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Research Article

Rediscovery and redescription of clinostomid trematodes from *Pelecanus onocrotalus*

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Abstract

This study reports the first rediscovery and redescription of *Euclinostomum lauroi* in more than 40 years, based on an adult specimen recovered from the oesophagus of a great white pelican (*Pelecanus onocrotalus*) in Lake Tana, Ethiopia. An integrative taxonomic approach combining detailed morphological examination and molecular analyses (ITS rDNA and COI mtDNA) was employed. Scanning electron microscopy revealed additional features, including the fine surface morphology of the cirrus and genital opening. The same host individual harboured 2 *Clinostomum* species, *C. phalacrocoracis* and *C. tilapiae*, for which updated morphological redescriptions are provided. The record of *C. tilapiae* represents a new geographical record for Ethiopia. These findings expand current knowledge of African clinostomid diversity and identify Lake Tana as an important hotspot of trematode biodiversity. The newly generated morphological and molecular data refine species boundaries within *Euclinostomum* and *Clinostomum* and support the use of integrative approaches to resolve persistent taxonomic uncertainties in clinostomid systematics.

Introduction

The members of the family Clinostomidae Lühe, 1901 are characterized by heteroxenous life cycle including fish-eating birds as preferential definitive hosts, except the subfamily Nephrocephalinae Travassos, 1928, which prefer reptiles (Kanev et al., 2002). In the last 25 years, the taxonomy has been heavily revised to reorder the high number of descriptions based only on few morphological features, as happened with species of *Clinostomum* Leidy, 1856 (Ukoli, 1966a; Yamaguti, 1971; Feizullaev and Mirzoeva, 1983). The advent of molecular methods, integrated with morphological observation, has substantially improved species delimitation, leading to the description and re-description of multispecies of *Clinostomum* and *Euclinostomum* Travassos, 1928, and in some instances prompting generic reassignment (Gustinelli et al., 2010; Caffara et al., 2011, 2016, 2019; Sereno-Uribe et al., 2013, 2018, 2025; Locke et al., 2015; Rosser et al., 2017, 2018; Truter et al., 2025). A clear biogeographical separation have been observed in the distributions of species of *Clinostomum* (Caffara et al., 2011; Locke et al., 2015 and references therein; Sereno-Uribe et al., 2025; Truter et al., 2025), but such separation has not been identified for *Euclinostomum* (Caffara et al., 2016). Finally, it is clear that species cannot be distinguished based on host species, because host associations of all developmental stages are broad or poorly known (Lo et al., 1982; Matthews and Cribb, 1998; Hoffman, 1999; Bullard and Overstreet, 2008; Locke et al., 2015).

Compared with the closely related genus *Clinostomum*, species of *Euclinostomum* have been reported less frequently in the literature, and several aspects of their taxonomy and biology remain insufficiently documented (Caffara et al., 2016). The taxonomic history of the genus *Euclinostomum* has been characterized by the erection of numerous nominal species; in total, 10 species have been proposed, 8 of which are currently regarded as invalid or of doubtful status. Caffara et al. (2016) revised the genus, recognizing as valid only the Old World species such as *Euclinostomum heterostomum* (Rudolphi, 1809) and *Euclinostomum multicaecum Tubangui & Masiluñgan, 1935*, a conclusion supported by the redescription of the type species (*E. heterostomum*) and the incorporation of molecular data. However, at that time, only a limited number of sequences linked to detailed morphological information were available (Athokpam et al., 2014; Senapin et al., 2014). More recently, the number of molecular reports on *E. heterostomum* has increased, although comprehensive morphological descriptions remain scarce (Shukla et al., 2024; Guz et al., 2025; Shigoley et al., 2025).

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A recent study by Truter et al. (2025) corroborates the previous reports by Caffara et al. (2016, 2017) regarding the descriptions of *Clinostomum* and *Euclinostomum* in African publications. Most of these studies report the 2 genera without providing a complete morphological description. In some recent papers (e.g., Mahdy et al., 2021), molecular analyses have been carried out using only 1 molecular marker, for instance ITS2 rDNA (Kaur et al., 2012; Zimik et al., 2019; Mahdy et al., 2021, 2023), which has shown high similarity among *Clinostomum* species (Gustinelli et al., 2010; Sereno-Uribe et al., 2013, 2018, 2025; Locke et al., 2015; Pérez-ponce de León et al., 2016; Briosio-Aguilar et al., 2018).

Ethiopia has many major water bodies, especially lakes, that have an estimated volume of in more than 335 billion cubic meters (Getaneh et al., 2022); many of these lakes are recognized for their outstanding biological diversity and societal significance (Belete et al., 2015). Lake Tana is the largest lake in Ethiopia and supports a large fishing industry mainly based on tilapias and catfish. Moreover, this country is an important resting and feeding ground for many Palearctic migrant waterbirds including the great white pelican *Pelecanus onocrotalus*, Linnaeus, 1758, which is a suitable definitive host for clinostomids trematodes. The co-occurrence of fish and waterbirds enables the life cycle of clinostomids for which the former are second intermediate hosts while the latter are definitive hosts.

Few studies on clinostomids in birds definitive host in Africa are old reports (Dollfus, 1932, 1950; Van der Kuyp, 1953; Ukoli, 1966a,b; Manter and Pritchard, 1969; Dennis and Sharp, 1973; Britz et al., 1984), but most provide exhaustive morphological description useful for identification. Few report of clinostomids in fish have been reported from Ethiopia in the last 25 years (Eshetu, 2000; Yimer, 2000; Eshetu and Mulualem, 2003; Yimer and Enyew, 2003; Zekarias and Yimer, 2007; Gulelat et al., 2013; Amare et al., 2014; Bekele and Hussien, 2015; Gebawo, 2015; Reshid et al., 2015; Gebremedhn and Tsegay, 2017). Most of these are epidemiological studies reporting quantitative data without any specific identification. Gustinelli et al. (2010) published the first comprehensive paper using an integrative taxonomic approach (morphology coupled with molecular analyses) to report *Clinostomum cutaneum* Paperna, 1964 metacercariae and adults collected in Kenya, representing the first input towards a systematic revision of this complex group of parasites. Zhokhov and Morozova (2020) published a study on clinostomids of fish from Lake Tana, providing exhaustive morphological descriptions but no molecular data.

The current study provides the first detailed descriptions of adult clinostomids isolated from *P. onocrotalus* in Ethiopia. By integrating new morphological data with phylogenetic analyses, we refine species boundaries within and between *Clinostomum* and *Euclinostomum*.

Materials and methods

Sampling and morphological study

Twenty-three adult clinostomids were collected from the oesophagus (Figure 1) of 1 specimen of *P. onocrotalus* collected from Bahar Dar Gulf, Tana Lake (11°35′ N 37°23′ E) and processed fresh, according to permits of the Addis Ababa University (ref. No. ALIPB IRERC/141/2016/24) and the Amhara National Regional State Environment and Forest Protection Authority (ref. No. Aka/T/B-10/01-18/03/2017). The clinostomids were washed in saline and preserved in 70% ethanol for morphological and molecular analysis. Measurements are given in micrometres unless otherwise stated and follows Matthews and Cribb (1998) and Caffara et al. (2016).

Figure 1. Oesophagus of *Pelecanus onocrotalus* with several adult clinostomids.

Some specimens were processed for scanning electron microscopy (SEM) analysis; they were dehydrated through a graded ethanol series, subjected to critical point drying, sputter-coated with gold palladium, and observed using a Phenom XL G2 Desktop SEM (Thermo Fisher Scientific, Eindhoven, The Netherlands) operating at 5 kV.

Molecular study

Before the clarification in Amman's lactophenol a small portion of the posterior end of the body were removed for DNA extraction using a PureLink Genomic DNA Kit (Life Technologies, Carlsbad, CA, USA) following the manufacturer's protocol. Amplification of ITS rDNA employed protocols and primers of Gustinelli et al. (2010); COI mtDNA employed those of Moszczynska et al. (2009). Amplified products were electrophoresed on a 1% agarose gel stained with SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, Carlsbad, CA, USA) in 0.5X TBE. For sequencing of both ITS and COI, amplicons were excised and purified by NucleoSpin Gel and PCR Cleanup (Mackerey-Nagel, Düren, Germany) and sequenced with an ABI 3730 DNA analyser at StarSEQ GmbH (Mainz, Germany). The DNA trace files were assembled with VectorNTI AdvanceTM 11 software (Invitrogen) and compared with published data by BLAST tools (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignments were performed using BioEdit 7.2.5 (Hall, 1999) (32 ITS sequences and 35 COI sequences in the final dataset), p-distance and maximum-likelihood (ML) tree (T93+I substitution model for ITS, and KHY+G+I for COI, bootstrap of 1000 replicates for both genes) were performed using MEGA version 12 (Kumar et al., 2024). Some published clinostomid sequences were excluded from the analyses, as previous studies have demonstrated a clear molecular separation between Old World and New World representatives of the genus; therefore, only taxa relevant to the present study were retained.

The sequences generated in this study were uploaded in GenBank under the accession numbers PX626916-PX626929 (ITS rDNA) and PX661153-PX661172 (cox1 mtDNA).

Results

Based on morphological features, the adults recovered from the great white pelican (*P. onocrotalus*) belonged to 2 genera: *Euclinostomum* and *Clinostomum*.

Morphological redescription

***Euclinostomum lauroi* Tendeiro, Travassos and Fazendeiro, 1974 (*n* = 1)** (Figures 2 and 3; Table 1)

Type host: *Pelecanus onocrotalus*

Type locality: Maniquenique, Chibuto, Gaza, Mozambique

New locality: Bahar Dar Gulf (11°35′ N, 37°23′ E), Tana Lake, Ethiopia

Body large, slipper-shaped, slightly constricted in ventral sucker region and widest in gonadic region (Figures 2 and 3A). Anterior end invaginated to form inconspicuous oral collar. Oral sucker smaller than ventral sucker, not well evident as folded back on itself. Pharyngeal chamber not discernible. Intestine bifurcates immediately posterior to pharynx. Intestinal ceca, run laterally to ventral sucker (Figures 2 and 3A) to posterior end of body. Posterior to ventral sucker main ceca give rise to thin secondary blind diverticula, variable in number (15 at right margin and 13 at left), extending latero-posteriorly to main ceca with their external margin almost perpendicular to body side (Figures 2 and 3A). Connection between intestinal ceca and excretory system not visible. Testes tandem, intercaecal, in posterior third of body. Anterior testis in anterior part of the posterior third of body, crescent or U-shaped, with rounded and symmetrical arms embracing posterior part of uterus and cirrus pouch (Figure 3F). Posterior testis completely in posterior third of body, Y-shaped with anterior margin concave (Figure 3A). Cirrus pouch round, within arm of anterior testis (Figure 3F); genital pore not visible. Cirrus well visible and everted, finely striated in posterior half (Figure 3B, C). Ovary smooth, round, smaller than cirrus pouch, intertesticular, dextrally contiguous with posterior margin of anterior testis. Uterus in the middle third of body. Uteroduct emerging from ootype runs around left margin of anterior testis running parallel and opens into uterine sac at middle portion. Metraterm not visible. Vitelline glands distributed from ventral sucker to posterior end scattered among intestinal caecae. Tegument surface devoid of spines. Eggs smooth (Figure 3E).

The other 2 species were adults of *C. phalacrocoracis* (20 specimens) and *C. tilapiae* (2 specimens) (Figures 4 and 5).

Clinostomum phalacrocoracis (Dubois, 1930) **adult (*n* = 18)** (Figure 4; Table 2)

Body stout, slightly wider in gonadic region (Figure 4A). Oral sucker smaller than ventral one (Figure 4A), surrounded by inconspicuous oral collar. Pharynx slightly developed (Figure 4B); intestine bifurcates immediately posterior to pharynx. Intestinal caeca run laterally to ventral sucker and genital complex. Testes in tandem between middle and posterior third of body. Anterior testis, in posterior part of middle third of body, lobed, consists of 5 blunt lobes, some of which are sub-lobed. Posterior testis, in anterior part of posterior third of body, fan-shaped with anterior margin concave and with 3 major lateral lobes on each side and 1 major posterior lobe. Cirrus pouch bean-shaped, in dextral intertesticular space, anterior to ovary, with genital pore opening laterally at posterior margin of anterior testis between right and posterior lobe. Cirrus well visible and everted in 1 subject (Figure 4E), with slightly visible longitudinal ridges and small blunt tubercles at its base. Genital opening with evident small blunt tubercles along its internal edge (Figure 4D). Ovary round, smaller than cirrus pouch, located in dextral intertesticular space. Uterus

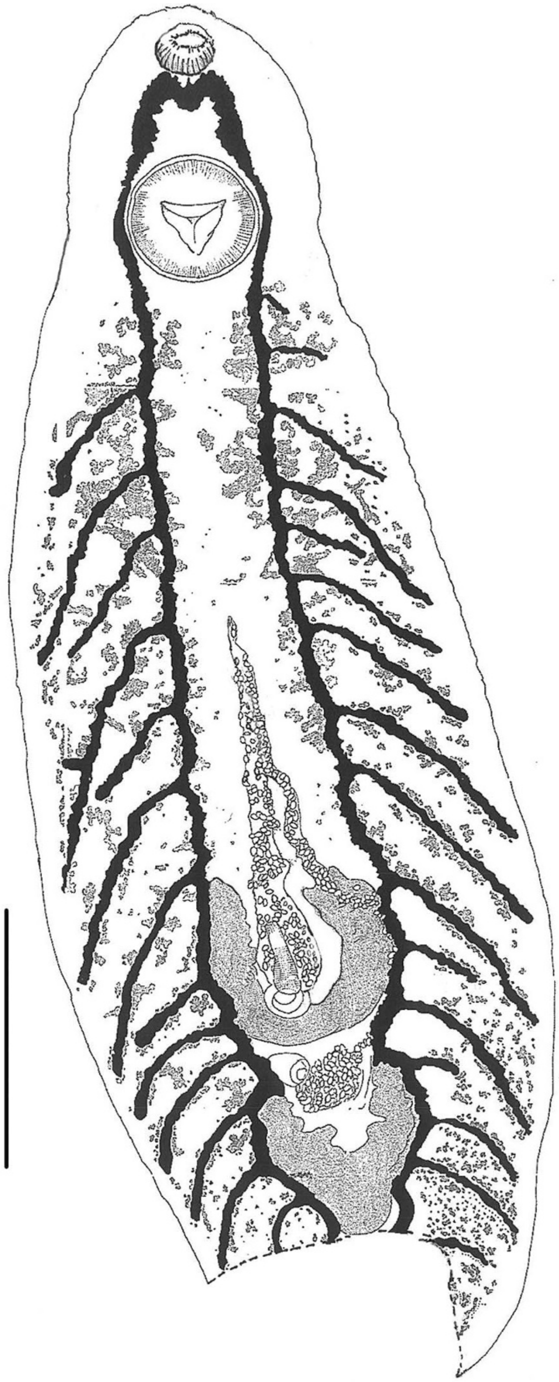

Figure 2. Line drawing of *Euclinostomum lauroi* mature specimen from *Pelecanus onocrotalus*. Scale bar = 370 μm.

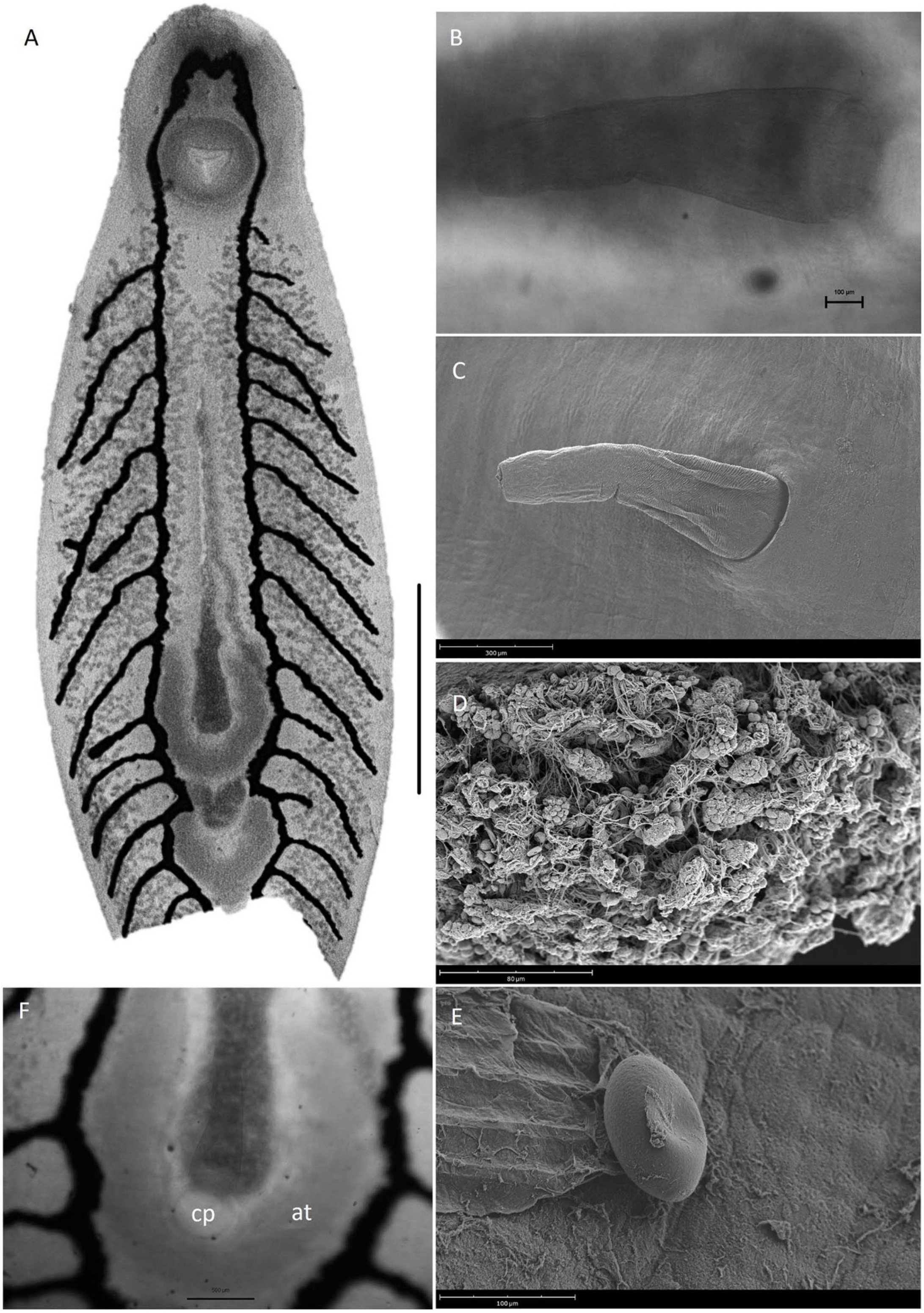

Figure 3. Morphological details of *E. lauroi* mature specimen from *P. onocrotalus*: (A) whole specimen, LM (Scale bar = 500 µm); (B) everted cirrus, LM; (C) detail of cirrus surface, SEM; (D) detail of sperm cells from sectioned posterior testis, SEM; (E) egg, SEM; (F) detail of cirrus pouch (cp) and anterior testis (at), LM.

runs straight from ventral sucker to anterior testis. Uteroduct runs around left margin of anterior testis and opens into uterine sac at its base. Metraterm, straight and overlapping right half of anterior testis, connects uterus. In some specimens, some of the structure composing the ootype complex were visible such as the vitelline reservoir and vitelline duct (Figure 4C). Tegument surface with numerous papillae on ventral side. Eggs smooth, operculated (Figure 4F).

Table 1. Measurements of *Euclinostomum lauroi* [Min–Max (Mean ± SD) μm]

Euclinostomum lauroi	*E. lauroi* (*n* = 1). Present study. *Pelecanus onocrotalus*.	*E. lauroi* (*n* = 5). (Tendeiro et al., 1974). *Pelecanus onocrotalus*. Mozambique	*Euclinostomum* sp. metacercariae (*n* = 6) (Zhokhov and Morozova, 2020). *Clarias gariepinus*. Lake Tana, Ethiopia Lake Tana, Ethiopia	*Euclinostomum* sp. metacercariae (*n* = 1) (Prudhoe and Hussey, 1977). *Clarias gariepinus*. Transvaal (South Africa)
Oral collar width	1284.9	–	–	–
Body length	20 000	9860–18210	13600–18000 (15800)	19 300
Body width	7000	3700–6250	4000–6000 (4930)	5370
Body length/Width	2.85	–		–
Oral sucker (OS) length	437.4	1100–1450	360–468 (396)	400
OS width	656.8	–	432–612 (510)	400
OS width/Body width	0.09	–	0.108–0.102 (1.05)	–
Ventral sucker (VS) length	1749.3	1320–2020	1800–2250 (2030)	1750
VS width	1843	1410–2110	1800–2250 (2030)	1750
VS width/OS width	2.80	–	4.16–3.67 (1.13)	–
VS width/Body width	0.26	–	0.45–0.37 (1.2)	–
Distance between suckers	2469	–	900–1620 (1162)	–
Anterior testis length	2288.1	–	1650–2250 (1936)	2200
Anterior testis width	2200	–	1250–1750 (1600)	300
AT width/Length	0.96	–	0.75–0.77 (0.97)	–
Posterior testis length	1905.3	–	1000–1350 (1207)	1060
Posterior testis width	2057.9	–	1100–1700 (1342)	200
PT width/Length	1.08	–	1.1–1.25 (0.87)	–
Distance between testes	2193.9	–	–	–
Ovary length	492.1	960–1250	252–360 (306)	340
Ovary width	440	640–710	216–288 (252)	160
Ovary width/Length	0.89	–	0.86–0.8 (1.08)	–
Cirrus sac (CS) length	341.3	–	288–360 (317)	430
CS width	445.9	–	252–432 (317)	430
CS length/Body length	0.01	–	0.02–0.02 (1.05)	–
Uterus length	6875.6	–	4250–6300 (5414)	6900
Uterus width	734.2	–	–	–
Number of right diverticula	15	14–17	13–17	16
Number of left diverticula	13	14–17	15–17	14
Eggs length (*n* = 50)	115.3 (103.6–135.3 ± 7.98)	96–125	–	–
Eggs width (*n* = 50)	73.7 (60.3–80 ± 4.16)	64–71	–	–

Clinostomum tilapiae **Ukoli 1966. Adult (1 immature and 1 mature) (Figure 5; Table 3)**

Body thick, elongated, thinner at anterior and terminal parts. Oral sucker subterminal, small, round, surrounded by oral collar (Figure 5A). Pharynx not well visible. Ventral sucker larger than oral sucker, in middle of anterior third of body. Intestinal caeca run lateral to ventral sucker, reaching posterior end of body. Tandem testes strongly digitated, very close to each other (Figure 5A). Anterior testis asymmetrical in medial longitudinal axis, in posterior portion of middle third of body, irregularly lobed, more developed on left side. Posterior testis located in anterior portion of posterior third of body, symmetrical, triangular, with 2 major lateral lobes, subdivided into smaller lobes, and 1 posterior lobe. Cirrus pouch oval and large, on right side, under posterior border of anterior testis. Genital pore medial to cirrus pouch, at level of posterior margin of anterior testis (Figure 5B). In immature specimens, several small blunt tubercles are present at base of everted cirrus (Figure 5C, D). Vitelline glands developed from

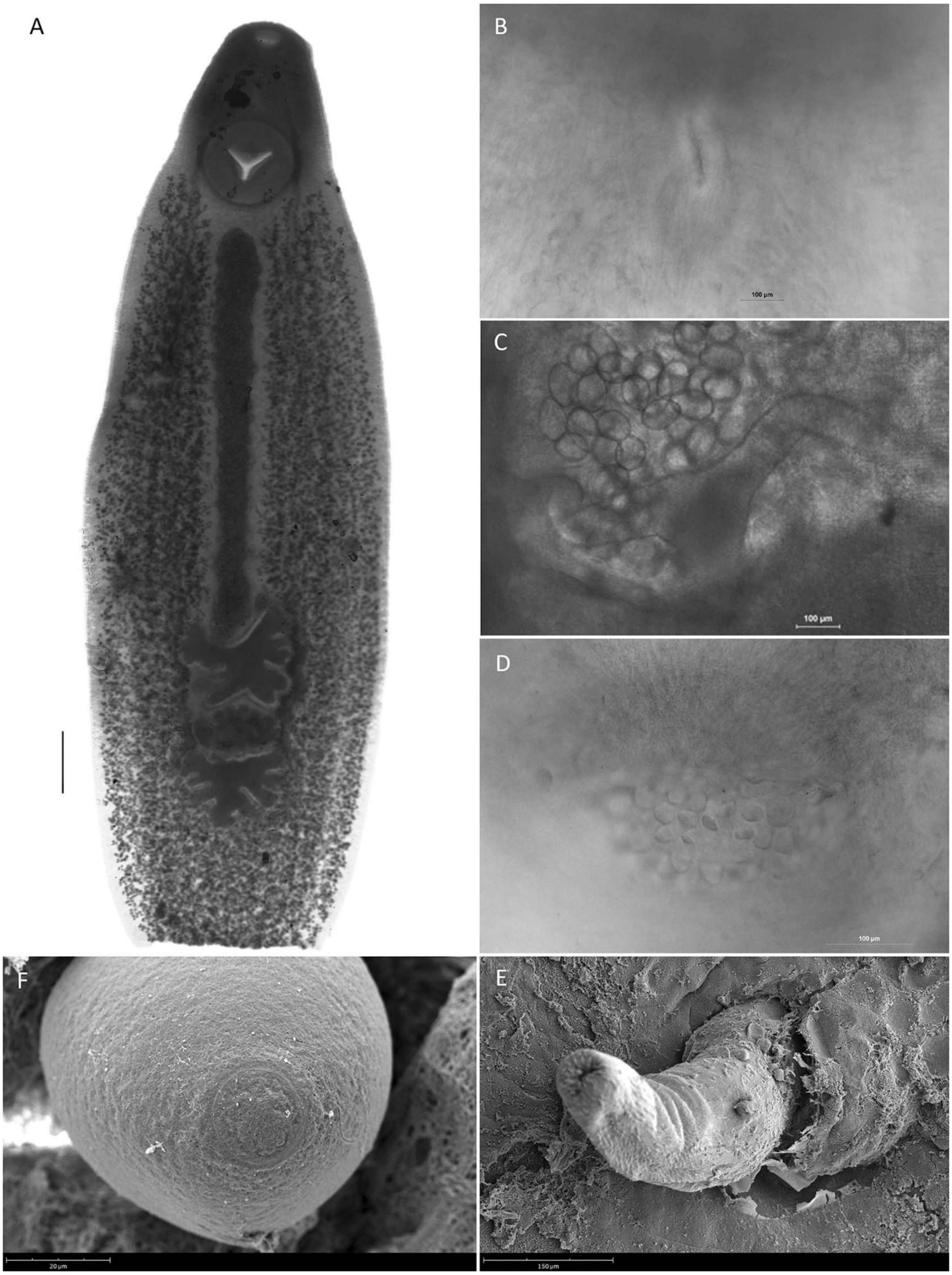

Figure 4. Morphological details of *C. phalacrocoracis* mature specimen from *P. onocrotalus*: (A) whole specimen, LM (scale bar = 100 µm); (B) pharynx, LM; (C) detail of vitelline reservoir and vitelline duct, LM; (D) detail of papilla-like structures at cirrus base, LM; (E) everted cirrus with small blunt tubercles at its base (arrow), SEM; (F), surface of egg showing apical operculum, SEM.

ventral sucker to posterior end of body. Ovary round, not median, in intertesticular space posterior to cirrus pouch. Uterine sac runs straight from ventral sucker to anterior testis, partially covering, right portion of testis when filled of eggs. Uterus runs around left margin of anterior testis, forming knee-like folding before opening into uterine sac close to anterior testis. Eggs ellipsoidal, operculate.

Molecular analyses

The ITS rDNA sequence of *E. lauroi* was 1025 bp long (681 bp ITS1, 159 bp 5.8S, 187 bp partial ITS2), showing 96.0–96.3% similarity with *Euclinostomum* sp. 1–4 (KC894798-KC894801, Osphoronemid fish from Thailand, Senapin et al., 2014) and 95.0–96.7% similarity with *E. heterostomum* (KP721439, Cichlids

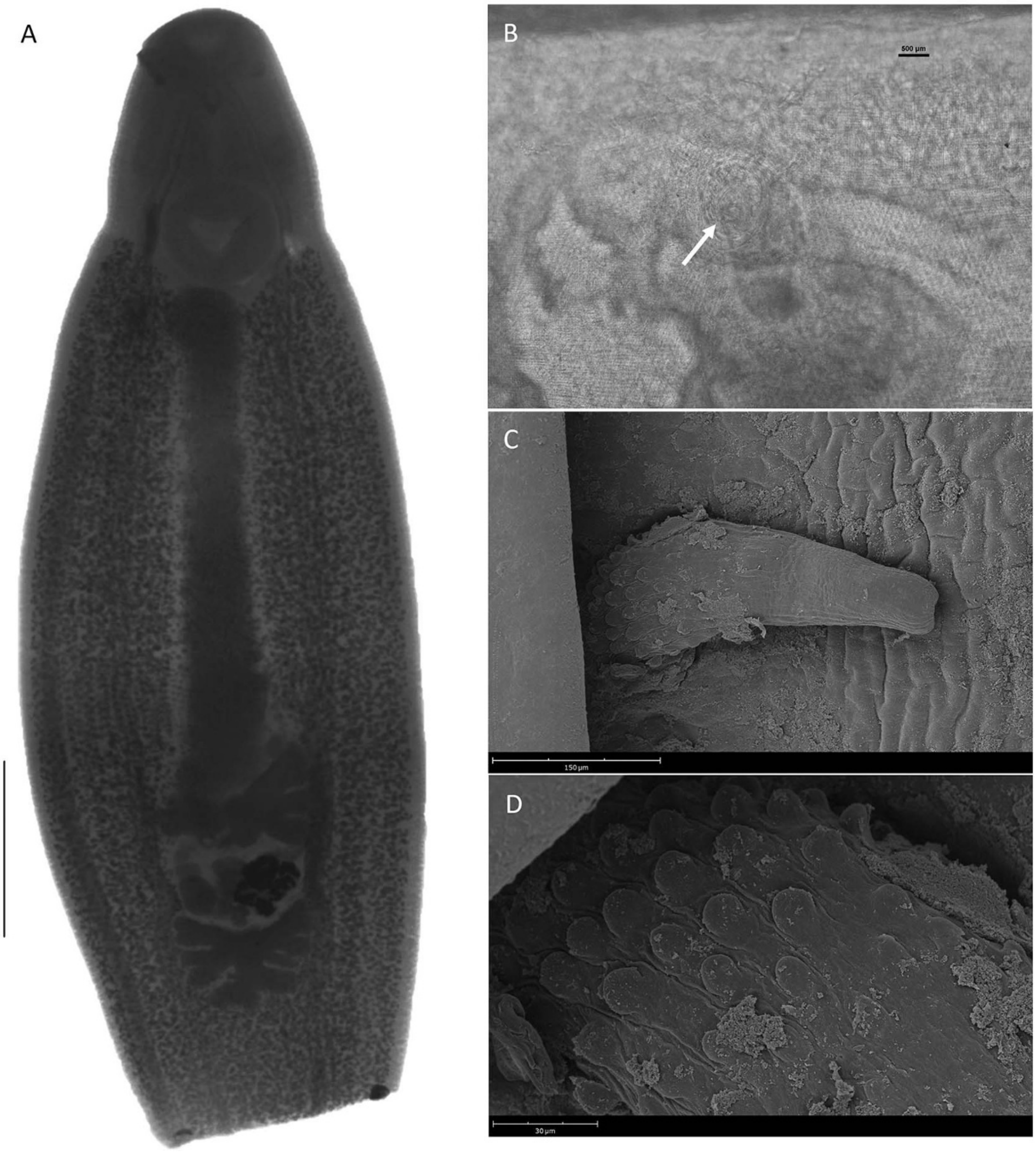

Figure 5. Morphological details of *C. tilapiae* from *P. onocrotalus*: (A) whole mature specimen, LM (scale bar = 400 µm); (B) detail of genital pore (arrow), LM; (C) everted cirrus with small blunt tubercles at its base, SEM; (D) detail of papilla-like structures at cirrus base, SEM.

from Israel, Caffara et al., 2016), based on BLAST search. The ITS rDNA p-distance between *E. lauroi* and *Euclinostomum* spp. sequences retrieved from GenBank was 0.03% with *E. heterostomum* and 0.04% with *Euclinostomum* sp. 1–4.

The COI mtDNA sequence was 621 bp long, showing similarity values ranging from 85% (*E. heterostomum*; Caffara et al., 2016) to 86% (*Euclinostomum* sp. 1–3, KC894795-97, Senapin et al., 2014). The p-distance observed were 18% relative to *Euclinostomum* sp. 1–3 (Senapin et al., 2014) and 19.6% relative to *E. heterostomum* (KP721421, Caffara et al., 2016).

Thirteen new ITS rDNA sequences and 17 COI mtDNA for *C. phalacrocoracis*, and 2 ITS rDNA and 2 COI mtDNA for *C. tilapiae* were obtained; BLAST analysis showed 99–100% identity with published sequences of *C. phalacrocoracis* (e.g. KP110569 ITS; KP110522 COI) and *C. tilapiae* (e.g. KY649356 ITS; KY649364 COI).

The ML trees of ITS rDNA (Figure 6) and cox1 mtDNA (Figure 7) showed the same topology with *Euclinostomum* spp. forming a well-supported (100% bootstrap) cluster, clearly separated from the *Clinostomum* group. Within *Euclinostomum, E. lauroi* is clearly distinct and basal to this group. The 2 *Clinostomum* species form well-supported clusters with sequences available in GenBank.

Discussion

The most species-rich clinostomids genus is *Clinostomum*, which has been the primary focus of taxonomic revision. Early studies synonymized all *Clinostomum* species under a single one, *Clinostomum complanatum* Rudolphi, 1814 (Ukoli, 1966a; Feizullaev and Mirzoeva, 1983), whereas in the molecular era, 24 valid species have now been described morphologically and

Table 2. Measurements of *Clinostomum phalacrocoracis* [Min–Max (Mean ± SD)]

	C. phalacrocoracis (*n* = 18). *Pelecanus onocrotalus*. Present study. Lake Tana Ethiopia	*C. phalacrocoracis* (Dubois 1950). *Phalacrocorax levaillanti* (syn *Anhinga rufa rufa*. Angola	*C. phalacrocoracis* (*n* = 1) (Ukoli 1966) *A. rufa rufa*. Ghana
Oral collar width	1238.9–2110.6 (1772.7 ± 276.2)		
Body length	9450–22000 (16756.9 ± 3310.25)	11000	12800
Body width	4000–7000 (5271.2 ± 967.21)	3600–3900	3900
Body length/Width	1.87–4.13 (3.21 ± 0.53)		3.28
Oral sucker (OS) length	550–806.2 (692.2 ± 61.5)	600–660	530
OS width	333.5–773.8 (630.3 ± 125.5)	480	610
OS width/Body width	0.08–0.16 (0.12 ± 0.02)		0.15
Ventral sucker (VS) length	1268.5–1714.4 (1456.1 ± 126.8)	1400–1560	1170
VS width	1279.2–1721.9 (1503.1 ± 112.59	1200–1260	1130
VS width/OS width	1.95–5.16 (2.53 ± 0.84)		0.96
VS width/Body width	0.22–0.41 (0.29 ± 0.05)		0.29
Distance between suckers	2232–3130 (2691.5 ± 318.4)		1220
Anterior testis length	1155.3–1956.3 (1587.1 ± 232.6)	1000–1050	1020
Anterior testis width	1013.2–2203.6 (1760 ± 327.9)	1500–1800	1580
AT width/Length	0.82–1.38 (1.12 ± 0.2)		1.55
Posterior testis length	958.4–1814.6 (1497.6 ± 243.6)	840–1050	900
Posterior testis width	1369.7–2111.5 (1744.6 ± 229.9)	1200–1500	1650
PT width/Length	0.91–1.66 (1.18 ± 0.18)		1.83
Distance between testes	578.1–1411.4 (1088.8 ± 211.7)		–
Ovary length	212.6–607.2 (459.2 ± 122.9)	300–350	330
Ovary width	202.8–535 (402 ± 79.2)	330–420	310
Ovary width/Length	0.66–1.47 (0.92 ± 0.22)		0.94
Cirrus sac (CS) length	572.4–950 (796.6 ± 115.3)		750
CS width	238.2–508.1 (393.5 ± 70.7)	150–450	380
CS length/Body length	0.03–0.06 (0.05 ± 0.01		0.06
Eggs length (*n* = 25)	97–115 (107.4 ± 4.8)	117	88–95
Eggs width (*n* = 25)	66.6–77.1 (72.3 ± 3.12)	72	54–61

characterized molecularly, along with additional putative species known only from molecular data (Sereno-Uribe et al., 2025). Moreover, the number of *Clinostomum* sequences available in GenBank has dramatically increased from 8 in 2010 to more than 1500 in 2025. For *Euclinostomum*, prior the present study, the only species described using a combined morphological and molecular approach was the type species, *E. heterostomum*. The present research provides the first molecular data for *E. lauroi* and additional morphological information for *C. phalacrocoracis* and *C. tilapiae*.

Ukoli (1966b) revised the genus *Euclinostomum*, outlining the unique morphological features to be considered for species identification: position of the genital complex, the arrangement of diverticula, and the position of the entrance of the uteroduct in the uterus. Based on these features Ukoli (1966b) considered only 2 species valid, *E. heterostomum* and *E. multicaecum* synonymizing all the other species described until then. Later, Tendeiro et al. (1974) described *E. lauroi* collected from the pharynx of *Pelecanus onocrotalus* in Mozambique to accommodate 5 specimens with morphological features different from the type species. These features observed in the single specimen herein described resemble those of Tendeiro et al. (1974): overall, our specimen is a little bit bigger than the Tendeiro specimens (20 000 vs 9860–18 200). Considering Ukoli (1966b) keys, the position of the genital complex of *E. lauroi* is in the posterior third of the body, while in *E. heterostomum* it is across the middle and posterior third of body; in *E. lauroi*, the morphology of the anterior testis is U-shaped with long symmetrical arms embracing the cirrus pouch and slightly anterior to, the posterior portion of the uterus; in *E. lauroi*, the anterior testis is more pronounced and bigger than in *E. heterostomum* (2288.15 × 2200 vs 410–460 × 430–440); in *E. lauroi*, the diverticula are fishbone like, while in *E. heterostomum* they are almost parallel to body side; the number are variable (15 right-13 left vs 7–15 right-6–14 left), but this is not considered a useful taxonomical feature for species discrimination. Finally, in *E. lauroi*, the anterior end of the uterus does not reach the ventral sucker

Table 3. Measurements of *Clinostomum tilapiae* [Min–Max (Mean ± SD)]

	C. tilapiae ($n = 1$ immature) *Pelecanus onocrotalus* Present study. Lake Tana Ethiopia	*C. tilapiae* ($n = 1$ immature) (Ukoli, 1966a) *Nycticorax nycticorax* Ghana	*C. tilapiae* ($n = 3$ immature) (Ukoli, 1966a). *Anhinga rufa* Ghana	*C. tilapiae* ($n = 1$ adult) *Pelecanus onocrotalus* Present study. Lake Tana Ethiopia	*C. tilapiae* adults (Ukoli, 1966a) *Bubulcus ibis* Ghana	*C. tilapiae* ($n = 2$ adult) (Öztürk and Umur, 2025) *Ardea purpurea* Turkey
Oral collar width	1100			1837		
Body length	9897	5840	5600–6330	18438	5250–5770	7800–7820
Body width	3139	2030	2120–2640	5900	1980–2260	2400–2410
Body length/Width	3.15		2.64–2.39	3.12		3.2
Oral sucker (OS) length	326	410	320–380	617	300–360	207–227
OS width	425	460	360–460	815	380–470	480–492
OS width/Body width	0.14		0.17	0.14		
Ventral sucker (VS) length	1214	900	860–980	1600	690–780	917–937
VS width	1214	890	870–1000	1653	690–830	858–866
VS width/OS width	2.85			2.03		0.23–0.25
VS width/Body width	2.59			0.27		
Distance between suckers	1987	750	680–830	2996	710–770	1200–1220
Anterior testis length	868	610	490–750	1442	460–680	454–466
Anterior testis width	785	580	680–919	2087	830–900	1014–1022
AT width/Length	0.90			1.45		
Posterior testis length	793	610	490–610	1552	470–560	444–448
Posterior testis width	841	610	810–1020	1927	900–1060	808–832
PT width/Length	1.06			1.24		
Distance between testes	514	90	80–150	1454	80–270	190–200
Ovary length	185	200	–	472	210–300	144–150
Ovary width	260	80	170–240	406	150–170	148–152
Ovary width/Length	1.40		–	0.86		
Cirrus sac (CS) length	510	330	350–380	787	300–350	350–390
CS width	261	230	230–260	398	230–260	270–290
CS length/Body length	0.05			0.04		
Eggs length ($n = 10$)			–	105.1–112.6 (109.2 ± 1.97)	88–102	89–103
Eggs width ($n = 10$)				65.5–75.1 (71.8 ± 2.62)	48–61	64–66

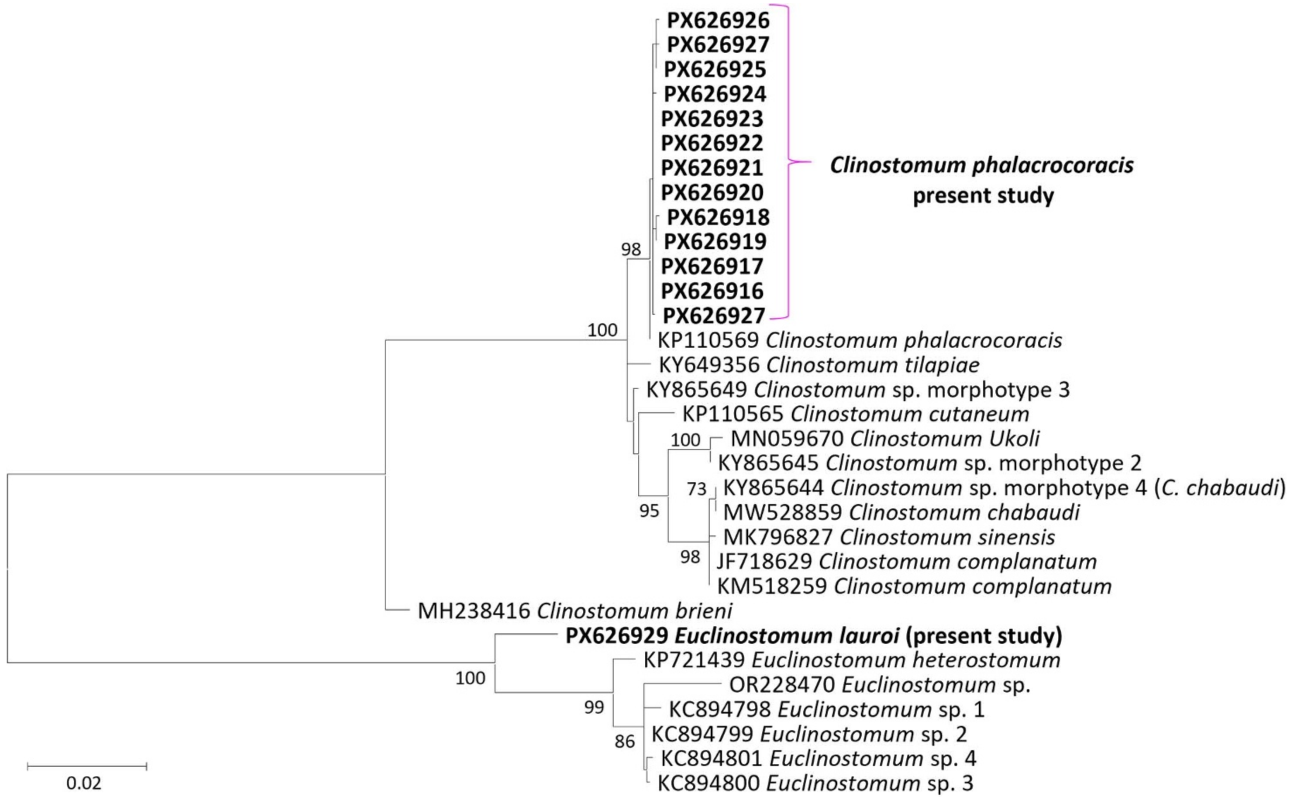

Figure 6. Phylogeny of *Euclinostomum* spp. and *Clinostomum* spp. based on ITS rDNA inferred using the maximum likelihood method and Tamura 3-parameter (+I) model of nucleotide substitutions. The percentage of replicate trees in which the associated taxa clustered together (1000 replicates) is shown next to the branches. The analytical procedure encompassed 32 sequences. Evolutionary analyses were conducted in MEGA12 utilizing up to 5 parallel computing threads.

(entirely in middle part of body), while in *E. heterostomum* it is very close to the ventral sucker; the uteroduct emerges from the ootype (not visible in our specimen due to the body's thickness), runs along the left margin of the anterior testis, reaches the middle portion of the uterus where it runs parallel to it, and opens subterminally into the uterine sac, whereas in *E. heterostomum* it opens at its anterior tip. In *E. lauroi* of Tendeiro et al. (1974), the uteroduct is reported to emerge at the tip of the uterine sac; however, in our specimens, this is clearly not the case. Prudhoe and Hussey (1977) described a metacercaria from *Clarias gariepinus* in South Africa whose morphology is consistent with of *E. lauroi*. Finkelman (1988) briefly described *Euclinostomum* sp. in the esophagus of some cormorants collected in Israel, identifying it as *E. heterostomum*. The author included 2 line drawings of the parasites and the larger (20 mm) resembles *E. lauroi*. The recent study by Zhokhov and Morozova (2020), carried out in fish from Lake Tana (Ethiopia), reported the morphological description of an undetermined species of *Euclinostomum* collected at the metacercarial stage from the orbital cavity of *Clarias gariepinus*. The authors provided a complete description, including morphometric data, a line drawing, and a stained image of the specimen. Based on all this evidence, we can state that these metacercariae represent *E. lauroi*. Unfortunately, neither study provided molecular data that could be compared with those obtained in our study. We exclude *E. multicaecum* due to its peculiar intestinal caeca, which extend into the posterior third of the body. Molecular analyses support the distinct phylogenetic position of *E. lauroi*, which is recovered as the basal lineage within *Euclinostomum*, consistent with its distinctive morphology.

Concerning the other *Clinostomum* species collected from the same bird, *C. phalacrocoracis* was first described in its adult stage from *Phalacrocorax levaillanti* (syn. *Anhinga rufa rufa*) in Angola (Africa) by Dubois (1930) later by Ukoli (1966a) from the same host in Ghana, and by Tendeiro et al. (1974) from *Pelecanus onocrotalus* in Mozambique. These authors provided a complete morphological description. Nevertheless, we noticed some morphological differences between our specimens and the previous reports. Despite the thickness of the specimens, the pharynx was visible as a vestigial structure of very fine fibres, mostly radial (Figure 4B) as reported by Dubois (1930) and Tendeiro et al. (1974), while Ukoli (1966a) stated its absence. The abovementioned authors reported the genital complex in the posterior third of the body; however, in all our specimens, the complex lies between middle and posterior end. The anterior testis extends across the posterior third and the middle portion, whereas the posterior one is entirely within the posterior third. This discrepancy could be due to the egg-filled uterus pushing the genital complex posteriorly, as seen in the line drawings of Dubois (1930), Ukoli (1966a), and Tendeiro et al. (1974), while in our specimens the eggs were partially released by the parasite after collection and before preservation. This arrangement was also observed by Caffara et al. (2014) in metacercariae which, being immature, do not contain eggs. The cirrus is well visible only in some of our specimens and is characterized by a strong muscular layer as described only by Dubois (1930). Moreover, in 1 subject, the cirrus was everted showing at light microscopy (LM) and SEM observation the presence of some tubercle-like structure at its base together with slightly visible longitudinal ridges on its surface; these characters have never been described before.

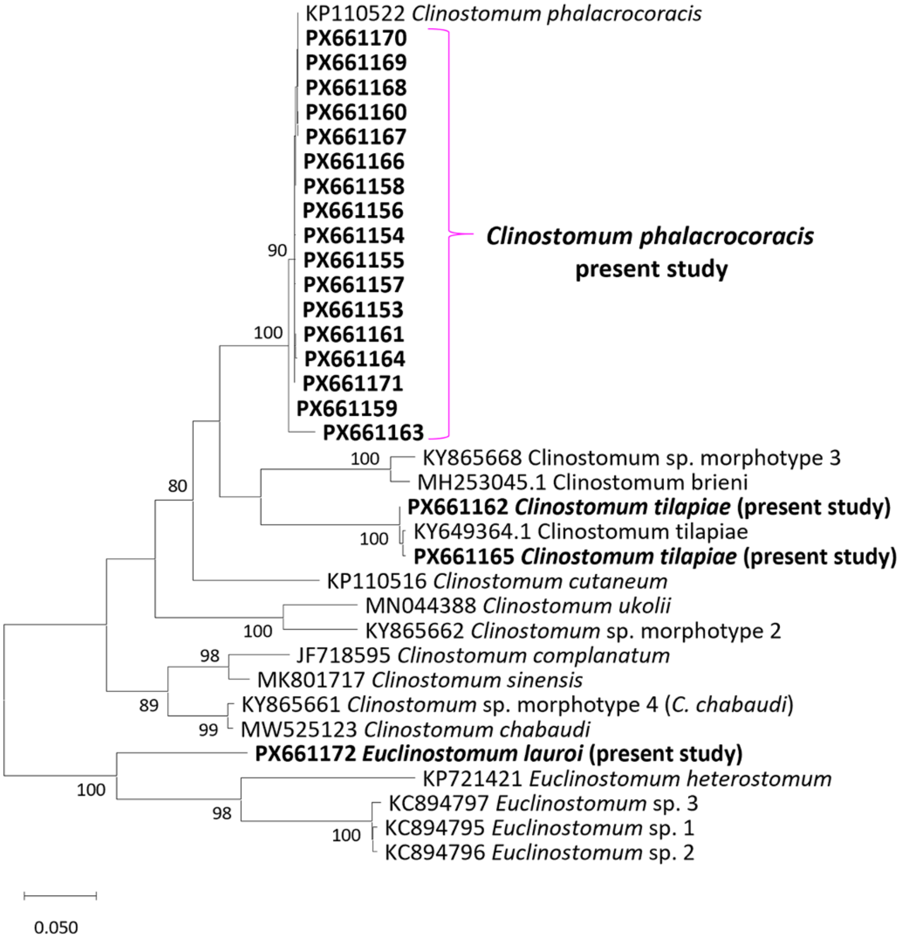

Figure 7. Phylogeny of *Euclinostomum* spp. and *Clinostomum* spp. based on cox1 mtDNA inferred using the maximum likelihood method and Hasegawa–Kishino–Yano (+G+I) model of nucleotide substitutions. The percentage of replicate trees in which the associated taxa clustered together (1000 replicates) is shown next to the branches. The analytical procedure encompassed 35 coding nucleotide sequences. Evolutionary analyses were conducted in MEGA12 utilizing up to 5 parallel computing threads.

Finally, the genital opening displayed small blunt tubercles along its internal edge, which were clearly visible in some specimens at LM.

Zhokhov and Morozova (2020) reported, among other clinostomids, *C. phalacrocoracis*, providing a detailed morphometric description. In our opinion, the specimens they described do not belong to *C. phalacrocoracis* but rather to *C. tilapiae*. Indeed, both the line drawing and the accompanying figure show anatomical structures that perfectly match the description provided by Caffara et al. (2017). Notably, these include the position of the genital complex in the middle third of the body, the ovary not being median, and the characteristic uteroduct forming a knee-like bend before opening into the uterine sac near the anterior testis. The overall measures are slightly larger than those reported by Caffara et al. (2017) but smaller than those given for *C. phalacrocoracis* by Caffara et al. (2014).

Clinostomum tilapiae has been reported in 5 species of waterbirds along their migratory routes, with the great majority of reports among the family Ardeidae. Immature adults of *C. tilapiae* were first described by Ukoli (1966a) in the oesophagus of night heron (*Nycticorax nycticorax*) and African darter (*Anhinga rufa*) from Ghana. The author described morphometric changes in maturation stages during an experimental infection of cattle egret (*Bubulcus ibis*) (1966a). Manter and Pritchard (1969) reported adults of *C. tilapiae* in Goliath heron (*Ardea goliath*) from Democratic Republic of Congo without providing any descriptions. Later, Britz et al. (1984) described 1 adult obtained after experimental infection of a grey heron chick (*A. cinerea*), providing detailed descriptions of the internal architectures. Recently, adults of *C. tilapiae* have been described from the oral cavity of a purple heron (*A. purpurea*) from Turkey (Öztürk and Umur, 2025), reporting morphological features that overlap with our description, except that their specimens are smaller. Finkelman (1988) described adults of *C. tilapiae* collected in the oral cavity and oesophagus of the great white pelican (*Pelecanus onocrotalus*) in Israel, but providing few morphological details.

The morphometries of *C. tilapiae* examined in this study are overall larger than the immature and adult parasites previously described (Ukoli, 1966a). Since the age of parasites obtained from naturally infected birds is unknown, morphological differences and size variations might depend on the age of the parasite and the host species (Ukoli, 1966a; Öztürk and Umur, 2025). In the present study we provided further details of the cirrus pouch, not described by the abovementioned authors together with specific details of the structure of the cirrus as the presence of small tubercle around the genital opening (Figure 5. LM, SEM). Contrary to Ukoli (1966a), in our specimens, the tegument spines were visible only in the ventral side of the body between the 2 suckers, probably due to the poor preservation of the specimen.

This research provides updated morphological re-descriptions and new molecular data for *E. lauroi* 40 years after the original report. The recognition of *E. lauroi* based on congruent morphological and molecular evidence provides the first robust framework for its systematic placement and contributes to resolving long-standing taxonomic uncertainty within *Euclinostomum*. Notably, since its initial description by Tendeiro et al. (1974), this species has not been recorded again, despite later studies (Prudhoe and Hussey, 1977; Finkelman, 1988; Zhokhov and Morozova, 2020) in which *Euclinostomum* were recorded but identified only at the genus level, although their morphological features were consistent with *E. lauroi*. For the previously described species *C. phalacrocoracis* and *C. tilapiae*, the present study refines and supplements the available morphological descriptions by incorporating additional characters, including SEM observations. Moreover, *C. tilapiae* is herein recorded for the first time from Lake Tana (Ethiopia).

Finally, as suggested also by Truter et al. (2025), we hope to encourage researchers, especially those working in this geographical area, to adopt an integrated approach (morphology combined with molecular analyses) to clarify the correct taxonomic placement of this complex family of trematodes.

Data availability statement. All the data that support the findings of this study are available from the corresponding author. Molecular sequences are available in GenBank under accession numbers PX626916-PX626929 (ITS rDNA) and PX661153-PX661172 (cox1 mtDNA).

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Author contributions. FM: methodology, data curation; MAM: sampling, data curation; PT: SEM analyses, writing; EM: review and editing; CT and AL: technical support, molecular analyses; AG: review and editing; MC: conceptualization, data curation, methodology, writing original draft. All authors reviewed the manuscript and approved the final version.

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Competing interests. The authors declare none.

Ethical standards. The permission to collect the great white pelican was obtained from the Amhara national Reginal State Environment and Forest Protection Authority (ref. No. Aka/T/B-10/01-18/03/2017). Moreover, this study was conducted with the approval from Addis Ababa University – Aklilu Lemma Pathobiology Institutional Research Ethics Review committee (reference number: ALIPB IRERC/141/2016/24).

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