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Molecular data show *Clinostomoides* Dollfus, 1950 is a junior synonym of *Clinostomum* Leidy, 1856, with redescription of metacercariae of *Clinostomum brieni* n. comb.

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Research Article

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Abstract

The genus *Clinostomoides* Dollfus, 1950 was erected to accommodate a single worm from *Ardea goliath* sampled in the Belgian Congo. The specimen was distinguished from other clinostomids by its large size and posterior genitalia. In the following years, metacercariae of *Clinostomoides brieni*, have been described in *Clarias* spp. in southern and western Africa. A few authors have referred to *Clinostomum brieni*, but all such usages appear to be *lapsus calami*, and the validity of *Clinostomoides* remains widely accepted. In this study our aim was: position *C. brieni* among the growing clinostomids molecular database, and redescribe the species with emphasis on characters that have emerged as important in recent work. We sequenced two nuclear (partial 18S and ITS) and one mitochondrial marker (partial cytochrome *c* oxidase I) and studied morphology in metacercariae from hosts and localities likely to harbour the type species (*Clarias* spp., Democratic Republic of the Congo, South Africa). Phylogenetic analysis shows *C. brieni* belongs within *Clinostomum* Leidy, 1856. We therefore transfer *C. brieni* to *Clinostomum*, amend the diagnosis for the genus *Clinostomum* and provide a critical analysis of other species in *Clinostomoides*, all of which we consider *species inquirendae*, as they rest on comparisons of different developmental stages.

Introduction

The genus *Clinostomoides* was erected by Dollfus (1950) to accommodate a single adult collected from the esophagus of *Ardea goliath* sampled in the Belgian Congo (now Democratic Republic of the Congo – DRC). The type species, *C. brieni* Dollfus, 1950, was distinguished from *Clinostomum* based on its large body size (30 mm), the genital complex in the posterior (rather than middle or across middle and posterior) third of body and the genital pore located ventral to the posterior testis (rather than lateral to the anterior testis). In the following years, metacercariae of *C. brieni* were described by Prudhoe (1957) from *Clarias lazera* collected in Belgian Congo, by Manter and Pritchard (1969) from *Clarias* sp. from Rwanda, by Fischthal and Thomas (1970) from *C. senegalensis* in Ghana, by Barson *et al.* (2008) and by Jansen van Rensburg *et al.* (2013) from *C. gariepinus* sampled in Zimbabwe and Botswana, respectively. Outside the African continent, Mirzoeva (1981) described one adult of *C. brieni* collected from the esophagus of *Ardea purpurea* in Azerbaijan, and metacercariae of *C. brieni* were also reported in the Philippines (Arthur and Lumanlan-Mayo, 1997).

Additional species of *Clinostomoides* have been described in Central India, the first being *C. dollfusi* Agarwal, 1958, followed by *C. ophicephali* (Tubangui and Masiluñgan, 1935) Agarwal, 1958 (transferred from genus *Clinostomum*), *C. chauhani* Pandey, 1971, *C. rai* Rai, 1970, *C. meerutensis* Pandey and Tyagi, 1986, *C. pandeyii* Singh and Sharma, 1994 and *C. baughi* Pandey, 1998. However, all were erected based either on comparisons among these regional species or with the earliest species in the region, *C. dollfusi*. Most importantly, all, including *C. dollfusi*, were based on metacercariae. This is problematic because in erecting *C. dollfusi*, Agarwal (1958) compared metacercariae with the adult described by Dollfus (1950), not with metacercariae described by Prudhoe (1957). Manter and Pritchard (1969) synonymized *C. dollfusi* with *C. brieni*, and their doubts about the morphological characters used to erect the junior species were confirmed by Fischthal and Thomas (1970). However, subsequent work in India has not taken the latter studies into account and continues to treat as valid *C. dollfusi* and other species erected in comparison with it (e.g. Pandey and Agrawal, 2013). Finally, the species described by Dollfus has occasionally been reported as *Clinostomum brieni*, in *lapsus calami*. Prudhoe (1957) and Douellou (1992) used both '*Clinostomum brieni*' and '*Clinostomoides brieni*' as names for the same species, and Lio-Po *et al.* (1983) listed

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'*Clinostomum brieni*,' but without taxonomic comment or support. Other than these isolated cases, the genus *Clinostomoides* and its type species *C. brieni* are widely considered valid (e.g. Kanev *et al.*, 2002).

A combination of molecular and morphological approaches is a useful way to resolve situations like this, as has already been shown in other clinostomids, i.e. *Clinostomum* Leidy, 1856 (Caffara *et al.*, 2011; Sereno-Uribe *et al.*, 2013), *Euclinostomum* Travassos, 1928 (Senapin *et al.*, 2014; Caffara *et al.*, 2016), *Odhneriotrema* Travassos, 1928 (Woodyard *et al.*, 2017) and *Ithyoclinostomum* Witenberg, 1925 (Briosio-Aguilar *et al.*, 2018). However, few DNA sequences from *Clinostomoides* are currently available for comparison. Athokpam *et al.* (2014) provided rDNA sequences from *C. brieni*, but without supporting morphological information. Moreover, the identification of *C. brieni* by these authors was questioned by Briosio-Aguilar *et al.* (2018) because of the close relationship of its 28S sequence to data from *Clinostomum*. It is also relevant that the material sequenced by Athokpam *et al.* (2014) was from *Heteropneustes* in Northeastern India, rather than the region or hosts (central and southern Africa, *Clarias*, *Ardea*) where the genus originated and is better known.

The aim of this work was to provide a redescription of *C. brieni* metacercariae based on morphological and molecular analyses, following Matthews and Cribb (1998), and to provide an updated critical analysis of previously described species. To this end, we collected in localities and hosts in which we were likely to encounter the same species as Dollfus (1950) and Prudhoe (1957), with the aim of characterizing the type species. Our results led us to transfer *C. brieni* to *Clinostomum* and to consider species of *Clinostomoides* from India as *species inquirendae*.

Materials and methods

Two metacercariae of *Clinostomoides* sp. were collected from *Clarias gariepinus* sampled at Phalaborwa barrage, Limpopo province (South Africa) and four from *C. ngamensis* sampled in the Democratic Republic of the Congo (one from Lake Tshangalele, Kapolowe Mission and three from Kiswishi River near Futuka Farm). All were recovered from the body cavity, cleaned in saline and preserved in 70% ethanol.

Total lengths of metacercariae were measured before cutting a small piece of the posterior end for molecular analyses. Morphometrics of hologenophores (*sensu* Pleijel *et al.*, 2008) were 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 measurements are given in micrometres following Matthews and Cribb (1998). DNA was extracted from hologenophore subsamples using a PureLink Genomic DNA Kit (Invitrogen) following the manufacturer's protocol. Amplification of 18S and Internal transcribed Spacer 1 – 5.8S – Internal Transcribed Spacer 2 (ITS) rDNA employed protocols and primers of Gustinelli *et al.* (2010), cytochrome *c* oxidase I (CO1) mtDNA those of Moszczynska *et al.* (2009).

Amplified products were resolved on a 1% agarose gel stained with SYBR Safe DNA Gel Stain in 0.5× TBE (Molecular Probes – Life Technologies). For sequencing of 18S, ITS and CO1, bands were excised and purified by NucleoSpin Gel and polymerase chain reaction Cleanup (Macherey-Nagel) and sequenced with an ABI 3730 DNA analyser at StarSEQ GmbH (Mainz, Germany). Contigs were assembled with Vector NTI Advance™ 11 software (Invitrogen) and sequences are published in GenBank under the following accession numbers: MH606186-90 (18S), MH238412-16 (ITS) and MH253044-48 (CO1).

Pairwise *p*-distances and models of nucleotide evolution (Bayesian Information Criterion) were calculated using MEGA 6.06 (Tamura *et al.*, 2013). For trees constructed with Bayesian Inference (BI), in MrBayes 3.2.6 (Ronquist *et al.*, 2012), nst = 2 + G was used for ITS and 18S, and GTR + G + I was used for CO1. The K2P + G-model was used for maximum likelihood (ML) analysis in MEGA of 18S and ITS rDNA while GTR + G + I was used for ML analysis of CO1 mtDNA.

The newly generated sequences of 18S, ITS and CO1 were aligned along with one or two representative sequences of *Clinostomum* species (*C. complanatum*, *C. cutaneum*, *C. phalacrocoracis*, *C. tilapiae*, *C. philippinensis*, *C. marginatum*, *C. tataxumui*, *C. album*, *C. poteae*, *C. heluans*, *C. attenuatum*, *C. detruncatum*, *C. arquus*, *C. caffarae*, *C. cichlidorum*) plus undescribed or unidentified species of *Clinostomum* (Locke *et al.*, 2015; Caffara *et al.*, 2017). *Euclinostomum heterostomum* (ITS: KP721422, CO1: KP721404), *Odhneriotrema incommodum* (ITS: MF766000, CO1: MF766003) and *Ithyoclinostomum* (ITS: MH159753, CO1: MH159752) were used as outgroup for the subfamily Clinostomatinae, while *Tylodelphys immer* (18S and ITS: MH521252; CO1: MH536513), *Cyathocotyle prussica* (18S and ITS: MH521249; CO1: MH536510), *Schistosoma mansoni* (18S: U65657; ITS: AY446082) as outgroup for Clinostomidae. All codon positions in the CO1 alignment were used in the analysis because of lack of evidence of nucleotide saturation (Iss = 0.237, Iss.c = 0.697, df = 472, $P = 0$; Xia *et al.*, 2003; Xia and Lemey, 2009).

Results

Among five 18S rDNA sequences 1826–1877 bp in length obtained from African samples of *C. brieni* in the current study, there were four variable sites, all transitions, i.e. mean divergence 0.1%, range 0–0.2%. All variation was in two sequences from South Africa; three 18S sequences from Congo were identical. An 18S sequence (KF781300, 1907 bp) of Athokpam *et al.* (2014) from *C. brieni* from *Heteropneustes fossilis* in Manipur differed at 32 positions (1.7%) from another 18S sequence by the same authors (KF811009, 1859 bp) from the same host in Meghalaya (the latter sequence is not mentioned in the paper of Athokpam *et al.*, 2014). Variation in 18S between the five African *C. brieni* sequences and the two Indian isolates averaged 1.4% (range 0.5–2.6%). Phylogenetic analysis showed that 18S sequences from Indian and African *C. brieni* form a well-supported clade nested within *Clinostomum* species. The *Clinostomum* + *C. brieni* clade is also well supported, and comparatively deeply divergent from *Euclinostomum*. Variation among 18S sequences of *Clinostomum* spp. averaged 0.9% (range 0.2–1.5%) and in the *Clinostomum* + *C. brieni* clade, 18S variation averaged 1.1% (range 0–3.6%). All the highest divergence values (⩾1.8%) in the latter clade were associated with the unpublished *C. brieni* sequence KF811009. Variation between *Euclinostomum* and members of the *Clinostomum* + *C. brieni* clade averaged 2.8% (range 2.4–5.0%).

The five ITS rDNA sequences 1005–1028 bp in length from African *C. brieni* were identical to each other and to the 300-bp ITS2 sequence of *C. brieni* (KF781298) of Athokpam *et al.* (2014). The ITS of *C. brieni* varied by a mean of 5.7% (range 4.7–7.1%) from species of *Clinostomum*. Variation in ITS among species of *Clinostomum* s.s. was of similar magnitude: mean 4.9% (range 0.1–8.6%). In contrast, ITS variation among members of the genera *Euclinostomum*, *Odhneriotrema* and *Clinostomum* + *Clinostomoides* averaged 15.2% (range 13.9–16.3%).

The CO1 sequences of four specimens of *C. brieni* were identical but that of one specimen (MH253045, from *C. gariepinus* in South Africa) differed by 11% from the other four. This specimen did not differ morphologically from the other five *C. brieni*

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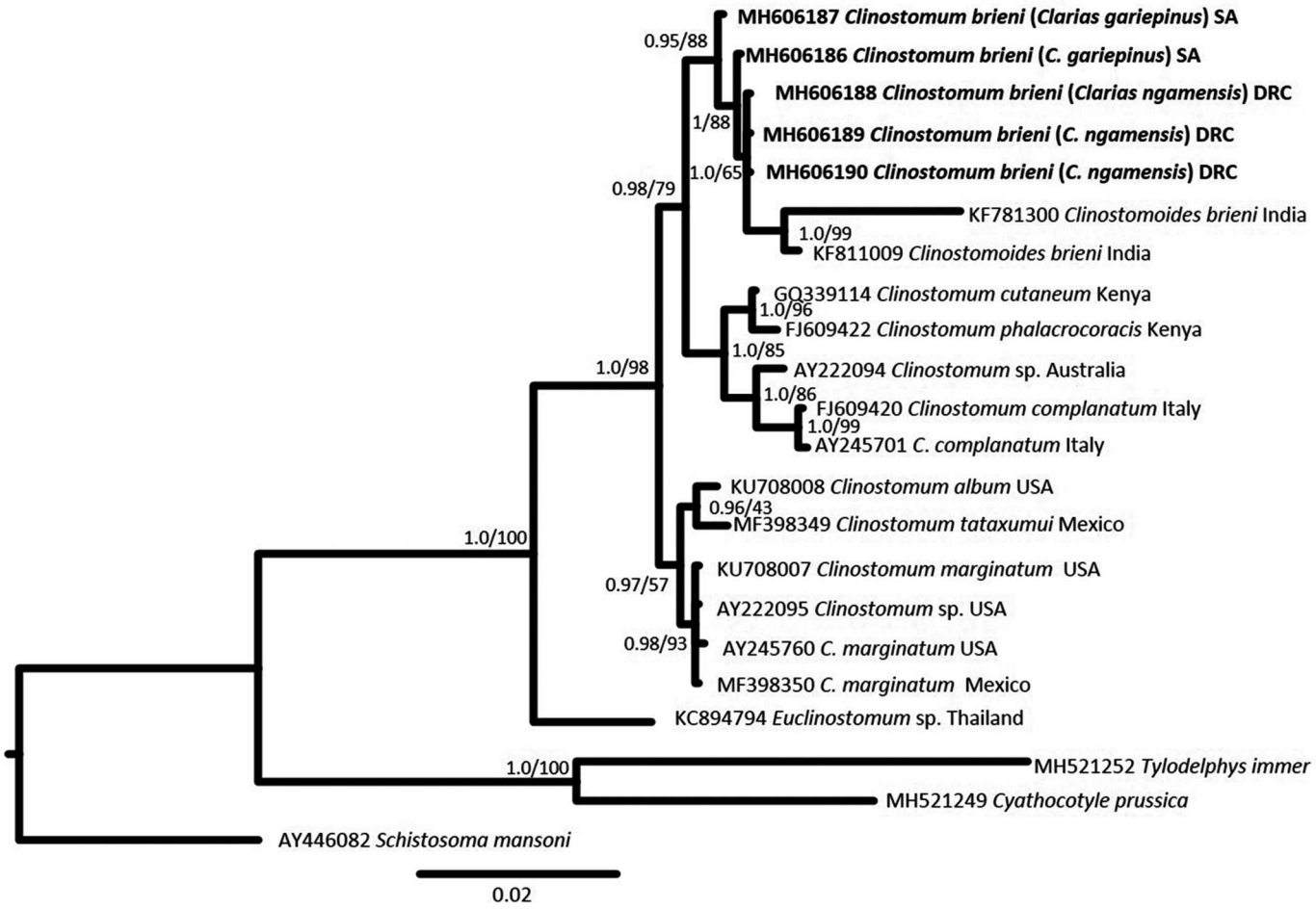

Fig. 1. Evolutionary history inferred using BI (nst = 2 + G) from 18S rDNA of *Clinostomum brieni* n. comb. generated in this study (in bold) with data from other studies (22 nucleotide sequences, 1663 positions). Nodes are labelled with posterior probability in BI analysis and, after the slash, percent of bootstrap support in 1000 replicates in ML. SA, South Africa; DRC, Democratic Republic of the Congo.

examined, and its 18S (MH606187) and ITS (MH238413) sequences were not similarly divergent (Figs 1 and 2). The CO1 of this specimen differed by 0.2% from *Clinostomum* morphotype 3 (KY865667, from the *Amphilius uranoscopus* in South Africa). The DNA from this specimen was amplified and sequenced an additional four times with the same results. The BI and ML trees were based on a 473 bp CO1 alignment and had little statistical support at deeper nodes, but both showed *C. brieni* within *Clinostomum*. The *C. brieni* specimen MH253045 grouped with *Clinostomum* sp. morphotype 3, while the other four *C. brieni* sequences form a monophyletic clade within the Old-World clade of *Clinostomum* species. The four monophyletic *C. brieni* sequences differ by mean 15.3% (range 13–19.5%) from other *Clinostomum* species. Interspecific CO1 variation in *Clinostomum* is similar, with mean 16.3% (range 3.5–22.1%). The mean intergeneric CO1 distances between members of *Odhneriotrema*, *Clinostomoides* + *Clinostomum*, *Euclinostomum* and *Ithyoclinostomum* is 19.8% (range 17.3–23.5%).

Analyses of three molecular markers indicate *Clinostomoides* should be regarded as junior synonym of *Clinostomum*, as amended below. Tree topologies (Figs 1–3) show relatively deep divergence between *Odhneriotrema*, *Euclinostomum* and *Clinostomum* whereas *Clinostomoides* falls within a clade of *Clinostomum* species. Genetic distances between *C. brieni* and *Clinostomum* species are comparable with those within *Clinostomum* s.s. and inferior to distances among other clinostomid genera.

Clinostomum Leidy, 1856
(Synonym *Clinostomoides* Dollfus, 1950)

Family Clinostomidae Lühe, 1901
Subfamily Clinostominae Lühe, 1901

Body medium to very large, linguiform, stout, convex dorsally and concave ventrally. Tegument smooth or with spines. Oral sucker may or may not be surrounded by collar-like fold when retracted. Ventral sucker muscular, well developed, always larger than oral sucker. Caeca long, simple, with more or less sinuous wall, particularly in anterior half of body, but lacking lateral branches or diverticula. Testes smooth or irregular in shape, in posterior half of body. Ovary intertesticular, to right of medial line. Vitelline follicles in lateral fields anteriorly, from the level of intestinal bifurcation or ventral sucker to posterior extremity, may remain lateral and extracaecal or become confluent posterior to genital complex. Uterus intercaecal, extending from Mehlis' gland to fill part of total distance to ventral sucker. Genital pore anterior, lateral or posterior to testicular-ovary complex. Cosmopolitan.

Type species *Clinostomum complanatum* (Rudolphi, 1819).

Morphological description of Clinostomum brieni (Dollfus, 1950) n. comb. (Fig. 4, Table 1) (based on five hologenophores and one paragenophore)

(Synonym *Clinostomoides brieni* Dollfus, 1950)

Body regularly elongated, narrow, tongue-shaped. Oral sucker small, with indistinct marginal limits. Pre-pharynx cup-shaped elongated, thick, muscular. Pharynx visible, muscular. Intestinal bifurcation anterior to ventral sucker, forming caecal shoulders before running laterally to ventral sucker to posterior end of body. Ventral sucker robust, larger than oral sucker, muscular, trilobed structure easily visible. Caeca provided with small lateral

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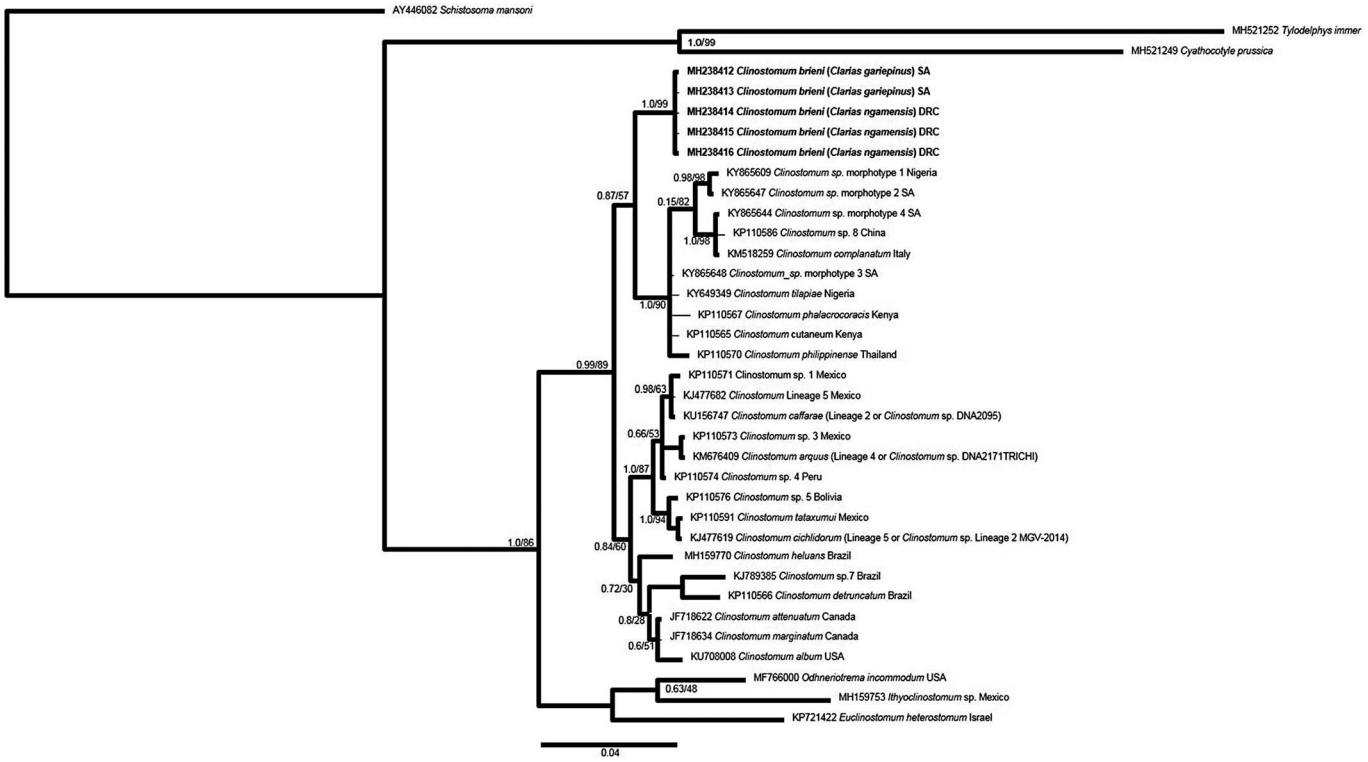

Fig. 2. Evolutionary history inferred using the BI analysis (nst = 2 + G) from ITS rDNA of *C. brieni* n. comb. generated in this study (in bold) with data from other studies (36 nucleotide sequences, 575 positions). Nodes are labelled posterior probability and, after the slash, with percent of bootstrap support in 1000 replicates in ML. SA, South Africa; DRC, Democratic Republic of the Congo.

pockets becoming more digitated posteriorly to ventral sucker. Whole genital complex in posterior part of posterior third of body. Testes two, tandem, intercaecal, transversely elongated. Anterior testis bow-tie shaped. Posterior testis Y- to crescent shaped with anterior margin concave. Cirrus sac comma-shaped, thick walled, intertesticular dextral, from right posterior margin of anterior testis to anterior margin of posterior testis, genital pore opening in concave margin of posterior testis. Ovary small, intertesticular dextrally to cirrus sac, close to right margin of posterior testis.

Uteroduct emerging from ootype complex runs around left margin of anterior testis, ascending sinistrally with some undulation to slightly above metraterm before looping posteriorly on itself entering directly into the proximal part of uteroduct and opening into uterine sac. Uterine sac median elongate narrow, tip reaching the posterior part of middle third of body. Tegument armed with spines (8–11 μm) from posterior part of oral sucker to posterior end of body. Excretory bladder Y-shaped, postcaecal, arms extending anteriorly in extracaecal position. Excretory pore terminal.

Discussion

Our original aim was redescribe metacercariae of *C. brieni* and assess the relationship of the species with other clinostomids using DNA. Unexpectedly, the molecular data strongly indicate the species belongs within *Clinostomum*, which led us to amend the diagnosis of the genus to accommodate *C. brieni* n. comb. The genus *Clinostomoides* was limited to Afrotropic and Indo-Malayan regions, and most reports are from *Clarias* or *Heteropneustes*, which are closely related siluriform genera occurring in the same regions (Froese and Pauly, 2000; Hardman, 2005; Kushwaha *et al.*, 2015). The phylogenetic association of *C. brieni* with a clade of Old-World *Clinostomum* species (Figs 1–3) is consistent with a biogeographic pattern that continues to be observed as data accumulate from more species of *Clinostomum* (Locke *et al.*, 2015; Pérez-Ponce de León *et al.*, 2016; Rosser *et al.*, 2018), which adds further evidence that *C. brieni* belongs to *Clinostomum*.

One specimen we collected was morphologically indistinguishable and shared identical rDNA sequences with other *C. brieni*, but its CO1 (MH253045) was highly divergent, and nearly identical to *Clinostomum* morphotype 3, which Caffara *et al.* (2017) obtained from mochokid and amphiliid catfishes in South Africa. We believe this can be most plausibly explained by hybridization. Both *C. brieni* and *Clinostomum* morphotype 3 infect siluriform second intermediate hosts in the same region, which are preyed upon by local ardeid definitive hosts, including the type host of *C. brieni*, *A. goliath* (Mock and Mock, 1980). Other than this particular specimen, *C. brieni* and *Clinostomum* morphotype 3 appear to be distantly related (Figs 1 and 2). This argues against another possible explanation for mitochondrial haplotype sharing between *C. brieni* and *Clinostomum* morphotype 3, incomplete sorting among recently separated species. In any event, when viewed together with the highly distinctive morphology of *C. brieni*, both hybridization and incomplete lineage sorting suggest species belonging to a single genus, which supports our main taxonomic conclusion.

The most distinctive characters of *Clinostomoides* were the size of the adult (30 mm) described in *A. goliath* by Dollfus (1950), the extremely posterior position of the genital complex, and the position of the cirrus sac and genital pore within the genital complex, characters considered also in regional descriptions of metacercariae (Prudhoe, 1957; Manter and Pritchard, 1969; Fischthal and Thomas, 1970; Jansen van Rensburg *et al.*, 2013). The most obvious distinction of *Clinostomoides*, its large size, is noteworthy in that the adult is known only from the largest of ardeids, *A. goliath* (Mock and Mock, 1980). In light of the molecular evidence that *Clinostomoides* belongs within *Clinostomum*, the other characters may be considered as size-related allometric changes.

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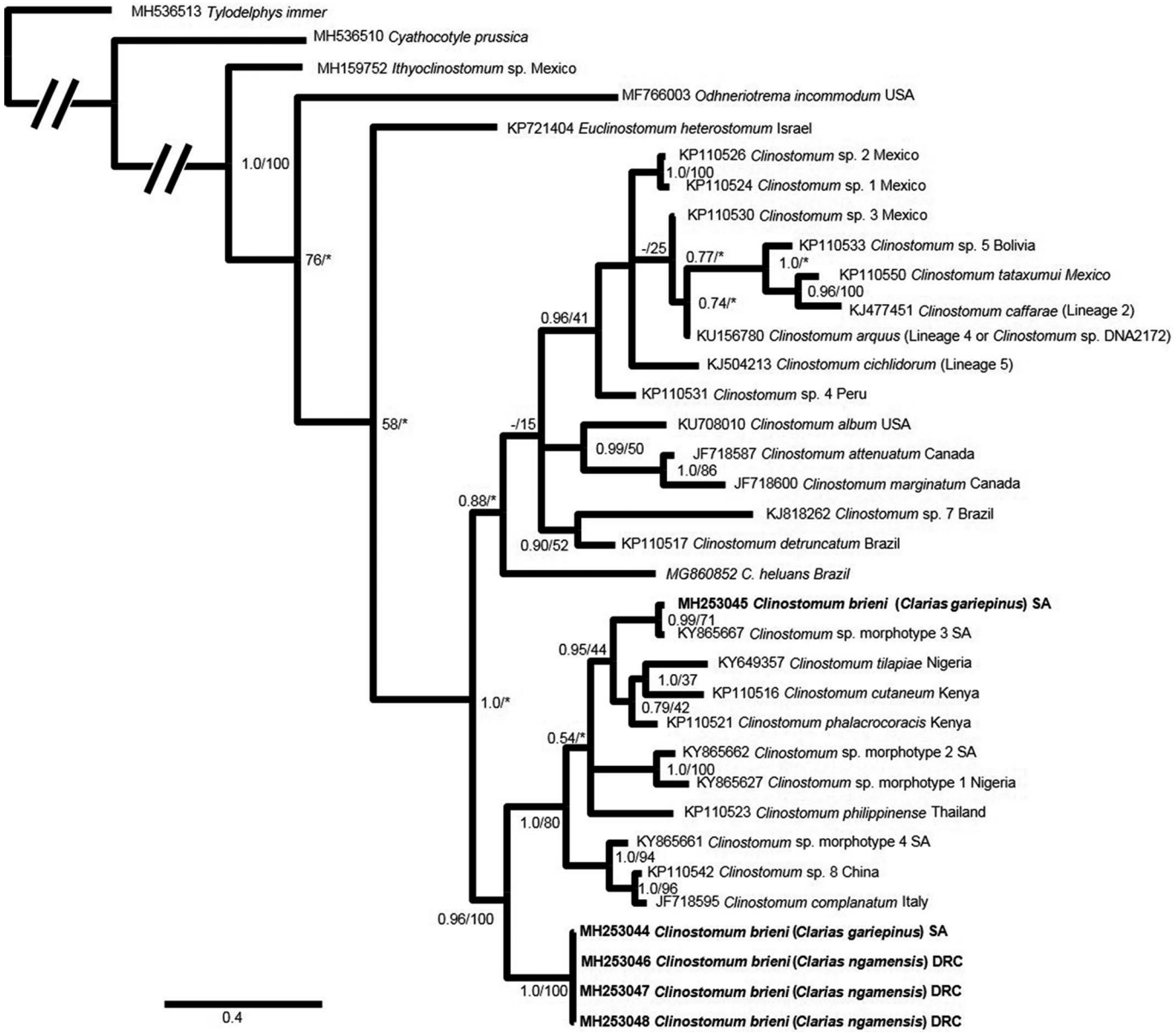

Fig. 3. Evolutionary history inferred in BI analysis (GTR + G + I) from CO1 mtDNA of *C. brieni* n. comb. generated in this study (in bold) with data from other studies (37 nucleotide sequences, 473 positions). Nodes are labelled with posterior probability and, after the slash, percent of bootstrap support in 1000 replicates in separate ML analysis; an asterisk indicates a clade that was not recovered with ML. SA, South Africa; DRC, Democratic Republic of the Congo.

The clinostomid metacercariae we collected from *Clarias* species in South Africa and Democratic Republic of the Congo were morphologically consistent with previous descriptions of *C. brieni* in the same host and region (Prudhoe, 1957; Manter and Pritchard, 1969; Fischthal and Thomas, 1970; Jansen van Rensburg *et al.*, 2013). The only inconsistency was the pharyngeal morphology. Fischthal and Thomas (1970) reported a thick-walled, muscular pre-pharynx and very muscular pharynx (as observed in our specimens), while Prudhoe (1957) reported the pharynx absent, Manter and Pritchard (1969) did not mention it, and Jansen van Rensburg *et al.* (2013) reported a short pre-pharynx and muscular pharynx. In our opinion, these structures are probably always present but not always visible.

As the genital complex provides reliable features for discriminating species of *Clinostomum* (i.e. Caffara *et al.*, 2017; Sereno-Uribe *et al.*, 2018, see Table 2), it can also shed light on prior records of *C. brieni*. The posterior testis in most descriptions is crescent-shaped, and showed this form in two of six subjects we examined, but in four worms the posterior testis was Y-shaped, as also reported by Fischthal and Thomas (1970). In metacercariae of *Clinostomum*, testes are more digitated than in pre-adults/adults (Ukoli, 1966). The only description of the adult of *C. brieni* is that of Dollfus (1950), who reported testes similar to those later described in metacercariae, except for small marginal lobules in the adult organs. Thus, in this species of *Clinostomum*, developmental variation in the morphology of the testicular margin appears to be reversed (going from smooth to more digitated), although data are needed from additional adults to confirm this. The cirrus pouch (CP) in *C. brieni* is well developed and lies between the testes, at a variable distance from the posterior border of the anterior testis and touching the concave part of the posterior testis where the genital pore opens. This pattern has been observed in all previous descriptions of *C. brieni* except that of Fischthal and Thomas (1970), in which the CP did not touch the posterior testis. Finally, in all metacercarial descriptions the uteroduct forms a similar loop devoid of eggs, which becomes filled with eggs in the adult (Dollfus, 1950).

Molecular data indicate a single species (albeit with potential capacity to hybridize) of *C. brieni* among samples spanning

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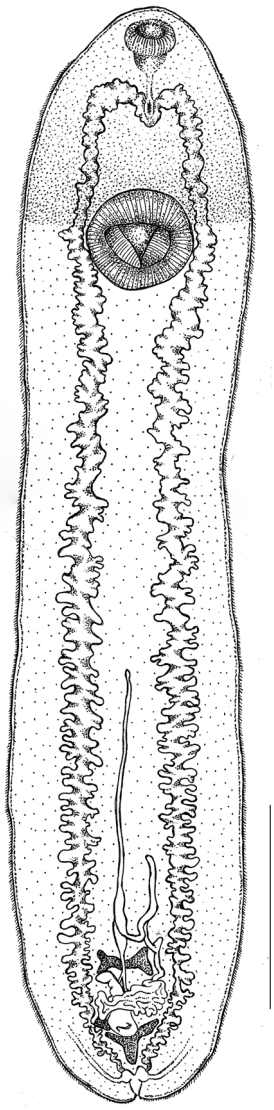

Fig. 4. Line drawing of metacercaria of *C. brieni* n. comb. Scale bar = 1000 μm.

Table 1. Morphological data of *Clinostomum brieni* [range (mean ± S.D.)]

Body length	6762–10 602 (8683 ± 1425)
Body width	1248–1527 (1392 ± 118.3)
Body length/width	4.80–8.40 (6.27 ± 1.17)
Oral sucker (OS) length	172–256 (218 ± 37.91)
OS width	172–282 (233 ± 35.99)
OS width/body width	0.11–0.19 (0.15 ± 0.033)
Ventral sucker (VS) length	622–749 (676 ± 54.08)
VS width	678–794 (733 ± 42.96)
VS width/OS width	2.91–4.61 (3.55 ± 0.71)
VS width/body width	0.48–0.57 (0.53 ± 0.03)
Distance between OS and VS	531–1709 (1277 ± 397.60)
Anterior testis (AT) length	104–128 (115 ± 10.53)
AT width	317–526 (426 ± 76.8)
AT width/length	2.92–4.99 (3.74 ± 0.87)
Posterior testis (PT) length	114–190 (145 ± 30.57)
PT width	254–444 (326 ± 74.81)
PT width/length	1.62–3.03 (2.28 ± 0.45)
Distance between testes	426–650 (494 ± 83.40)
Ovary length	165–200 (178 ± 12.17)
Ovary width	35–108 (75 ± 24.82)
Ovary width/length	0.20–0.60 (0.42 ± 0.14)
Cirrus sac (CS) length	437–652 (577 ± 76.76)
CS width	112–223 (168 ± 39.14)
CS length/body length	0.06–0.075 (0.06 ± 0.005)

approximately 1500 km of the known geographic range of this species, including the type region. Morphological differences among our specimens and those of prior regional accounts (Dollfus, 1950; Prudhoe, 1957; Manter and Pritchard, 1969; Jansen van Rensburg *et al.*, 2013) therefore likely represent intraspecific variation. This can provide a useful perspective for considering species of *Clinostomoides* described from the Indian subcontinent, the first of which was *C. dollfusi*, which Agarwal (1958) described from metacercariae from *Clarias* sp. and *Heteropneustes* (=*Saccobranchus*) sp. collected in Jabalpur. Agarwal (1958) emphasized body length (7.8–9.8 mm in the Indian metacercariae *vs* the 30 mm in adult holotype of *C. brieni*), the absence of lateral sacculations of the uterus, and distance between suckers. However, all these characters are in fact typical of metacercariae of *C. brieni* (Manter and Pritchard, 1969; Fischthal and Thomas, 1970; Jansen van Rensburg *et al.*, 2013; present study), if not of the larger adult holotype. All other species of *Clinostomoides* in this region are from *Heteropneustes*. *Clinostomoides chauhani* Pandey, 1971, was described from the body cavity and viscera of *H. fossilis* collected in Lucknow, based on comparison with *C. dollfusi* and *C. ophicephali*, which were both synonymized with *C. brieni* by Manter and Pritchard (1969) and Fischthal and Thomas (1970). The species *C. chauhani* is said to possess an aspinose body but spinose cuticle (Pandey and Agrawal, 2013). *Clinostomoides rai* was proposed by Rai (1970) for metacercariae from the intestine of *Clarias batrachus* collected in Mathura, on the basis of distance between suckers and limbs of uterus (Pandey and Agrawal, 2013). However, Pandey and Agrawal (2013) appear to have mistranscribed morphological values (i.e. acetabulum 1.16 × 0.78 mm *vs* acetabulum at distance of 1.16 mm behind anterior end of body and 0.78 mm in diameter) and host tissues (muscle *vs* intestine) recorded by Rai (1970). *Clinostomoides meerutensis* Pandey and Tyagi, 1986 and *C. pandeyii* Singh and Sharma, 1994, were both created for metacercariae from the body surface of *H. fossilis* in Meerut based on a spinose tegument and ovary opposite to cirrus sac. Pandey and Kiran (2002) synonymized *C. rai*, *C. meerutensis* and *C. pandeyii* with *C. dollfusi*. *Clinostomoides baughi* Pandey, 1998 was described from metacercariae in skin near the operculum of *H. fossilis* in Lucknow, based only on comparisons with the species of Indian *Clinostomoides* mentioned above. In the descriptions of *C. meerutensis*, *C. pandeyii* and *C. baughi*, the ovary is described as opposite to the cirrus sac. However, to our knowledge the ovary in the genus *Clinostomoides* and/or *Clinostomum* is always on the same side as the CP. Line drawings in Pandey and Agrawal (2013) appear to show specimens of the latter three species transposed, with the uteroduct in the right side of the body.

In our opinion, these species of *Clinostomoides* (*C. dollfusi*, *C. rai*, *C. chauhani*, *C. meerutensis*, *C. pandeyii*, *C. baughi*) described from India are *species inquirendae* because the morphological basis of each may be an artefact of development, beginning with the comparison of larval *C. dollfusi* with adult *C. brieni* and cascading through later descriptions. The characters considered in these studies vary with parasite development and may

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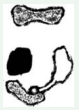
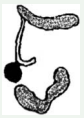
Table 2. Morphological data and line drawings of the genital complex of *C. brieni* n. comb. and in species described from the genus *Clinostomoides*

	Clinostomum brieni present study	*C. brieni* Dollfus, 1950 (A)	*C. brieni* Mirzoeva, 1981 (A)	*C. brieni* Prudhoe, 1957	*C. brieni* Manter and Pritchard, 1969	*C. brieni* Fischthal and Thomas, 1970	*C. brieni* Jansen van Rensburg et al., 2013	*C. dollfusi* Agarwal, 1958	*C. chauhani* Pandey, 1970	*C. rai* Rai, 1970	*C. meerutensis* Pandey and Tyagi, 1986	*C. pandeyii* Singh and Sharma, 1994	*C. baughi* Pandey, 1998
													
Locality	SA, DRC	BC (DRC)	Azerbaijan	BC (DRC)	Rwanda	Ghana	Botswana	Jabalpur (India)	Lucknow (India)	Mathura (India)	Meerut (India)	Meerut (India)	Lucknow (India)
Host	*Clarias gariepinus*, *Clarias ngamensis*	*Ardea goliath*	*Ardea purpurea*	*Clarias lazera*	*Clarias* sp.	*Clarias senegalensis*	*C. gariepinus*	*Clarias* sp., *Heteropneustes* sp.	*Heteropneustes fossilis*	*Clarias batrachus*	*H. fossilis*	*H. fossilis*	*H. fossilis*
Location	Body cavity	Esophagus	Esophagus	Gills	Gills	Pharyngeal region	Gill chamber/ branchial region		Body cavity and viscera	Intestine	Body surface	Body surface	Skin near operculum
BL	6762– 10 602	30 700	17 000	6000–9000	6198– 10 138	5980–9085		7800–9800	5470	7410	5670	8500– 10 200	5050–5850
BW	1248–1527	3700	4000	1250–1650	1443–1850	1075–1670		1220–1990	1200	1370	1350	1500–2300	1450–1500
OSL	172–256	Ø 400	410	Ø 250–350	278	175–195		156–260	200	120	260	250–350	160–180
OSW	172–282		430		463	295–340		234–312	160	190	400	40–500	250–280
VSL	622–749	Ø 1660	710	600–650	574	475–675		589–754	440	1160	520	500–600	500–530
VSW	678–794		980	700–750	822	590–895		650–780	260	780	510		450–500
ATL	104–128		510	60		75–150		130–150	20	86	70	350–440	90–100
ATW	317–526		1060	150		375–620		280–620	150	430	300	150–220	350–400
PTL	114–190		310			55–110		70–130	220	68	60	210–230	100–400
PTW	254–444		670			345–500		230–330	160	340	230	350–440	420
OL	165–200	1060	950	250		200–287		150–220	100	65	120	220–260	120–130
OW	35–108	540	330	50		92–144		70–130	40	170	110	110–130	90–100
CSL	437–652			300–500		380–600		390–620	390		200	500–600	180–200
CSW	112–223			120–150		105–260		100–180	50		70	200–300	90

BL, body length; BW, body; OSL, oral sucker length; OSW; oral sucker width; VSL, ventral sucker length; VSW, ventral sucker width; ATL, anterior testis length; ATW, anterior testis width; PTL, posterior testis length; PTW, posterior testis width; OL, ovary length; OW, ovary width; CPL, cirrus pouch length; CPW, cirrus pouch width; SA, South Africa, DRC, Democratic Republic of Congo; BC, Belgian Congo; (A), adult. The line drawings were based on figures in the original publications.

also be influenced by fixation and mounting (Manter and Pritchard, 1969; Fischthal and Thomas, 1970). Until such species can be verified through morphological comparisons at equivalent stages of development, preferably with detailed accounts of the genital complex and with supporting molecular data, we consider only *C. brieni* to be valid. Essentially, this view follows Manter and Pritchard (1969) and Fischthal and Thomas (1970). Further work may reveal additional species of *Clinostomum* with the *Clinostomoides*-morphotype on the Indian subcontinent or elsewhere, but currently this is supported with neither molecular (Figs 1 and 2) nor morphological data. Connectivity between Indian and African populations of *C. brieni* could be maintained by species of *Ardea* which occur in both regions (e.g. *A. cinerea*, *A. purpurea*, *A. goliath*, BirdLife International, 2018).

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