BioInvasions Records (2024) Volume 13, Issue 1: 281–291

Rapid Communication

First taxonomic description of a gyrodactylid, *Gyrodactylus cichlidarum* Paperna, 1968 (Monogenoidea) infecting Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae) in the United States

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Citation: Brule JH, Warren MB, Dutton HR, Truong TN, Ksepka SP, Curran SS, Shurba JA, Lawson LL, Bullard SA (2024) First taxonomic description of a gyrodactylid, *Gyrodactylus cichlidarum* Paperna, 1968 (Monogenoidea) infecting Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Cichlidae) in the United States. *BioInvasions Records* 13(1): 281–291, https://doi.org/10.3391/bir.2024.13.1.25

Received: 9 October 2023
Accepted: 2 December 2023
Published: 19 February 2024

Handling editor: Thomas Therriault
Thematic editor: Andrew Davinack

Abstract

The parasites of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Cichliformes: Cichlidae) are poorly documented in the U.S. Gyrodactylids can be pathogens of cultured and wild fish populations and their infections can be relevant to the captive maintenance of fishes as well as wild fish health monitoring programs. As part of a survey of the parasites of Nile tilapia cultured within high density flow-through raceways (hydrologically linked to Sougahatchee Creek, Tallapoosa River), we observed numerous specimens of *Gyrodactylus cichlidarum* Paperna, 1968 (38% prevalence; 2.5 mean intensity) attached to the skin, fins, and gill filaments. This parasite was originally described from the skin and gill of mango tilapia, *Sarotherodon galilaeus* (Linnaeus, 1758) (Cichlidae) from pools and streams in the Accra Plains, Ghana. Live specimens of *G. cichlidarum* intended for morphology were heat-killed, formalin-fixed, routinely stained, cleared, and whole-mounted on glass slides. Additional specimens were preserved in 95% ethanol for DNA extraction and sequencing of the internal transcribed spacers 1 and 2 (ITS1, ITS2) and the 5.8S ribosomal DNA. We identified our specimens as *G. cichlidarum* by having a ventral bar with a distinctive dorsal posteromedial knob and marginal hooks that have a wide aperture and flat sickle base. Our two identical sequences of the ITS1-5.8S-ITS2 differed from a sequence of *G. cichlidarum* from the United Kingdom by 1 bp. Although this parasite was mentioned in a vaccine study of Nile tilapia in the U.S. in 2011, the present study is the first to diagnose the morphology of *G. cichlidarum* infecting Nile tilapia in the U.S. (and thereby also the first to deposit whole-mounted voucher specimens of *G. cichlidarum* from this host and locality). The present study also provides the first nucleotide sequences tethered to a morphological voucher of *G. cichlidarum* in the U.S., and these resulting sequences were included in a phylogenetic analysis.

Key words: Africa, Alabama, aquaculture, exotic, parasite, Polyonchoinea, taxonomy

Introduction

Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Cichliformes: Cichlidae) is among the most commercially important intensively cultured fishes worldwide following grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) and

silver carp, *Hypophthalmichthys molitrix* (Valenciennes, 1844) (both Cypriniformes: Xenocyprididae) (FAO 2022). Nile tilapia has a natural distribution in Africa and was first introduced to the U.S. from Israel by Auburn University in 1957 for pond aquaculture (Swingle 1960). The hardiness and adaptability of Nile tilapia make it both a robust aquaculture species and likewise a probable invasive species (Grammer et al. 2012). Invasive Nile tilapia concern natural resource managers because of their potential to displace and increase predation of native fishes (Martin et al. 2010; Sanches et al. 2012). Cold intolerance of Nile tilapia has evidently prevented its spread within the U.S. where water temperatures fall below 10.8 °C (Henson et al. 2018), but Nile tilapia is established in southern Mississippi and the Florida peninsula and could overwinter in natural waterways elsewhere in the southeastern U.S. (Zambrano et al. 2006; Grammer et al. 2012; Hill 2017). The parasites and diseases of invasive Nile tilapia concern fisheries managers, fish culturists, and fish disease diagnosticians because they comprise potential introduced pathogens that can harm cultured tilapias and could theoretically harm sympatric wild fishes (Shinn et al. 2023).

Little is known about the parasites of Nile tilapia in the U.S. The only report of a metazoan parasite infecting Nile tilapia there comes from a vaccine study that mentioned "*Gyrodactylus*" von Nordmann, 1832 (Gyrodactylidae Cobbold, 1864) and "*Gyrodactylus cichlidarum*" Paperna, 1968 (see Martins et al. 2011). Those authors did not diagnose the morphology of the specimens, deposit a voucher specimen or generate a nucleotide sequence (making the study not repeatable because there is no way to retroactively confirm the identity of the monogenoid specimens in that study). We herein rectify that ambiguity by providing the first taxonomic description, morphological diagnosis, voucher specimens, and nucleotide sequences that support our identification of *G. cichlidarum* from Nile tilapia in the U.S.

Materials and methods

During May 2022, 5,600 hatchery-reared Nile tilapia fingerlings were stocked (200 fish/raceway) into 28 raceways (0.71 m × 0.61 m × 3.00 m; ~ 1 m^3 working volume) at the E.W. Shell Fisheries Center (EWSFC), Auburn, Alabama. Fish were fed to satiation twice daily. Each raceway was supplied with flow-through water (single pass) at 40 L/min (2 turnovers/hr). Aeration was supplied by low pressure air blowers with 2 diffuser air stones per raceway. Ten (10) Nile tilapia per month per raceway (280 Nile tilapia per month total for all raceways) were haphazardly selected for parasitological examination, removed from the raceways, transported to the laboratory alive in aerated containers, euthanized using tricaine methanesulphonate (MS-222), and necropsied. In total, we examined 1,400 Nile tilapia from June to November of 2022 (comprising 5 sample events).

The skin was examined for monogenoid infection by pouring 60 °C water over the body surface of the euthanized fish. The resulting fluid (and potential monogenoid specimens) was transferred to a settling column, allowed to sit for several minutes, and partially decanted before examining the sediment in a petri dish using stereo dissecting microscopes (Meiji RZ 3288) fitted with fiber optic light sources, light and dark field sub-stage illumination, and a digital camera (Jenoptik HDD076-CMT, Jenoptik AG, Jena, Germany). Specimens were wet-mounted upon collection and examined with a compound microscope (Olympus BX-51, Olympus, Tokyo, Japan) equipped with differential interference (DIC) optical components. The heat-killed monogenoids extracted from the sediment were fixed in 10% neutral buffered formalin (n. b. f.) for morphology or preserved in 95% EtOH for DNA extraction. The gill was examined by excising the anterior-most sinistral gill arch, placing it in water, and examining it for monogenoids with the aforementioned stereo-dissecting microscope. Live monogenoids were removed from the gill filaments using fine forceps and a minutien pin mounted on a wooden dowel before being heat-killed on glass slides under a coverslip without pressure. Specimens intended for morphology were fixed in 10% n. b. f. or preserved in 95% ethanol (EtOH) for DNA extraction.

Monogenoids intended for morphology were stained overnight in Van Cleave's hematoxylin, dehydrated using a graded EtOH series, made basic in 70% EtOH with lithium carbonate and n-butylamine, dehydrated in 100% EtOH, cleared in clove oil, and permanently whole-mounted on glass slides using Canada balsam. Additional specimens for morphology were dehydrated, cleared in clove oil, and mounted unstained to show sclerotized features. Whole-mounted specimens were drawn (Figure 1) with the aid of an Olympus BX-51 compound microscope equipped with DIC and a drawing tube. Measurements were obtained by using a Jenoptik Gryphax camera (Jenoptik AG, Jena, Germany). Lengths and angles are reported as a range in micrometers (µm) or degrees (°) followed by the mean, standard deviation, and sample size. Voucher specimens of *G. cichlidarum* were deposited in the National Museum of Natural History's Invertebrate Zoology Collection (Smithsonian Institution, USNM Collection Nos. 1606908–1606919). The haptoral sclerite measuring scheme and terminology we use herein follows that of Shinn et al. (2004). Scientific names, including taxonomic authorities and dates, for fishes follow Eschmeyer et al. (2016; online version updated 2023). Common names for fishes follow Froese and Pauly (2023). The fish were identified as Nile tilapia by having 23 gill rakers and 18 dorsal fin spines as per Boschung and Mayden's (2004) key to the cichlids.

Two EtOH-preserved specimens of *G. cichlidarum* (one infecting the gill filaments and one infecting the skin) intended for DNA extraction were again temporarily wet-mounted on a glass slide with coverslip to morphologically confirm their identity. Each of these specimens was then digested to extract

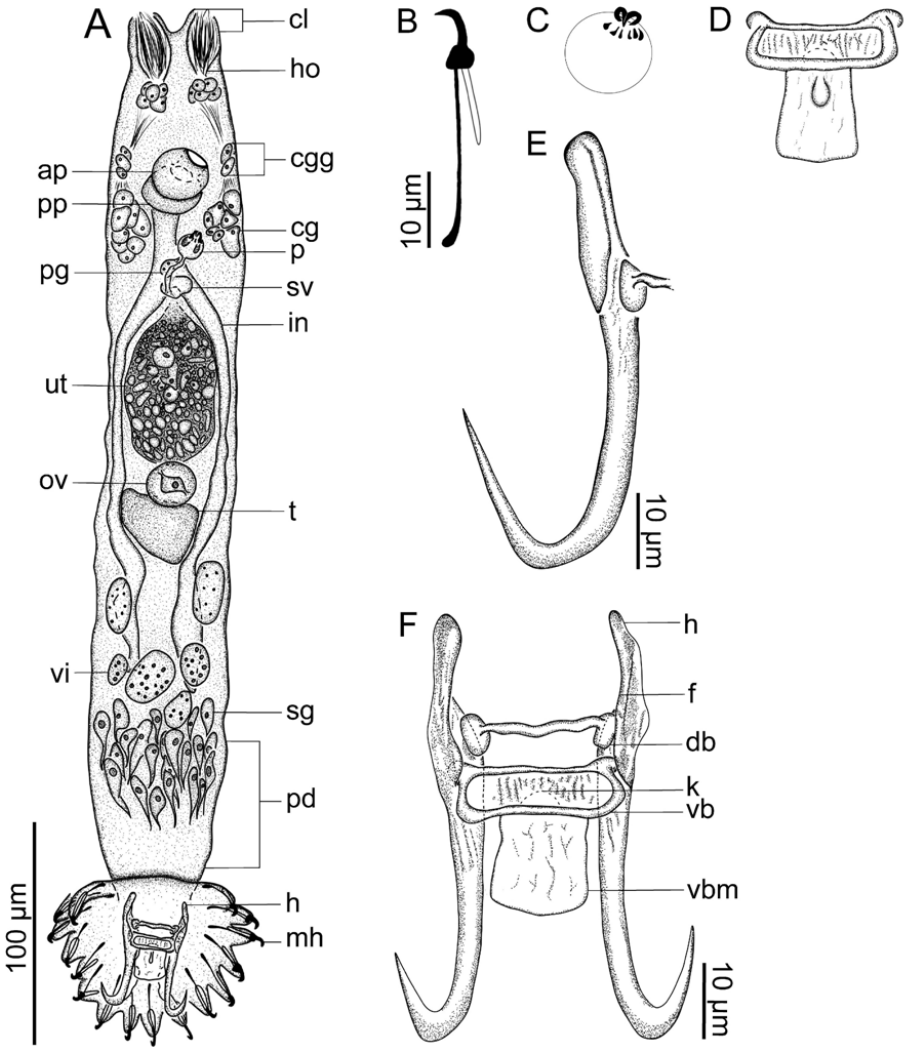

Figure 1. *Gyrodactylus cichlidarum* Paperna, 1968 (Monogenoidea: Gyrodactylidae) from the fins, skin, and gill filaments of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Cichliformes: Cichlidae) from flow-through raceways in the E.W. Shell Fisheries Center, Auburn, Alabama, U.S.A. Scale values aside bars. Ventral views unless noted otherwise. (A) Illustration of the body of voucher (USNM 1606915). (B) Marginal hook of voucher (USNM 1606917) lateral view. (C) Penis of voucher (USNM 1606916). (D) Ventral transverse bar of voucher (USNM 1606917). (E) Hamulus of voucher (USNM 1606908) lateral view. (F) Central hook complex of voucher (USNM 1606915). Abbreviations: anterior pharyngeal bulb (ap), cephalic gland (cg), cephalic gland group (cgg), cephalic lobe (cl), dorsal transverse bar (db), fold (f), hamulus (h), head organ (ho), intestinal cecum (in), knob (k), marginal hook (mh), ovary (ov), peduncle (pd), penis (p), posterior pharyngeal bulb (pp), prostatic gland (pg), secretion granule (sg), seminal vesicle (sv), testis (t), uterus (ut), ventral transverse bar (vb), ventral transverse bar membrane (vbm), vitellarium (vl).

complete genomic DNA using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, California) following the manufacturer's protocol. A DNA fragment spanning the 3' end of the small subunit ribosomal DNA (18S) gene to the 5' end of the large subunit ribosomal DNA (28S) gene (containing the internal transcribed spacer 1 [ITS1], 5.8S ribosomal DNA gene, and internal transcribed spacer 2 [ITS2] [ITS1-5.8S-ITS2]) was amplified using the primers ITS1A (5'-GTAACAAGGTTTCCGTAGGTG-3') and ITS2 (5'-TCCTCCGCTTAGTGATA-3') (García-Vásquez et al. 2007). PCR thermocycling protocol follows García-Vásquez et al. (2007). DNA

amplification was verified with a 5% agarose gel stained with 1% ethidium bromide. PCR product was purified using the QIAquick PCR Product Purification Kit (Qiagen, Valencia, California). Sequencing was performed by Genewiz, Incorporated (South Plainfield, New Jersey) with the additional internal primers ITS1R (5'-ATTTGCGTTCGAGAGACCG-3') and ITS2F (5'-TGGTGGATCACTCGGCTCA-3') used in García-Vásquez et al. (2007). Chromatograms were assembled based on sequence overlap, proofread by eye, and had low-quality read ends trimmed in Geneious version 2023.2.1 (http://www.geneious.com), resulting in fragments of 783 base pairs (bp) and 723 bp. Our sequences and in-group taxa sequences from Zahradníčková et al. (2016) were aligned with the multiple alignment using fast Fourier transform (MAFFT) tool (Katoh and Standley 2013) and trimmed to the length of our shortest sequence (723 bp [ITS1-5.8S-ITS2]). JModelTest 2 version 2.1.10 (Darriba et al. 2012) was implemented to perform statistical selection of the best-fit models of nucleotide substitution based on Bayesian Information Criterion (BIC). Aligned sequences were reformatted (from .fasta to .nexus) using the web application ALTER (Glez-Peña et al. 2010) to run Bayesian inference (BI). BI was performed in MrBayes version 3.2.7a (Ronquist and Huelsenbeck 2003) using substitution model averaging ("nst-mixed") and a gamma distribution to model rate-heterogeneity. Defaults were used in all other parameters. Three independent runs with 4 Metropolis-coupled chains were run for 5,000,000 generations, sampling the posterior distribution every 1,000 generations. Convergence was checked using Tracer v1.6.1 (Rambaut et al. 2014) and the "sump" command in MrBayes: all runs appeared to reach convergence after discarding the first 25% of generations as burn-in. A majority rule consensus tree of the post burn-in posterior distribution was generated with the "sumt" command in MrBayes. The inferred phylogeny was visualized using FigTree v1.4.4 (Rambaut et al. 2014) and further edited for visualization purposes with Adobe Illustrator (Adobe Systems) (Figure 2).

Description

Gyrodactylus cichlidarum Paperna, 1968

(Figure 1)

Based on 20 whole mounted specimens from the skin, fins, and gill filaments of Nile tilapia: Body (including haptor) elongate, fusiform, 353–526 (463 ± 58.6; 10) long, 53–79 (64 ± 10.1; 9) wide at maximum width usually in mid-forebody (Figure 1A). Cephalic lobes two in number, each containing one head organ and one spike sensillum (Figure 1A). Cephalic glands consisting of three bilateral groups, posterior to cephalic lobes, located near lateral body margin (Figure 1A). Pharynx consisting of two tandem bulbs (Figure 1A); anterior pharyngeal bulb spherical, 23–28 (25 ± 1.9; 10) long, 24–28 (28 ± 1.3; 10) wide (Figure 1A); posterior pharyngeal bulb 20–28 (22 ± 3; 10) long, 23–28 (26 ± 1.8; 10) wide (Figure 1A). Intestinal ceca lacking cyclocoel,

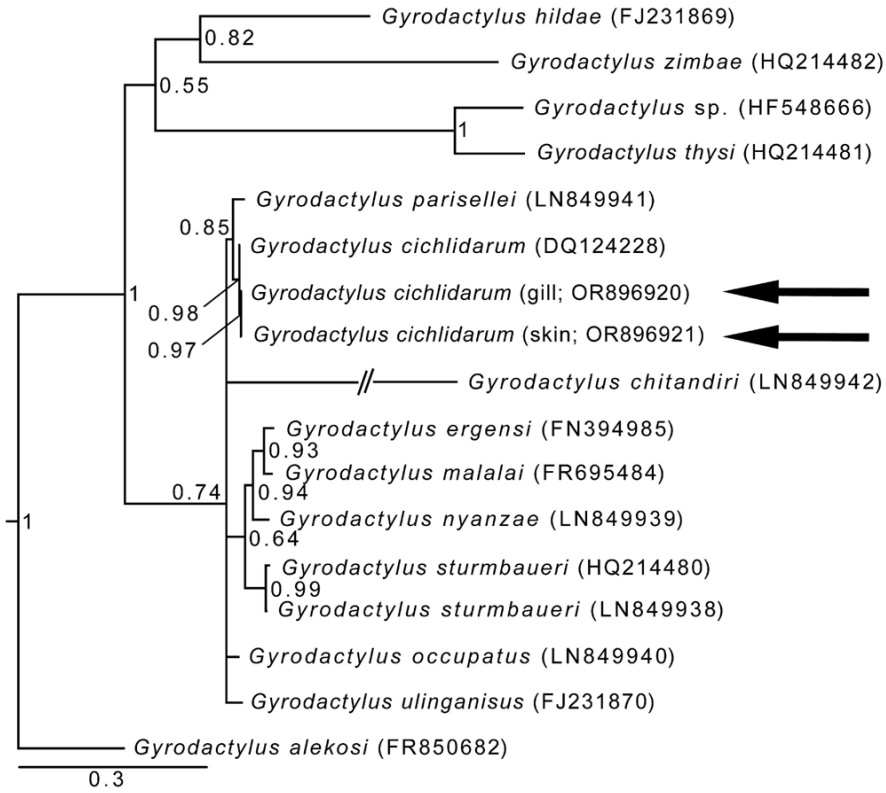

Figure 2. Internal transcribed spacer 1 (ITS1), 5.8S, and internal transcribed spacer 2 (ITS2) Bayesian phylogeny. Values aside nodes are posterior probability. Scale bar is in substitutions per site. GenBank numbers are in parentheses following each taxon. Arrows indicate sequences of *Gyrodactylus cichlidarum* from the present study (Auburn, Alabama).

terminating anterior to peduncle (Figure 1A). Secretory granules 13–25 in number, post-cecal, each containing one nucleus, tapering posteriad (Figure 1A). Haptor 1.0%–1.3% (1.2 ± 0.2; 5) broader than long, 65–83 (68 ± 8.2; 7) long, 69–96 (84 ± 10.1; 6) wide (Figure 1A). Haptoral sclerites comprising 16 marginal hooks, one pair of hamuli, one dorsal transverse bar, and one ventral transverse bar (Figures 1A, B, D–F). Marginal hooks with flat sickle base and knob-like shaft base (Figures 1A, B). Marginal hook 29–30 (29 ± 1; 11) long; shaft 20–23 (21 ± 0.9; 11) long; sickle 7–8 (7 ± 0.5; 11) long; sickle proximal width 3–5 (4 ± 0.8; 11); sickle distal width 4–6 (5 ± 0.7; 11); toe 1–2 (2 ± 0.2; 11) long; aperture 6–7 (7 ± 0.3; 11) long; filament loop 11–16 (14 ± 1.9; 7) long (Figures 1A, B). Hamuli roots containing inward folds (Figures 1A, E, F). Hamulus 55–58 (56 ± 1.2; 9) long; root 17–25 (20.3 ± 2.3; 9) long; shaft 30–37 (35 ± 2; 9) long; proximal shaft 19–23 (21 ± 1.2; 9) wide; distal shaft 4–5 (4 ± 0.4; 9) wide; point 23–26 (25 ± 1.1; 9) long; aperture distance 19–23 (21 ± 1.2; 9); inner curve 2–5 (3 ± 1; 9) long; aperture angle 38.6–43.6 (41.4 ± 1.5; 9); point curve angle 4.3–8.6 (6.4 ± 1.4; 9); inner aperture angle 43.5–50.7 (46.6 ± 2.1; 9) (Figures 1A, E, F). Dorsal transverse bar narrow, much wider than long, attaching to roots of hamuli with widened, approximately spherical ends (Figures 1A, F). Ventral transverse bar with uneven surface surrounded by a smooth and elevated rim, containing a dorsal posteromedial knob (Figures 1A, D, F). Ventral bar membrane containing folds and wrinkles, approximately square in outline,

posterior margin rounded (Figures 1A, D, F). Ventral transverse bar 19–23 (2.5 ± 1.3; 11) long; 21–25 (23 ± 1.4; 11) wide; process to mid length 1–2 (2 ± 0.2; 11); median length 6–9 (8 ± 1; 11); process 2 (2 ± 0; 11) long; membrane 11–15 (13 ± 1.5; 11) long (Figures 1A, D, F).

Testis lacking lobes, 20–31 (25 ± 4; 6) long or 4–7% of body length, 24–34 (29 ± 4.5; 6) wide or 39–51% of maximum body width, intercaecal, bordering intestinal ceca, approximately circular in outline, subovate, slightly dorsal to ovary at intersection, 183–324 (251 ± 55.3; 6) or 50–62% of body length from anterior body end, 133–201 (177 ± 23.7; 6) or 33–52% of body length from posterior body end (Figure 1A). Vas deferens not observed. Seminal vesicle 10–19 (15 ± 6.4; 2) long, 10–14 (12 ± 2.8; 2) wide, at level of intestinal cecal bifurcation, containing a duct extending anteriad to union with penis (Figure 1A). Prostatic glands one (50% of specimens) or two in number (50% of specimens), each possessing one (40% of specimens) or two nuclei (60% of specimens), immediately anterior to seminal vesicle, each containing a short duct extending to union with penis (Figure 1A). Penis spheroid, located in mid-forebody, slightly dextral or sinistral, 11–16 (13 ± 1.8; 9) long, 10–13 (11 ± 1; 9) wide, having a ring of seven spinelets; apical spinelet largest, 85–106 (97 ± 8.8; 6) or 19–25% of body length from anterior body end, 284–410 (342 ± 54.9; 6) or 74–81% of body length from posterior body end (Figures 1A, C).

Ovary 12–27 (20 ± 5.5; 6) long, 18–28 (24 ± 3.8; 6) wide, 171–308 (232 ± 56.2; 6) or 43–59% of body length from anterior body end, approximately spheroid, containing one large oocyte (Figure 1A). Oocyte 6–8 (7 ± 1.4; 2) long, 7–11 (9 ± 2.8; 2) wide (Figure 1A). Oviduct not observed. Uterus 54–176 (110 ± 53; 6) long or 15–34% of body length, 38–65 (52 ± 9.5; 6) wide or 61–89% of maximum body width, containing zero to two generations of embryos, 24–34 (29 ± 4.5; 6) or 27–36% of body length from anterior body end, 182–251 (216 ± 23.2; 6) or 40–60% of body length from posterior body end (Figure 1A). Vitellaria spheroid to ovoid, possessing an irregular outline, six to nine in number, aggregated in space between posterior intestinal ceca terminus and anterior extension of secretion granules (Figure 1A).

Taxonomic summary

Host: *Oreochromis niloticus* (Linnaeus, 1758) (Cichliformes: Cichlidae), Nile tilapia.

Locality: E. W. Shell Fisheries Center, Auburn, Alabama, U.S.A. (Sougahatchee Creek, Tallapoosa River, Mobile-Tensaw Basin).

Specimens and sequences deposited: Voucher specimens (USNM 1606908–1606919); 18S ribosomal DNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal DNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal DNA gene, partial sequences (OR896920; OR896921 GenBank Nos.).

Prevalence and intensity of infection: 524 of 1,400 (37%) Nile tilapia were infected by *G. cichlidarum* on the external body surface with a mean

intensity of 2.47; 25 of 1,400 (2%) Nile tilapia were infected by *G. cichlidarum* on the gill filaments with a mean intensity of 1.2; including both sites of infection, 537 of 1,400 (38%) of Nile tilapia were infected by *G. cichlidarum* with a mean intensity of 2.47.

Site on host: Skin, fins, and gill filaments.

Taxonomic remarks

The redescription of *G. cichlidarum* by García-Vázquez et al. (2007) corrected inaccuracies in Paperna's (1968) original description; in specific demonstrating that the posteromedial knob of the ventral bar is dorsal and that each marginal hook contains a flat sickle base and wide aperture (not a curved sickle base and narrow aperture, as described by Paperna [1968]). These comprise the species-level diagnostic features of *G. cichliadrum* (see García-Vázquez et al. 2007). Our specimens matched the redescription of *G. cichlidarum* by having these features and by the measurements of all sclerites overlapping with those provided therein.

To date, ten species assigned to *Gyrodactylus* have been reported from Nile tilapia (*G. cichlidarum* [syn. *Gyrodactylus niloticus* Cone, Arthur & Bondad-Reantaso, 1995]; *Gyrodactylus ergensi* Přikrylová, Matějusová, Musilová & Gelnar, 2009; *Gyrodactylus hildae* García-Vásquez, Hansen, Christison, Bron & Shinn, 2011; *Gyrodactylus malalai* Přikrylová, Radim & Gelnar, 2012; *Gyrodactylus nyanzae* Paperna, 1973; *Gyrodactylus occupatus* Zahradníčková, Barson, Luus-Powell & Přikrylová, 2016; *Gyrodactylus parisellei* Zahradníčková, Barson, Luus-Powell & Přikrylová, 2016; *Gyrodactylus shariffi* Cone, Arthur & Bondad-Reantaso, 1995; *Gyrodactylus shinni* García-Vásquez, Pinacho-Pinacho, Guzmán-Valdivieso, Calixto-Rojas & Rubio-Godoy, 2021; and *Gyrodactylus yacatli* García-Vásquez, Hansen, Christison, Bron & Shinn, 2011). *Gyrodactylus cichlidarum* was the only gyrodactylid recovered herein.

Phylogenetic results

Our sequences (783 bp and 723 bp fragments of the 18S ribosomal DNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal DNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal DNA gene, partial sequence) comprised 723 nucleotides after alignment, were identical to each other, and were recovered sister to a sequence (differing by 1 bp) ascribed to *G. cichlidarum* (DQ124228 GenBank No.; 861 bp; ex. *Oreochromis niloticus* [see García-Vázquez et al. 2007]) from the United Kingdom. All sequences ascribed to *G. cichlidarum* were recovered sister to a sequence ascribed to *G. parisellei* (LN849941 GenBank No.; 781 bp; differing from our sequences by 53 [3.3%] nucleotides) from the fins of southern mouthbrooder, *Pseudocrenilabrus philander* (Weber, 1897) (Cichlidae) from Lake Kariba, Zimbabwe and from the fins of Nile tilapia and *Tilapia* sp. (Cichlidae) from Lake Chivero, Zimbabwe (Zahradníčková et al. 2016) (Figure 2).

A low level of intraspecific variation in ITS1-5.8S-ITS2 sequences exists between populations of *G. cichlidarum* and are possibly a function of geographic distance or host identity (or both) (García-Vázquez et al. 2010). For example, morphologically identified ITS1-5.8S-ITS2 sequences of *G. cichlidarum* from Mexico (KX512807 GenBank No.; 792 bp; ex. *Poeciliopsis gracilis* [Heckel, 1848] [Cyprinodontiformes: Poeciliidae], porthole livebearer [García-Vázquez et al. 2017]) and the United Kingdom (DQ124228 GenBank No.; 861 bp; ex. *Oreochromis niloticus* [see García-Vázquez et al. 2007]) have two nucleotide differences. The single nucleotide difference between the ITS1-5.8S-ITS2 sequences generated herein and a sequence from the United Kingdom (DQ124228 GenBank No.; 861 bp; ex. *Oreochromis niloticus* [see García-Vázquez et al. 2007]) could be attributed to their geographic distance (Dmitrieva et al. 2022).

Discussion

The present report is the first taxonomic description of *G. cichlidarum* in the U.S. We herein supply the first taxonomic description of the complete soft anatomy of this species, including the first description of the peduncle secretory granules (Figure 1A). Little is known about the gyrodactylids of Nile tilapia in the U.S. and what other hosts are infected by *G. cichlidarum* there. *Gyrodactylus cichlidarum* was introduced into Mexico by the aquaculture industry, where it infects three species of livebearers (Cyprinodontiformes: Poeciliidae). García-Vásquez et al. (2017) found *G. cichlidarum* infecting the skin and fins of two introduced poeciliids: shortfin molly, *Poecilia mexicana* Steindachner, 1863; and two-spot livebearer, *Pseudoxiphophorus bimaculatus* (Heckel, 1848) (both Lerma River, Michoacán, Mexico), and the skin and fins of one native poecilid: porthole livebearer (Tecolutla River, Puebla, Mexico). The host specificity of *G. cichlidarum* to non-cichlids is unknown apart from these three records (García-Vázquez et al. 2017).

To our knowledge, no taxonomic survey of the parasites of wild Nile tilapia in the U.S. has been conducted and published. Of interest to us is exploring the parasites of established wild Nile tilapia populations and how their parasites are distributed in the sympatric fish community. Nile tilapia have been periodically anecdotally observed in Sougahatchee Creek (Tallapoosa River, Mobile-Tensaw Basin) since 1986. During July 2023, we electroshocked a reach of that creek downstream of the facility (EWSFC) that cultured the infected Nile tilapia, but no tilapia was caught.

Authors' contribution

JHB: sample design and methodology, investigation and data collection, writing – original draft, and writing – review and editing. MBW: investigation and data collection. HRD: investigation and data collection. TNT: investigation and data collection. SPK: investigation and data collection. SSC: investigation and data collection. JAS: investigation and data collection. LLL: investigation and data collection. SAB: research conceptualization, sample design and methodology, investigation and data collection, writing – original draft, and writing – review and editing.

Acknowledgements

We thank Anna Phillips, Chad Walter, Kathryn Ahlfeld, and William Moser (all Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC) for curating our museum specimens. This study was supported by The Southeastern Cooperative Fish Parasite and Disease Project, the U.S. Fish and Wildlife Service (Department of Interior), National Sea Grant (National Oceanic and Atmospheric Administration), United States Department of Agriculture (National Institute of Food and Agriculture), Federal Aid in Sport Fish Restoration (Alabama Department of Conservation and Natural Resources, Inland and Marine Resources Divisions), and the Alabama Agricultural Experiment Station (Auburn University, College of Agriculture).

Ethics and permits

Ethical guidelines followed Auburn University's IACUC protocol 2022–4094. All research pertaining to this article did not require any research permit.

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