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1	A new species of Clinostomum Leidy, 1856 based on molecular and morphological analysis of
2	metacercariae from African siluriform fishes
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24	

26 Abstract

In the Afrotropic region, the genus *Clinostomum* is represented by four valid and four putative 27 species distinguished using molecular data. Here we describe one of the putative species as 28 Clinostomum ukolii n. sp. based on metacercariae from siluriform fishes (Synodontis batensoda, 29 Schilbe intermedius) collected in Nigeria and South Africa. The new species is distinguished by 30 31 molecular data (39 new sequences of partial cytochrome c oxidase I $\geq 6.7\%$ divergent from those of other species) and morphological differences from valid and putative species in the same region. 32 33 Metacercariae of C. ukolii n. sp. can be distinguished based on size, tegumental spines, and various aspects of the genital complex, including its position, lobation of the anterior testis, and the 34 disposition and shape of the cirrus pouch. Although descriptions of new species of digeneans are 35 36 typically based on the morphology of adults, we argue that in cases where data are available from metacercariae from regionally known species, new species can be described based on 37 metacercariae, particularly when supported by molecular data, as here. Moreover, sub-adult 38 reproductive structures can be clearly visualized in metacercaria of *Clinostomum*. Considering 39 40 metacercariae as potential types for new species could advance clinostome systematics more rapidly, because metacercariae are encountered much more often than adults in avian definitive 41 hosts. 42

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48 Introduction

The genus *Clinostomum* Leidy, 1856 (Digenea: Clinostomidae) was first reported in Africa in 49 1930 when Dubois described C. phalacrocoracis from Angola. In Nigeria, Ukoli (1966) 50 provided the first revision of the genus together with the description of the species C. tilapiae 51 52 from Ghana, which was recently redescribed by Caffara et al. (2017). The latter morphological 53 redescription was supported with molecular comparisons to one named and four unnamed, genetically distinguished species from Africa, along with a review of African reports (see table 1 54 55 in Caffara et al. 2017) highlighting incomplete or absent morphological regional descriptions of Clinostomum. 56 Following a pioneering study by Matthews and Cribb (1998), since 2010, morphological 57 data coupled to molecular analyses have been used to characterize species of *Clinostomum* and 58 other clinostomids in Africa and elsewhere. This approach has now been applied to 14 named 59 60 species, namely C. cutaneum Paperna, 1964 (Gustinelli et al. 2010), C. complanatum (Rudolphi, 1814) and C. marginatum (Rudolphi, 1819) (Caffara et al. 2011), C. tataxumui Sereno-Uribe et 61 al. 2013, C. phalacrocoracis Dubois, 1930 (Caffara et al. 2014), C. detruncatum Braun, 1899 62 63 (Acosta et al. 2016), C. album Rosser et al. 2017, C. tilapiae Ukoli, 1966 (Caffara et al. 2017), C. poteae Rosser et al. 2018, C. heluans Braun, 1899 (Briosio-Aguilar et al. 2018), and C. 64 65 caffarae Sereno-Uribe et al. 2018, C. arquus Sereno-Uribe et al. 2018, C. cichlidorum Sereno-66 Uribe et al. 2018, and the recent combination C. brieni (Dollfus, 1950) (Caffara et al. 2019). In addition to these species, however, a number of lineages await description or identification. Most 67 68 pertinent here are four putative species distinguished by Caffara et al. (2017) based on genetic 69 diversity and a brief account of morphological differences in the genital complex.

In the present study, we provide a complete morphological description of the

- 71 metacercariae previously identified as *Clinostomum* morphotype 1 (Caffara et al. 2017) collected
- from *Synodontis batensoda* and *Schilbe intermedius* from Nigeria and South Africa respectively,
- 73 which we erect as a new species, namely *Clinostomum ukolii* n. sp.
- 74

75 Materials and methods

76 Sixty-two metacercariae of *Clinostomum* sp. morphotype 1, were removed from fresh skin tissue of Synodontis batensoda (Siluriformes: Mochokidae) collected in the Anambra River Basin, 77 78 Nigeria, and 6 from the abdominal cavity or gill chambers of Schilbe intermedius (Siluriformes: Schilbeidae) sampled in different areas of Limpopo province, South Africa. Of these 24 (18 from 79 Nigeria and 6 from South Africa) were morphologically and molecularly described as 80 *Clinostomum* sp. morphotype 1 in our previous work (Caffara et al. 2017). The new specimens 81 were excysted, washed in saline and preserved in 70% ethanol for morphological analysis, all at 82 83 room temperature. The posterior end was removed for molecular analysis (Caffara et al. 2017). Thirty-eight new ITS rDNA and 39 CO1mtDNA sequences were generated and published in 84 GenBank (COI: MN044350-MN044388, ITS: MN059670-MN059707). Morphometrics were 85 86 taken after clarification with Amman's lactophenol and staining by Malzacher's method (Pritchard and Kruse 1982). Line drawings were made with the aid of a drawing tube, and 87 88 measurements are given in micrometers following Matthews and Cribb (1998). Specimens of C. 89 ukolii n. sp. were morphologically compared with those of C. complanatum (data from Caffara et 90 al. 2011; Locke et al. accepted), Clinostomum morphotypes 2-4, C. tilapiae (Caffara et al. 2017), 91 C. brieni (Caffara et al. 2019) and C. phalacrocoracis (Caffara et al. 2014) using principal 92 components analysis (PCA) of morphometrics normalized to range from -1 to 1. Analysis of

- 93 similarities (ANOSIM) was used to test for differences in morphometric variation among species
- 94 based on Euclidean distances in normalized morphometrics.

95 **Results**

- 96 Morphological description
- 97 *Clinostomum ukolii* n. sp. (Fig. 1, Table 1)
- 98 Type host: *Synodontis batensoda*
- 99 Type locality: Anambra River Basin, Nigeria
- 100 Other host and locality: *Schilbe intermedius*, Limpopo Province, South Africa
- 101 GenBank Accession numbers: ITS KY865609-26, KY865656-60 and MN059670-MN059707;
- 102 CO1 KY865626-43, KY865676-81 and MN044350-MN044388
- 103 Type specimens deposited in the Museum of the Southwestern Biology, Division of Parasites,
- 104 University of New Mexico (Accessions MSB: Para: 29098-29101)
- 105 Etymology: Clinostomum ukolii n. sp. is named after Professor F.M.A. Ukoli who provided the
- 106 first important revision of the genus *Clinostomum*.

107

- 108 Morphological features of the metacercariae (n=54) from *S. batensoda* and *Sch. intermedius*
- 109 from Nigeria and South Africa. Body stout, widest in gonadic region. Oral sucker small,
- surrounded by oral collar (not always visible). Pharynx small, opening into pharyngeal bulb
- 111 (visible only in some specimens). Ventral sucker larger than oral sucker. Intestinal caeca with
- small lateral pouches from ventral sucker to posterior end of body. Testes digitated. Anterior
- 113 testis across middle and posterior third of body, irregularly lobed, slightly displaced to left.
- 114 Posterior testis in anterior part of posterior third of body, symmetrical, triangular, with sublobes
- 115 more or less evident. Efferent ducts from lateral right margin of testes to left margin of cirrus

116	pouch (Fig. 2d). Cirrus pouch bean-shaped with tapering anterior margin, embracing right
117	margin of anterior testis, overlapping it, with well evident longitudinal and radial muscle fibers
118	and hair-like structures (Fig. 2a-c). Genital pore medial to cirrus sac, close to right anterior
119	margin of anterior testis. Ovary small, irregular, sometimes slightly lobed, not median, in
120	intertesticular space dextrally alongside cirrus pouch. Uterus running straight from ventral sucker
121	to anterior testis. Uteroduct passing around left margin of anterior testis, forming knee-like bend
122	before opening into uterine sac above anterior testis very close to metraterm. Metraterm
123	muscular, sometimes cup-like, connecting uterus to genital atrium. Tegument completely
124	covered with minute spines. Dome-like structures on tegument surface, between suckers, in some
125	specimens.
126	Remarks
127	In an alignment of partial sequences of CO1 overlapping by at least 554 bp, 39 newly generated
128	sequences averaged 99.58 (range 98.14-100%) similarity to the 23 sequences of Clinostomum
129	morphotype sp. 1 published by Caffara et al. (2017) (KY865627-43, KY865676-81), with 20 of
130	the newly generated sequences identical to two or more of those of Caffara et al. (2017). The
131	CO1 sequences of C. ukolii n. sp. differ by at least 6.74% from those of other species, the most
132	similar being from Clinostomum sp. morphotype 2 (KY865662-6).
133	Twenty-five of 38 newly generated ITS rDNA sequences were identical to over half the
134	23 sequences of <i>Clinostomum</i> morphotype sp. 1 published by Caffara et al. (2017) (KY865609-
135	26, KY865656-60). The new ITS sequences differed by average 0.12% (range 0-0.5%) from
136	those previously published from Clinostomum morphotype sp. 1. The most similar ITS
137	sequences were those of <i>Clinostomum</i> sp. morphotype 2 (KY865645-7), which differed by 0.1-
138	0.4% from those of <i>C. ukolii</i> n. sp.

139	Among 117 metacercariae, including 54 of C. ukolii n. sp., substantial morphometric
140	variation was attributable to species (global ANOSIM R=0.636, p=0.0001). Metacercariae of C.
141	ukolii n. sp. differed morphometrically from all other species (Table 2). The pairwise ANOSIM
142	results, which are based on ranks of Euclidean distances, correspond well to the metric
143	ordination of Euclidean distances in PCA (Fig. 3), in which C. ukolii n. sp. was well separated
144	from C. brieni, C. phalacrocoracis, and C. complanatum (pairwise ANOSIM R values 0.728-
145	0.853, Table 2), but less so from Clinostomum sp. morphotypes 2 and 3 and C. tilapiae (pairwise
146	ANOSIM R values 0.275-0.338). Along PC1, all characters scored between 0.211 and 0.283,
147	indicating that no single measurement is particularly discriminating along this dimension, which
148	explained 70.2% of morphometric variation.
149	
150	Discussion
151	In this study we describe C. ukolii n. sp., which was provisionally identified as morphotype 1 based
152	mainly on molecular data (Caffara et al. 2017). The new species is based on phylogenetic analysis
153	of 62 CO1 and 61 ITS sequences (39 CO1 and 38 ITS sequences newly generated here, see also
154	Caffara et al. 2017), and morphological and morphometric analysis of 54 metacercariae that in
155	combination show C. ukolii n. sp. to be distinct from those of other valid and putative species in
156	the region.
157	Matthews and Cribb (1998), Sereno-Uribe et al. (2018) and two anonymous reviewers of an
158	earlier version of this communication argued that species of <i>Clinostomum</i> (or other digeneans)
158 159	earlier version of this communication argued that species of <i>Clinostomum</i> (or other digeneans) should rest on the morphology of adults, not metacercariae. We agree that the morphology of
158 159 160	earlier version of this communication argued that species of <i>Clinostomum</i> (or other digeneans) should rest on the morphology of adults, not metacercariae. We agree that the morphology of metacercariae is often not comparable to that of adults (e.g., Caffara et al. 2019). Consequently, in

can be problematic. However, in the present case, the metacercarial morphology of all regionally 162 known species is well characterized, and C. ukolii n. sp. is distinct from them both morphologically 163 (see below, Fig. 3) and genetically (Caffara et al. 2017). In this special circumstance of a regional 164 fauna in which both developmental stages are well characterized, naming a new species is an 165 appropriate course. The new name is accompanied by molecular and morphological data from a 166 167 substantial and representative sample of isolates collected in different hosts and localities. These data will allow identification of other developmental stages of C. ukolii n. sp. and comparisons 168 with other regional species, known and new, thus increasing biological knowledge. Ferris (1928) 169 cautioned against undue haste in naming new species, but the value of the stability that a valid 170 name provides should not be underestimated in the context of modern molecular surveys. We 171 believe that erection of C. ukolii n. sp. will reduce the accumulation of confusing, conflicting 172 provisional names that arise in molecular prospecting studies of poorly known taxa. For example, 173 most sequence records of a putative species called *Clinostomum* lineage 5 (Pérez-Ponce de Léon 174 175 et al. 2016) or *Clinostomum* L5 (Briosio-Aguilar et al. 2018) are labelled *Clinostomum* sp. lineage 2 on GenBank, and this is a different species from what Locke et al. (2015b) had earlier called 176 Clinostomum spp. 2 or 5 (see Locke et al. 2015a, for other examples from Diplostomum). While 177 178 data from adults of C. ukolii n. sp. would be valuable, such data are unnecessary for the establishment of C. ukolii n. sp., and it is unclear when such information will be obtained. 179 180 Collecting definitive hosts of *Clinostomum* is logistically challenging and highly regulated due to 181 conservation concerns (e.g., seven ardeid species are critically endangered, endangered, or vulnerable, IUCN, 2019). Consequently, metacercariae from fish and amphibians will continue to 182 183 be encountered and studied with greater frequency than adults. Like Ukoli (1966), we believe that 184 metacercariae merit full consideration from a taxonomic perspective, and that this practice could

allow clinostome systematics to advance reliably and more rapidly than limitation to adult 185 morphology in all circumstances. Moreover, the sub-adult morphology of clinostome metacercaria 186 187 presents some advantages over the adult form. Nearly all taxonomically important reproductive structures (testes, cirrus poach, ovary and uterus) are well developed and more clearly visible when 188 the parasite is not sexually mature. Comparisons among these and other structures in metacercariae 189 190 have been used to discriminate several species of Clinostomum (Caffara et al. 2011, 2017). The principal morphological structures unique to adults, eggs and vitellaria, do not play a critical role 191 192 in distinctions of most species (Matthews and Cribb, 1998; Gustinelli et al. 2010; Caffara et al. 2011; Sereno-Uribe et al. 2018), although they do provide a clear demarcation of maturity. Under 193 experimental conditions, clinostome metacercariae show slower growth over a longer period than 194 adults, which are shorter-lived (Jhansilakshmibai and Madhavi, 1997; Liao, 1992). In natural 195 infections, however, it is our experience that the majority of metacercariae display fairly uniform 196 size distributions, which likely reflects an accumulation of specimens with stable sub-adult 197 198 morphology. For example, adults body lengths are more variable than metacercariae in C. marginatum and C. cichlidorum, but the opposite holds for C. complanatum, C. arquus, and C. 199 caffarae (see morphometrics in Caffara et al. 2011 and Sereno-Uribe et al. 2018). In other words, 200 201 there is no compelling empirical basis for rejecting morphometric analysis of metacercariae because of allegedly greater variability ascribable to unknown specimen age. Most importantly, as 202 203 discussed below, the metacercariae of C. ukolii n. sp. can be morphologically (and genetically) 204 distinguished from all others in the Afrotropic region, which leaves no doubt that it represents a new species. 205

206 Metacercariae of *C. ukolii* n. sp. can be morphologically distinguished from valid and putative 207 species in Africa and Europe based on the genital complex. We view detailed morphological

comparisons with species in other regions as unnecessary because there is little evidence of 208 transcontinental distributions in Clinostomum (Locke et al. 2015b) and the molecular distinction 209 of C. ukolii n. sp. from such species is clear from prior phylogenetic analysis (Caffara et al. 2017). 210 In C. ukolii n. sp., the genital complex lies between middle and posterior third of the body, while 211 in C. tilapiae (Caffara et al. 2017) it occupies the posterior portion of the middle third of the body, 212 213 with the posterior lobe of the posterior testis extending into the posterior third of body; the genital complex is entirely in the middle third in C. cutaneum (Gustinelli et al. 2010) and Clinostomum 214 sp. morphotype 3 (Caffara et al. 2017), and entirely in the posterior third of body in C. brieni 215 (Caffara et al. 2019). The genital complex in C. ukolii n. sp. is similar in position to that of C. 216 phalacrocoracis (Caffara et al. 2014), C. complanatum (Caffara et al. 2011) and Clinostomum sp. 217 morphotypes 2 and 4 (Caffara et al. 2017), but different in structure. The irregular lobation of the 218 anterior testis of C. ukolii n. sp. is unlike the fan shape of the anterior testis of C. phalacrocoracis 219 or the triangular, digitated anterior testis of Clinostomum sp. morphotypes 2 and 4 and C. 220 221 cutaneum. In C. ukolii n. sp., the anterior testis is also less lobed than the posterior, while in C. tilapiae, the anterior is more lobed, with two main lateral and one posterior lobe on the posterior 222 testis; in C. complanatum, the anterior testis is strongly left-dislocated by the cirrus pouch. In C. 223 224 brieni, the anterior testis is bow-tie shaped while the posterior varies from Y to crescent shaped. Only in *Clinostomum* sp. morphotype 3 does the structure of the testes resemble that of *C. ukolii* 225 226 n. sp.

The cirrus pouch of *C. ukolii* n. sp. overlaps the anterior testis, as in *Clinostomum* sp. morphotypes 2 and 3, while in *Clinostomum* sp. morphotype 4, the cirrus pouch is in the intertesticular space close to the right posterior margin of anterior testis. In *C. tilapiae*, the cirrus pouch is oval and lies between the testes, almost in contact with the right cecum, while in *C.* *phalacrocoracis* it is bean-shaped in the dextral intertesticular space; in *C. cutaneum* it is round with a deep cleft forming two lobes, and in *C. complanatum*, it is wide, extending from the intertesticular space to the posterior right margin of the anterior testis; in *C. brieni* it is commashaped, intertesticular and in close contact to both testes. Interestingly, in *C. ukolii* n. sp. we were able to see the longitudinal muscular fibers of the cirrus pouch described previously only by Maccagno (1934) in *C. complanatum*.

The tegument of metacercariae of *C. ukolii* n. sp. is completely covered with minute spines over
the whole body, as in *C. tilapiae* and *Clinostomum* sp. morphotypes 3 and 4. In *C. brieni*, the spines
are thicker and present from oral sucker to posterior end of body, while other African species are
devoid of spines. Metacercariae of *C. ukolii* n. sp. are smaller (mean total length 6169, range 37268804 µm) than those of *C. brieni* (mean 8683, range 6762-10602 µm, data from Caffara et al. 2019)
and *C. phalacrocoracis* (mean 12061, range 9500–15200 µm, data from Caffara et al. 2014).

243 Clinostomum ukolii n. sp. is now added to the list of species of Clinostomum in the Afrotropic 244 ecozone that have been validated with a combined molecular and morphological approach, namely C. cutaneum, C. phalacrocoracis, C. tilapiae, and the recent combination C. brieni (Gustinelli et 245 246 al. 2010, Caffara et al. 2014, 2017, 2019). We do not include C. complanatum in this fauna because its presence in the Afrotropic region has not been confirmed with molecular data, although it has 247 often been reported in Africa (Batra, 1984; Barson et al. 2008; Oliver et al. 2009; Ejere et al. 2014; 248 Echi et al. 2012; Aboel Hadid and Lofty, 2007). Many of these morphology-based records are 249 250 open to other interpretation. For example, El-Shahawy et al. (2017) and El-Dakhly et al. (2018) recently reported C. complanatum in Egypt, but the distinctive, fan-shaped testes of the specimens 251 figured in these studies clearly differ from those of C. complanatum and resemble C. 252 phalacrocoracis, although the total lengths of the worms in both papers is far smaller or bigger 253

254	than any record of either C. complanatum or C. phalacrocoracis of which we are aware. This
255	situation illustrates the ongoing need to reassess the diversity of Afrotropic clinostomes with both
256	DNA and morphology in both larvae and adults.
257	
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268	
269	References
270	
271	
272	Aboel Hadid SM, Lotfy HS (2007) Some studies on helminth parasites of buff backed heron
273	(Ardeola ibis ibis) with special reference to its role in transmission of Clinostomum
274	complanatum in Beni-Suef Governorate. Beni -Suef Vet Med J 135-141.
275	Acosta AA, Caffara M, Fioravanti ML, Utsunomia R, Zago AC, Franceschini L, Josè da Silva R
276	(2016) Morphological and molecular characterization of Clinostomum detruncatum

- 277 (Trematoda: Clinostomidae) metacercaria infecting *Synbranchus marmoratus*. J Parasitol
 278 102:151-156. doi: 10.1645/15-773
- 279 Barson M, Bray R, Ollevier F, Huyse T (2008) Taxonomy and faunistics of the helminth
- 280 parasites of *Clarias gariepinus* (Burchell, 1822), and *Oreochromis mossambicus* (Peters,
- 1852) from temporary pans and pools in the Save-Runde River Floodplain, Zimbabwe. Comp
 Parasitol 75:228-240. doi: 10.1654/4337.1
- Batra V (1984) Prevalence of helminth parasites in three species of cichlids from a man-made
 lake in Zambia. Zool J Linn Soc 82:319-333. doi: 10.1111/j.1096-3642.1984.tb00646.x
- Briosio-Aguilar R, Pinto HA, Rodríguez-Santiago MA, López-García K, García-Varela M,
- Pérez-Ponce de León G (2018) Link between the adult and the metacercaria of *Clinostomum*
- 287 *heluans* Braun, 1899 (Trematoda: Clinostomidae) through DNA sequences, and its
- phylogenetic position within the genus *Clinostomum* Leidy, 1856. J Parasitol 104: 292-296.
- doi: 10.1645/17-183
- 290 Caffara M, Locke SA, Gustinelli A, Marcogliese DJ, Fioravanti ML (2011) Morphological and
- 291 molecular differentiation of *Clinostomum complanatum* and *Clinostomum marginatum*
- (Digenea: Clinostomidae) metacercariae and adults. J Parasitol 97:884-891. doi: 10.1645/GE2781.1
- 294 Caffara M, Davidovich N, Falk R, Smirnov M, Ofek T Cummings D, Gustinelli A, Fioravanti
- 295 ML (2014) Redescription of *Clinostomum phalacrocoracis* metacercariae (Digenea:
- 296 Clinostomidae) in cichlids from Lake Kinneret, Israel. Parasite 21, 32. doi:
- 297 10.1051/parasite/2014034
- 298 Caffara, M, Locke SA, Echi PC, Halajian A, Benini D, Luus-Powell WJ, Tavakol S, Fioravanti
- 299 ML (2017) A morphological and molecular study of Clinostomid metacercariae from African

300	fish with a redescription of Clinostomum tilapiae. Parasitology 144:1519-1529. doi:
301	10.1017/S0031182017001068
302	Caffara M, Locke SA, Halajian A, Luus-Powell WJ, Benini D, Tedesco P, Kapepula Kasembele
303	G, Fioravanti ML (2019) Molecular data show Clinostomoides Dollfus, 1950 is a junior
304	synonym of Clinostomum Leidy, 1856, with redescription of metacercariae of Clinostomum
305	brieni n comb. Parasitology 146: 805-813. doi: 10.1017/S0031182018002172
306	Dubois G (1930) Materiaux de la mission scientifique Suisse en Angola. Bull Soc Neuchl Sci
307	Nat 55:73-88.
308	Echi PC, Eyo JE, Okafor FC, Onyishi GC, Ivoke N (2012) First record of co-infection of three
309	clinostomatid parasites in cichlids (Osteichthyes: Cichlidae) in a tropical freshwater lake. Iran
310	J Public Health 41, 86-90.
311	Ejere VC, Aguzie OI, Ivoke N, Ekeh FN, Ezenwaji NE, Onoja US, Eyo JE (2014) Parasitofauna
312	of five freshwater fishes in a Nigerian freshwater ecosystem. Ribarstvo 72:17-24. doi:
313	10.14798/72.1.682
314	El-Dakhly KM, Hussein NM, El-Nahass E (2018) Occurrence of helminths in the great
315	cormorants, Phalacrocorax carbo, in Qena, Egypt. J Adv Vet Anim Res 8:6-11
316	El-Shahawy IS, El-Seify MO, Metwally AM, Fwaz MM (2017) Survey on endoparasitic fauna of
317	some commercially important fishes of the River Nile, southern of Egypt (Egypt). Rev Med
318	Vet 168:126-134.
319	Gustinelli A, Caffara M, Florio D, Otachi EO, Wathuta EM, Fioravanti ML (2010) First
320	description of the adult stage of Clinostomum cutaneum Paperna, 1964 (Digenea:
321	Clinostomidae) from grey herons Ardea cinerea L. and a redescription of the metacercaria

322	from the Nile tilapia Oreochromis niloticus niloticus (L.) in Kenya. Sys Parasitol 76:39-51.
323	doi: 10.1007/s11230-010-9231-5
324	Jhansilakshmibai K, Madhavi R (1997) Euclinostomum heterostomum (Rudolphi, 1809)
325	(Trematoda): Life-cycle, growth and development of the metacercaria and adult. Sys Parasitol
326	38:51–64.
327	Liao XH (1992) The biology of Clinostomum complanatum (Rud.) life history. Wuyi Sci J 9:99-
328	134
329	Locke SA, Al-Nasiri FS, Caffara M, Drago F, Kalbe M, Lapierre AR, McLaughlin JD, Nie P,
330	Overstreet RM, Souza GTR, Takemoto RM, Marcogliese DJ (2015a) Diversity, specificity
331	and speciation in larval Diplostomidae (Platyhelminthes: Digenea) in the eyes of freshwater
332	fish, as revealed by DNA barcodes. Int J Parasitol 45: 841-55. doi:
333	10.1016/j.ijpara.2015.07.001
334	Locke SA, Caffara M, Marcogliese DJ, Fioravanti ML (2015b) A large-scale molecular survey
335	of Clinostomum (Digenea, Clinostomidae). Zool Scr 44:203-217. doi: 10.1111/zsc.12096
336	Locke SA, Caffara M, Barčák D, Tedesco P, Fioravanti ML, Li W (2019) A new species of
337	Clinostomum Leidy, 1856 in East Asia based on genomic and morphological data. Par Res
338	(accepted in press)
339	Maccagno T (1934) Osservazioni intorno a Clinostomum complanatum. Ital J Zool 5:45-60. doi:
340	10.1080/11250003409426258
341	Matthews D, Cribb TH (1998) Digenetic trematodes of the genus Clinostomum Leidy, 1856
342	(Digenea: Clinostomidae) from birds of Queensland, Australia, including C. wilsoni from
343	Egretta intermedia. Sys Parasitol 39:199-208. doi: 10.1023/A:1005982530560

344	Olivier PAS, Luus-Powell WJ, Saayman JE (2009) Report on some monogenean and
345	clinostomid infestations of freshwater fish and waterbird hosts in Middle Letaba Dam,
346	Limpopo Province, South Africa. Onderstepoort J Vet Res 76:187–199. doi:
347	10.4102/ojvr.v76i2.44
348	Pérez-Ponce de León G, García-Varela M, Pinacho-Pinacho CD, Sereno-Uribe AL, Poulin R
349	(2016) Species delimitation in trematodes using DNA sequences: Middle-American
350	Clinostomum as a case study. Parasitology 143:1773-1789. doi: 10.1017/S0031182016001517
351	Pritchard MH, Kruse G (1982) The collection and preservation of animal parasites. University of
352	Nebraska Press, Lincoln, Nebraska.
353	Rosser TG, Alberson NR, Woodyard ET, Cunningham FL, Pote LM, Griffin MJ (2017)
354	Clinostomum album n. sp. and Clinostomum marginatum (Rudolphi, 1819), parasites of the
355	great egret Ardea alba L. from Mississippi, USA. Sys Parasitol 94:35-49. doi:
356	10.1007/s11230-016-9686-0
357	Rosser TG, Baumgartner WA, Alberson NR, Noto TW, Woodyard ET, King DT, Wise DJ,
358	Griffin MJ (2018) Clinostomum poteae n. sp. (Digenea: Clinostomidae), in the trachea of a
359	double-crested cormorant Phalacrocorax auritus Lesson, 1831 and molecular data linking the
360	life-cycle stages of Clinostomum album Rosser, Alberson, Woodyard, Cunningham, Pote &
361	Griffin, 2017 in Mississippi, USA. Sys Parasitol 95:543–566. doi: 10.1007/s11230-018-9801-
362	5
363	Sereno-Uribe AL, Pinacho-Pinacho CD, Garcia-Varela M, Perez-Ponce de Leon G (2013) Using
364	mitochondrial and ribosomal DNA sequences to test the taxonomic validity of Clinostomum
365	complanatum Rudolphi, 1814 in fish-eating birds and freshwater fishes in Mexico, with the
366	description of a new species. Par Res 112:2855-2870. doi: 10.1007/s00436-013-3457-5

367	Sereno-Uribe AL, García-Varela M, Pinacho-Pinacho CD, Pérez-Ponce de León G (2018) Three
368	new species of Clinostomum Leidy, 1856 (Trematoda) from Middle American fish-eating
369	birds. Par Res 117:2171-2185. doi: 10.1007/s00436-018-5905-8
370	Ukoli FMA (1966) On Clinostomum tilapiae n. sp., and C. phalacrocoracis Dubois, 1931 from
371	Ghana, and a discussion of the systematics of the genus Clinostomum Leidy, 1856. J
372	Helminthol 40:215–226.
373	

374	Fig. 1 Line drawing of metacercaria of <i>Clinostomum ukolii</i> n. sp. Scale bar = $1000 \mu m$.
375	Fig. 2 Cirrus pouch of <i>Clinostomum ukolii</i> n. sp. from <i>Synodontis batensoda</i> : (A) <i>in toto</i> ; (B)
376	longitudinal muscle fiber (*), (C) hair-like structures (arrows) (D) efferent ducts (ef) connected
377	to cirrus pouch (cp).
378	Fig. 3 PCA. Principal Components Analysis (PCA) of variation in 16 morphometrics among 102
379	metacercariae of Clinostomum Leidy, 1856. The first two axes of PCA explained 76.8% of
380	morphometric variation among (PC1 70.2%, PC2 6.6%). Vectors show the direction and
381	magnitude of correlations of the morphometric features along both axes, with the circle
382	representing correlation of maximum strength.
383	