-Olmo, 2014).

84 Here, with the aid of molecular evidence, we provide the first known comprehensive
85 phylogeny for the Echinostomatoidea. Using 28S rRNA gene sequences for a broad diversity of
86 taxa (80 species representing eight families and 40 genera), we evaluate the consistency of the
87 present morphology-based classification system of the Echinostomatoidea with the phylogenetic
88 relationships of its members. In particular, we: (i) test the cohesion of the Echinostomatoidea and its
89 constituent families; (ii) evaluate the monophyly of and relationships between subfamilies and
90 genera of the largest and most complex family, the Echinostomatidae; (iii) provide systematic and
91 nomenclatural changes consistent with the phylogenetic estimates of generic and suprageneric
92 boundaries; and (iv) suggest a new phylogeny-based classification of the superfamily. Our results
93 revealed a picture departing significantly from the currently accepted morphology-based systematic
94 framework for the Echinostomatoidea.

95

96 **2. Materials and methods**

97 *2.1. Sampling and morphological studies*

98 In order to reconstruct a representative, large-scale phylogeny of the Echinostomatoidea,
99 adult and larval stages of echinostomatoid digeneans were collected between 1997 and 2015 from
100 various vertebrates (predominantly birds) and snails sampled in Europe, Africa, North America and

101 South America. The resulting set of taxa included 80 species representing 40 echinostomatoid
102 genera and numerous suprageneric taxa. A list of species used in this study with their hosts,
103 geographical origin and the corresponding museum and GenBank accession numbers is provided in
104 Table 1. Most of the digeneans were collected by the authors from freshly killed vertebrate hosts
105 under appropriate collecting permits, or from snails emitting cercariae. Several specimens for this
106 study have been provided by our colleagues (see Acknowledgements).

107 For morphological studies and scanning electron microscopy, the worms were removed
108 from the host intestine, rinsed in saline, killed with hot water and fixed in 70% ethanol. When
109 numerous specimens of certain species were available, some of them were fixed in 95% ethanol.

110 For light microscopical examination and identification, specimens were stained with
111 aqueous alum carmine or Mayer's haematoxylin, dehydrated in a graded ethanol series, cleared in
112 clove oil (after carmin staining) or methyl salicylate (after haematoxylin staining), and mounted
113 permanently in Damar gum or Canada balsam. All specimens used for generation of the new
114 sequences were identified to the species level, except for three (*Echinoparyphium* sp.,
115 *Typhlocoelum* sp. and cercariae of *Sphaeridiotrema* sp.) and a number of new species within the
116 Psilostomidae, *Acanthoparyphium* Dietz, 1909, *Echinochasmus* Dietz, 1909, *Isthmiophora* Lühe,
117 1909 and *Neopetasiger* Bashkirova, 1941. The following abbreviations for the features used in the
118 generic diagnoses by Kostadinova (2005a) are used: BW (%), maximum body width as a proportion
119 of body length; FO (%), forebody width as a proportion of body length; T (%), length of post-
120 testicular field as a proportion of body length.

121 Hologenophores and paragenophores *sensu* Pleijel et al. (2008) are deposited in the
122 collection of the Harold W. Manter Laboratory of Parasitology (HWML) at the University of
123 Nebraska, Lincoln, USA and in the Helminthological Collection of the Institute of Parasitology
124 (HCIP), Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
125 (see Table 1 for details).

126

127 *2.2. DNA extraction, amplification and sequencing*

128 For molecular analysis, live worms recovered from the host were rinsed thoroughly in saline
129 and fixed in 70% or 95% ethanol. New DNA sequences were generated for 47 species of the
130 Echinostomatoidea. Genomic DNA was extracted from partial or, rarely, complete single adult
131 specimens or metacercariae and 5 – 15 cercariae according to the protocol described by Tkach and
132 Pawlowski (1999) or Georgieva et al. (2013).

133 Approximately 1,350 bp long fragment at the 5' end of the 28S rRNA was amplified by PCR
134 on an Eppendorf EP gradient thermal cycler using the forward primer dig12 (5'-AAG CAT ATC
135 ACT AAG CGG-3') and the reverse primer 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3')
136 (Tkach et al., 2003). PCRs were performed in a total volume of 25 µl using New England Biolabs
137 (USA) One-Taq quick load PCR mix according to the manufacturer's instructions. The
138 thermocycling profile was as follows: 30 s denaturation hold at 94 °C; 40 cycles of 30 s at 94 °C, 30
139 s at 53 °C, 2 min at 68 °C, and a 5 min extension hold at 68 °C.

140 PCR products were purified using QIAquick PCR purification Kit (Qiagen, Germantown,
141 Maryland, USA). PCR primers and an additional internal forward primer 300F (5'-CAA GTA CCG
142 TGA GGG AAA GTT G-3') were used in the sequencing reactions. PCR products were cycle-
143 sequenced directly using ABI BigDye™ (Applied Biosystems, Foster City, California, USA)
144 chemistry, alcohol-precipitated and run on an ABI Prism® 3100 automated capillary sequencer
145 (Life Technologies, Grand Island, New York, USA). Contiguous sequences were assembled using
146 Sequencher ver. 4.2 (GeneCodes Corp., Ann Arbor, Michigan, USA) and submitted to GenBank
147 under accession numbers **KT956912** – **KT956960** (Table 1).

148

149 *2.3. Sequence alignment and phylogenetic analyses*

150 The partial 28S rDNA sequences generated in the study were aligned with sequences of
151 representatives of the Echinostomatoidea *sensu* Olson et al. (2003) available in GenBank (Table 1).

152 A sequence for *Notocotylus attenuatus* (Rudolphi, 1809) (**AF184259**) was used as an outgroup
153 based on the topologies in the phylogenetic trees of the Digenea published by Olson et al. (2003).

154 Sequences were initially aligned with the aid of ClustalW as implemented in the BioEdit
155 program, version 7.0.1 (Hall, 1999). The alignment was then trimmed to the length of the shortest
156 sequence, manually refined using BioEdit, saved in FASTA format and imported into the MacClade
157 ver. 4.02 software (<http://macclade.org/macclade.html>). Upon selection of the exclusion sets the
158 alignments were saved in NEXUS format for subsequent analyses. Positions which could not be
159 unambiguously aligned were excluded from the analysis.

160 The final alignment yielded was 1,166 nucleotide (nt) long of which 31 nt positions were
161 excluded, leaving 1,135 characters for analyses. Bayesian inference (BI) and Maximum Likelihood
162 (ML) analyses of the 28S rDNA data were conducted to explore relationships between the taxa. The
163 software jModelTest version 2.1.6 (Darriba et al., 2012) was used to estimate the best nucleotide
164 substitution model for the dataset. Bayesian inference analysis was conducted using the GTR + I +
165 Γ model predicted as the best estimator by the Akaike Information Criterion (AIC) in jModelTest,
166 and the closest approximation of this model was implemented in the subsequent ML analysis. BI
167 analysis was performed using MrBayes version 3.2.3 (Ronquist et al., 2012) run on the CIPRES
168 Science Gateway v. 3.1. (http://www.phylo.org/sub_sections/portal/) with the following nucleotide
169 substitution model settings: lset nst = 6, rates = invgamma, samplefreq = 100, ncat = 4, shape =
170 estimate, inferrates = yes and basefreq = empirical. Markov chain Monte Carlo (MCMC) chains
171 were run for 10,000,000 generations, log-likelihood scores were plotted and only the final 75% of
172 trees were used to produce the consensus trees by setting the "burn-in" parameter at 2,500. This
173 number of generations was considered sufficient because the SD dropped well below 0.01 at the end
174 of the run. ML analysis was performed using PhyML version 3.0 (Guindon et al., 2010) run on the
175 ATGC bioinformatics platform (<http://www.atgc-montpellier.fr/>). Nodal support in the ML analysis
176 was estimated by performing 100 bootstrap pseudoreplicates. Trees were visualised using the
177 FigTree ver. 1.4 software (<http://tree.bio.ed.ac.uk/software/figtree/>).

178

179 **3. Results**180 *3.1. Taxonomic coverage*

181 Table 1 provides a list of the taxa analysed following the most recent classifications based
182 on morphology (Kanev et al., 2002; Kostadinova, 2005a, b, c; Kostadinova and Jones, 2005;
183 Dronen, 2007; see Supplementary Table S1). The echinostomatoid dataset comprises 85 sequences
184 for 80 species belonging to 40 genera and includes representatives of eight of the 10 families and 17
185 of the 32 subfamilies currently recognised within the superfamily using the concept of Olson et al.
186 (2003) based on molecular evidence. Importantly, type species of 23 genera (marked with asterisks
187 in Table 1 and stars in Fig. 1) are represented in the dataset and sequence data for 47 species were
188 generated de novo with most intensive sampling within the most diverse family Echinostomatidae
189 (33 species).

190

191 *3.2. Molecular phylogeny*

192 Both BI and ML analyses yielded trees with identical, generally strongly supported
193 topologies. The posterior probability support in BI analyses had overall higher values than the
194 bootstrap support in ML across the whole tree. Throughout the Results and Discussion, we will
195 refer to BI branch support values, although both BI and ML values are shown on the tree (Fig. 1).

196 At higher taxonomic levels, our phylogenetic analysis provided strong support for eight
197 family-level lineages (designated as Clades 1 – 8 in Fig. 1). Based on the analysis, three of these
198 lineages (*Caballerotrema* Prudhoe, 1960 and the subfamilies Himasthlinae Odhner, 1910 and
199 Echinochasminae Odhner, 1910) clearly needed to be elevated to the family level. In contrast,
200 representatives of two independent echinostomatoid families (Cathaemasiidae and Rhopaliidae), as
201 well as of the psilostomid subfamily Ribeiroinae Travassos, 1951, were clustered together as a
202 100% supported sub-clade within the Echinostomatidae. These topologies and their systematic
203 implications are discussed in detail below.

204 Clade 1 was 100% supported and comprised 17 genera sampled within the
205 Echinostomatidae, Psilostomidae, Cathaemasiidae and Rhopaliidae, including *Echinostoma*
206 Rudolphi, 1809, the type genus of the Echinostomatidae. It split into two major strongly supported,
207 multi-taxon sub-clades, one including *Echinostoma*, *Neoacanthoparyphium* Yamaguti, 1958,
208 *Patagifer* Dietz, 1909, *Artyfechinostomum* Lane, 1915, *Moliniella* Hübner, 1939, *Echinoparyphium*
209 Dietz, 1909 and *Hypoderaeum* Dietz, 1909, and the other including *Ribeiroia* Travassos, 1939,
210 *Cathaemasia* Looss, 1899, *Rhopalias* Stiles & Hassall, 1898, *Paryphostomum* Dietz, 1909,
211 *Petasiger* Dietz, 1909, *Isthmiophora*, *Drepanocephalus* Dietz, 1909, *Euparyphium* Dietz, 1909
212 (type species) and *Chaunocephalus* Dietz, 1909. A majority of the generic level lineages within this
213 clade that were represented in our analysis by more than a single species (*Echinostoma*, *Patagifer*,
214 *Isthmiophora* and *Echinoparyphium*) were strongly supported. However, the type species of
215 *Hypoderaeum* was nested within the 100% supported clade of *Echinoparyphium* + *Euparyphium*,
216 and *Petasiger* proved to be polyphyletic. *Euparyphium* cf. *murinum* Tubanguui, 1931 was nested
217 within the strongly supported clade of *Echinoparyphium* within the first major sub-clade, whereas
218 the type species *Euparyphium capitaneum* Dietz, 1909 appeared as sister to *Drepanocephalus*
219 *auritus* Kudlai, Kostadinova, Pulis & Tkach, 2015 within the second major sub-clade. Two species
220 of *Petasiger* possessing 27 collar spines, including the type species of the genus, *P. exaeretus* Dietz,
221 1909, clustered with *Paryphostomum radiatum* (Dujardin, 1845), the type species of
222 *Paryphostomum*, with strong support, whereas the remaining six *Petasiger* spp. represented in our
223 analysis (all possessing 19 collar spines) formed a tight, distinct, 100% supported clade. Two of the
224 currently accepted subfamilies of the Echinostomatidae, the Echinostomatinae Odhner, 1910 and
225 Himasthlinae, appeared in our tree as non-monophyletic and there was no support for the subfamily
226 status of two additional subfamilies, the Nephrostominae Mendheim, 1943 (represented by
227 *Patagifer* spp.) and Chaunocephalinae Travassos, 1922 (represented by *Chaunocephalus ferox*
228 (Rudolphi, 1795), the type species of the type genus). Most remarkably, representatives of three

229 echinostomatoid families, i.e. *Ribeiroia* (Psilostomidae), *Cathaemasia* (Cathaemasiidae) and
230 *Rhopalias* (Rhopaliidae), formed a 100% supported clade nested within the Echinostomatidae.

231 Clade 2 includes a single genus *Caballerotrema* the species of which are parasitic in South
232 American freshwater fishes, predominantly *Arapaima gigas* (Schinz).

233 Clade 3 represents five out of the six genera of the Fasciolidae. *Protofasciola* Odhner, 1926
234 (and the Protofasciolinae Skrjabin, 1948) appeared somewhat distant from the remaining genera and
235 subfamilies. There was strong support for the subfamily Fasciolinae Railliet, 1895 comprising two
236 species each of *Fasciola* Linnaeus, 1758 (*Fasciola hepatica* Linnaeus, 1758 and *Fasciola gigantica*
237 Cobbold, 1855) and *Fascioloides* Ward, 1917 (*Fascioloides magna* Bassi, 1875 and *Fascioloides*
238 *jacksoni* (Cobbold, 1869)), the latter being positioned basal to *Fasciola*. It should be noted,
239 however, that *F. magna* and *F. jacksoni* did not cluster with each other. The same is true for the two
240 genera of the Fasciolopsinae Odhner, 1910 (*Fasciolopsis* Looss, 1899 and *Parafasciolopsis*
241 Ejsmont, 1932) (Fig. 1).

242 Clades 4 and 5 represent two closely related clades, the Cyclocoelidae + Typhlocoelidae
243 Harrah, 1922 and the Philophthalmidae. The three philophthalmid subfamilies, i.e. the
244 Philophthalminae Looss, 1899, Parorchinae Lal, 1936 and Cloacitreminae Yamaguti, 1958, were
245 well supported in our analysis (Fig. 1).

246 Clade 6 includes representatives of the two most diverse genera of the echinostomatid
247 subfamily Himasthlinae, *Himasthla* Dietz, 1909 and *Acanthoparyphium*, which form two well-
248 supported clades. The maximum support for Clade 6, coupled with its earlier divergence in relation
249 to the clades representing most of the diversity of the Echinostomatidae, indicates that the
250 subfamily Himasthlinae (*sensu stricto*) should be elevated to full family rank. The finding that the
251 previously recognised subfamily Himasthlinae is polyphyletic in the present analysis, with
252 *Artyfechinostomum* nested among the genera belonging to the Echinostomatidae (Clade 1) and
253 *Caballerotrema* exhibiting an earlier divergence than the latter (Clade 2) (Fig. 1), support this
254 suggestion.

255 The remaining two family level clades (7 and 8) appear as two closely related lineages, one
256 of them including representatives of several genera of the Psilostomidae and the other comprising
257 members of four genera belonging to the Echinochasminae. The Psilostomidae (Clade 7) included
258 two currently recognised subfamilies, the Psilostominae Looss, 1900 and Sphaeridiotrematinae
259 Yamaguti, 1958, whose monophyly received maximum support, and a 100% supported group of
260 five new species sampled in various species of duck predominantly from North America (Fig. 1;
261 Table 1). These species likely represent at least three new genera of a distinct subfamily which will
262 be formally established and named separately, concurrently with the descriptions of these new
263 species. The situation in Clade 8, comprising representatives of *Echinochasmus* (type species),
264 *Uproctepisthmium* Fischthal & Kuntz, 1976, *Microparyphium* Dietz, 1909 (type species) and
265 *Stephanoprora*, is rather complex due to the non-monophyletic nature of *Echinochasmus* which was
266 split into two clusters. Some of the *Echinochasmus* spp. sampled here were associated with
267 members of *Stephanoprora*, whereas the others represented derived taxa in the clade that also
268 included *Uproctepisthmium* and *Microparyphium* (Fig. 1).

269

270 3.3. Systematic and nomenclatural changes

271 3.3.1. Family Echinostomatidae Looss, 1899 (*sensu stricto*)

272 *Synonyms*: Balfouriidae Travassos, 1951; Cathaemasiidae Fuhrmann, 1928 (new synonym);
273 Chaunocephalidae Travassos, 1922; Chaunocephalinae Travassos, 1922; Echinocollidae Odening,
274 1961; Nephrostominae Mendheim, 1943 (new synonym); Rhopaliidae Looss, 1899 (new synonym);
275 Ribeiroiinae Travassos, 1951 (new synonym)

276 *Diagnosis* (modified after Kostadinova, 2005a): Digenea: Echinostomatoidea. Body small to
277 large, elongate-oval to fairly elongate, occasionally foliate or subdivided into two or three regions.
278 Tegument usually armed. Apical end armament present, occasionally absent (*Ribeiroia*), typically
279 in the form of circumoral head-collar, armed with one or two crowns of spines interrupted ventrally
280 and uninterrupted dorsally or two anteriorly directed retractile proboscides armed with spines, one

281 on each side of oral sucker (*Rhopalias*). Oral sucker subterminal, typically smaller than ventral
 282 sucker, occasionally larger (*Ruffetrema*) or rudimentary (*Pegosomum*). Ventral sucker muscular,
 283 spherical, usually close to anterior extremity, occasionally in second quarter of body. Prepharynx
 284 short or absent. Pharynx muscular, elongate-oval. Oesophagus short to long, occasionally with
 285 lateral diverticula (*Ribeiroia*). Intestinal bifurcation in forebody. Caeca two, simple or with outer
 286 lateral diverticula, reach fairly close to posterior extremity, usually blind, rarely forming uroproct.
 287 Testes two, in hindbody, usually tandem, may be oblique or symmetrical. Cirrus-sac muscular, in
 288 forebody, anterodorsal to ventral sucker, occasionally extending into hindbody (*Artyfechinostomum*,
 289 *Pelmatostomum*, *Rhopalias*) or apparently absent (*Pelmatostomum*). Internal seminal vesicle
 290 saccular or tubular, simple or bipartite. Pars prostatica usually well developed, tubular or vesicular.
 291 Male copulatory organ an evaginable cirrus. Ovary anterior to testes, median or dextral. Mehlis'
 292 gland contiguous with ovary and anterior testis. Canalicular seminal receptacle absent; uterine
 293 seminal receptacle present. Laurer's canal opens dorsally. Uterus intercaecal, pre-ovarian.
 294 Metraterm present. Eggs operculate, numerous to few, normally unembryonated, occasionally may
 295 contain fully developed miracidium with distinct eye spots. Vitellarium follicular, in two lateral
 296 fields usually in hindbody, may extend into forebody. Excretory system Y-shaped, diverticulate;
 297 pore usually terminal. In birds, reptiles, fishes and mammals; cosmopolitan.

298 *Type genus: Echinostoma* Rudolphi, 1809.

299 *Included genera: Balfouria* Leiper, 1908; *Bashkirovitrema* Skrjabin, 1944; *Cathaemasia*
 300 Looss, 1899; *Chaunocephalus* Dietz, 1909; *Drepanocephalus* Dietz, 1909; *Echinodollfusius* Skrjabin
 301 & Bashkirova, 1956; *Echinoparyphium* Dietz, 1909; *Echinostoma* Rudolphi, 1809; *Euparyphium*
 302 Dietz, 1909; *Guaicaipuria* Nasir, Diaz & Marcato, 1970; *Hypoderaeum* Dietz, 1909; *Ignavia*
 303 Freitas, 1948; *Isthmiophora* Lühe, 1909; *Kostadinovatrema* Dronen, 2009; *Longicollia*
 304 Bykhovskaya-Pavlovskaya, 1954; *Lyperorhis* Travassos, 1921; *Moliniella* Hübner, 1939;
 305 *Neoacanthoparyphium* Yamaguti, 1958; *Neopetasiger* Bashkirova, 1941; *Nephrostomum* Dietz,
 306 1909; *Pameileenina* Wright & Smithers, 1956; *Parallelotestis* Belopol'skaya, 1954; *Patagifer* Dietz,

1909; *Pegosomum* Ratz, 1903; *Pelmatostomum* Dietz, 1909; *Petasiger* Dietz, 1909; *Prionosoma*
Dietz, 1909; *Prionosomoides* Freitas & Dobbin, 1967; *Protechinostoma* Beaver, 1943;
Pseudocathaemasioides Saito & Fukumoto, 1972; *Pulchrosoma* Travassos, 1916; *Rhopalias* Stiles
& Hassall, 1898; *Ribeiroia* Travassos, 1939; *Ruffetrema* Saxena & Singh, 1982; *Singhia* Yamaguti,
1958; *Sodalis* Kowalewski, 1902; and *Trifolium* Travassos, 1923.

Remarks: The present diagnosis both narrows the family diagnosis of the Echinostomatidae
given by Kostadinova (2005a) to reflect the exclusion of the Himasthlinae, Echinochasminae and
Caballerotrema and slightly expands it to reflect the inclusion of the Cathaemasiidae, Rhopaliidae
and *Artyfechinostomum* (see Discussion).

3.3.2. Family Caballerotrematidae n. fam.

Diagnosis (modified after Kostadinova, 2005a): Digenea: Echinostomatoidea. Body medium
to large, elongate to considerably attenuated, with maximum width at level of collar. Forebody very
short. Tegument armed with small, scale-like spines in anterior half of body. Collar muscular, very
wide, dorsoventrally flattened, constricted off from body laterally but without ventral ridge;
ventrolateral edges curved ventrally and medially, each with finger-like muscular process. Collar-
spines 29, small, conical; two pairs of somewhat larger spines on each ventral lappet. Oral sucker
small, oval. Ventral sucker funnel-shaped, fairly close to anterior extremity. Prepharynx short.
Pharynx well developed. Oesophagus relatively short. Intestinal bifurcation dorsal to middle of
ventral sucker. Caeca two, simple, blind, reach fairly close to posterior extremity. Testes oblique or
tandem, oval or rather elongate, winding, contiguous or separated, in third quarter of body. Cirrus-
sac large, very elongate, folded between intestinal bifurcation and anterior uterine loop or at least
extending well posterior to ventral sucker. Internal seminal vesicle very long, tubular. Pars
prostatica long, tubular, lined with small anuclear blebs; prostatic cells in dense mass occupying
entire dilated anterior portion of cirrus-sac. Ejaculatory duct short. Cirrus short, muscular, smooth.
Ovary rounded or transversely oval, dextral or median, postequatorial or equatorial. Mehlis' gland

compact, transversely oval. Uterine seminal receptacle expected (not observed). Uterus long to very long, with intercaecal loops; eggs numerous. Vitelline fields non-confluent, extend anteriorly to mid-distance between ovary and ventral sucker, reach close to posterior extremity; follicles small. Excretory pore terminal. In intestine of freshwater fishes (*Arapaima*, *Osteoglossum*, *Zungaro*); South America.

Type- and only genus: Caballerotrema Prudhoe, 1960.

Etymology: The family name refers to the name of the type genus.

3.3.3. Genus *Caballerotrema* Prudhoe, 1960

Diagnosis: With characters of family.

Type species: Caballerotrema brasiliense Prudhoe, 1960.

Other species: Caballerotrema aruanense Thatcher, 1980 and *C. piscicola* (Stunkard, 1960) Kostadinova & Gibson, 2001.

Remarks: Caballerotrema Prudhoe, 1960, erected for *C. brasiliense* Prudhoe, 1960 from *Arapaima gigas* in Brazil (Prudhoe, 1960), was placed in the Singhiinae Yamaguti, 1958 by Yamaguti (1958, 1971) based solely on the parasitism of a piscine host. The genus was subsequently assigned to the subfamily Himasthlinae (see Odening, 1963; Thatcher, 1980; Kostadinova and Gibson, 2001; Kostadinova, 2005a). Species of *Caballerotrema*, however, exhibit characters that are unique for the Himasthlinae (very wide and dorsoventrally flattened collar that possesses finger-like muscular process on each ventrolateral edge; intestinal bifurcation dorsal to ventral sucker; short, muscular unarmed cirrus; extensive pars prostatica). With regard to the above peculiar characters combined with the parasitism of a fish host and the present phylogenetic hypothesis, we propose to distinguish *Caballerotrema* at the family level.

3.3.4. Family *Himasthlidae* Odhner, 1910

Diagnosis (modified after Kostadinova, 2005a): Body small to large, elongate-oval to markedly elongate and slender. Forebody extremely short to short. Tegument armed. Collar

360 reniform. Collar spines in single row uninterrupted dorsally; angle spine-groups differentiated or
361 not; ventral ridge present or absent. Oral sucker subspherical, small. Ventral sucker muscular, small,
362 fairly close to anterior extremity or more posterior. Prepharynx short. Pharynx subequal to or
363 smaller than oral sucker. Oesophagus short to long, narrow. Intestinal bifurcation just anterior to
364 ventral sucker. Caeca blind, reach fairly close to posterior extremity. Testes tandem, postequatorial,
365 fairly close to posterior extremity or more anterior. Cirrus sac very long, extends into hindbody far
366 beyond posterior margin of ventral sucker. Internal seminal vesicle long, tubular, with dilate
367 posterior part. Pars prostatica tubular, small. Ejaculatory duct short. Cirrus muscular, usually long,
368 tubular, armed or occasionally smooth (*Aporchis*). Genital pore median, just postbifurcal. Ovary
369 small, median, closely anterior to anterior testis. Mehlis' gland conspicuous, comparable in size to
370 ovary or larger. Uterine seminal receptacle conspicuous. Uterus extremely long to short. Metraterm
371 distinct. Eggs abundant to few, may possess long polar filament (*Aporchis*). Vitellarium in form of
372 two lateral fields of follicles, in hindbody. Arms of excretory vesicle usually with numerous lateral
373 diverticula; pore subterminal or terminal. In intestine of birds; cosmopolitan.

374 *Type genus: Himasthla* Dietz, 1909.

375 *Included genera: Acanthoparyphium* Dietz, 1909; *Aporchis* Stossich, 1905; *Cloeophora*
376 Dietz, 1909; and *Curtuteria* Reimer, 1963.

377 *Remarks:* The present diagnosis narrows the subfamily diagnosis of the Himasthlinae given
378 by Kostadinova (2005a) to reflect the exclusion of *Artyfechinostomum* and *Caballerotrema* and the
379 elevation of the Himasthlinae to full family status.

380

381 3.3.5. Family Echinochasmidae Odhner, 1910

382 *Synonym:* Saakotrematidae Odening, 1962 (new synonym)

383 *Diagnosis:* With characters of subfamily Echinochasminae Odhner, 1910 (see Kostadinova,
384 2005a).

385 *Type genus: Echinochasmus* Dietz, 1909.

386 *Included genera: Dissurus* Verma, 1936; *Stephanoprora* Odhner, 1902; *Mehrastomum*
387 *Saksena*, 1959; *Microparyphium* Dietz, 1909; *Pulchrosomoides* Freitas & Lent, 1937;
388 *Echinochasmus* Dietz, 1909; and *Saakotrema* Skrjabin & Bashkirova, 1956.
389
390 3.3.6. *Genus Neopetasisiger Bashkirova, 1941*
391 *Diagnosis* (modified after Kostadinova, 2005a): Body small, fusiform, broadly oval to
392 elongate (BW = 18 – 48%), with maximum width at level of ventral sucker or testes. Forebody very
393 long (FO = 25 – 50%), with more or less constricted ‘neck region’ posterior to collar. Tegument
394 armed with small, scale-like spines, dense in forebody. Collar well developed, reniform; ventral
395 ridge distinct. Collar spines 19; lateral spines in single row; dorsal spines in double row; angle
396 spines 2 × 4, longer than marginal spines. Oral sucker subspherical. Ventral sucker prominent,
397 spherical, usually equatorial. Prepharynx distinct. Pharynx muscular, smaller than oral sucker.
398 Oesophagus long. Intestinal bifurcation at some distance anterior to ventral sucker. Caeca two,
399 simple, blind, reach fairly close to posterior extremity. Testes tandem, oblique or symmetrical,
400 subglobular, smooth or slightly lobed, contiguous, usually in mid-hindbody (T = 10 – 35%). Cirrus-
401 sac large, elongate or broadly oval, between intestinal bifurcation and ventral sucker or extending
402 dorsally to its middle. Internal seminal vesicle voluminous, bipartite. Pars prostatica well
403 developed. Cirrus tubular or bulb-like, unarmed (except in *N. combesi*). Ovary oval, dextral, close
404 to ventral sucker, postequatorial. Mehlis’ gland compact, larger than ovary. Uterine seminal
405 receptacle conspicuous. Uterus very short (U = 0 – 7%), with few eggs. Metraterm short. Fields of
406 follicular vitellarium reach into forebody anteriorly to anterior margin of ventral sucker, genital
407 pore or intestinal bifurcation, converge towards median line or confluent in post-testicular field,
408 may be confluent in forebody; follicles large. Stem of excretory vesicle with lateral diverticula; pore
409 terminal. In intestine of grebes (Podicipedidae), occasionally Anatidae, Anhingidae, Laridae,
410 Phalacrocoracidae and Phoenicopteridae; cosmopolitan. Cercariae in Planorbidae; metacercariae in
411 oesophagus or pharynx of freshwater teleosts.

412 *Type species: Neopetasiger skrjabini* (Bashkirova, 1941) (syn. of *P. neocomense* Fuhrmann,
413 1927).

414 *Included species: Neopetasiger australis* (Johnston & Angel, 1941) n. comb.; *N. combesi*
415 (*Zamparo, Overstreet & Brooks, 2005*) n. comb.; *N. floridus* (*Premvati, 1968*) n. comb.; *N.*
416 *grandivesicularis* (*Ishii, 1935*) n. comb.; *N. islandicus* (*Kostadinova & Skírnisson, 2007*) n. comb.;
417 *N. johnstoni* (*Faltýnková, Gibson & Kostadinova, 2000*) n. comb.; *N. megacanthus* (*Kotlán, 1922*)
418 n. comb.; *N. neocomense* (*Fuhrmann, 1927*) n. comb.; *N. nitidus* (*Linton, 1928*) n. comb.; *N.*
419 *novemdecim* (*Lutz, 1928*) n. comb.; *N. oschmarini* (*Kostadinova & Gibson, 1998*) n. comb.; *N.*
420 *pseudoneocomense* (*Bravo-Hollis, 1969*) n. comb.; and *N. pungens* (*Linstow, 1894*) n. comb.

421 *Remarks:* The present concept represents a revision of the concepts of *Petasiger* of
422 *Kostadinova (2005a)* and *Faltýnková et al. (2008)* to include only species with 19 collar spines,
423 predominantly parasitising grebes (*Podicepididae*), and to reflect the exclusion of *P. exaeretus*, *P.*
424 *phalacrocoracis* (*Yamaguti, 1939*) and *P. variospinosus* (*Odhner, 1910*) retained within *Petasiger*.
425 The name *Petasiger* cannot be used for the group of species with 19 collar spines, since, based on
426 the present molecular analysis, the type species of *Petasiger* does not belong to this group. We have
427 refrained from providing a new name for this group, because it contains most of the species of
428 *Petasiger* and the name is well established in the literature. *Bashkirova (1941)* erected the subgenus
429 *Neopetasiger* *Bashkirova, 1941* (with the type species *P. skrjabini* *Bashkirova, 1941*, possessing 19
430 collar spines) for a much smaller group of species known at that time with oblique or symmetrical
431 testes and allocated the forms with tandem testes to the nominal subgenus (with the type species *P.*
432 *exaeretus*, possessing 27 collar spines) (*Bashkirova, 1941*; *Faltýnková et al., 2008*). The position of
433 the testes has been considered a variable character, inadequate for distinguishing the two subgenera
434 (*Faltýnková et al., 2008*) and the concept of *Bashkirova (1941)* was not accepted by *Yamaguti*
435 (*1971*) and *Kostadinova (2005a)*. However, the clear separation between *Petasiger* spp. with 19 and
436 27 collar spines in the present analysis, and the strongly supported association of *P. exaeretus* and
437 *P. phalacrocoracis* with *Paryphostomum radiatum*, reflect differences at the generic level, other

438 than collar armament, that have to be re-evaluated. Therefore, the elevation of *Neopetasiger* to full
439 generic rank with a different content appears most parsimonious. Two species possessing 23 collar
440 spines, *Petasiger soochowensis* Ku, Chiu, Li & Zhu, 1977 and *P. minutissimus* Gogate, 1934, were
441 not considered in the generic diagnosis.

442

443 3.3.7. Genus *Petasiger* Dietz, 1909

444 *Synonym: Paryphostomum* Dietz, 1909

445 *Diagnosis* (modified after Kostadinova, 2005a): Body small to medium, elongate, elongate-
446 oval, with nearly parallel margins, to fusiform (BW = 14 – 48%), with maximum width at level of
447 testes or ventral sucker. Forebody short to very long (FO = 15 – 54%). Tegument armed with small
448 scale-like spines, dense in forebody. Collar well developed, reniform; ventral incision deep; ventral
449 ridge present. Collar spines 27, large; lateral spines in single row; dorsal spines in double row; angle
450 spines 2×4 , considerably larger than marginal spines. Oral sucker spherical. Ventral sucker
451 spherical to cup-shaped, with deep cavity, between first and second quarters of body or equatorial.
452 Prepharynx short. Pharynx subglobular to elongate-oval, similar in size to oral sucker or slightly
453 smaller. Oesophagus short to long. Intestinal bifurcation at significant distance anterior to ventral
454 sucker. Caeca two, simple, blind, reach fairly close to posterior extremity. Testes tandem to oblique,
455 large, deeply lobed or smooth, contiguous or slightly separated, in mid-hindbody or more posterior
456 (T = 10 – 37%). Cirrus sac elongate-oval, anterior to or extending to mid-level of ventral sucker
457 dorsally. Internal seminal vesicle voluminous, bipartite. Pars prostatica well developed. Cirrus
458 tubular, large, spined or unarmed. Ovary small, spherical, dextral, pre-equatorial, occasionally just
459 post-equatorial. Mehlis' gland compact, larger than ovary. Uterine seminal receptacle conspicuous.
460 Uterus very short to short (U = 0 – 11%), with few coils. Metraterm muscular, short to long. Eggs
461 not numerous. Vitellarium follicular; lateral fields extend between intestinal bifurcation, anterior or
462 posterior margin of ventral sucker and posterior extremity, non-confluent, approach median line
463 posteriorly to testes; follicles large. Arms of excretory vesicle with lateral diverticula; pore terminal.

464 In intestine mainly of Phalacrocoracidae in Europe, Asia, Africa, Australia, North America and
465 South America, Anhingidae in Africa and Ciconiidae [or Cathartidae?] and Sulidae in South
466 America. Cercariae in Planorbidae and Lymnaeidae; metacercariae in cyprinid fishes and tadpoles.

467 *Type species: Petasiger exaeretus* Dietz, 1909.

468 *Included species: Petasiger azerbaijanicus* (Sailov, 1963) n. comb.; *P. carbonis*
469 (Mendheim, 1940) n. comb.; *P. exaeretus* Dietz, 1909; *P. lobulatus* Odhner, 1910 n. comb.; *P.*
470 *mexicanus* (Lamothe-Argumedo & Pérez-Ponce de León, 1989) n. comb.; *P. parvicephalus*
471 (Rietschel & Werding, 1978) n. comb.; *P. phalacrocoracis* (Yamaguti, 1939); *P. radiatus*
472 (Dujardin, 1845) n. comb.; *P. segregatus* (Dietz, 1909) n. comb.; *P. testitriifolius* (Gogate, 1934) n.
473 comb.; and *P. variospinosus* (Odhner, 1910).

474 *Remarks:* The present concept represents a revision of the concept of *Petasiger* of
475 Kostadinova (2005a) to reflect the inclusion of the species of *Paryphostomum* considered valid by
476 Kostadinova et al. (2002) listed above.

477

478 3.4. Phylogeny-based classification of the Echinostomatoidea

479 Order Plagiorchiida La Rue, 1957

480 Suborder Echinostomata La Rue, 1926

481 Superfamily Echinostomatoidea Looss, 1899 (new syn. Cyclocoeloidea Stossich, 1902)

482 Family Echinostomatidae Looss, 1899 (new syns Rhopaliidae Looss, 1899; Cathaemasiidae
483 Fuhrmann, 1928; Nephrostominae Mendheim, 1943; Ribeiroiinae Travassos, 1951)

484 Family Caballerotrematidae n. fam.

485 Family Cyclocoelidae Stossich, 1902 (new syn. Typhlocoelidae Harrah, 1922)

486 Family Echinochasmidae Odhner, 1910 (new syn. Saakotrematidae Odening, 1962)

487 Family Fasciolidae Railliet, 1895

488 Family Himasthlidae Odhner, 1910

489 Family Philophthalmidae Looss, 1899

Family Psilostomidae Looss, 1900

? Family Rhytidodidae Odhner, 1926

? Family Calycodidae Dollfus, 1929

4. Discussion

This dense sampling of family level lineages and the wide taxonomic coverage achieved in this first comprehensive molecular phylogeny for the Echinostomatoidea provided important insights into the evolutionary relationships and classification system of this large digenean superfamily. The results have provided strong support for three of the traditionally recognised major echinostomatoid lineages, but challenge the current systematic framework based on comparative morphology in several ways, most importantly concerning the content and structure of the largest family, the Echinostomatidae. The morphology-based evaluation of the new molecular framework resulted in a number of systematic and nomenclatural changes consistent with the phylogenetic estimates of the generic and suprageneric boundaries and a new phylogeny-based classification of the Echinostomatoidea.

The present analysis confirmed the monophyly of each of the families Fasciolidae, Philophthalmidae and Psilostomidae. The inclusion of the Typhlocoelidae in the analyses resulted in its association with the Cyclocoelidae (previously shown to belong to the Echinostomatoidea; see Olson et al., 2003) with maximum levels of support within the Echinostomatoidea. The features used by Kanev et al. (2002) to distinguish the two families in their key to families of the Cyclocoeloidea exhibit overlapping states. We find no reason to recognise these taxa at the family level and consider the Typhlocoelidae synonymous with the Cyclocoelidae.

Perhaps the most unexpected result of the phylogenetic analysis is that the representative genera of three echinostomatoid families, i.e. *Rhopalias* (Rhopaliidae), *Cathaemasia* (Cathaemasiidae) and *Ribeiroia* (Psilostomidae), appeared in a strongly supported clade nested among the echinostomatids of Clade 1. Based on the very strong support for their phylogenetic

516 placement and the lack of other significant morphological differences, the three genera are
517 transferred to the Echinostomatidae (*s. str.*); and the family diagnosis is emended to reflect this
518 action.

519 Of these three genera, *Cathaemasia* is the only taxon characterised by the presence of a
520 "rudimentary collar" armed with small spines in both the sexual adult and the cercarial stages. All
521 other features of the morphology of the sexual adult agree well with the concept of the
522 Echinostomatidae.

523 *Rhopalias* has been traditionally separated in its own family based on the unique armament
524 of the apical end. Species of *Rhopalias* possess two anteriorly directed retractile proboscides armed
525 with spines, one on each side of the oral sucker. Otherwise, their general morphology corresponds
526 with that of the members of the Echinostomatidae (*s. str.*) with the exception of the somewhat
527 longer cirrus-sac extending beyond the posterior margin of the ventral sucker. The unusual structure
528 of the apical armament appears to be a highly specialised but secondary adaptation. The family
529 Rhopaliidae is regarded here as synonymous with the Echinostomatidae (*s. str.*); the name
530 Echinostomatidae is well established in the literature and is associated with a vast species diversity
531 (more than 450 species versus four species; see Kostadinova and Gibson, 2000). In conflict with the
532 diagnosis of the superfamily Echinostomatoidea (see Kostadinova and Jones, 2005), a canalicular
533 seminal receptacle (likely an erroneous interpretation of a uterine seminal receptacle) was included
534 in the generic diagnosis of *Rhopalias* by Radev et al. (2005); this feature has not been considered in
535 the amended diagnosis of the Echinostomatidae.

536 The position of *Ribeiroia* among the echinostomatids was also unexpected. Travassos
537 (1951) allocated this genus to the subfamily Ribeiroiinae in the Cathaemasiidae; this was supported
538 by Yamaguti (1958, 1971). Kostadinova (2005b) reduced the Cathaemasiidae to include only those
539 genera that possess a rudimentary collar and/or rudimentary spines and transferred the Ribeiroiinae
540 to the Psilostomidae. Wilson et al. (2005) carried out an analysis based on sequences of the nuclear
541 ribosomal internal transcribed spacer 2 (ITS2) region, a marker too variable for phylogenetic

analyses at higher taxonomic levels, for a limited number of taxa and concluded that “given the current data, species of either *Cathaemasia* or *Isthmiophora* are the likely sisters to *Ribeiroia* spp., but additional interpretation on the placement of *Ribeiroia* spp. into either Psilostomidae or Cathaemasiidae cannot be made at this time”. The position of *Ribeiroia* within the Echinostomatidae (*s. str.*) is unequivocally resolved in our phylogenetic analysis, as indicated by the maximum support values (Fig. 1). Furthermore, comparative analysis of the known life cycles of *Ribeiroia* spp. and representatives of the Psilostomidae (species of *Astacatreumatula* Macy & Bell, 1968, *Grysoma* Byrd, Bogitsh & Maples, 1961, *Psilochasmus* Lühe, 1909, *Psilostomum* Looss, 1899, *Psilotrema* Odhner, 1913 and *Sphaeridiotrema* Odhner, 1913) indicate that the biology of *Ribeiroia* spp. does indeed fit its present phylogenetic placement. Species of *Ribeiroia* utilise a three-host life cycle, with cercariae developing in pulmonate gastropods (Planorbidae) and metacercariae encysting in amphibian and fish second intermediate hosts (Beaver, 1939). In contrast, species of the psilostomid genera listed above develop in orthogastropods (Bithyniidae, Hydrobiidae, Littorinidae, Thiaridae and Lithoglyphidae) and metacercariae encyst either between the mantle and shell or within snail tissues (Kostadinova, 2005c). Cercariae of *Ribeiroia* spp. differ from those of species of the Psilostomidae by the possession of an oesophagus with two saccular lateral diverticula, crista on the suckers and finger-like processes on the main collecting ducts at the level of the pharynx (a character shared with the closely related *Cathaemasia hians* (Rudolphi, 1809)) (Beaver, 1939; our unpublished data) (Fig. 1).

Our study strongly indicates that the lack of collar spines in *Ribeiroia* is a result of secondary loss which is a rather common phenomenon among the Echinostomatoidea. The remaining morphological features are similar to other members of the clade in which it was placed in the present analysis. Therefore, we consider the subfamily Ribeiroiinae to be a synonym of the Echinostomatidae. The re-allocation of *Cathaemasia*, *Rhopalias* and *Ribeiroia* resulted in the abolition of the Cathaemasiidae Fuhrmann, 1928, Rhopaliidae Looss, 1899 and Ribeiroiinae Travassos, 1951 as synonyms of the Echinostomatidae (*s. str.*).

568 The most important outcome of the present analysis is that the largest and best sampled
569 family of the Echinostomatoidea, its main family, the Echinostomatidae, appears to be a
570 polyphyletic taxon split into three major, strongly supported, family-level clades, plus a branch of
571 *Caballerotrema* that was clustered together with the members of Clade 1 with very weak support
572 (only 0.72 in BI and lower than 50% in ML) (Fig. 1). Furthermore, there was no support for the
573 monophyly of all of the subfamilies sampled within the Echinostomatidae, i.e. the
574 Echinostomatinae, Chaunocephalinae, Echinochasminae and Himasthlinae. In the current
575 systematic treatment, the rank of the three family-level lineages, i.e. the Himasthlinae,
576 Echinochasminae and an elevated *Caballerotrema*, and the content and diagnosis of the
577 Echinostomatidae (*s. str.*) were revised to reflect the phylogeny.

578 Of the three clades representing the family according to the concept of Kostadinova (2005a),
579 the Echinostomatidae (*s. str.*) is the largest group and includes members of the subfamily
580 Echinostomatinae, represented in our analysis by 11 genera plus the himasthline
581 *Artyfechinostomum*, nephrostomine *Patagifer* and chaunocephaline *Chaunocephalus*, and the three
582 genera (*Cathaemasia*, *Rhopalias* and *Ribeiroia*) discussed above. The lack of support for the
583 subfamilial status of *Patagifer* and *Chaunocephalus* resulted in the abolition of the Nephrostominae
584 Mendheim, 1943 and Chaunocephalinae Travassos, 1922 as synonyms of the Echinostomatidae (*s.*
585 *str.*). The clade of the Echinostomatidae (*s. str.*) was further divided into two strongly supported
586 sub-clades (Fig. 1), which appear to correlate with the life histories of their constituent taxa. The
587 first sub-clade unites seven genera, representing species which typically utilise molluscs as both
588 first and second intermediate hosts, whereas the second sub-clade includes representatives of nine
589 genera representing species which predominantly utilise fishes as second intermediate hosts (Fig.
590 2). Interestingly, *Isthmiophora* spp. *Paryphostomum* spp., *Cathaemasia* spp. and *Ribeiroia* spp.
591 have acquired the ability to use amphibians as second intermediate hosts, in addition to fish (except
592 for *Cathaemasia* spp.). This shift in intermediate host specificity has allowed some species to infect
593 a new group of definitive hosts, mammals (including humans, e.g. *Isthmiophora* spp.) (Fig. 2).

594 *Caballerotrema* represents a unique group, comprising three species considered valid, all
595 parasitic in freshwater fishes of South America (for details, see Kostadinova and Gibson, 2001). In
596 the phylogenetic tree, this genus appeared as a separate branch closest to the Echinostomatidae (*s.*
597 *str.*) and very distant from the Himasthlinae *sensu* Kostadinova (2005a) (Clade 6 in Fig. 1), within
598 which this genus was placed previously (see Odening, 1963; Thatcher, 1980; Kostadinova and
599 Gibson, 2001; Kostadinova, 2005a). Based on this result, the distinct morphological features and
600 the definitive host range, *Caballerotrema* is distinguished at the family level.

601 The second lineage of the polyphyletic Echinostomatidae (*sensu lato*) (*s.l.*) includes
602 representatives of two genera of the former subfamily Himasthliinae, *Himasthla* (type genus) and
603 *Acanthoparyphium*. Notably, *Artyfechinostomum*, the third genus of this subfamily sampled in our
604 study, clustered within the Echinostomatidae (*s. str.*). Species of the Himasthlinae, with the
605 exception of *Artyfechinostomum* spp. and *Caballerotrema* spp., are among the few
606 echinostomatoids with a marine life cycle. Cercarial morphology is also very specific, particularly
607 the excretory system, with the main collecting ducts forming numerous lateral branches in the
608 forebody filled with excretory concretions (Loos-Frank, 1967). In defining the Himasthlidae, we
609 have accepted the boundaries proposed for the Himasthlinae by Odening (1963) and Kostadinova
610 (2005a) to the exclusion of *Caballerotrema* (elevated to family rank) and *Artyfechinostomum*,
611 allocated in the present study to the Echinostomatidae (*s. str.*). The latter action is in agreement with
612 Yamaguti (1958, 1971).

613 The third family-level group of the polyphyletic Echinostomatidae (*s.l.*) includes members
614 of four genera representing the subfamily Echinochasminae *sensu* Kostadinova (2005a). In this
615 clade, the type species of *Echinochasmus* is united in a clade with three other species (Fig. 1).
616 Odening (1962, 1963) elevated the Echinochasminae to full family rank. This action was accepted
617 by Sudarikov and Karmanova (1977) but not followed by Skrjabin and Bashkistrova (1956),
618 Yamaguti (1971) or Kostadinova (2005a). The distinct separation of the Echinochasminae in the
619 present analysis clearly supports the opinion of Odening (1962, 1963).

620 With respect to generic boundaries, our analysis revealed a complex picture of relationships.
621 The monophyly of the majority of the genera represented in the tree by at least two species, i.e.
622 *Echinostoma*, *Echinoparyphium*, *Patagifer* and *Isthmiophora* within the Echinostomatidae (s. str.),
623 *Himasthla* and *Acanthoparyphium* within the Himasthlidae, *Sphaeridiotrema* within the
624 Psilostomidae, and *Parorchis* and *Cloacitrema* within the Philophthalmidae, was strongly
625 supported. However, within the Echinostomatidae (s. str.): (i) *Hypoderaeum* was placed within a
626 strongly supported clade of *Echinoparyphium* spp.; (ii) *Euparyphium* and *Petasisger* were recovered
627 as polyphyletic; and (iii) the position of *Echinoparyphium aconiatum* Dietz, 1909 indicated a
628 possible distinction at the generic level. The close relationships between *H. conoideum* (Bloch,
629 1782) and *Echinoparyphium* spp., and the basal position of *E. aconiatum*, have been reported and
630 discussed in previous phylogenetic studies using less conserved molecular markers. In an analysis
631 of the ITS1 region of rDNA, Grabda-Kazubska et al. (1998) suggested a close relationship between
632 *H. conoideum* and three *Echinoparyphium* spp. Subsequently, Kostadinova et al. (2003) recovered
633 *Hypoderaeum* as a monophyletic sister clade to *Echinoparyphium* and *E. aconiatum* as a sister
634 group to the "revolutum" group of *Echinostoma* in an analysis of a larger group of echinostomatid
635 taxa based on the mitochondrial *nad1* gene, and suggested treating *Echinoparyphium aconiatum* as
636 a member of a separate genus. Our results support this solution, but *E. aconiatum* exhibited a close
637 relationship with *Echinoparyphium* rather than with *Echinostoma*. Although our data reinforce the
638 synonymy of *Hypoderaeum* and *Echinoparyphium*, and the need to elevate the rank of *E.*
639 *aconiatum*, we prefer to await the gathering more sequences for these taxa.

640 However, our results warrant a decision in relation to the two currently recognised
641 echinostomatid genera that proved to be polyphyletic. Recently, Kudlai et al. (2015b) commented
642 on the rather mechanistic generic diagnosis of *Euparyphium* given by Kostadinova (2005a) due to
643 the specific features of the type species and highlighted the morphological and molecular similarity
644 of *Euparyphium albuferensis* Esteban, Toledo, Sanchez & Munoz-Antoli, 1997 with
645 *Echinoparyphium* spp. Although the 28S rDNA sequence of *E. albuferensis* available in the

646 GenBank is too short to be included in the present analysis, this species is transferred to
647 *Echinoparyphium* (as *Echinoparyphium albuferensis* (Esteban, Toledo, Sanchez & Munoz-Antoli,
648 1997) n. comb.) based on the data of Kudlai et al. (2015b) and Heneberg (2013). The morphology
649 and the close association of *Euparyphium* cf. *murinum* with *Echinoparyphium* spp. rather than with
650 the type species *Euparyphium capitaneum*, also support its transfer to *Echinoparyphium*. We
651 anticipate that the sequencing of *Euparyphium guerreroi* Tubangui, 1931, the fourth species
652 currently allocated to *Euparyphium*, may prove that the latter is a monotypic genus.

653 The polyphyly of *Petasiger* required a revision of the concepts of *Petasiger* presented by
654 Kostadinova (2005a) and Faltýnková et al. (2008) by narrowing the concept of *Petasiger* to include
655 only species possessing 27 collar spines and species of *Paryphostomum* considered valid by
656 Kostadinova et al. (2002) and elevating *Neopetasiger* to full generic rank in order to accommodate
657 those species possessing 19 collar spines. Two features of the life-cycle stages also tend to support
658 our concepts of *Neopetasiger* and *Petasiger*. First, all known cercariae of species allocated to
659 *Neopetasiger* belong to the "magnacauda" group (large-tailed cercariae), whereas the cercaria of
660 *Petasiger variospinosus* is characterised by having a substantially shorter tail in comparison to
661 *Neopetasiger* spp. (tail to body length ratio 1:1.32 – 1.52 vs 1: 3.5 – 20; see Faltýnková et al., 2008;
662 King and Van As, 2000; Selbach et al., 2014) and more similar to that in the cercaria of *Petasiger*
663 *radiatus* (tail to body length ratio 1:1.89; see Našincová et al., 1993). Secondly, the cercariae of
664 *Neopetasiger* spp. lack the "prepharyngeal sac" described in *P. variospinosus* and illustrated in *P.*
665 *radiatus* (see Našincová et al., 1993; King and Van As, 2000). Finally, it appears that the cercariae
666 of *Petasiger* spp. utilise an active, direct penetration route of infection, whereas those of
667 *Neopetasiger* spp. may utilise a passive, trophic transmission route (see Faltýnková et al., 2008).

668 Within the Echinochasmidae, *Echinochasmus coaxatus* Dietz, 1909 (type species) was
669 united in a strongly supported clade with two other *Echinochasmus* spp. and *Uroproctepisthmium*
670 *bursicola*. The latter, previously allocated to *Episthmium* Lühe, 1909, was transferred to
671 *Uroproctepisthmium* by Kostadinova (2005a) based on the presence of an uroproct in the sexual

adult. The results of the present phylogenetic analysis suggest that this feature cannot be considered significant at the generic level. Therefore, we consider *Uroproctepisthmium* a synonym of *Echinochasmus*.

Echinochasmus (including *E. bursicola*) appeared in our analysis as paraphyletic. The clade that includes the type species, *E. coaxatus*, formed a well-supported clade with *Microparyphium facetum*, but the remaining four species of *Echinochasmus* fell into a cluster that also included three species of *Stephanoprora*. Notably, these two clusters contained species with different numbers of collar spines. All members of the clade containing the type species of *Echinochasmus* possess 24 collar spines, whereas the members of the *Stephanoprora* + *Echinochasmus* clade possess 20 and 22 collar spines. It is worth noting that the morphological description of the cercaria of *Echinochasmus* sp. VS-2012 (**JQ088098**) (Stanevičiūtė et al., 2008, 2015) indicates a misidentification of *Stephanoprora*. Much wider sampling and combined morphological and molecular characterisation, preferably of the adult stage, is required within the Echinochasmidae (currently comprising more than 120 nominal species) in order to satisfactorily resolve the complex problems of the validity and composition of its genera. We suggest that the clade containing *E. coaxatus* (type species) represents *Echinochasmus* and that the second clade represents *Stephanoprora* (*Stephanoprora*) Odhner, 1902 (type species *S. (S.) ornata* Odhner, 1902) and *Stephanoprora* (*Monilifer*) Dietz, 1909 (type species *S. (M.) spinulosa* (Rudolphi, 1809) Dietz, 1909). It is also worth noting that sequencing of *S. ornata*, described from *Crocodilus niloticus* Laurenti in Africa (Odhner, 1902) and currently representing the only species of the genus possessing 26 collar spines, appears crucial for solving the long-standing debate on the validity and composition of *Stephanoprora*, *Monilifer* and *Mesorchis* Dietz, 1909 (syn. of *Stephanoprora*; see Kostadinova, 2005a).

Although the monophyly of the family Fasciolidae was strongly supported, as in previous molecular phylogenetic studies, our analysis highlighted some existing problems in the generic and suprageneric classification within the family. One of them is the non-monophyly of the genus *Fascioloides* in some of the analyses of Lotfy et al. (2008) and Heneberg (2013). Although the

698 monophyly of *Fascioloides* was not supported in the analyses of the 28S rDNA by these authors
699 (and in the ITS1 trees published by Heneberg (2013)), Lotfy et al. (2008) suggested that the
700 placement of *Fasciola jacksoni* (Cobbold, 1869) in *Fascioloides* should be considered, pending life-
701 cycle details, and Heneberg (2013) went further and made a new combination. In our 28S tree (Fig.
702 1), *Fascioloides jacksoni* and *F. magna* did not form a monophyletic group (a result similar to those
703 in Lotfy et al. (2008) and Heneberg (2013)), thus providing no support for the transfer by Heneberg
704 (2013). On the other hand, the clade uniting species of *Fasciola* and *Fascioloides* is monophyletic
705 and well supported. This situation allows for two possible taxonomic solutions: (i) the separation of
706 *F. jacksoni* in its own genus, thus creating yet another monotypic genus within the Fasciolidae; and
707 (ii) grouping the current members of *Fasciola* and *Fascioloides* into a single genus (*Fasciola*). In
708 our opinion the second solution is preferable due to the rather feeble morphological differences
709 between *Fascioloides* and *Fasciola* (i.e. absence versus presence of a distinct cephalic cone, and
710 symmetrical vs tandem testes) and between *Fascioloides jacksoni* and either of these genera.

711 In a similar situation, but at the subfamily level, the genera *Fasciolopsis* and
712 *Parafasciolopsis*, currently belonging to the Fasciolopsinae Odhner, 1910, did not form a
713 monophyletic clade (similar to the phylogeny in Lotfy et al., 2008). This suggests that the current
714 subfamily structure of the Fasciolidae, as outlined by Jones (2005), may need to be reconsidered or
715 abandoned upon a detailed comparison of morphology of different developmental stages of these
716 digeneans and additional molecular phylogenetic studies.

717 The wide range of taxa sampled in our molecular phylogenetic analysis allowed a
718 preliminary assessment of the evolutionary associations of members of the Echinostomatoidea with
719 their intermediate and definitive hosts and tendencies in the evolution of their life-cycles. Based on
720 our phylogenetic data, it can be concluded that echinostomatoids have evolved as parasites of birds
721 with several secondary host-switching events resulting in parasitism in non-avian vertebrates.
722 Higher-level host groupings for the genera analysed here are indicated in the schematic tree in Fig.
723 2. Echinostomatoids are primarily parasitic in birds, with species of three families, the

724 Philophthalmidae, Psilostomidae and Himasthlidae, almost exclusively utilising avian hosts. Only a
725 limited number of species of ten genera within the Echinostomatidae (*s. str.*), Echinochasmidae and
726 Himasthlidae included in the present phylogeny have incorporated mammalian hosts in their life-
727 cycles, typically in parallel with bird hosts. The Fasciolidae is an example of a lineage that has
728 entirely transitioned to parasitism in mammals, and this contrasts with the limited number of
729 echinostomatid genera (*Rhopalias* and *Isthmiophora*) with species exclusively parasitic in
730 mammals. Parasitism in cold-blooded vertebrates appears exceptional within the superfamily
731 (*Artyfechinostomum*, *Caballerotrema* and *Stephanoprora*).

732 Associations with the first intermediate hosts demonstrated a distinct pattern. As shown by
733 Cribb et al. (2003), the Echinostomatoidea is among digenean superfamilies with the highest
734 diversity of mollusc intermediate hosts. Based on the phylogenetic tree, it is evident that all basal
735 lineages of the superfamily, i.e. the Echinochasmidae, Psilostomidae, Himasthlidae and
736 Philophthalmidae, have evolved in association with "prosobranch" gastropods (subclass
737 Orthogastropoda Ponder & Lindberg, 1995), whereas the remaining more derived groups have
738 evolved to utilise freshwater pulmonate gastropods (subclass Pulmonata Cuvier in Blainville, 1814),
739 with a few (single species of *Echinostoma*, *Echinoparyphium* and *Neacanthoparyphium*) evidently
740 representing secondary reversals to parasitism in orthogastropods (Fig. 2).

741 The situation with the second intermediate host associations is more complicated (Fig. 2)
742 due to the apparent large number of secondary host-switching events and changes in transmission
743 patterns in this large group of digeneans (Cribb et al., 2003). A denser low level taxon sampling is
744 needed to adequately assess the evolutionary adaptations to second intermediate hosts and cercarial
745 encystment patterns among the Echinostomatoidea. Nevertheless, our current tree shows that the
746 ability to encyst in the open (on aquatic vegetation) appears among members of two large clades of
747 the group, the Fasciolidae and Philophthalmidae (Fig. 2). The Cyclocoelidae is characterised by an
748 abbreviated life cycle in which the cercaria encysts within the first intermediate host, and cercariae
749 of some psilostomids encyst in association with the first intermediate hosts. Our data strongly

750 suggest that second intermediate hosts were adopted early in the evolution of the echinostomatoids
751 and that the transition to two-host life cycles, with the encystment of cercariae in the open, resulted
752 from independent evolutionary events (see also Cribb et al., 2003).

753 Although our study represents the first known exhaustive molecular analysis of the
754 Echinostomatoidea, several important taxa at different levels are yet to be included in a
755 phylogenetic framework (see Supplementary Table S1 for details). At the family level, only two
756 echinostomatoid families, the Rhytidodidae and Calycodidae, both comprising parasites of marine
757 turtles, were not represented in our analysis. At lower taxonomic levels, 18 currently recognised
758 subfamilies are lacking in our phylogenetic tree. Notably, half of these (see Supplementary Table
759 S1) represent monotypic subfamilies which have proliferated under previous classifications and
760 require a clarification of their phylogenetic interrelationships. As demonstrated above for the
761 Echinostomatidae (*s.l.*), a number of the subfamilies have proven to be non-monophyletic, thus
762 strongly indicating that the subfamilial taxonomic structure of this and other families, especially the
763 Cyclocoelidae and Typhlocoelidae *sensu* Kanev et al. (2002), need to be reconsidered based on a
764 combination of morphological, biological and molecular phylogenetic data. At the generic level, 70
765 out of 106 echinostomatoid genera were not sampled in our analysis. Of these, 26 (37%) are
766 monotypic and very rare, the great majority having been reported only once in the original
767 descriptions of the type species. We anticipate that it would be impossible to obtain sequences for
768 representatives of most of these genera. Nevertheless, an increased molecular sampling within the
769 subfamilies elevated to full family rank appears to be a required and realistic task, at least for
770 species of the echinochasmid genera *Saakotrema* Skrjabin & Bashkistrova, 1956 and
771 *Pulchrosomoides* Freitas & Lent, 1937, the himasthlid genera *Aporchis* Stossich, 1905 and
772 *Curtuteria* Reimer, 1963, and for the type species of the Nephrostominae Mendheim, 1943,
773 Pegosominae Odhner, 1910, Guaicaipuriinae Nasir, Diaz & Marciano, 1971, Psilostominae Looss,
774 1900 and Grysominae Byrd, Bogitsh & Maples, 1961. Furthermore, increased molecular sampling
775 within the Cyclocoelidae and Typhlocoelidae *sensu* Kanev et al. (2002) and the inclusion of several

776 of the missing genera, particularly those comprising parasites of non-avian hosts such as species of
777 *Rhytidodes* Looss, 1901, *Rhytidodoides* Price, 1939 and *Calycodes* Looss, 1901, parasitic in marine
778 turtles, *Prionosomoides* Freitas & Dobbin, 1967 from freshwater turtles, *Pulchrosomoides* Freitas &
779 Lent, 1937 from iguanas and *Singhia* Yamaguti, 1958 from freshwater fish, would contribute
780 significantly to our understanding of the taxonomic content, phylogenetic interrelationships, and the
781 evolution of life-cycles and host associations within the Echinostomatoidea. Our phylogenetic
782 estimates and nomenclatural actions provide a much needed evolutionary framework for future
783 molecular and comparative morphology assessments at several nested levels.

784

785 **Acknowledgements**

786 We are grateful to Eric Pulis (Institute for Marine Mammal Studies, Gulfport, USA),
787 Stephen Curran (Gulf Coast Research Laboratory, University of Southern Mississippi, USA),
788 Stephen Greiman (University of North Dakota, USA) and James Flowers (North Carolina State
789 University, USA) for providing specimens and/or assistance with field collection. We are very
790 grateful to David Gibson (The Natural History Museum, London, UK) for corrections and insightful
791 comments on an earlier draft of the manuscript. We also thank Simona Georgieva (Institute of
792 Parasitology, Biology Centre of the Czech Academy of Sciences, Czech Republic) for providing
793 sequences for important taxa. We gratefully acknowledge two anonymous reviewers for their
794 valuable comments and important suggestions. This study was supported by the US National
795 Science Foundation (grants DEB-1120734 and DEB 1021431 to VT) and by the Czech Science
796 Foundation (AK, OK, grant P505/12/G112). OK was also supported by the European Union
797 Structural Funds project "Postdoctoral Fellowship Implementation in Lithuania" (VP1-3.1-ŠMM-
798 01-V-02-004).

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978 **Legends to figures**

979

980 **Fig. 1.** Phylogenetic relationships between the taxa of the Echinostomatoidea resulting from
981 Bayesian inference (BI) and Maximum likelihood (ML) analyses based on the partial sequences of
982 the nuclear 28S rRNA gene. BI posterior probability values and ML bootstrap values associated
983 with the branches are shown as BI/ML; support values lower than 0.70 (BI) and 50 (ML) are not
984 shown. The scale bar indicates the number of substitutions per site. Type species are indicated by
985 stars; newly generated sequences are highlighted in bold; shaded areas indicate the taxa belonging
986 to the family Echinostomatidae in the concept of Kostadinova (2005a). The eight strongly supported
987 family-level clades discussed in the text are indicated by numbers. Labels along the vertical bars
988 reflect the classification proposed herein.

989

990 **Fig. 2.** Schematic representation of the phylogenetic tree shown in Fig. 1 with higher level host
991 groupings for the genera analysed here indicated by silhouettes. The scale bar indicates the number
992 of substitutions per site. Labels within the vertical bars reflect the classification proposed herein.
993 Absence of a silhouette indicates a lack of life cycle data. IH1, first intermediate host; IH2, second
994 intermediate host; DH, definitive host; Cab, Caballerotrematidae n. fam.; Cyc, Cyclocoelidae; Him,
995 Himasthlidae.

Table 1

Table 1. Summary data for the echinostomatoid taxa included in molecular analyses. Taxa are listed following the most recent classifications of Gibson et al. (2002), Kostadinova and Jones (2005) and Kostadinova (2005a, b, c). The type species are marked with asterisks.

Taxon	Life cycle stage	Host	Country	Museum No.	GenBank No.	Source
Family Echinostomatidae Looss, 1899						
Subfamily Echinostomatinae Looss, 1899						
<i>Drepanocephalus auritus</i> Kudlai et al., 2015	A	<i>Phalacrocorax auritus</i> (Lesson)	USA	HWML 75310–75312	<u>KP683124</u>	Kudlai et al. (2015a)
<i>Echinoparyphium aconiatum</i> Dietz, 1909	C	<i>Lymnaea stagnalis</i> (L.)	Czech Republic	–	<u>KT956912</u>	Present study
<i>Echinoparyphium cinctum</i> (Rudolphi, 1803)	A	<i>Anas platyrhynchos</i> L.	Ukraine	–	<u>AF184260</u>	Tkach et al. (2001)
<i>Echinoparyphium recurvatum</i> (Linstow, 1873)	C	<i>Radix ovata</i> (Draparnaud)	Slovakia	–	<u>KT956913</u>	Present study
<i>Echinoparyphium rubrum</i> (Cort, 1914)	C	<i>Planorbella trivolvis</i> (Say)	USA	–	<u>JX262943</u>	Tkach et al. (2012)
<i>Echinoparyphium</i> sp.	A	<i>Anas clypeata</i> L.	USA	HWML 101848	<u>KT956914</u>	Present study
<i>Echinostoma revolutum</i> (Frölich, 1802) (<i>sensu lato</i>)	A	<i>Aythya collaris</i> (Donovan)	USA	HWML 101849	<u>KT956915</u>	Present study
<i>Echinostoma revolutum</i> (Frölich, 1802) (<i>sensu stricto</i>)*	A	<i>Aythya fuligula</i> (L.)	Czech Republic	HCIP D-130/13	<u>KP065596</u>	Georgieva et al. (2014)
<i>Echinostoma bolschewense</i> (Kotova, 1939)	C	<i>Viviparus acerosus</i> (Bourguignat)	Slovakia	HCIP D-288	<u>KP065592</u>	Georgieva et al. (2014)
<i>Echinostoma miyagawai</i> Ishii, 1932	A	<i>Anas platyrhynchos</i> L.	Ukraine	HWML 101850	<u>KT956916</u>	Present study
<i>Echinostoma nasincovae</i> Faltýnková et al., 2015	C	<i>Planorbarius corneus</i> (L.)	Czech Republic	HCIP D-289	<u>KP065603</u>	Georgieva et al. (2014)
<i>Echinostoma paraensei</i> Lie & Basch, 1967	A	"hamster" ^a	USA	–	<u>EU025867</u>	Lotfy et al. (2008)
<i>Echinostoma paraulum</i> Dietz, 1909	C	<i>Lymnaea stagnalis</i> (L.)	Germany	HCIP D-711/1	<u>KP065604</u>	Georgieva et al. (2014)
<i>Echinostoma trivolvis</i> (Cort, 1914)	A	<i>Mesocricetus auratus</i> Waterhouse (exp.)	?USA	–	<u>AY222246</u>	Olson et al. (2003)
<i>Echinostoma</i> sp. IG	C	<i>Radix auricularia</i> (L.)	Germany	–	<u>KP065606</u>	Georgieva et al. (2014)
<i>Euparyphium capitaneum</i> Dietz, 1909*	A	<i>Anhinga anhinga</i> (L.)	USA	HWML 75112–75114	<u>KP009620</u>	Kudlai et al. (2015b)
<i>Euparyphium</i> cf. <i>murinum</i> Tubangui, 1931	A	<i>Malacomys longipes</i> Milne-Edwards	Uganda	HWML 101851	<u>KT956917</u>	Present study
<i>Hypoderaeum conoideum</i> (Bloch, 1782)*	A	<i>Anas platyrhynchos</i> L.	Ukraine	HWML 101854	<u>KT956918</u>	Present study
<i>Hypoderaeum conoideum</i> (Bloch, 1782)*	A	<i>Anas acuta</i> L.	USA	HWML 101855	<u>KT956919</u>	Present study
<i>Isthmiophora hortensis</i> (Asada, 1926)	A	<i>Procyon lotor</i> (L.)	Japan	–	<u>AB189982</u>	Sato and Suzuki (2006)
<i>Isthmiophora melis</i> (Schrank, 1788)*	A	<i>Nyctereutes procyonoides</i> (Gray)	Ukraine	–	<u>AF151941</u>	Tkach et al. (2000)
<i>Isthmiophora</i> n. sp.	A	<i>Neovison vison</i> (Schreber)	USA	– ^b	<u>KT956920</u>	Present study
<i>Moliniella anceps</i> (Molin, 1859)	M	<i>Planorbarius corneus</i> (L.)	Lithuania	–	<u>KT956921</u>	Present study
<i>Neoacanthoparyphium echinatoides</i> (Filippi, 1854)*	C	<i>Viviparus acerosus</i> (Bourguignat)	Slovakia	–	<u>KT956922</u>	Present study
<i>Paryphostomum radiatum</i> (Dujardin, 1845)*	A	<i>Phalacrocorax carbo</i> (L.)	Ukraine	HWML 101858	<u>KT956927</u>	Present study
<i>Petasiger exaeretis</i> Dietz, 1909*	A	<i>Phalacrocorax carbo</i> (L.)	Ukraine	HWML 101856	<u>KT956923</u>	Present study
<i>Petasiger islandicus</i> Kostadinova & Skírnisson, 2007	A	<i>Aechmophorus occidentalis</i> (Lawrence)	USA	HCIP D-720	<u>KT956924</u>	Present study
<i>Petasiger islandicus</i> Kostadinova & Skírnisson, 2007	C	<i>Gyraulus</i> cf. <i>laevis</i>	Iceland	BMNH 2012.1.9.1-4	<u>JO425592</u>	Georgieva et al. (2012)

<i>Petasiger phalacrocoracis</i> (Yamaguti, 1939)	A	<i>Phalacrocorax carbo</i> (L.)	Ukraine	HWML 101857	<u>KT956926</u>	Present study
<i>Neopetasiger</i> n. sp.	A	<i>Podiceps grisegena</i> (Boddaert)	USA	– ^b	<u>KT956925</u>	Present study
<i>Petasiger</i> sp. 1	C	<i>Planorbis planorbis</i> (L.)	Czech Republic	HCIP D-707	<u>KM191800</u>	Selbach et al. (2014)
<i>Petasiger</i> sp. 2	C	<i>Gyraulus albus</i> (O. F. Müller)	Germany	HCIP D-708	<u>KM191802</u>	Selbach et al. (2014)
<i>Petasiger</i> sp. 3	C	<i>Planorbis planorbis</i> (L.)	Germany	HCIP D-709	<u>KM191804</u>	Selbach et al. (2014)
<i>Petasiger</i> sp. 4	M	<i>Gasterosteus aculeatus</i> L.	Canada		<u>KM191807</u>	Selbach et al. (2014)
Subfamily Chaunocephalinae Travassos, 1922						
<i>Chaunocephalus ferox</i> (Rudolphi, 1795)*	A	<i>Ciconia nigra</i> (L.)	Ukraine	HCIP D-133; SIZ 1-012	<u>KT447522</u>	Greben et al. (unpublished data)
Subfamily Echinochasminae Odhner, 1910						
<i>Echinochasmus beleocephalus</i> (Linstow, 1873)	A	<i>Ardea alba</i> L.	Ukraine	HWML 101860	<u>KT956929</u>	Present study
<i>Echinochasmus coxatus</i> Dietz, 1909*	A	<i>Podiceps nigricollis</i> Brehm	Ukraine	HWML 101859	<u>KT956928</u>	Present study
<i>Echinochasmus donaldsoni</i> Beaver, 1941	A	<i>Podilymbus podiceps</i> (L.)	USA	HWML 101861	<u>KT956930</u>	Present study
<i>Echinochasmus japonicus</i> Tanabe, 1926	A	<i>Gallus gallus</i> dom. (exp.)	Vietnam	–	<u>JO890579</u>	Besprozvannykh et al. (2013)
<i>Echinochasmus mordax</i> (Looss, 1899)	A	<i>Podiceps nigricollis</i> Brehm	Ukraine	HWML 101862	<u>KT956931</u>	Present study
<i>Echinochasmus</i> n. sp.	A	<i>Anhinga anhinga</i> (L.)	USA	– ^b	<u>KT956932</u>	Present study
<i>Echinochasmus</i> sp. VS-2012	C	<i>Lithoglyphus naticoides</i> (Pfeiffer)	Lithuania	–	<u>JO088098</u>	Stanevičiūtė et al. (2015)
<i>Micropharyngium fasciatum</i> Dietz, 1909*	A	<i>Anhinga anhinga</i> (L.)	USA	HWML 101863	<u>KT956933</u>	Present study
<i>Stephanoprora pseudoechinata</i> (Olsson, 1876)	A	<i>Larus fuscus</i> L.	USA	HWML 101864	<u>KT956934</u>	Present study
<i>Stephanoprora pseudoechinata</i> (Olsson, 1876)	A	<i>Larus genei</i> (Brème)	Ukraine	HWML 101865	<u>KT956935</u>	Present study
<i>Stephanoprora</i> sp. 1	A	<i>Gavia immer</i> (Brünnich)	USA	HWML 101866	<u>KT956936</u>	Present study
<i>Stephanoprora</i> sp. 2	A	<i>Aechmophorus occidentalis</i> (Lawrence)	USA	HWML 101867	<u>KT956937</u>	Present study
<i>Uroproctepisthium bursicola</i> (Creplin, 1837)	A	<i>Ardea alba</i> L.	Ukraine	HWML 101868	<u>KT956938</u>	Present study
Subfamily Himasthlinae Odhner, 1910						
<i>Acanthoparyphium spinulosum</i> Johnston, 1917	A	<i>Pluvialis squatarola</i> (L.)	Ukraine	HWML 101869	<u>KT956939</u>	Present study
<i>Acanthoparyphium</i> n. sp.	A	<i>Haematopus palliatus</i> Temminck	USA	– ^b	<u>KT956940</u>	Present study
<i>Artyfechinostomum sufrartylfex</i> Lane, 1915*	A	<i>Sus scrofa</i> dom.	India	–	<u>KF781303</u>	Tandon et al. (unpublished data)
<i>Caballerotrema</i> sp.	A	<i>Arapaima gigas</i> (Schinz)	Peru	HCIP D 634	<u>KT956941</u>	Present study
<i>Himasthla leptosoma</i> (Creplin, 1829)	A	<i>Calidris alpina</i> (L.)	Ukraine	HWML 101870	<u>KT956942</u>	Present study
<i>Himasthla limnodromi</i> Didyk & Burt, 1997	A	<i>Limnodromus griseus</i> (Gmelin)	USA	HWML 101871	<u>KT956943</u>	Present study
<i>Himasthla militaris</i> (Rudolphi, 1802)	A	<i>Gallinago gallinago</i> (L.)	Ukraine	HCIP D-571	<u>KT956944</u>	Present study
Subfamily Nephrostominae Mendheim, 1943						
<i>Patagifer bilobus</i> (Rudolphi, 1819) *	A	<i>Plegadis falcinellus</i> (L.)	Ukraine	HWML 101853	<u>KT956945</u>	Present study
<i>Patagifer vioscai</i> Lumsden, 1962	A	<i>Eudocimus albus</i> (L.)	USA	HWML 101852	<u>KT956946</u>	Present study
Family Cathaemasiidae Fuhrmann, 1928						
Subfamily Cathaemasiinae Fuhrmann, 1928						
<i>Cathaemasia hians</i> (Rudolphi, 1809)*	C	<i>Planorbis planorbis</i> (L.)	Czech Republic	–	<u>KT956947</u>	Present study
Family Fasciolidae Railliet, 1895						
Subfamily Fasciolinae Railliet, 1895						

<i>Fasciola gigantica</i> Cobbold, 1855	A	"domestic cow" ^a	Kenya	—	<u>EU025873</u>	Lotfy et al. (2008)
<i>Fasciola hepatica</i> Linnaeus, 1758*	A	<i>Capra hircus</i> (L.)	Saudi Arabia	—	<u>AY222244</u>	Olson et al. (2003)
<i>Fascioloides jacksoni</i> (Cobbold, 1869)	A	"Asian elephant" ^a	Sri Lanka	—	<u>EU025871</u>	Lotfy et al. (2008)
<i>Fascioloides magna</i> (Bassi, 1875)*	A	"white-tailed deer" ^a	USA	—	<u>EU025872</u>	Lotfy et al. (2008)
Subfamily Fasciolopsinae Odhner, 1910						
<i>Fasciolopsis buski</i> (Lankester, 1857)*	A	"domestic pig" ^a	Vietnam	—	<u>EU025870</u>	Lotfy et al. (2008)
<i>Parafasciolopsis fasciolaemorphia</i> Ejsmont, 1932*	A	"European bison" ^a	Poland	—	<u>EU025869</u>	Lotfy et al. (2008)
Subfamily Protofasciolinae Skrjabin, 1948						
<i>Protofasciola robusta</i> (Lorenz, 1881)*	A	"African forest elephant" ^a	Africa	—	<u>EU025868</u>	Lotfy et al. (2008)
Family Philophthalmidae Looss, 1899						
Subfamily Philophthalminae Looss, 1899						
<i>Philophthalmus gralli</i> Mathis & Leger, 1910	A	<i>Rhea americana</i> (L.)	USA	USNPC 104259	<u>JO246435</u>	Church et al. (2013)
Philophthalmidae gen. sp.	C	<i>Batillaria australis</i> (Quoy & Gaimard)	Australia	—	<u>AY222247</u>	Olson et al. (2003)
Subfamily Cloacitrematinae Yamaguti, 1958						
<i>Cloacitrema michiganensis</i> McIntosh, 1938	A	<i>Limnodromus scolopaceus</i> (Say)	USA	HWML 101879	<u>KT956948</u>	Present study
<i>Cloacitrema narrabeenensis</i> Howell & Bearup, 1967	C	<i>Batillaria australis</i> (Quoy & Gaimard)	Australia	—	<u>AY222248</u>	Olson et al. (2003)
Subfamily Parorchiniinae Lal, 1936						
<i>Parorchis avitus</i> Linton, 1914	A	<i>Calidris alpina</i> (L.)	USA	HCIP D-721	<u>KT956949</u>	Present study
<i>Parorchis</i> sp. NZ	C	<i>Austrolittorina antipodum</i> (Philippi)	New Zealand	HCIP D-700	<u>KJ868209</u>	O'Dwyer et al. (2014)
<i>Parorchis</i> sp. CG-2013	—	—	Argentina	—	<u>KF451929</u>	Tkach et al. (unpublished)
Family Psilostomidae Looss, 1900						
Subfamily Psilostominae Looss, 1900						
<i>Psilochasmus oxyurus</i> (Creplin, 1825)*	A	<i>Anas platyrhynchos</i> L.	Ukraine	—	<u>AF151940</u>	Tkach et al. (2000)
<i>Psilostomum brevicolle</i> (Creplin, 1829)*	A	<i>Haematopus ostralegus</i> L.	Ukraine	HCIP D-609	<u>KT956950</u>	Present study
New subfamily						
Psilostomidae n. g., n. sp. 1	A	<i>Anas crecca</i> L.	Ukraine	— ^b	<u>KT956951</u>	Present study
Psilostomidae n. g., n. sp. 2	A	<i>Aythya affinis</i> (Eyton)	USA	— ^b	<u>KT956952</u>	Present study
Psilostomidae n. g., n. sp.	A	<i>Bucephala albeola</i> (L.)	USA	— ^b	<u>KT956953</u>	Present study
Psilostomidae n. g., n. sp.	A	<i>Aix sponsa</i> (L.)	USA	— ^b	<u>KT956954</u>	Present study
Psilostomidae n. g., n. sp.	A	<i>Aix sponsa</i> (L.)	USA	— ^b	<u>KT956955</u>	Present study
Subfamily Ribeiroiinae Travassos, 1951						
<i>Ribeiroia ondatrae</i> (Price, 1931)*	A	<i>Pelecanus erythrorhynchos</i> Gmelin	USA	—	<u>KT956956</u>	Present study
Subfamily Sphaeridiotrematinae Yamaguti, 1958						
<i>Sphaeridiotrema pseudoglobulus</i> McLaughlin et al., 1993	A	<i>Aythya affinis</i> (Eyton)	USA	HWML 101872; HCIP D-722	<u>KT956957</u>	Present study
<i>Sphaeridiotrema</i> sp.	C	<i>Bithynia tentaculata</i> (L.)	Lithuania	—	<u>KT956958</u>	Present study
Family Rhopalidae Looss, 1899						
<i>Rhopalias macracanthus</i> Chandler, 1932*	A	<i>Didelphis virginiana</i> (Kerr)	USA	HWML 101877	<u>KT956959</u>	Present study
Family Cyclocoelidae Stossich, 1902						
Subfamily Cyclocoelinae Stossich, 1902						

<i>Cyclocoelum mutabile</i> (Zeder, 1800)*	A	<i>Calidris canutus</i> (L.)	UK	BMNH 1997.1.3.1	<u>AY222249</u>	Olson et al. (2003)
Family Typhlocoelidae Harrah, 1922						
Subfamily Typhlocoelinae Harrah, 1922						
<i>Typhlocoelum</i> sp.	A	<i>Anas platyrhynchos</i> L.	USA	HWML 101878	<u>KT956960</u>	Present study
Family Notocotylidae (outgroup)						
<i>Notocotylus attenuatus</i> (Rudolphi, 1809)	A	<i>Aythya ferina</i> (L.)	Ukraine	–	<u>AF184259</u>	Tkach et al. (2001)

^a Hosts as in Lotfy et al. (2008)

^b The type material will be deposited in the collection of the Harold W. Manter Laboratory of Parasitology (HWML) at the University of Nebraska, Lincoln, USA upon the description of the new species.

A, adult; C, cercaria; M, metacercaria; BMNH, Natural History Museum, London, UK; HCIP, Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic; SIZ, Helminthological Collection of the Schmalhausen Institute of Zoology, Ukrainian National Academy of Sciences, Kiev, Ukraine.

Figure 1

Figure 2