PARASITES OF FRESHWATER FISH FROM THE EASTERN EVERGLADES

BY

KATERINA DEMEITROVNA SAWICKIJ

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THESIS

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Approved by: ___________________________________
FLORIAN B. REYDA
17 MAY 2024
DIRECTOR
State University of New York,
College at Oneonta, Oneonta, New York

____
DANIEL S. STICH
17 MAY 2024
ASSOCIATE PROFESSOR
State University of New York,
College at Oneonta, Oneonta, New York

____
V. ALEX SOTOLA
17 MAY 2024
ASSISTANT PROFESSOR
State University of New York,
College at Oneonta, Oneonta, New York

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Parasites of the Eastern Everglades, Florida Fishes: Bangham 1940 Revisited

ABSTRACT

A survey of fish parasites was conducted in the Everglades, Florida and examined 31 species of fish from 15 locations across Lake Okeechobee, Stormwater Treatment Area 3/4 (STA-3/4) and Water Conservation Areas (WCA) 3A and 3B. We documented 30 species of parasites, 19 of which were found as adults from the intestines of fishes. We collected 15 of the fish species and 16 of the parasite species that were examined/documented in Bangham's 1938 survey. Thirteen established non-native fish species were examined and were found to host 14 species of parasites. There was 1 new species, 19 new host records for 5 species of parasites, and 4 new locality records documented. These new records demonstrate the need for parasite surveys in understudied areas of North America (like the southeastern region) to better understand what species are present and what species are utilized as hosts. The changes in the parasite communities in the area demonstrate the extensive environmental changes of the region.

INTRODUCTION

Many parasites tend to have a complex life cycle in which multiple hosts are utilized. Parasites with complex life cycles often rely on trophic (i.e., food web) interactions for transmission in order to complete their life cycle (Marcogliese, 2005). Many parasites are also host specific, meaning that the parasite is found in only one or a few species of hosts and will not be found in other host species. Ecosystem disturbances can often have impacts on biodiversity and in turn the food web. If vertebrate or invertebrate animals disappear from an ecosystem in which they are hosts, then the parasite that uses that host species cannot persist in the ecosystem. Parasites reliance on the food web to transmit between hosts makes them sensitive to environmental changes that may only affect one of its intermediate or definitive hosts (Marcogliese, 2005). This connection between parasites, their host specificity, and the biotic factors of an ecosystem in which they occur makes parasites good indicators of ecosystem health (Marcogliese, 2005).

This study aims to use parasites to evaluate environmental changes in a previously studied ecosystem where extensive environmental changes have occurred. Bangham (1939 & 1940) reported the species occurrence and abundance of parasites in southern Florida fishes. His survey included 1,380 fish of 45 species collected in 1938 from the Englewood region, Lake Okeechobee, the Myakka River, and unspecified Everglades canals (Bangham, 1939 & 1940). Since the time of the study, two species Bangham examined (*Fundulus majalis* and *Fundulus similis*) were synonymized into one species, *F. similis* (Robins et al., 2018). Of the fish species examined, 34 were exclusively freshwater species found in south Florida, 10 were saltwater or brackish, and 3 were found exclusively in the northwestern region of Florida (Robins et al., 2018). Bangham's survey was the first study to extensively document the parasites of the region, listing 72 different species (larval and adult) of monogenes, trematodes, cestodes, nematodes, acanthocephalans, copepods, leeches, and a pentastome (Bangham, 1940). In terms of adult parasites, Bangham (1939 & 1940) found 6 monogenes, 14 trematodes, 8 cestodes, 14 nematodes, and 6 acanthocephalans. One of the unknown adult acanthocephalans found was later described as *Neoechinorhynchus doryphorus* (Van Cleave & Bangham, 1949). Since the time of the study extensive changes in invertebrate and vertebrate animal populations have occurred in the region, but few studies have been conducted on freshwater fish parasites in the region (Scholz & Choudhury, 2014).

This study aims to use parasites to evaluate environmental changes in a previously studied ecosystem where extensive abiotic and biotic environmental changes have occurred. Lake Okeechobee is a freshwater lake in Florida, part of the Kissimmee-Okeechobee-Everglades

(KOE) ecosystem, and historically was the headwaters of the Everglades (Johnson et al., 2007; Lodge, 2017; Canfield et al., 2021). Anthropogenic impacts in the Everglades include habitat destruction and fragmentation, changes in water flow, eutrophication, and introduced species (Chimney & Goforth, 2001; Johnson et al., 2007; Canfield et al., 2021). Historically, Lake Okeechobee would overflow along its southern shore during the wet season, creating the characteristic sheet flow of freshwater through the Everglades southwest to Florida Bay (Lodge, 2017). Unsuccessful attempts to drain the Everglades for human development occurred as early as 1913, but the major changes in water flow occurred with the Central and South Florida Project (C&SF) in 1948 (Lodge, 2017). The C&SF project constructed a series of canals, dikes, and dams for water management and flood control (Lodge, 2017). This disrupted sheet flow resulting in less water reaching the southern Everglades and Florida Bay, contributing to habitat fragmentation (Lodge, 2017). The first documented changes in water quality were in 1938 (Chimney & Goforth, 2001). The C&SF Project established the Everglades Agricultural Area (EAA) south of Okeechobee leading to increased nutrient levels in the water entering the Everglades (Lodge, 2017), further decreasing water quality (Chimney & Goforth, 2001; Lodge, 2017).

The widespread changes associated with these drainage efforts, especially habitat fragmentation and destruction as well as eutrophication, have facilitated the establishment of non-native species (Lodge, 2017; Howell et al., 2021; Searcy et al., 2023). Introduced plants, mollusks, reptiles, and fish have established and negatively impacted the native species through competition and predation (Howell et al., 2021). As of 2018, it was documented that 48 non-native freshwater fishes have established reproducing populations across Florida, with 36 foreign invaders establishing in South Florida (Robins et al., 2018). Declines in wading bird populations, large-bodied fishes, and small mammal abundances have been documented in the Everglades and are associated with increased anthropogenic impacts (Howell et al., 2004).

The goal of this project was to document the current parasite community of freshwater fishes in the Everglades region and compare it to Bangham's 1938 survey. Parasites rely on the food web to complete their life cycles, and given the changes in invertebrate and vertebrate animal populations, we expect that some parasite life cycles have been interrupted as a result of those environmental changes. We expect a decrease in parasite diversity due to these changes in the food web that have potentially interrupted the complex life cycles of the parasites documented in 1938.

METHODS

Specimen collection

A total of 715 fish were collected from 15 locations across Lake Okeechobee, Stormwater Treatment Area 3/4 (STA 3/4), Water Conservation Areas (WCA) 3A and 3B, and the eastern Everglades between May 30, 2023, and August 30, 2023 (Table 1 and Fig. 1). Fish species not of conservation concern were collected using seine net, cast net, minnow traps, electrofishing, and traditional hook and line fishing. Fish carcasses and guts were donated to the study from local anglers under permit S-23-09. Of the 715 fish collected there were 31 species as shown in Table 2. Fish were identified using Robins et al. (2018). Fish collected were euthanized in the field in accordance with SUNY Oneonta IACUC protocol 2023-60 and kept on ice or refrigerated until time of necropsy. Fish were photographed with an identification number before necropsy. A ventral incision was made to extract the digestive tract, which was examined for parasites using a Zeiss dissecting microscope (6.5-50x) for all fish collected. External surfaces and gills were examined (under a dissecting microscope) opportunistically. Parasites encountered were preserved and saved in individually-labeled vials for morphological and molecular analysis. Acanthocephalans were relaxed in tap water for 24-48 hours and then preserved in 70% ethanol. Nematodes were relaxed using near-boiling tap water and then preserved in 70% ethanol. Cestodes and trematodes were relaxed using near-boiling tap water, fixed in 5-10% neutral-buffered formalin, and then transferred to 70% ethanol after 2-12 weeks. Arthropods were preserved in 70% ethanol. Specimens from all groups saved for DNA extraction were preserved in 95% molecular-grade ethanol and stored at 4 °C.

Morphological Identification and Scanning Electron Microscopy (SEM)

Parasites were stained and mounted as permanent whole mount microscope slides for observation with a light microscope using standard methods based on the group of parasites to which they belonged (Pritchard & Kruse, 1982). Acanthocephalans intended for morphology were cleared of debris using a fine brush, poked with an acupuncture needle, stained using filtered Semichon's acetocarmine stain, de-stained using acidic and basic 70% ethanol, dehydrated using a graded ethanol series, cleared in methyl-salicylate, and permanently mounted in Damar gum. Cestodes and trematodes intended for morphology were dehydrated using a graded ethanol series, stained using filtered and diluted Delafield's hematoxylin, de-stained using acidic and basic 70% ethanol, dehydrated using a graded ethanol series, cleared in methyl salicylate, and permanently mounted in Damar gum. Large nematodes were observed under a Zeiss dissecting microscope (6.5-60x) in 70% ethanol and identified using morphological features. Small nematodes were kept in vials of 5:1 95% glycerine to 70% ethanol. Nematodes were placed in 10 parts glycerine and 9 parts 70% ethanol before a wet mount was made and the worm was observed under a Leica DM 2500 microscope (Buffalo Grove, Illinois). Parasite species identification was made based on morphological characteristics using current literature and guides. Prevalence data was calculated and reported for each host species as the number of individuals infected divided by number of individuals uninfected for each parasite species. All comparisons made between Bangham's 1938 survey and the 2023 survey will only be done for the parasite species documented from the 15 fish species examined in both surveys. Slides were deposited in the United States National Museum, Smithsonian Institute, Washington, D.C. (USNM) accession numbers can be found in Table 4.

Genetic Analysis

Specimens saved for molecular analysis were kept at -70°C in 95% molecular-grade ethanol. All worms were photographed prior to sequencing. Before sequencing, acanthocephalans were processed according to Doolin & Reyda (2018) so that the anterior portion of the worm was whole-mounted to serve as a voucher for the portion of the worm that was sequenced. For trematodes, the whole worm was used for the molecular analysis while another worm of the same species from the same host individual was saved as a paragenophore (*sensu* Pleijl, 2008). Specimens were prepared for DNA isolation, PCR, and sequencing according to Doolin & Reyda (2018). The posterior portion of acanthocephalans or in the case of trematode specimens, the entire specimen, was digested overnight at 56 °C in solution with Buffer AE and proteinase K (Doolin & Reyda, 2018). Extractions were carried out individually using QiagenÒ DNeasy tissue kits (Qiagen, Inc., Valencia, California), according to the manufacturer's instructions. Two regions of nuclear DNA, the internal transcribed space (ITS) region (ITS1+5.8S+ITS2) and the near-complete large ribosomal subunit (28S rDNA), were amplified for each specimen. Four separate overlapping PCR fragments of 700-800 bp each were used for the 28S rDNA using the following primers; amplicon 1 forward 5'-CAAGTACCGTGAGGGAAAGTTGC-3' and reverse 5'-CAGCTATCCTGAGGGAAAC-3'; amplicon 2 forward 5'-ACCCGAAAGATGGTGAACTATG-3' and reverse 5'-CTTCTCCAACKTCAGTCTTCAA-3'; amplicon 3 forward 5'-CTAAGGAGTGTGTAACAACTCACC-3' and reverse 5'-AATGACGAGGCATTTGGCTACCTT-3'; and amplicon 4 forward 5'-GATCCGTAACTTCGGGAAAAGGAT-3' and reverse 5'-CTTCGCAATGATAGGAAGAGCC-3' (Garcia-Varela and Nadler, 2005; Doolin and Reyda, 2018). The forward primer BD1 5'-GTCGTAACAAGGTTTCCGTA-3' and reverse primer BD2 5'-TATGCTTAAATTCAGCGGGT-3' were used to amplify the ITS region. Sequence contigs were edited and assembled using 4Peaks and notepad++ software. Edited sequences were compared to DNA sequences available on GenBank using the standard nucleotide basic local alignment search tool (nBLAST) from the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/genbank).

RESULTS

A total of 715 fish of 31 species were collected from 15 locations across Lake Okeechobee and the eastern Everglades between May 30, 2023, and August 30, 2023 (Table 1 and 2, and Fig. 1). Of the fish species examined 13 species were established non-native species and 18 species were native (Robins et al., 2018). Fifteen fish species encountered in this survey were also in Bangham's original 1938 survey (Table 3). We documented 3 of Bangham's original 4 species of acanthocephalan, 3 of the original 7 species of cestode, 6 of the original 12 species of trematode, and 4 of the original 13 species of nematode (Table 3). There were adults of 19 species of parasite found: six were acanthocephalans, three species were cestodes, seven species were trematodes, and three species were nematodes. The host-parasite checklist can be found in Table 4. We documented a total of 30 species of parasites which are listed in the parasite-host checklist in Table 5. Overall, larval parasites of 13 species were found and accounted for 43% of species found. The most common parasite, or group of parasites, in native species was trematodes which occurred in 15 of 18 fish species at the metacercaria stage. Non-native fish species hosted 14 species of parasites and native fish hosted 26 species of parasites.

Genetic sequencing of the ITS region of two *Neoechinorhynchus* c.f. *golvani* specimens from *Mayaheros urophthalmus* and *Astronotus ocellatus* matched 99.8% with *Neoechinorhynchus golvani* lineage 10 ITS region on GenBank (MG870811.1 and MG870800.1). Genetic sequencing of the 28S amplicon 1 from a Derogenidae trematode from *Channa marulius* matched 99.63% with *Thometrema patagonica* partial 28S gene on GenBank (LC586091.1).

There were six new host records for *N. doryphorus*: *Astronotus ocellatus, Fundulus chrystotus, Fundulus seminolis, Lepomis macrochirus, Lucania goodei,* and *Micropterus salmoides*. There was two new host records for *Octospiniferoides chandleri*: *Labidesthes vanhyningi* and *Astronotus ocellatus*. There were nine new host records for *Leptorhynchoides apoglyphicus*: *Astronotus ocellatus, Channa marulius, Cichla ocellaris, Fundulus seminolis, Hemichromis letourneuxi, Lepomis gulosus, Lepomis macrochirus, Lucania goodei,* and *Oreochromis niloticus*. There was one new host record for *N. golvani*, *Astronotus ocellatus*, and one new host record for *Thometrema overstreeti*, *Channa marulius*. The Everglades Florida is a new locality record for *N.* c.f. *tenellus, N.* c.f. *golvani, Diegoglossidium* sp., and *Thometrema overstreeti*.

DISCUSSION

1938 vs 2023

The high number of larval parasites documented during this survey was consistent with Bangham's 1938 survey. In the 15 fish species examined in 1938, that were also examined in 2023, 38% of species were found in their larval stages (Bangham, 1940). Larval parasites accounted for a higher percent of total species in 2023 (44%), but this could be due to less species found (29 vs 37). The Everglades supports a large wading bird population which hosts several species of trematodes (also known as digeneans) that use fish as an intermediate host which could explain the large number of larval species found (Lodge, 2017; Hoffman, 1999). These results are consistent with Bangham's survey in 1938 in which he noted the high number of larval parasites (Bangham 1939 & 1940).

For the rest of this paper, we will be addressing only those parasite species found in their adult stages unless otherwise stated. We documented two species of *Paramacroderoides* from Florida Gar that were not documented in 1938. The only adult trematode documented from Florida Gar in 1938 was *Macroderoides spiniferus* which infected 57% of gar (Bangham, 1940). *Paramacroderoides* spp. And *Macroderoides* spp. Share the same intermediate hosts, snail *Planorbella duryi* and *Gambusia* sp., and definitive host, Florida Gar, in their life cycle (Leigh & Hollman, 1956; Leigh, 1958; Hoffman, 1999). The presence of adult species of *Paramacroderoides* indicates the intermediate hosts, *Gambusia affinis* and *Planorbella duryi*, are both still present in sufficient numbers in the ecosystem to support the life cycle of *Paramacroderoides*. It is likely the reason we did not document *Macroderoides spiniferus* was due to a smaller sample size of Florida Gar in 2023 (n=5) than in 1938 (n=88) because other trematode species that use the same intermediate and definitive hosts are still present.

Paramacroderoides echinus was not described until after Bangham's survey by Venard in 1941 (Leigh, 1956). Bangham did not deposit any slides from his survey in 1938 to a museum and so we do not know if he observed *Paramacroderoides echinus*. We documented three species of trematode during our current survey that were also found in 1938 in fish species that were examined in both surveys, out of a total five observed by Bangham (1940). This difference could be explained in two ways. One is that this is a real difference and the trematode species are no longer found in the ecosystem due to an environmental change or a missing host to complete the life cycle. This is most likely the case for *Creptotrema funduli*, which was found to infect 76% of Golden Silversides (n=17) in 1938 (Bangham, 1940). In 2023, despite looking at 147 Golden Silversides, we did not find any *Creptotrema funduli*. The lifecycle of *C. funduli* is unknown, but because the parasite is no longer present in the definitive host we suspect the life cycle of *C. funduli* is disrupted at the intermediate host stage. We suspect that the mollusk intermediate host is no longer present in the ecosystem in sufficient enough numbers to support *C. funduli*. The other explanation is that these missing trematodes are still present but were not encountered because of some aspect of our survey, such as sample size. Many of the species of adult trematodes from Bangham's survey were found to be rare (<10%) or occasional (10-25%) (Bauer and Whipps, 2015). Thus, to detect them large sample sizes of fish are needed. For example, in 2023 we did not find *Plagiocirrus primus,* which infected 2.3% of Golden Shiner (n=43) in 1938 (Bangham, 1940), but our sample size of 13 Golden Shiner in 2023 would not have been large enough to find *P. primus*. In the case of *Homalometron* sp., it was found in 6 of 104 Bluegill (Bangham, 1939 & 1940) in 1938 but was not found in the 34 Bluegill examined in 2023. In these cases, the trematode species may still be in the area but we may not have sampled enough fish to detect it at such a low prevalence. For some trematode species we had good sample sizes of fish species and were able to draw conclusions about the presence/absence of the parasite (i.e. Golden Silverside), but for others we were unable to draw any conclusions due to low sample size of certain fish species (i.e. Bluegill and Golden Shiner). More research would need to be conducted to determine if these species we did not observe are in fact gone from the ecosystem or just undetected due to low sample sizes.

In 2023 we documented a total of seven acanthocephalan species, including 6 native and 1 non-native, but in 1938 only 4 species were documented from the 15 fish species that were collected. The common and widespread centrarchid acanthocephalan *Neoechinorhynchus cylindratus* was documented in several fish species in 1938, but we did not encounter it in 2023 (Bangham, 1939 & 1940). We suspect a combination of other *Neoechinorhynchus* species may have been mistaken by Bangham as *N. cylindratus* from Largemouth Bass, Bluegill, and Brown Bullhead. A possibly new species of *Neoechinorhynchus* was found in Florida Bass that resembles *N. cylindratus*. But it has a wider proboscis and a unique egg morphology. Another similar species, *Neoechinorhynchus doryphorus*, was documented by us from Seminole Killifish, Golden Topminnow, Largemouth Bass, and Bluegill, the last two of which were reported to host *N. cylindratus* in Bangham's survey (Bangham, 1939 & 1940). *Neoechinorhynchus doryphorus* was first collected by Bangham during the 1938 survey from American Flagfish and later described by Van Cleave and Bangham in 1949. In Brown Bullhead we documented one specimen that we report as *Neoechinorhynchus* c.f. *tenellus*. It differs from *N. cylindratus* by having a thicker base of the anterior lateral hook (Van Cleave, 1919). The one individual of *Neoechinorhynchus* c.f. *tenellus* recovered was a juvenile from the intestine of one *Ameiurus nebulosus*. Unfortunately, Bangham did not deposit any of his *N. cylindratus* slides from the 1938 survey so we cannot confirm our suspicions of that possible misidentification. It is possible that *N. cylindratus* actually does occur in Florida and was present in the other fish species from Bangham's survey that we did not examine. Bangham noted an unidentified acanthocephalan species found in *Gambusia holbrooki*, which we suspect was *Octospiniferoides chandleri* given we found this acanthocephalan in *G. holbrooki*. We examined 153 *Gambusia holbrooki* and found only *O. chandleri* and no other species of acanthocephalan. One species of acanthocephalan we documented for which Bangham made no record is *Southwellina hispida*. Cystacanths of *S. hispida* are found in fishes while the adult stages are found in birds (Hoffman, 1999). *Southwellina hispida* has circumglobal distribution and we documented this species from three native fish and four non-native fish (Hoffman, 1999). When considering the native species of *Neoechinorhynchus* examined we do not believe any are new to the ecosystem, they were most likely present during Bangham's survey but not documented due to misidentification or sampling bias.

When comparing the nematodes observed between 1938 and 2023, we found fewer species of adult nematodes. We found one of Bangham's original six species of adult nematodes, *Camallanus oxycephalus* from Bluegill (N=34). We did not find: *Capillaria 12lavicep* from Channel Catfish (N=6); *Spinitectus carolini* from *Micropterus* sp. (N=15) and Bluegill (N=34); *Dichelyne cotylophora* from Bluegill (N=34); *Dichelyne robusta* from Brown Bullhead (N=12); *Dichelyne* sp. from Florida Gar (N=5), Warmouth (N=14), Redear Sunfish (N=12), and Bass (N=15); or *Philometra cylindracea* from Bluegill (N=34). Much like the trematodes, the three species of *Dichelyne* were found rarely in 1938, meaning a larger sample size of fish would be needed to determine whether these nematodes are still present in the ecosystem. We did not find *Spinitectis carolini* but we did find *Spinitectus micracanthus*. Based on this information we can infer that burrowing mayflies, the intermediate host for *Spinitectus* species, are still present in the ecosystem (Hoffman, 1999).

We found only one of four species of adult cestode documented in 1938. *Corallobothrium* sp. was found in *Ameiurus nebulosus* consistent with Bangham's original survey. *Proteocephalus singularis* from *Lepisosteus platyrhincus* and *Bothriocestus 13laviceps* from *Micropterus salmoides* were found in less than 5% of fish examined by Bangham (Bangham, 1939 & 1940). Given this low prevalence it is possible that these cestodes are still present in the environment but we did not have a large enough sample size of either fish species to encounter them.

The largest scale survey of parasites in freshwater fishes of Florida was conducted by Bangham in 1938 (Bangham 1939 & 1940). In the more than 80 years since, no substantial surveys have been conducted on freshwater fish parasites in this region. Bangham's survey included large numbers of centrarchid fishes, which we were unable to match. When collecting fish for the survey, centrarchids and ictalurids were surprisingly hard to obtain. Our sampling was limited to easily accessible boat launches and canals that were accessible by car. Many of the canals sampled were too deep to seine and traditional hook-and-line angling or minnow trapping was used instead. Canals are one of the deep-water refuges used by large-bodied fishes and this limitation may explain the low sample size of some fish species in our survey when compared to Bangham (Chick et al., 2004). The number of species sampled and sample sizes of each species may have increased if the interior of the Everglades was sampled. Overall we documented adults of 8 of Bangham's 19 parasite species found in the 15 fish species both surveys examined. Due to survey constraints we cannot determine if all of these 11 undocumented parasites are gone from the ecosystem or just not observed due to the parasite being rare or occasional.

Non-native Species

During our study, we found several species of non-native fish species which were not introduced at the time of Bangham's 1938 survey. We found less species of parasites in non-native fish species than in the native fish species. Invasive species are often thought to have few parasites in their introduced ranges as predicted by the parasite-escape phenomenon (Torchin et al., 2001) in which they are more resistant to pathogens, are introduced with fewer parasites than their original range, or the parasites that they brought over fail to establish due to their complex life cycles (Torchin et al., 2003; Dunn et al., 2012; Gendron et al., 2012). Dead or degraded helminths that could not be identified were found in several invasive fish species. Most notably, 11 of 24 examined Mayan Cichlids contained dead nematodes and acanthocephalan cystacanths within their body cavity and mesenteries. This indicates that although Mayan Cichlids have been ingesting a diet including infected invertebrate animals the presumably native parasites are not able to successfully use the Mayan Cichlid as a host. Dead or degraded helminths were also found in the Black Acara, Peacock Bass, Goldline Snakehead, and Blue Tilapia, but not in as high numbers (Table 4). Dead, native helminths found in non-native fish hosts may impair the populations of these parasites because the fish hosts may serve as a sink if they are not the definitive host (Gendron and Marcogliese, 2016). This could affect the populations of parasites species that we found dead in invasive fish, including the nematodes *Eustrongyloides* or *Contracaecum* species as well as the acanthocephalan *Southwellina hispida*, all of which use birds as the definitive host.

In this survey, we found four species of exotic parasites that were likely introduced with their host species. Gravid individuals of a *Thometrema* species were found in the invasive channid, *Channa marulius* in high prevalence (66.6%) from several locations. The 28S gene matched 99.63% to *Thometrema patagonica* which is native to Argentina where it is known to parasitize a percichthyid, *Percichthys trucha* (Tsuchida et al., 2021a). There are five valid members of the genus *Thometrema* Amato, 1968: *Thometrema dissimilis* Moravec et Prouza, 2024, *Thometrema lotzi* Curran Overstreet et Font, 2002, *Thometrema magnifica* (Szidat, 1954), *Thometrema overstreeti* (Brooks Mayes et Thorston, 1979), and *Thometrema patagonica* (Szidat, 1956). The recovered specimens of *Thometrema* were more consistent in their morphology with *T. overstreeti*. *Thometrema lotzi*, the only species known from North America, was ruled out because the sinus organ in our specimens is too short and there is only one polar filament on the eggs (Curran et al., 2002). *Thometrema patagonica* was ruled out because the vitellarium of our specimens are medium to deeply lobate (Tsuchida et al., 2021a). *Thometrema overstreeti* is known from several hosts in South America including several within the characiform and siluriform fish families (Lunaschi, 1968; Brooks et al., 1979; Kohn & Fernandez, 1988; Kohn et al., 1990). Of the nine known hosts of *T. overstreeti*, two (*Hoplias malabaricus* and *Rhamdia* sp.) are known to have been released but not established in Florida (Robins et al., 2018). This raises several questions on how *T. overstreeti* made it to Florida. The life cycle is unknown for this species, but most likely it uses a snail as an intermediate host given that others in the family Derogenidae do (Hoffman, 1999; Tsuchida et al., 2021b). This species may have been brought over in its definitive host, another unknown definitive host, or in a snail intermediate host. The presence of this species in Florida indicates an infected host from South America was introduced at some point in Florida and was infected with this parasite. Two known hosts of *T. overstreeti* are known to have been released in Florida, but none of the known hosts are known to have an established reproducing population in Florida (Robins et al., 2018). It is not documented in any of the native species investigated in this survey.

The other three non-native species of parasite documented were not found in as high prevalence as *Thometrema patagonica*. *Schizocotyle acheilognathi* is a well-known invasive tapeworm that has already been documented in Florida, along with 19 other states (Hoffman, 1999; Reyda et al., 2019). Neither of the specimens we obtained of the other two exotic parasites found, *Neoechinorhynchus* c.f. *golvani* and *Diegoglossidium* sp., were gravid. *Neoechinorhynchus golvani* is an acanthocephalan species known to infect fresh-water cichlids (Pinacho-Pinacho et al., 2018). We found specimens tentatively identified as *N.* c.f. *golvani* in Mayan Cichlid and Oscar in this survey. In their native ranges *Mayaheros urophthalmus* (Central America) is known to host *N. golvani*, but *Astronotus ocellatus* (South America) is not (Santos et al., 2008; Pinacho-Pinacho et al., 2018; Santos et al., 2018). Recently through genetic sequencing it was determined *N. golvani* is actually a species complex of about 10 cryptic species (Pinacho-Pinacho et al., 2018). Species within this species complex will be named once morphological characteristics are determined to identify the new species, this work is ongoing (Pinacho-Pinacho et al., 2018; Pinacho-Pinacho et al., 2020). Both *M. urophthalmus* and *A. ocellatus* were infected with *Neoechinorhynchus* c.f. *golvani*, identified as lineage 10 via genetic sequences that were compared to those in Pinacho-Pinacho et al. (2018). *Astronotus ocellatus* is native to South America, but not Central America where lineage 10 of *Neoechinorhynchus golvani* is known to occur (Robins et al., 2018; Pinacho-Pinacho et al., 2018). *Astronotus ocellatus* most likely became infected with *N.* c.f. *golvani* in Florida given the lack of parasitism by acanthocephalans in its native range. This indicates there is an ostracod species acting as a suitable intermediate host present in South Florida to support the life cycle of *N. golvani* lineage 10.

We also found evidence of native parasite species colonizing invasive fish species. The most common species found in invasive host species was *Leptorhyncoides apoglyphicus*. We found adults of *L. apoglyphicus* from *Channa marulius*, *Astronotus ocellatus*, *Cichla ocellaris,* and *Oreochromis Niloticus* and cystacanth stages from *Hemichromis letourneuxi* and *Cichlasoma bimaculatum,* all six of which are new host records. These data suggest that *L. apoglyphicus,* previously only known to infect three *Micropterus* species could be a generalist, able to colonize non-native hosts (Steinauer and Nickol, 2015). Of all the invasive species investigated, Oscar was found to host the highest number of parasite species (n = 7) and had the highest parasitized individuals (4/4). Oscar was found to host one species of non-native acanthocephalan, three species of native acanthocephalan, and a native species of nematode, *Camallanus* species. The only acanthocephalans Oscar is known to host in its native range are cystacanth stages of *Polymorphus* sp. and Acanthocephala gen. sp. with no documented adults (Santos et al., 2008; Santos et al., 2018; Pinheiro et al., 2019).

New Host and Locality Records

Since we did not examine any of the known fish hosts of *Neoechinorhynchus doryphorus*, all six fish species in which we documented *N. doryphorus* are new host records (Van Cleave and Bangham, 1949). *Neoechinorhynchus tenellus* has never been documented in Florida. The one individual of *Neoechinorhynchus* c.f. *tenellus* we recovered was a juvenile from the intestine of *Ameiurus nebulosus*. More individuals would need to be collected to confirm its identity as *Neoechinorhynchus tenellus*. This is important because *N. tenellus* has never been documented from the southern USA (Hoffman, 1999). *Lepomis macrochirus* was documented with gravid females of *Leptorhynchoides apoglyphicus*, which is not a previously documented host of gravid adults (Steinauer and Nickol, 2015). Several individuals of *Octospiniferoides chandleri* were documented from *Labidesthes vanhyningi* which also constitutes a new host record (Hoffman, 1999; McCallister and Cloutman, 2016).

When a non-native fish can act as a host for a native parasite and the parasite can reproduce, then the non-native fish species can serve as a reservoir host for the native parasite (Kelly et al., 2009). This can lead to parasite spillback where an invasive species can help introduce native parasites to new areas and can increase infection of the native parasite (Kelly et al., 2009). A gravid female of *Octospiniferoides chandleri* was documented from *Astronotus ocellatus* which is a new host record of a native parasite in a non-native host species (Hoffman, 1999). This is an interesting case of a native parasite not only colonizing an invasive host species, but also able to reproduce and complete its life cycle in an invasive species. More work would be needed to determine if spillback is occurring between the invasive fish *Astronotus ocellatus* and the definitive native host *Gambusia holbrooki* for *Octospiniferoides chandleri*. The potential for spillback is lower for *L. apoglyphicus* because none of the seven new host records from non-native fish were gravid. More research would need to be done to determine if gravid individuals of *L. apoglyphicus* are present in any of the non-native fish species due to low sample sizes in this survey.

Neoechinorhynchus c.f. *golvani* has never been documented in the USA making this a new locality record. This is the first time *Astronotus ocellatus* has been documented as a host for *Neoechinorhynchus* c.f. *golvani* despite them co-occurring in their native ranges (Santos et al., 2008; Pinacho-Pinacho et al., 2018; Santos et al., 2018). This is the first time *Diegoglossidium* sp. and *Thometrema patagonica* was documented in North America making both of these new locality records. The presence of gravid *T. patagonica* in *Channa marulius* is a new host record and also indicates the parasite has found a suitable intermediate host to be able to complete its life cycle.

Conclusions

Overall we documented a total of 19 new host records for 5 species of parasite and a total of 4 new locality records, 3 of which were new to the USA. This survey has documented many new host and new locality records demonstrating the importance of surveys especially in understudied areas of North America (Sholz and Choudhury, 2014). Several species of parasite known to have been in the region in 1938 were not documented during this survey, demonstrating the need for further research with larger sample sizes. Overall, the presence/absence of both parasite and host species shows the effects of the extensive ecological changes in the area. We documented adults of 19 species of parasite across the 31 fish we examined, but we found only of 8 of the 40 species of adult parasites documented from 45 fish species in 1938. These differences are due to both sampling bias, less fish species examined and low sample size of some fishes, as well as due to environmental changes, non-native fish and parasites. Our survey showed that while Bangham's 1938 survey was extensive, some native species of parasites were not documented during it either due to some form of sampling bias or even potentially parasite misidentification (i.e. *Neoechinorhynchus cylindratus*, *Neoechinorhynchus doryphorus*, and *Paramacroderoides echinus*). While conclusions on the invertebrate and vertebrate host populations can be drawn from the presence/absence of parasites, survey work targeting these species would need to be done to determine their population status. Although more research on the parasites and their invertebrate and vertebrate hosts across the Everglades is needed to fully document the extent of change in this ecosystem; our results demonstrate that the change is extensive.

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LITERATURE CITED

Bangham, R.V. 1939. Parasites of centrarchidae from Southern Florida. Transactions of the American Fisheries Society 68: 263-268.

Bangham, R. V. 1940. Parasites of fresh-water fish of Southern Florida. Florida Academy of Sciences 5: 289–307.

Bauer, E.F. and C.M. Whipps. 2015. The bass parasites of Oneida Lake, 80 years later. Journal of Parasitology 101: 505-513.

Brooks, D.R., M.A. Mayers, and T.B. Thorson. 1979. *Paravitellotrema overstreeti* Sp. n. (Digenea: Hemiuridae) from the Colombian freshwater stingray *Potamotrygon magdalenae* dumeri. Proceedings of the Helminthological Society of Washington 46: 52-54.

Canfield, D. E., R. W. Bachmann, and M. V. Hoyer. 2021. Restoration of Lake Okeechobee, Florida: Mission impossible? Lake and Reservoir Management 37: 95–111. Doi: 10.1080/10402381.2020.1839607.

Chick, J.H., C.R. Ruetz, and J.C. Trexler. 2004. Spatial scale and abundance patterns of large fish communities in freshwater marshes of the Florida E verglades. Wetlands 24: 652-664.

Chimney, M.J., and G. Goforth. 2001. Environmental impacts to the Everglades ecosystem: A historical perspective and restoration strategies. Water Science and Technology 44: 11-12.

Curran, S. S., R.M. Overstreet, and W.F. Font. 2002. *Thometrema lotzi* sp. nov. (Digenea, Derogenidae) from freshwater and brackish water fishes of Louisiana and Mississippi (USA). Acta Parasitologica 47: 14-19.

Dunn, A.M., M.E. Torchin, M.J. Hatcher, P.M. Kotanen, D.M. Blumenthol, J.E. Byers, C.A.C. Coon, V.M. Frankel, R.D. Holt, R.A. Hufbauer, A.R. Kanarek, K.A. Schierenbeck, L.M. Wolfe, and S.E. Perkins. 2012. Indirect effects of parasites in invasions. Functional Ecology 26: 1262-1274.

García-Varela, M., and S.A. Nadler. 2005. Phylogenetic relationships of Palaeacanthocephala (Acanthocephala) inferred from SSU and LSU rDNA gene sequences. Journal of Parasitology 91:1401-1409.

Gendron, A. and D. Marcogliese. 2016. Reduced survival of a native parasite in the invasie round goby: evidence for the dilution hypothesis? Aquatic Invasions 11: 189-198.

Gendron, A.D., D.J. Marcogliese, and M. Thomas. 2012. Invasive species are less parasitized than native competitors, but for how long? The case of the round goby in the Great Lakes-St. Lawrence Basin. Biological Invasions 14: 367-384.

Hoffman, G. L. 1999. Parasites of North American freshwater fishes, 2nd ed. Comstock Publishing Associates, Ithaca, New York, U.S.A. 576 p.

Howell, H.J., G.L. Delgado, A.C. Wood, L.M. Thompson, E.A. Cline, and C.A. Searcy. 2021. A dry future for the Everglades favors invasive herpetofauna. Biological Invasions 23: 3119-3133. Doi: 10.1007/s10530-021-02562-z.

Johnson, K. G., M. S. Allen, and K. E. Havens. 2007. A review of littoral vegetation, fisheries, and wildlife responses to hydrologic variation at Lake Okeechobee. Wetlands 27: 110–126. Doi: 10.1672/0277-5212(2007)27[110:AROLVF]2.0.CO;2

Kelly, D.W., R.A. Paterson, C.R. Townsend, R. Poulin, and D.M. Tompkins. 2009. Parasite spillback: A neglected concept in invasion ecology? Ecology 90: 2047-2056.

Kohn, A. and B.M.M. Fernandes. 1988. Revision of the Brazilian species of the genus *Halipegus* Looss, 1899 (Trematoda: Derogenidae). Systematic Parasitology 11: 129-137.

Kohn, A., B.M.M. Fernandes, D.I. Gibson, and O.M. Fróes. 1990. On the Brazilian species of halipegine genera (Trematoda: Derogenidae) from fishes, with new morphological data, hosts and synonyms. Systematic Parasitology 16: 201-211.

Leigh, W.H. 1975. Observations on the life histories of *Paramacroderoides pseudoechinus* sp. n. (Digenea: Plagiorchioidea) and *P. echinus* Venard 1941, trematodes from the Florida Gar. The Journal of Parasitology 61: 873-876.

Leigh, W.H. and R.B. Hollman. 1956. The life-history of Paramacroderoides echinus Venard, 1941 a trematode of the Florida Gar, Lepisosteus platyrhincus. The Journal of Parasitology 42: 400-407.

Lodge, T.E. 2017. The Everglades handbook: Understanding the ecosystem, 4th ed. CRC Press, Boca Raton, Florida, 440 p.

Lunaschi, L.I. 1968. Helmintos parasitos de peces de agua dulce de la Argentina X: Tres nuevas especies del género *Thometrema* Amato, 1968 (Trematoda-Derogenidae). Neotropica 34: 23-32.

Marcogliese, D. J. 2005. Parasites of the superorganism: Are they indicators of ecosystem health? International Journal for Parasitology 35: 705–716. Doi: 10.1016/j.ijpara.2005.01.015

McAllister, C.T. and D.G. Cloutman. 2016. Parasites of Brook Silversides, *Labidesthes sicculus*, and Golden Silversides, *Labidesthes vanhyningi* (Atheriniformes: Atherinopsidae), from Arkansas and Oklahoma, U.S.A. Comparative Parasitology 83: 250-254.

Moravec, F. and A. Prouza. 2024. Some trematodes including three new species from freshwater fishes of Venezuela. Folia Parasitologica 71: 007. Doi: 10.14411/fp.2024/007

Pinheiro, R.H.S., M. Taveres-Dias, and E.G. Giese. 2019. Helminth parasites in two populations of *Astronotus ocellatus* (Cichliformes: Cichlidae) from the eastern Amazon, Northern Brazil. Brazilian Journal of Veterinary Parasitology 28: 425-431. Doi: 10.1590/S1984-29612019052

Pinacho-Pinacho, C.D., M. García-Varela, A.L. Sereno-Uribe, G. Pérez-Ponce de León. 2018. A hyper diverse genus of acanthocephalans revealed by tree-based and non-tree-based species delimitation methods: Ten cryptic species of *Neoechinorhynchus* in Middle American freshwater fishes. Molecular Phylogenetics and Evolution 127: 30-45.

Pleijel, R., U. Jondelius, E. Norlinder, A. Nygeren, B. Oxelman, C. Schander, P. Sundberg, and M. Thollesson. 2008. Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. Molecular Phylogenetics and Evolution 48: 369–371.Pritchard, M. H., and G. O. W. Kruse. 1982. The collection and preservation of animal parasites. University of Nebraska Press, Lincoln, NE. 141 pp.

Reyda, F., C.P. Pomelle, and M.L. Doolin. 2019. Asian fish tapeworm (*Schyzocotyle acheilognathi*) found in New York state for the first time after a long-term fish-parasite survey. Comparative Parasitology 86: 108-113.

Robins, R.H., L.M. Page, J.D. Williams, Z.S. Randall, and G.E. Sheehy. 2018. Fishes in the fresh waters of Florida: An identification guide and atlas. University of Florida Press, Gainesville, Florida, 467 p.

Santos, G.G., M.S.B. Oliveira, L.R. Neves, and M. Tavares-Dias. 2018. Parasites community of *Astronotus crassipinnis* (Cichlidae), a fish from the Brazilian Amazon. Annals of Parasitology 64: 121-128. Doi: 10.17420/ap6402.143

Santos, C.P., D.I. Gibson, L.E.R. Taveres, and J.L. Luque. 2008. Checklist of Acanthocephala associated with the fishes of Brazil. Zootaxa 6: 1-22.

Scholz, T. and A. Chaudhury. (2014). Parasites of freshwater fishes in North America: Why so neglected? Journal of Parasitology 100: 26-45.

Searcy, C.A., H.J. Howell, A.S. David, R.B. Rumelt, and S.L. Clements. 2023. Patterns of non-native species introduction, spread, and ecological impact in South Florida, the world's most invaded continental ecoregion. Annual Review of Ecology, Evolution, and Systematics 54: 195-218.

Steinauer, M.L. and B.B. Nickol. 2015. Revision of *Leptorhynchoides apoglyphicus* (Acanthocephala: Illiosentidae), with morphometric analysis and description of six new species. Journal of Parasitology 101: 193-211.

Szidat L. 1954. Trematódes nuevos de peces de agua dulce de la República Argentina y un intento para aclarar su carácter marino. Revista del Instituto National de Investigacion de las Ciencias Naturales, Ciencias Zoológicas 3: 1–85.

Szidat, L. Über die parasitenfauna von *Percichthys trucha* (Cuv. & Val.) Girard der patagonischen Gewässer und die Beziehungen des Wirtsfisches und seiner parasite. Archiv für Hydrobiologie 51: 542-577.

Torchin, M.E., K.D. Lafferty, & A.M. Kuris. 2001. Release from parasites as natural enemies: Increased performance of a globally introduced marine crab. Biological Invasions 3: 333-345. Doi: 10.1023/A:1015855019360

Torchin, M.E., K.D. Lafferty, A.P. Dobson, V.J. McKenzie, and A.M. Kuris. Introduced species and their missing parasites. Nature 421: 628-630.

Tsuchida, K., V. Flores, G. Viozzi, C. Rauque, and M. Urabe. 2021a. Hemiuroidean trematodes from freshwater paragonian fishes: description of a new species, distribution and molecular phylogeny. Parasitology Research 120: 1219-1232.

Tsuchida, K., M. Urabe, G. Viozzi, C. Rauque, and V. Flores. 2021b. A new species of hemiuroidean trematode from *Hatcheria macraei* (Siluriformes, Trichomycteridae) and *Heleobia hatcheri* (Gastropodae, Cochliopidae) in a Patagonian river. Parasitology Research 120: 2523-2532.

Van Cleave, H J. 1919. Acanthocephala from the Illinois River, with descriptions of species and a synopsis of the family Neoechinorhynchidae. Bulletin of the Illinois Natural History Survey, 13, 225-257.

Van Cleave, H.J., and R.V. Bangham. 1949. Four new species of acanthocephalan family Neoechinorhynchidae from fresh-water fishes of North America, one representing a new genus. Journal of the Washington Academy of Sciences 39: 398-409.

Venard, C.E. 1941. Studies on parasites of Reelfoot Lake fish. III. A new genus and species of trematode (Plagiorchioidea; Macroderoididae) from *Lepisosteus platostomus*. Journal of Tennessee Acadamy of Science 16: 379-383.

Water Body	Collection Site	Coordinates	Fish Sample Size
Lake Okeechobee	Slim's Fish Camp	26.705428, -80.714691	13
Lake Okeechobee	Torry Island Campground	26.703348, -80.718186	93
Lake Okeechobee	Lomax Harrelle Pavilion	26.706559, -80.719700	25
Lake Okeechobee	Joey's Site 9 and 12	26.741231, -80.758556	7
STA-3/4	Harold Campbell Public Use Area	26.338514, -80.627962	196
WCA-3A	Levee 5 ramp at 7 miles	26.338250, -80.628023	158
WCA-3A	Weasel Trail	26.233340, -80.462240	2
WCA-3B	Mack's Fish Camp Boat Ramp	25.941856, -80.441413	143
WCA-3B	Tamiami Trail at mile 30	25.763564, -80.673732	41
Greyknoll Lake	Jacob's Spot	25.950398, -80.163092	4
SW 70th Ave Canal	Davie, Florida	26.091337, -80.238977	4
SW 36th St canal	Davie, Florida	26.076395, -80.268592	1
Unnamed pond	Davie, Florida behind Laspada Hoagie House	26.085960, -80.252800	19
E1 canal near WCA-2	West Delray Regional Park	26.454216, -80.218252	13

Table 1. Geographic coordinates and number of fish caught at each of 15 sites. The sites with the highest number of fish examined were Harold Campbell Public Use Area in STA-3/4, Levee 5 ramp at 7 miles in WCA-3A, and Mack's Fish Camp in WCA-3B.

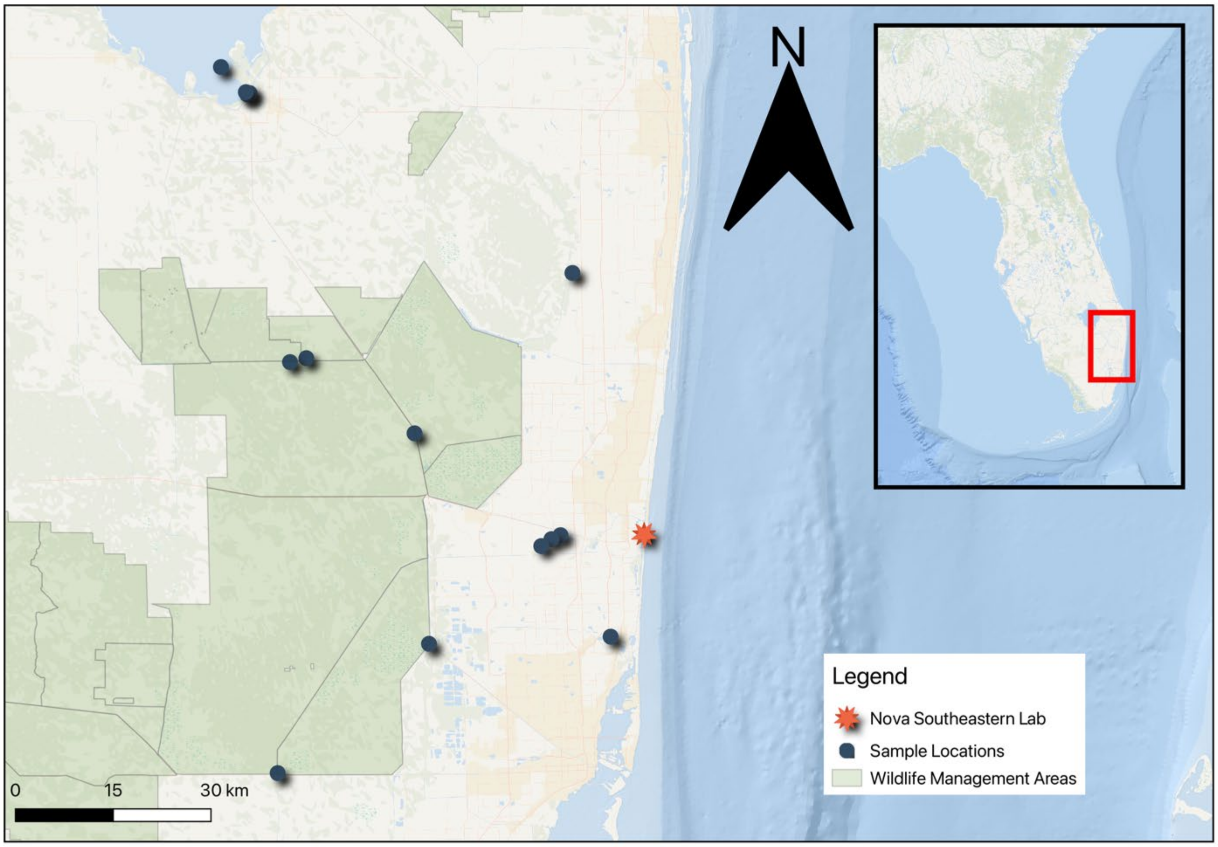
Family Name	Scientific Name	Common Name	Number	Parasitized
Atherinopsidae	*Labidesthes vanhyningi*	Golden Silverside	147	25
Callichthyidae	*Hoplosternum littorale**	Brown Hoplo	17	6
Centrarchidae	*Lepomis gulosus*	Warmouth	14	12
Centrarchidae	*Lepomis macrochirus*	Bluegill	34	23
Centrarchidae	*Lepomis marginatus*	Dollar Sunfish	1	1
Centrarchidae	*Lepomis microlophus*	Redear Sunfish	12	9
Centrarchidae	*Lepomis punctatus*	Spotted Sunfish	2	2
Centrarchidae	*Micropterus sp.*	LM Bass	15	15
Channidae	*Channa marulius**	Goldline Snakehead	9	8
Cichlidae	*Astronotus ocellatus**	Oscar	4	4
Cichlidae	*Cichla ocellaris**	Peacock Bass	5	2
Cichlidae	*Cichlasoma bimaculatum**	Black Acara	2	1
Cichlidae	*Hemichromis letourneuxi**	African Jewelfish	46	10
Cichlidae	*Mayaheros urophthalmus**	Mayan Cichlid	24	21
Cichlidae	*Oreochromis aureus**	Blue Tilapia	4	1
Cichlidae	*Oreochromis niloticus**	Nile Tilapia	11	1
Cichlidae	*Parachromis managuensis**	Jaguar Guapote	1	1
Clariidae	*Clarias batrachus**	Walking Catfish	2	1
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner	13	7
Fundulidae	*Fundulus chrysotus*	Golden Topminnow	10	2
Fundulidae	*Fundulus seminolis*	Seminole Killifish	30	26
Fundulidae	*Lucania goodei*	Bluefin Killifish	50	31
Lepisosteidae	*Lepisosteus platyrhincus*	Florida Gar	5	5
Loricariidae	*Pterygoplichthys sp.**	Sailfin Catfish	3	0
Percidae	*Etheostoma fusiforme*	Swamp Darter	5	0
Poeciliidae	*Gambusia holbrooki*	Eastern Mosquitofish	153	123
Poeciliidae	*Heterandria formosa*	Least Killifish	28	5
Poeciliidae	*Poecilia latipinna*	Sailfin Molly	49	30
Ictaluridae	*Ameiurus nebulosus*	Brown Bullhead	12	11
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	6	5
Synbranchidae	*Monopterus javanensis**	Asian Swamp Eel	1	0

Table 2. List of Fish Species Examined. Asterisk (*) indicates non-native species. Number of each species examined and parasitized listed.

		1938			2023		
Fish Family	Fish Scientific Name	Ex.	Inf.	Spp par.	Ex.	Inf.	Spp par.
Atherinopsidae	*Labidesthes vanhyingi*	17	16	6	147	25	4
Centrarchidae	*Lepomis gulosus*	143	143	19	14	12	3
Centrarchidae	*Lepomis macrochirus*	104	103	15	34	23	6
Centrarchidae	*Lepomis microlophus*	90	88	14	12	9	7
Centrarchidae	*Lepomis punctatus*	49	49	10	2	2	2
Centrarchidae	*Micropterus floridanus*	86	86	14	15	15	7
Cyprinidae	*Notemigonus crysoleucas*	43	27	4	13	7	2
Fundulidae	*Fundulus chrysotus*	84	76	8	10	2	2
Fundulidae	*Fundulus seminolis*	14	11	4	30	26	4
Ictaluridae	*Ameiurus nebulosus*	22	22	9	12	11	5
Ictaluridae	*Ictalurus punctatus*	13	13	9	6	5	5
Lepisosteidae	*Lepisosteus platyrhincus*	82	77	10	5	2	3
Poeciliidae	*Gambusia holbrooki*	79	61	10	153	123	3
Poeciliidae	*Heterandria formosa*	21	10	5	28	8	1

Table 3. Fish Species examined in Bangham's 1938 survey and the 2023 survey in Florida. This table shows the number of individuals of each species examined (Ex.), number infected with parasites (Inf.), and number of parasite species documented (Spp par.). The number of individuals of each Centrarchid species was much higher in 1938 than in 2023.

Fig 1. Survey map showing a close-up section of Florida where the sample sites were located. All sample sites are marked with blue circles and the lab where fish were processed is marked with a red star.



Family Name	Scientific Name	Common Name	Parasite Species	Number Examined	Prevalence (%)
Atherinopsidae	*Labidesthes vanhyningi*	Golden Silverside		147	
			Octospiniferoides chandleri		7.5
			Camallanus sp.*		0.7
			Contracaecum sp.*		4.1
			Diplostomoidea sp. *		2.7
			Metacercaria sp.*		4.8
Callichthyidae	*Hoplosternum littorale**	Brown Hoplo		17	
			Diegoglossidium sp.		11.8
Centrarchidae	*Lepomis gulosus*	Warmouth		14	
			*Leptorhyncoides apoglyphicus**		7.1
			Contracaecum sp.*		35.7
			Diplostomoidea sp. *		78.6
Centrarchidae	*Lepomis macrochirus*	Bluegill		34	
			Leptorhyncoides apoglyphicus		5.9
			Proteocephalus ambloplitis		8.8
			Spinitectus micracanthus		2.9
			*Leptorhyncoides apoglyphicus**		11.8
			*Neoechinorhynchus doryphorus**		8.8
			Proteocephalus sp.*		14.7
			Contracaecum sp.*		8.8
			Gnathostoma sp.*		2.9
			Diplostomoidea sp. *		32.4
			Metacercaria sp.*		17.6

Centrarchidae	*Lepomis marginatus*	Dollar Sunfish		1	
			Piscamphistoma stunkardi		100.0
			Nematoda sp.		100.0
			Diplostomoidea sp. *		100.0
Centrarchidae	*Lepomis microlophus*	Redear Sunfish		12	
			Leptorhyncoides apoglyphicus		8.3
			Crepidostomum sp.		8.3
			Nematoda sp.		8.3
			*Leptorhyncoides apoglyphicus**		58.3
			*Contracaecum sp.**		33.3
			Diplostomoidea sp. *		33.3
			Sebekia sp.*		8.3
Centrarchidae	*Lepomis punctatus*	Spotted Sunfish		2	
			Leptorhyncoides apoglyphicus		50.0
			*Leptorhyncoides apoglyphicus**		100.0
			Proteocephalus sp.*		50.0
Centrarchidae	*Micropterus sp.*	Largemouth Bass		15	
			Neoechinorhynchus doryphorus		40.0
			Neoechinorhynchus n. sp.		33.3
			Proteocephalus ambloplitis		20.0
			Camallanus oxycephalus		20.0
			Proteocephalus sp.*		26.7
			Contracaecum sp.*		33.3
			*Southwellina hispida**		6.7

			Diplostomoidea sp. *		46.7
			Metacercaria sp.*		6.7
Channidae	*Channa marulius**	Goldline Snakehead		9	
			Leptorhyncoides apoglyphicus		22.2
			Thometrema patagonica		66.7
			*Southwellina hispida**		11.1
			*Contracaecum sp.**		33.3
			Sebekia sp.*		22.2
			Dead/degraded helminths		22.2
Cichlidae	*Astronotus ocellatus**	Oscar		4	
			Octospiniferoides chandleri		25.0
			Leptorhyncoides apoglyphicus		25.0
			Neoechinorhynchus doryphorus		75.0
			Neoechinorhynchus c.f. *golvani*		25.0
			*Camallanus sp.**		25.0
			*Contracaecum sp.**		100.0
			Eustrongyloides sp.*		100.0
Cichlidae	*Cichla ocellaris**	Peacock Bass		5	
			*Leptorhyncoides apoglyphicus**		20.0
			Dead cystacanths		20.0
Cichlidae	*Cichlasoma bimaculatum**	Black Acara		2	
			Dead *Leptorhynchoides* sp.*		50.0
Cichlidae	*Hemichromis letourneuxi**	African Jewelfish		46	
			*Leptorhyncoides apoglyphicus**		10.9

			*Southwellina hispida**		4.3
			Contracaecum sp.*		8.7
			Cestoda sp.*		2.2
Cichlidae	*Mayaheros urophthalmus**	Mayan Cichlid		24	
			Neoechinorhynchus c.f. *golvani*		4.2
			*Southwellina hispida**		12.5
			*Contracaecum sp.**		41.7
			Dead helminths		45.8
Cichlidae	*Oreochromis aureus**	Blue Tilapia		4	
			*Contracaecum sp.** (degraded)		25.0
Cichlidae	*Oreochromis niloticus**	Nile Tilapia		11	
			Leptorhyncoides apoglyphicus		9.1
Cichlidae	*Parachromis managuensis**	Jaguar Guapote		1	
			*Southwellina hispida**		100.0
			*Contracaecum sp.**		100.0
			Eustrongyloides sp.*		100.0
Clariidae	*Clarias batrachus**	Walking Catfish		2	
			*Contracaecum sp.**		50.0
			Cestoda sp.*		50.0
Cyprinidae	*Notemigonus crysoleucas*	Golden Shiner		13	
			Schizocotyle acheilognathi		7.7
			Contracaecum sp.*		38.5
			Metacercaria sp.*		7.7
Fundulidae	*Fundulus chrysotus*	Golden Topminnow		10	

			Neoechinorhynchus doryphorus		10.0
			Diplostomidea sp.*		10.0
Fundulidae	*Fundulus seminolis*	Seminole Killifish		30	
			Camallanus sp.		3.3
			*Clinostomum marginatum**		3.3
			*Leptorhyncoides apoglyphicus**		13.3
			*Neoechinorhynchus doryphorus**		56.7
			Diplostomidea sp.*		30.0
			Metacercaria sp.*		30.0
Fundulidae	*Lucania goodei*	Bluefin Killifish		50	
			Nematoda sp.		4.0
			*Leptorhyncoides apoglyphicus**		6.0
			*Neoechinorhynchus doryphorus**		46.0
			Contracaecum sp.*		4.0
			Diplostomidea sp.*		2.0
			Metacercaria sp.*		10.0
Lepisosteidae	*Lepisosteus platyrhincus*	Florida Gar		5	
			Paramacroderoides echinus		40.0
			Paramacroderoides pseudoechinus		20.0
			Contracaecum sp.*		100.0
			Sebekia sp.*		20.0
Loricariidae	*Pterygoplichthys sp.**	Sailfin Catfish		3	
Percidae	*Etheostoma fusiforme*	Swamp Darter		5	
Poeciliidae	*Gambusia holbrooki*	Eastern Mosquitofish		153	

			Octospiniferoides chandleri		14.4
			Contracaecum sp.*		2.0
			Diplostomidea sp.*		26.8
			Metacercaria sp.*		32.7
			Echinostome metacercaria*		11.8
			Cyclophyllidean sp.*		3.9
			Cestoda sp.*		1.3
Poeciliidae	*Heterandria formosa*	Least Killifish		28	
			Diplostomidea sp.*		14.3
			Metacercaria sp.*		3.6
Poeciliidae	*Poecilia latipinna*	Sailfin Molly		49	
			*Clinostomum marginatum**		2.0
			Contracaecum sp.*		2.0
			Diplostomidea sp.*		36.7
			Echinostome metacercaria*		20.4
			Metacercaria sp.*		8.2
Ictaluridae	*Ameiurus nebulosus*	Brown Bullhead		12	
			Alloglossidium kenti		8.3
			Coralloobothrium sp.		16.7
			Neoechinorhynchus c.f. *tenellus*		8.3
			*Southwellina hispida**		91.7
			Contracaecum sp.*		83.3
			Cestoda sp.*		16.7
			Metacercaria sp.*		16.7
Ictaluridae	*Ictalurus punctatus*	Channel Catfish		6	
			*Southwellina hispida**		16.7
			Contracaecum sp.*		66.7

			Eustrongyloides sp.*		66.7
			Proteocephalus sp.*		16.7
			Diplostomidea sp.*		16.7
Synbranchida e	*Monopterus javanensis**	Asian Swamp Eel		1	

Table 4. Host-Parasite checklist in alphabetical order by fish family. Number of individuals examined for each fish species is given. Parasite species found in each fish species are given with their prevalence. Asterisk indicates a parasite found in a larval stage.

Phylum	Parasite Species	Host
Acanthocephala	*Leptorhynchoides apoglyphicus*	*Astronotus ocellatus*[α], *Channa marulius*[α], *Cichla ocellaris*[α], *Fundulus seminolis**[α], *Hemichromis letourneuxi*[α], *Lepomis gulosus**[α], *Lepomis macrochirus*[α], *Lepomis microlophus**, *Lepomis punctatus**, *Lucania goodei**[α], *Oreochromis niloticus*[α]
Acanthocephala	*Leptorhynchoides* sp.	*Cichlasoma bimaculatum**
Acanthocephala	*Neoechinorhynchus doryphorus*	*Astronotus ocellatus*[α], *Fundulus seminolis**[α], *Fundulus chrystotus*[α], *Lepomis macrochirus**[α], *Lucania goodei**[α], *Micropterus salmoides*[α]
Acanthocephala	*Neoechinorhynchus* c.f. *golvani*[Δ]	*Astronotus ocellatus*[α], *Mayaheros urophthalmus*
Acanthocephala	*Neoechinorhynchus* c.f. *tenellus*[Δ]	*Ameiurus nebulosus*
Acanthocephala	*Neoechinorhynchus* n. sp.	*Micropterus salmoides*
Acanthocephala	*Octospiniferoides chandleri*	*Astronotus ocellatus*[α], *Gambusia holbrooki*, *Labidesthes vanhyningi*[α]
Acanthocephala	*Southwellina hispida**	*Ameiurus nebulosus*, *Channa marulius*, *Hemichromis letourneuxi*, *Ictalurus punctatus*, *Mayaheros urophthalmus*, *Micropterus salmoides*, *Parachromis managuensis*
Cestoda	*Corallobothrium* sp.	*Ameiurus nebulosus*
Cestoda	*Proteocephalus ambloplitis*	*Lepomis macrochirus*, *Micropterus salmoides*
Cestoda	*Schizocotyle acheilognathi*	*Notemigonus crysoleucas*
Cestoda	*Proteocephalus* sp.*	*Ictalurus punctatus*, *Lepomis macrochirus*, *Lepomis punctatus*, *Micropterus salmoides*
Cestoda	Cestoda sp.*	*Ameiurus nebulosus*, *Clarias batrachus*, *Gambusia holbrooki*, *Hemichromis letourneuxi*
Trematoda	*Alloglossidium kenti*	*Ameiurus nebulosus*
Trematoda	*Crepidostomum* sp.	*Lepomis microlophus*
Trematoda	*Diegoglossidium* sp. [Δ]	*Hoplosternum littorale*
Trematoda	*Thometrema patagonica*[Δ]	*Channa marulius*[α]
Trematoda	*Pisciamphistoma stunkardi*	*Lepomis marginatus*
Trematoda	*Paramacroderoides echinus*	*Lepisosteus platyrhinchus*
Trematoda	*Paramacroderoides pseudoechinus*	*Lepisosteus platyrhinchus*

Trematoda	*Clinostomum marginatum**	*Fundulus seminolis, Gambusia holbrooki, Poecilia latipinna*
Trematoda	Diplostomoidea sp.*	*Fundulus chrystotus, Fundulus seminolis, Gambusia holbrooki, Heterandria formosa, Ictalurus punctatus, Labidesthes vanhyningi, Lepomis gulosus, Lepomis macrochirus, Lepomis marginatus, Lepomis microlophus, Lucania goodei, Micropterus salmoides, Poecilia latipinna*
Trematoda	Trematoda sp.*	*Ameiurus nebulosus, Fundulus seminolis, Gambusia holbrooki, Heterandria formosa, Labidesthes vanhyningi, Lepomis macrochirus, Lucania goodei, Micropterus salmoides, Notemigonus crysoleucas, Poecilia latipinna*
Nematoda	*Camallanus* sp.	*Astronotus ocellatus, Fundulus seminolis, Labidesthes vanhyningi*
Nematoda	*Camallanus oxycephalus*	*Micropterus salmoides*
Nematoda	*Spinitectus micracanthus*	*Lepomis macrochirus*
Nematoda	*Gnathostoma* sp.*	*Lepomis macrochirus*
Nematoda	*Contracaecum* sp.*	*Astronotus ocellatus, Clarias batrachus, Gambusia holbrooki, Hemichromis letourneuxi, Ictalurus punctatus, Labidesthes vanhyningi, Lepisosteus platyrhinchus, Lepomis gulosus, Lepomis macrochirus, Lepomis microlophus, Lucania goodei, Mayaheros urophthalmus, Micropterus salmoides, Notemigonus crysoleucas, Oreochromis aureus, Parachromis managuensis, Poecilia latipinna*
Nematoda	*Eustrongyloides* sp.*	*Astronotus ocellatus, Ictalurus punctatus, Parachromis managuensis*
Arthropoda	*Sebekia* sp.*	*Channa marulius, Lepisosteus platyrhinchus, Lepomis microlophus*

Table 5. Parasite-Host Checklist. Every fish species a parasite was documented out of is listed next to the parasite species. Parasites are listed in alphabetical order by phylum. An asterisk* indicates only larval stages were found, a delta$^{\Delta}$ indicates a new locality record, and an alpha symbol$^{\alpha}$ indicates a new host record. Host species are listed for each parasite in alphabetical order.

Finding Dory (*Neoechinorhynchus doryphorus*): Redescription of *Neoechinorhynchus doryphorus* (Acanthocephala: Neoechinorhynchidae) and a new species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) from Largemouth Bass (*Micropterus salmoides*) in the Everglades, Florida

ABSTRACT

The only published account of *Neoechinorhynchus doryphorus* is the description by Van Cleave and Bangham (1949) from *Jordanella floridae* and the report by Bullock (1960) of it from *Fundulus majalis, Lucania parva,* and *Notropis* species. The original description was adequate to identify specimens collected from a survey conducted in the Everglades, Florida, but the description and drawings of proboscis armature was either not complete or in error. The original type specimens was examined but determined to be of little use due to degradation of the hooks on the proboscis. For this reason *N. doryphorus* is redescribed from *Micropterus salmoides* and *Astronotus ocellatus*. *Neoechinorhynchus bulborostris* n. sp. is described from *Micropterus salmoides* from the Everglades in Florida following an extensive fish parasite survey. *Neoechinorhynchus bulborostris* n. sp. is distinguished from similar fish-infecting neoechinorhynchids by the size and shape of the proboscis and the shape of the eggs. Specimens of both *Neoechinorhynchus* species were examined for morphological study using light microscopy and scanning electron microscopy (SEM).

INTRODUCTION

The genus *Neoechinorhynchus* is a diverse genus within Eoacanthocephala found in fishes and turtles with 121 described species (Amin, 2002; Sarabeev et al., 2020). Although the vast majority of species of the genus are from fish, the multiple species in North American turtles exhibit morphological diversity in a couple of ways. The most reliable identifying features of *Neoechinorhynchus* species found in turtles include the egg morphology and shape of the posterior end of female worms, both of which vary extensively (Barger, 2005). Despite the diversity of egg morphologies of turtle *Neoechinorhynchus* species, eggs of fish

Neoechinorhynchus species have only two basic forms. They either possess polar prolongations of the fertilization membrane or have concentric membranes (Amin, 2002; Barger, 2005). In fish *Neoechinorhynchus* species these features are used in conjunction with other morphological features like hook morphology in order to distinguish species (Doolin & Reyda, 2018). We recognize a species as having unique combinations or sets of morphological features and then tested with 1-2 molecular markers.

Neoechinorhynchus doryphorus was originally described by Van Cleave and Bangham in 1949. The species was discovered in Flagfish, *Jordanella floridae*, during Bangham's 1938 survey of fish parasites in South Florida (Bangham, 1939 and 1940). The species was described from only four specimens: three adults from Flagfish and one juvenile from Striped Killifish, *Fundulus majalis* (Van Cleave & Bangham, 1949). One of the defining characteristics of this *Neoechinorhynchus* species is the size and shape of the lateral hooks, which were grossly larger than the dorsal and ventral hooks. In addition, Van Cleave and Bangham (1949) reported that the right and left lateral hooks were morphologically different, i.e. that they deviated from bilateral symmetry. This purported unusual asymmetry that differs from bilateral symmetry, paired with a limited set of poor-quality type specimens has led some to question whether *N. doryphorus* is a valid species (Bullock in Hoffman, 1999). Adults of *N. doryphorus* have only been reported during the 1938 survey from *Jordanella floridae* (reported as *Neoechinorhynchus* sp.) from which it was described. In addition, the cystacanth stage of the species were reported by Van Cleave and Bangham (1949) and Bullock (1960) from *Fundulus majalis, Lucania parva,* and *Notropis* sp. The species *Neoechinorhynchus dimorphospinus*, *Neoechinorhynchus tenellus*, and *Neoechinorhynchus carinatus* are the only other fish *Neoechinorhynchus* species reported to have lateral terminal proboscis hooks markedly larger than dorsal and ventral hooks (Buckner &

Buckner, 1993; Amin & Sey, 1996; Amin & Muzzall, 2009). During the species description of *N. dimorphospinus* they noted the museum specimens of *N. doryphorus* were of little use due to poor processing and mounting (Amin & Sey, 1996).

We completed a survey in South Florida in the summer of 2023 and encountered three species of *Neoechinorhynchus*. Adults of two of these species of *Neoechinorhynchus* were from Largemouth Bass, *Micropterus salmoides*. One of the species had asymmetrical hook morphology of the proboscis leading us to compare it to the type material of *N. doryphorus*. The other species of *Neoechinorhynchus* recovered from Largemouth Bass is described as a new species of *Neoechinorhynchus* due to its unique egg morphology and unusually wide proboscis. In this study we provide a redescription of *Neoechinorhynchus doryphorus* and a species description of *Neoechinorhynchus bulborostris* n. sp. Species delineation of *Neoechinorhynchus bulborostris* n. sp. is based on morphological data from whole mounts of the new species and several of its congeners in combination with DNA sequence data for two molecular markers the 28S rDNA of the large ribosomal subunit (LSU) and the internal transcribed space (ITS).

METHODS

Specimen Collection

Upon collection specimens chosen for morphological study from 4 individual *Micropterus* sp. (FR23_311, FR23_620, FR23_738, and FR23_931) and 3 individual *Astronotus ocellatus* (FR23_890, FR23_914, and FR23_915) were relaxed in tap water and then transferred to 70% ethanol for morphological identification. In addition, specimens collected from a single *Micropterus* sp. (FR23_657) were heat-fixed and saved in formalin before being transferred to 70% ethanol. One *Micropterus* sp. (FR23_738) individual contained only *Neoechinorhynchus* doryphorus, the other 5 individual *Micropterus* sp. had coinfections of *N. bulborostris* n. sp. and

N. doryphorus. All individuals of *A. ocellatus* contained only *N. doryphorus*. *Neoechinorhynchus doryphorus* specimens from 2 individual *Micropterus* sp. (FR23_737 and FR23_931) and *N. bulborostris* n. sp. from 2 individual *Micropterus* sp. (FR23_620 and FR23_931) intended for molecular study were saved in 95% molecular-grade ethanol and stored at -70°C.

Morphological Study

Specimens were cleared of debris using a fine brush, poked with an acupuncture needle, stained using filtered Semichon's acetocarmine stain, de-stained using acidic 70% ethanol, neutralized with basic 70% ethanol, dehydrated using a graded ethanol series, cleared in methyl-salicylate, and permanently mounted in Damar gum. The Leica application suite software was used for all measurements which are reported in micrometers and as range, mean, and standard deviation unless otherwise stated. We followed measurement techniques outlined in Richardson (2005) for the proboscis and its armature. Trunk length measurements do not include the proboscis or the bursa in the case of males (Doolin & Reyda, 2018). All width measurements were taken at the widest point. All measured females were gravid and all males were mature with sperm in the seminal vesicle (Doolin & Reyda, 2018). Egg measurements were taken from the body cavity of whole mounted females (Doolin & Reyda, 2018). Clopton (2004) used for shape terminology of morphological structures. Two individuals of each species from FR23_657 were prepared and examined with scanning electron microscopy (SEM). Specimens were cut in half and the proboscides were prepared for SEM following the methods of Reyda and Caira (2006). Slides will be deposited in the United States National Museum, Smithsonian Institute, Washington, D.C. (USNM).

Preparation of Hologenophores

Preparation of hologenophores (i.e. molecular vouchers) followed the methods outlined in Doolin & Reyda (2018) *sensu* Pleijel et al. (2018). Specimens were photographed using a Luminera Infinity 2 digital microscope camera on an Optivision SZ 6745 Stereomicroscope and were cut in half. Proboscides were prepared as whole mounts to serve as vouchers for the sequenced portion of the worms and deposited at the USNM (Table 4).

DNA Isolation, PCR, and Sequencing

We followed the DNA extraction and sequencing procedure outlined in Doolin & Reyda (2018). The posterior portion of the worms were digested overnight at 56 °C in solution with Buffer AE and proteinase K. Extractions were carried out individually using Qiagen® DNeasy tissue kits (Qiagen, Inc., Valencia, California), according to the manufacturer's instructions. We amplified the ITS region and the near complete 28S rDNA. Four separate overlapping PCR fragments of 700-800 bp each were used for the 28S rDNA using the following primers; amplicon 1 forward 5'-CAAGTACCGTGAGGGAAAGTTGC-3' and reverse 5'-CAGCTATCCTGAGGGAAAC-3'; amplicon 2 forward 5'-ACCCGAAAGATGGTGAACTATG-3' and reverse 5'-CTTCTCCAACKTCAGTCTTCAA-3'; amplicon 3 forward 5'-CTAAGGAGTGTGTAACAACTCACC-3' and reverse 5'-AATGACGAGGCATTTGGCTACCTT-3'; and amplicon 4 forward 5'-GATCCGTAACTTCGGGAAAAGGAT-3' and reverse 5'-CTTCGCAATGATAGGAAGAGCC-3' (Garcia-Varela and Nadler, 2005; Doolin and Reyda, 2018). The forward primer BD1 5'-GTCGTAACAAGGTTTCCGTA-3' and reverse primer BD2 5'-TATGCTTAAATTCAGCGGGT-3' were used to amplify the ITS region.

PCRs contained 10 μL total volume with final concentration of 0.3 pmol of each primer, 500 μM dNTPs, 3 mM MgCl, 1 μL of genomic DNA, 0.5 U Biolase DNA Polymerase (Bioline, Inc., Taunton, Massachusetts), manufacturer provided buffers, and nuclease-free distilled water (Doolin & Reyda, 2018). Amplification protocols from Doolin & Reyda (2018) were followed for the ITS region and 28S rDNA. Edited sequences were compared to DNA sequences available on GenBank (NCBI, https://www.ncbi.nlm.nih.gov/genbank) using the BLASTn suite of the BLAST function of the database. Sequences will be deposited in GenBank.

REDESCRIPTION

Neoechinorhynchus doryphorus Van Cleave and Bangham, 1949

(Figs. 1-6)

General description: With characters of the genus *Neoechinorhynchus* as defined by Amin (2002). Trunk of both sexes elongate, widest point approximately one-fourth distance down body, then tapering posteriorly. Dorsal body wall typically with 5 subcuticular giant nuclei, but occasionally 6. Ventral body wall with 1 subcuticular giant nucleus. Dorsal and ventral body wall of approximately equal thickness. Proboscis small relative to body, proboscis excluding neck somewhat wider than long, with 3 rows of 6 hooks each. Anterior lateral hooks markedly large, approximately 1.8 times the length of anterior medial hooks, somewhat posterior to anterior medial hooks. Middle and posterior hooks approximately of equal length. Anterior lateral hook extends to tip of posterior hook. Proboscis receptacle 3-4.7 times length of proboscis, with deeply deltoid to ovoid cerebral ganglion at posterior end of receptacle. Both lemnisci extend past proboscis receptacle. Lemnisci of approximately equal length, 15.5-30.9% length of trunk, averaging 24.1% in males and 19.4% in females. Eggs elliptical with polar prolongations of the fertilization membrane.

Males (measurements based on 19 mature specimens unless otherwise noted; Figs. 1, 3): Trunk 4-7 (5.79±1.03) mm long by 325.75-594.19 (503.7±70.1) wide; maximum width between first and second dorsal giant nuclei (Fig. 2). Dorsal body wall 19.78-64.89 (39.8±12.1) and ventral body wall 22.6-68.1 (43.0±13.0) thick at maximum widths. Proboscis 105.2-131.2 (116.6±6.9) long by 126.4-150.8 (137.1±10.1, n=20) wide (Figs. 1, 6). Neck 27.2-59.4 (46.6±9.5) long by 115.9-144.1 (133.2±8.6) wide. Anterior lateral hooks 88.91-122.45 (109.3±9.0, n=20) long, anterior medial hooks 48.4-76.8 (62.7±7.6, n=20) long; middle hooks 20.3-27.8 (23.1±1.7) long; posterior hooks 10.9-30.5 (21.7±5.7) long (Fig. 1). Proboscis receptacle 3.0-4.7 (3.9±0.5) times proboscis length, 339.8-519.7 (452.6±48.5) long by 116.0-139.5 (129.1±7.0) wide. Uninucleate lemniscus 932.4-2,004.7 (1,385.8±262.3) long by 44.8-141 (89.7±23.3) wide, occupying 15.5-30.9% (24.1%) of trunk length. Posterior margin of uninucleate lemniscus 403.6-1976.9 (1,105.6±410.8) in distance from anterior margin of anterior testis. Testes contiguous, or with slight gap. Anterior testis dolioform to elliptoid, 293.6-793.6 (588.5±146.3) long by 127.6-291.6 (207.9±43.2) wide; posterior testis dolioform, 260.3-708.5 (511.0±124.9) long by 123.8-252.8 (201.6±37.0) wide. Posterior testis usually contiguous with cement gland. Cement gland 408.9-1,482.0 (961.6±306.5) long by 110.7-242.0 (191.9±36.9) wide. Seminal vesicle 226.1-533.2 (388.8±86.2) long by 36.8-108.0 (68.6±18.0) wide. Saefftigen's Pouch 199.5-229.3 (214.4±21.1, n=2) long by 87.7-118.7 (103.2±21.9, n=2) wide, extending into bursa. Bursa 77.9-343.4 (175.9±145.7, n=3) long by 90.6-173.4 (125.2±43.0, n=3) wide.

Females (measurements based on 8 gravid specimens unless otherwise noted; Fig. 2): Trunk 7-11 (9.1±1.1) mm long by 441.0-738.3 (575.8±92.4) wide; maximum width between second and third dorsal giant nuclei. Dorsal body wall 26.1-59.6 (40.0±12.5) and ventral body wall 27.0-57.4 (41.1±11.7) thick at maximum widths. Proboscis 108.4-148.7 (127.3±12.5, n=11) long by 124.1-169.0 (150.2±13.5, n=12) wide. Neck 21.0-63.3 (42.7±13.4, n=11) long by 125.0-173.1 (145.6±12.7, n=11) wide. Anterior lateral hooks 98.1-146.2 (119.8±14.1, n=12) long; anterior medial hooks 46.4-82.9 (64.2±8.5, n=13) long; middle hooks 18.7-28.1 (24.3±3.2, n=10) long; posterior hooks 20.0-30.6 (25.2±3.3, n=10) long. Proboscis receptacle 3.4-4.5 times (4.1±0.4) proboscis length, 418.3-565.5 (514.7±54.5) long by 135.2-163.3 (146.2±9.1) wide. Uninucleate lemniscus 1,425.5-2,090.0 (1724.9±216.8) long by 71.8-127.3 (103.0±10.3) wide, occupying 15.8-21.7% (19.4%) of trunk length. Uterine bell 291.9-446 (387.1±55.9) long; uterus 233.2-386.6 (295.8±48.0) long; vagina long, with rounded posterior end of trunk and terminal genital pore. Reproductive system 579.5-1,024.9 (742.6±160.1) long, occupying 6.4-11.4% (8.4%, n=7) of total body length. Eggs 37.7-56.4 (47.6±5.6, n=10) long by 12.9-19.5 (15.8±1.9, n=10) wide.

Molecular data: Successful sequence data was obtained for the 28S (2,841 bp) and ITS (818 bp consensus) from 2 *N. doryphorus* specimens.

TAXONOMIC SUMMARY

Type host: Jordanella floridae Goode and Bean, 1879

Additional hosts: Micropterus salmoides Lacapède, 1802, *Astronotus ocellatus* Agassiz, 1831; *Fundulus chrysotus* Günther, 1866; *Lepomis macrochirus* Rafinesque, 1819. Cystocanths occur in the viscera of *Fundulus majalis* (Walbaum 1792), *Fundulus seminolis* Girard 1859, *Lepomis macrochirus*; *Lucania goodei* Jordan, 1880.

Type locality: Englewood area, Florida

Other localities: WCA-3B, Everglades, Florida (25°56'30.7" N, 80°26'29.1" W); Lake Okeechobee, Belle Glade, Florida (26°42'12.1" N, 80°43'5.5"); STA-3/4, Everglades, Florida (26°20'18.7" N, 80°37'40.7" W); Chester Lake, South Carolina (34°40'44.4" N, 81°14'34.8")

Site of infection: Intestine.

Type material: Holotype (USNM 1337830); paratypes (USNM 1338315).

Additional material examined: Vouchers FR23_311, FR23_620, FR23_657, FR23_738, FR23_890, FR23_914, FR23_915, and FR23_93; molecular voucher (hologenophore, *sensu* Pleijel et al., 2008) MK238069.

REMARKS

We conducted a survey of freshwater fish parasites in South Florida in an attempt to obtain *N. doryphorus* from its type host, *Jordanella floridae*, near the type locality, south Florida. We were unable to collect *J. floridae* during our survey which included examination of >700 fish representing >30 species. Pintar et al. (2023) documented declines in *J. floridae* numbers across WCA-3A, Taylor Slough, and Shark River Slough. In some cases, this was in association with high numbers of *Monopterus javanensis* and *Hemichromis letourneuxi*, both of which we documented at sites within our survey (Pintar et al., 2023). At several of our sites, African Jewelfish was the only fish species we caught in our minnow traps. Due to documented changes in the *J. floridae* populations, it was unsurprising we were unable to find any. We were able to find *N. doryphorus* in other fish species in our survey. Here we document the occurrence of adult *N. doryphorus* in *Micropterus* sp., *Astronotus ocellatus*, and *Fundulus chrysotus* and the occurrence of cystacanths in *Lucania goodei*, *Fundulus seminolis*, and *Lepomis macrochirus*. All of these fish species are new host records for *N. doryphorus*.

The original description of *N. doryphorus* described a "cone" extending from the neck to the wider trunk (Fig. 6 in Van Cleave and Bangham, 1949; see also Fig. 4). This cone is visible in the original specimens submitted to the museum (USNM 1338315 and others). Specimens of *N. doryphorus* that were heat-fixed and then placed in formaldehyde from our survey (Fig. 5) exhibited a structure consistent with the cone that is visible in the museum specimens (Fig. 4). When Bangham first collected *N. doryphorus* in 1938 he preserved the fish specimens in formaldehyde before examining them for their parasites. In specimens properly fixed, relaxed in cold tap water and then stored in 70% ethanol, there is no cone present. For these reasons we believe the cone in the original description is not a real morphological feature of *N. doryphorus*, but an artifact of the preservation method. During Bangham's 1938 Florida survey only 3 of 71 Flagfish were infected with *N. doryphorus*, and of those only 4 individual worms, 3 adults and 1 juvenile, had their proboscis extended enough to contribute to the description (Van Cleave and Bangham, 1949). Van Cleave and Bangham (1949) did not mention any other *Neoechinorhynchus* species that most closely resembled *N. doryphorus* as the morphological characters used to distinguish *N. doryphorus* as its own species are "unparalleled" and as follows: markedly longer lateral hooks than medial and asymmetry between the right and left lateral hooks. The right lateral hook is described as being longer and more robust than the left lateral hook, which is described as "a fine spinelike blade" (Van Cleave and Bangham, 1949). Due to the dissolved hooks in the museum specimens, we did not observe both lateral hooks on any one individual to confirm what the original description stated. We did not observe any asymmetry in the size or shape between the right and left lateral hooks in our specimens. The report of different shaped lateral hooks in the original description seems to be incorrect and may have been due to damaged proboscis and/or hooks. The proboscis of the *N. doryphorus* holotype

(USNM 1337830) had no hooks present. The paratypes (USNM 1338315) all had little to no hooks present on their proboscis. The few hooks that were still present on a few paratypes were degraded. The voucher specimens that we provide here will complement the type series given that the hooks are degraded in the holotype and paratypes and that various specimens in the type series are otherwise damaged, incomplete, or immature. This species is herein depicted via SEM (Fig. 6) for the first time.

Neoechinorhynchus doryphorus can be distinguished from most of the other species of *Neoechinorhynchus* from fish in North America in the asymmetry of the anterior hooks in which the 2 anterior lateral hooks are markedly larger than each of the 4 anterior medial hooks. It can be distinguished from the 2 species of *Neoechinorhynchus* from fishes in North America that have markedly larger anterior lateral hooks as follows. It can be distinguished from *N. tenellus* and from *N. carinatus* in that its anterior lateral hook lengths are longer (89-146 vs. 80-92 and 31-50, for males and females pooled together, respectively) (Buckner and Buckner, 1993; Amin and Muzzall, 2009).

DNA sequencing of the ITS revealed a match to an unknown *Neoechinorhynchus* species recovered from *Lepomis macrochirus* in Chester Lake, South Carolina. After examining the hologenophore, hook measurements are consistent with *N. doryphorus*. This is a new host record of an adult in *Lepomis macrochirus* and the furthest north that *N. doryphorus* has been documented. This specimen was collected during previous survey work in 2013, but not enough specimens were collected at the time to facilitate a redescription.

DESCRIPTION

Neoechinorhynchus bulborostris n. sp.

(Figs. 7; 9-12)

General description: With characters of the genus *Neoechinorhynchus* as defined by Amin (2002). Trunk of both sexes elongate, with widest point approximately one-third the distance down the body, then tapering posteriorly. Females longer than males. Dorsal body wall with 5 subcuticular giant nuclei, ventral body wall with 1 subcuticular giant nucleus. When mounted worm tends to twist so that the subcuticular giant nuclei are inconspicuous. Dorsal and ventral body wall about equal thickness. Proboscis unusually wide, 1.5 to 2.1 times wider than long, with 2 rows of 6 hooks each. Anterior hooks about 3 times as long as middle hooks. Posterior hooks shortest. Proboscis receptacle approximately 2.1 to 3.5 times proboscis length, with orbicular to elliptoid cerebral ganglion at end of receptacle. Both lemnisci extend past proboscis receptacle and are of equal length. Lemnisci occupy 21.1-42.3% of the trunk, averaging 37% in males and 22.9% in females. Eggs (Fig. 12) elliptical with polar prolongations that expand out into terminal crown-like formations and a rod-like extension in the middle.

Males (measurements based on 6 specimens unless otherwise noted; Figs. 10): Trunk 2.0-4.0 (3.0±0.8, n=4) mm long by 267.4-407.2 (327.0±60.4, n=4) wide; maximum width anterior. Dorsal body 19.0-38.1 (28.5±7.9, n=4) and ventral body wall 11-40.0 (27.0±12.0, n=4) thick at maximum widths. Proboscis 90.9-117.2 (103.3±11.7) long by 170.1-205.9 (186.4±16.5) wide. Neck 34.0-48.4 (41.4±5.2) long by 101.0-150 (130.1±20.4) wide. Proboscis 1.5-2.1 (1.8±0.2) times wider than long and proboscis width 1.3-1.7 (1.4±0.1) times neck width. Anterior lateral hooks 61.2-87.1 (76.5±8.2) long, anterior medial hooks 59.5-81.6 (69.4±6.1) long, middle hooks 16.9-27.7 (22.4±4.3, n=5) long, and posterior hooks 11.9-21.2 (18.2±3.8, n=5) long (Fig. 5). Proboscis receptacle 2.1-3.5 (2.9±0.5) times proboscis length, 242.2-368.2 (301.6±50.1) long by 69.7-142.6 (106.5±28.3) wide. Uninucleate lemniscus 971.3-1693.5 (1250.9±387.7, n=4) long by

34.8-100.5 (66.5±32.9, n=4) wide, occupying 32.3-42.3% (37.0%) of trunk length. Posterior margin of uninucleate lemniscus 0-953.5 (407.8±491.5, n=3) in distance from anterior margin of anterior testis. Testes contiguous, or with slight gap. Anterior testes dolioform, 201.9-500.7 (297.1±121.8, n=5) long by 111.7-180.0 (135.2±27.8, n=5) wide; posterior testes dolioform, 158.0-456.8 (277.1±125.2, n=5) long by 109.1-227.0 (144.6±49.9, n=5) wide. Posterior testes usually contiguous with cement gland. Cement gland 351.8-1001.2 (609.3±240.1, n=5) long by 98.3-232.2 (137.4±53.8, n=5) wide. Seminal vesicle 189.9-367.8 (267.9±76.1, n=4) long by 46.5-123.5 (73.8±34.5, n=4) wide. Saefftigen's Pouch 135.6-147.4 (141.5±8.3, n=2) long by 68.5-98.5 (83.5±21.3, n=2) wide, extending into bursa. Bursa 91.8-106.4 (99.1±10.4, n=2) long by 82.8-149.2 (116.0±47.0, n=2) wide. Total reproductive system 1369.3-3291.7 (2095.1±832.3, n=4) long, occupying 59.8-82.3% (68.7%, n=4) of trunk length.

Females (measurements based on 4 specimens unless otherwise noted; Fig. 11): Trunk 6 mm long by 280.3-425.3 (357.4±71.7) mm wide (n=2); maximum width between second and third dorsal giant nuclei. Dorsal body wall 21.9-30.8 (26.3±6.3, n=2) and ventral body wall 19.9-33.1 (26.5±9.3, n=2) thick at maximum widths. Proboscis 95.4-135.9 (111.3±17.2) long by 176.6-224.2 (198.0±19.6) wide. Neck 35.4-56.0 (44.8±9.0) long by 106.1-155.4 (130.6±22.3) wide. Proboscis 1.6-2.1 (1.8±0.2) wider than long, and proboscis width 1.4-1.7 (1.5±0.1) times neck width. Anterior lateral hooks 72.2-96.1 (84.4±8.8) long, anterior medial hooks 57.2-75.3 (67.6±7.0) long, middle hooks 20.9-28.7 (24.1±3.3) long, and posterior hooks 18.5-22.6 (20.2±1.8) long. Proboscis receptacle times 2.1-2.8 (2.4±0.3) proboscis length, 228.3-319.8 (271.2±46.3) long by 70.5-136.11 (107.1±31.9) wide. Uninucleate lemniscus 1269.4-1474.3 (1371.83±144.9, n=2) long by 61.8-91.3 (76.6±20.9, n=2) wide, occupying 21.1-24.5% (22.9%, n=2) of trunk length. Uterine bell 305.2-451.4 (378.3±103.4, n=2) long, uterus 208.9-211.8 (210.4±2.0, n=2) long; vagina long, with rounded posterior end of trunk and terminal genital pore. Reproductive system 562.4-763.8 (663.1±142.4) long, occupying 9.4-12.7% (11.1%, n=2) of total body length. Eggs 42.4-60.8 (51.5±7.9) long by 12.0-19.4 (15.4±3.2) wide (Fig. 7).

Molecular data: Successful sequence data was obtained for the 28S (2,841 bp) and ITS (818 bp consensus) from 2 *N. bulborostris* specimens.

TAXONOMIC SUMMARY

Type Host: Micropterus salmoides (Lacepède, 1802).

Additional Hosts: None.

Prevalence: 4 of 15 *M. salmoides* examined.

Type Locality: WCA-3B, Everglades, Florida (25°56'30.7" N, 80°26'29.1" W)

Other Localities: Lake Okeechobee, Belle Glade, Florida (26°42'12.1" N, 80°43'5.5"); STA-3/4, Everglades, Florida (26°20'18.7" N, 80°37'40.7" W)

Site of infection: Intestine.

Type material: FR23_311, FR23_620, FR23_657, FR23_931

Material examined: N. cylindratus Van Cleave 1919, USNM 1396441

Etymology: The species is named for the unusually wide proboscis with *bulbo*- meaning bulbous and -*rostris* indicating the proboscis.

REMARKS

During our survey, *Micropterus salmoides* was found to host two species of *Neoechinorhynchus*: *N. doryphorus* and *N. bulborostris*. *Neoechinorhynchus bulborostris* has markedly shorter anterior lateral hooks than *N. doryphorus* (70 vs. 113) and a wider proboscis.

Given its globular proboscis and body shape, *N. bulborostris* most closely resembles *Neoechinorhynchus cylindratus* (Van Cleave 1913) a widely reported species for which *M. salmoides* is also the type host.

Neoechinorhynchus bulborostris is easily distinguished from all but 14 members of the genus in fishes in North America by its possession of equal length lemnisci (i.e. *Neoechinorhynchus tenellus, N. doryphorus, Neoechinorhynchus saginatis, Neoechinorhynchus strigosis, Neoechinorhynchus crassus, Neoechinorhynchus tumidus, Neoechinorhynchus rutilli, Neoechinorhynchus salmonis, Neoechinorhynchus didelphus, Neoechinorhynchus limi, Neoechinorhynchus pungitius, Neoechinorhynchus cylindratus,* and *Neoechinorhynchus rostratum*). *Neoechinorhynchus bulborostris* can be distinguished from *N. tenellus* and *N. doryphorus* by having symmetrical anterior hooks vs. enlarged anterior lateral hooks (Van Cleave and Bangham, 1949; Amin and Muzzall, 2009). *Neoechinorhynchus bulborostris* can be distinguished from *N. saginatus, N. tumidus*, and *N. crassus,* by body shape and size. *Neoechinorhynchus bulborostris* is 0.267-0.425 mm wide while *N. saginatis* is 1.25-2.1 mm and *N. tumidus* is 0.87-2.5 mm (Van Cleave and Bangham, 1949). *Neoechinorhynchus bulborostris* has a thinner body wall (11-40 μm) than *N. crassus* (80-100 μm) which is described as short and thick (Van Cleave, 1919). Additionally, *N. crassus* has longer lemnisci which overlap the testes in males while the lemnisci of *N. bulborostris* stop 407 μm before the anterior testes.

Neoechinorhynchus bulborostris can be distuinguished from *N. strigosis*, *N. salmonis*, and *N. rutilli* based on trunk length. *Neoechinorhynchus strigosis* (males 3.7-5.5 mm and females 9-14 mm), *N. salmonis* (males 4.5-7.8 mm and females 5.8-14.5 mm), and *N. rutilli*, (males 7 mm and females 10 mm) are longer than *N. bulborostris*, (males 2-4 mm and females 6 mm) (Van Cleave, 1949; Ching, 1984). *Neoechinorhynchus bulborostris* can be distuinguished from

N. didelphis by having one uterine bell instead of two (Amin, 2001). *Neoechinorhynchus bulborostris* differs from *Neoechinorhynchus limi* and *Neoechinorhynchus pungitius* by having longer anterior hooks. Anterior hooks for *N. bulborostris* measure 61-96 µm compared with 29-39 µm for *N. limi* and 36-56 µm for *N. pungitius* (Muzzal and Buckner,1982; Dechtiar, 1971).

Neoechinorhynchus bulborostris (Fig. 7) differs from *Neoechinorhynchus cylindratus* (Fig. 8) and *Neoechinorhynchus rostratum* by having a shorter and wider proboscis. The length and width of *N. bulborostris* proboscis is 111x198 µm for females and 103x186 µm for males in comparison with 140x176 µm for females and 136x165 µm for males of *N. cylindratus* and 150x174 µm for females and 137x155 for males of *N. rostratum* (Amin and Bullock, 1998). The new species further differs from *N. cylindratus* and other species of fish parasitizing *Neoechinorhynchus* in North America in that its proboscis is both wide and fleshy, with thickened walls (Figs. 7, 9).

In addition, *N. bulborostris* can be distinguished from each previously mentioned species with equal length lemnisci as well as all of the North American *Neoechinorhynchus* species with unequal length lemnisci (i.e. *N. bullocki, N. cristatus, N. carinatus, N. buckneri, N. prolixus, N. prolixoides, N. carpiodi, N. distractus, N. idahoensis, N. notemigoni, N. robertbaueri,* and *N. venustus*) in its possession of a unique egg morphology (Fig. 12). Of all species of *Neoechinorhynchus* that parasitize fish in North America *N. bulborostris* most closely resembles *N. cylindratus* in its body shape and size, host use, and its proboscis shape. *Neoechinorhynchus bulborostris* also differs from *N. cylindratus* in egg morphology as *N. cylindratus* eggs have no polar prolongations of the fertilization membrane. In fact, the egg morphology of *N. bulborostris* is so unique it not only distinguishes it from all fish *Neoechinorhynchus* but it also distinguishes it from turtle *Neoechinorhynchus* species which have highly variable egg morphology (see Barger and Nickol, 2004).

During the survey conducted in Florida, *N. bulborostris* was found only in *Micropterus salmoides* despite extensive survey work including many other fish species (n=31) that hosted other *Neoechinorhynchus* species, indicating it may have strict host specificity (*sensu* Euzet Combes, 1980). The life cycle of this species is unknown as no cystacanth stages of the species was found in any of the fish species examined during the survey.

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LITERATURE CITED

Amin, O.M. 2001. *Neoechinorhynchus didelphis* sp. n. (Acanthocephala: Neoechinorhynchidae) from the Redfin Pickeral, *Esox americanus*, in Georgia, U.S.A. Comparative Parasitology 68: 103-107.

Amin, O.M. 2002. Revision of *Neoechinorhynchus* Stiles & Hassall, 1905 (Acanthocephala: Neoechinorhynchidae) with keys to 88 species in two subgenera. Systematic Parasitology 53: 1-18.

Amin, O.M. and W.L. Bullock. 1998. *Neoechinorhynchus rostratum* sp. n. (Acanthocephala: Neoechinorhynchidae) from the Eel, *Anguilla rostrata*, in estuarine waters of Northeastern North America. Journal of the Helminthological Society of Washington 65: 169-173.

Amin, O.M. and O. Sey. 1996. Acanthocephala from Arabian Gulf fishes off Kuwait, with descriptions of *Neoechinorhynchus dimorphospinus* sp. n. (Neoechinorhynchidae), *Tegorhynus holospinosus* sp. n. (Illiosentidae), *Micracanthorhynchina kuwaitensis* sp. n. (Rhadinorhynchidae), and *Slendrorhynchus breviclaviproboscis* gen. n., sp. n. (Diplosentidae); and key to species of the genus *Micracanthorhynchina*. Journal of the Helminthological Society of Washington 63: 201-210.

Amin, O.M. and P.M. Muzzall. 2009. Redescription of *Neoechinorhynchus tenellus* (Acanthocephala: Neoechinorhynchidae) from *Esox Lucius* (Esocidae) and *Sander vitreus* (Percidae), among other Percid and Centrarchid fish, in Michigan, U.S.A. Comparative Parasitology 76: 44-50.

Bangham, R.V. 1939. Parasites of centrarchidae from Southern Florida. Transactions of the American Fisheries Society 68: 263-268.

Bangham, R. V. 1940. Parasites of fresh-water fish of Southern Florida. Florida Academy of Sciences 5: 289–307.

Barger, M.A. 2005. A new species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) from turtles in Florida, USA. Comparative Parasitology 72: 6-9. doi: 10.1654/4167.

Barger, M. A., and B. B. Nickol. 2004. A key to the species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) from turtles. Comparative Parasitology. 71: 4-8.

Bucker, R.L. and S.C. Buckner. 1993. Description of *Neoechinorhynchus carinatus* n. sp. (Acanthocephala: Neoechinorhynchidae) from the Sharpfin Chubsucker, *Erimyzon tenuis*, of Louisiana and Mississippi. The Journal of Parasitology 79: 32-36.

Bullock, W. L. 1960. Some acanthocephalan parasites of Floridae fishes. Bulletin of Marine Science of the Gulf and Caribbean 10: 481-484.

Ching, H.L. 1984. Description of *Neoechinorhynchus salmonis* sp. n. (Acanthocephala: Neoechinorhynchidae) from freshwater fishes of British Columbia. Journal of Parasitology 70: 286-291.

Clopton, R.E. 2004. Standard nomenclature and metrics of plane shapes for use in gregarine taxonomy. Comparative Parasitology 71: 130-140.

Dechtiar, A.O. 1971. *Neoechinorhynchus pingitius* n. sp. (Acanthocephala: Neoechinorhynchidae) from ninespine stickleback of Lake Huron. Canadian Journal of Zoology 49: 483-486.

Doolin, M.L. and F.B. Reyda. 2018. A new species of *Neoechinorhynchus* (Acanthocephala: Neoechinorhynchidae) from White Sucker (*Catostomus commersonii*) in New York. Journal of Parasitology 104: 671-678. doi: 10.1645/18-94.

Fricke, R., Eschmeyer, W. N. & Van der Laan, R. (eds) 2024. ESCHMEYER'S CATALOG OF FISHES: GENERA, SPECIES, REFERENCES. (http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Ele ctronic version accessed 05 MAY 2024.

Hoffman, G. L. 1999. Parasites of North American freshwater fishes, 2nd ed. Comstock Publishing Associates, Ithaca, New York, U.S.A. 576 p.

Muzzall, P.M. and R.L. Buckner. 1982. *Neoechinorhynchus limi* sp. n. (Acanthocephala: Neoechinorhynchidae) from the Central Mudminnow, *Umbra limi*. Proceedings of the Helminthological Society of Washington 49: 231-234.

Pleijel, R., U. Jondelius, E. Norlinder, A. Nygeren, B. Oxelman, C. Schander, P. Sundberg, and M. Thollesson. 2008. Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. Molecular Phylogenetics and Evolution 48: 369–371.

Sarabeev, V., Ie. Tkach, R.A. Sueiro, and J. Leiro. 2020. Molecular data confirm the species status of *Neoechinorhynchus personatus* and *N. yamagutii* (Acanthocephala, Neoechinorhynchidae) from the Atlantic and Pacific grey mullets (Teleostei, Mugilidae). Zoodiversity 54: 1-10.

Van Cleave, H J. 1919. Acanthocephala from the Illinois River, with descriptions of species and a synopsis of the family Neoechinorhynchidae. Bulletin of the Illinois Natural History Survey, 13, 225-257.

Van Cleave, H.J. 1949. The acanthocephalan genus *Neoechinorhynchus* in the catastomid fishes of North America, with descriptions of two new species. The Journal of Parasitology 35: 500-512.

Van Cleave, H.J. and R.V. Bangham. 1949. Four new species of acanthocephalan family Neoechinorhynchidae from freshwater fishes of North America, one representing a new genus. Journal of the Washington Academy of Sciences 39: 398-409.

Figure legend.

Figure 1. Line drawing of proboscis of *Neoechinorhynchus doryphorus.* Line drawing of proboscis of FR23_931-2.

Figure 2. Composite series of light micrograph of female *N. doryphorus.* Light micrograph of complete female *N. doryphorus*.

Figure 3. Composite series of light micrograph of male *N. doryphorus.* Light micrograph of complete male *N. doryphorus*.

Figure 4. Proboscis and "cone" of *N. doryphorus* (paratype, USNM 1338415). Light micrograph of proboscis and "cone" of USNM paratype of *N. doryphorus*.

Figure 5. Proboscis and "cone" of *N. doryphorus* (voucher, FR23_657).

Figure 6. Scanning electron micrograph of proboscis of *N. doryphorus* with anterior lateral hook in center.

Figure 7. Scanning electron micrograph of proboscis of *N. bulborostris* with anterior lateral hook in center.

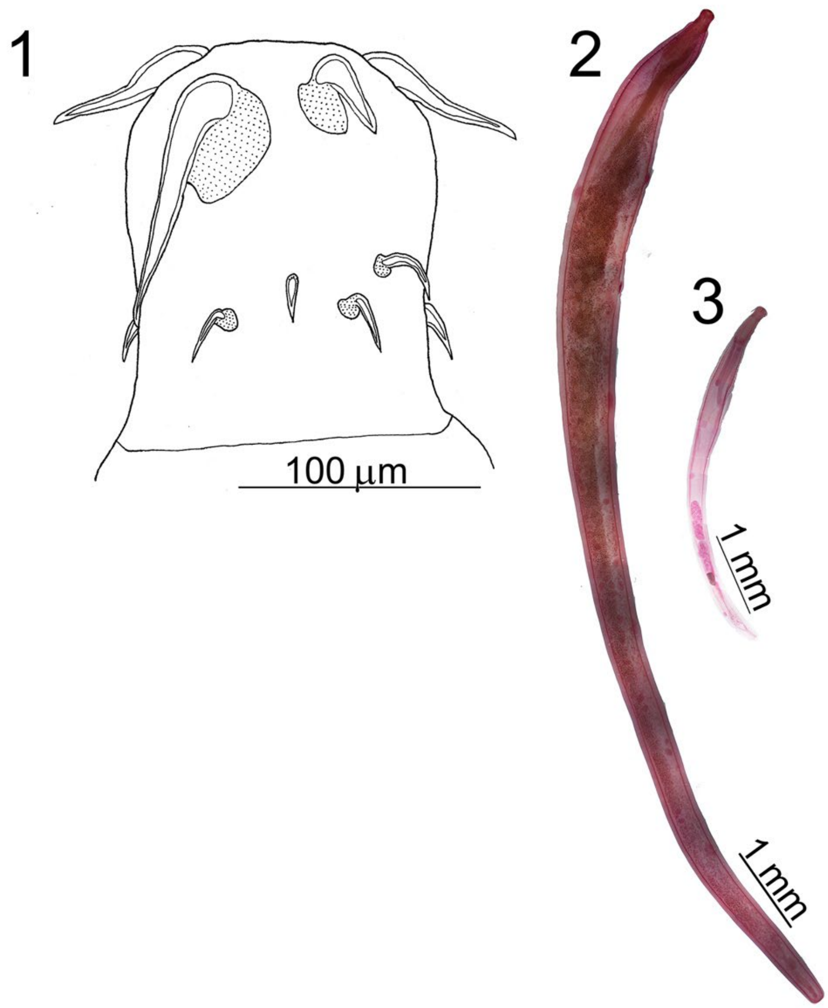
Figure 8. Scanning electron micrograph of proboscis of *N. cylindratus* with anterior lateral hook in center.

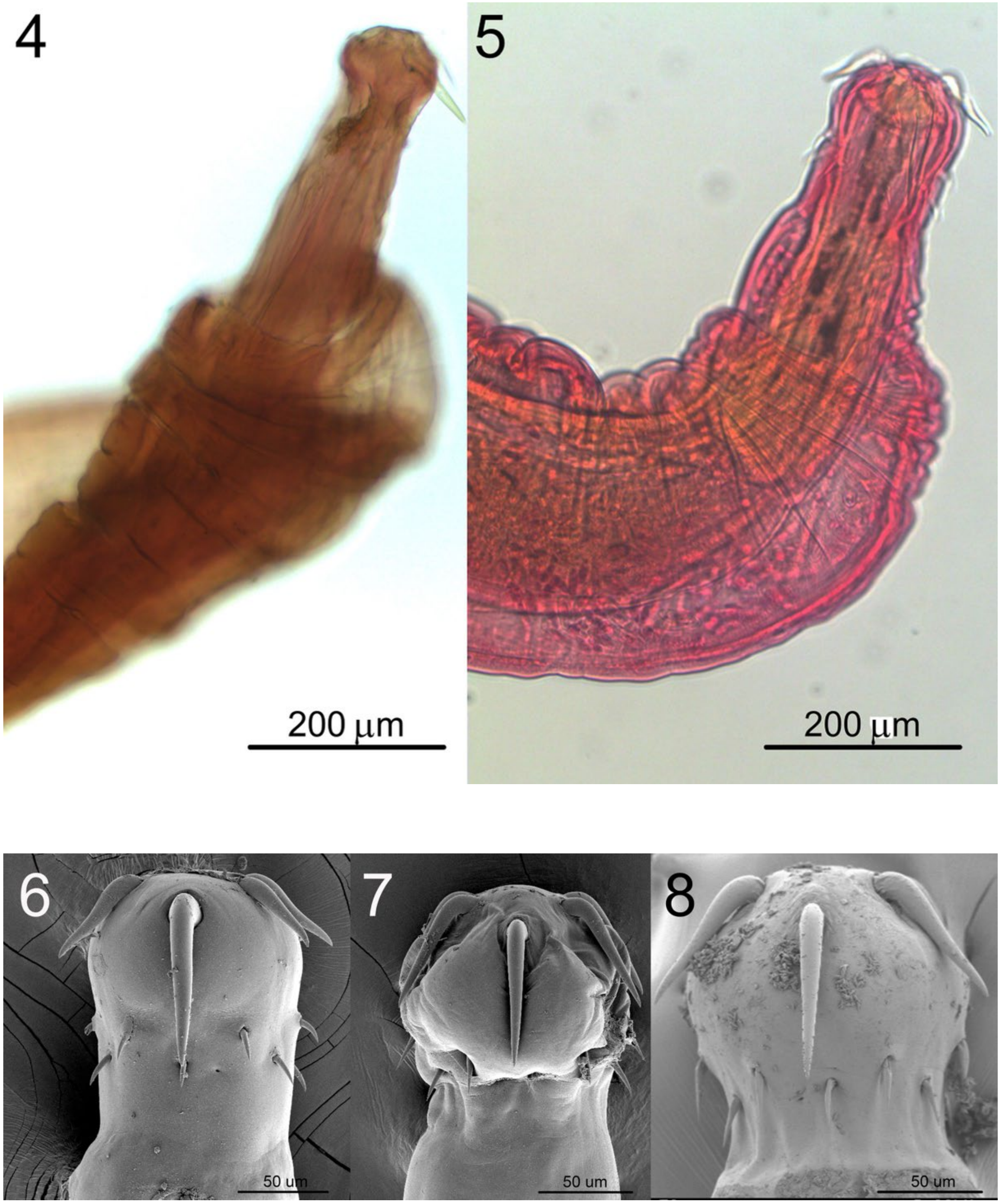
Figure 9. Line drawing of proboscis of *Neoechinorhynchus bulborostris.* Line drawing of proboscis of FR23_620-1.

Figure 10. Composite series of light micrograph of female *N. bulborostris.* Light micrograph of complete female *N. bulborostris*.

Figure 11. Composite series of light micrograph of male *N. bulborostris.* Light micrograph of complete male *N. bulborostris*.

Figure 12. Line drawing of eggs of *N. bulborostris* as viewed within body cavity. Three eggs from the body cavity of *N. bulborostris* are drawn. Appearance differs slightly with focus and position of eggs.

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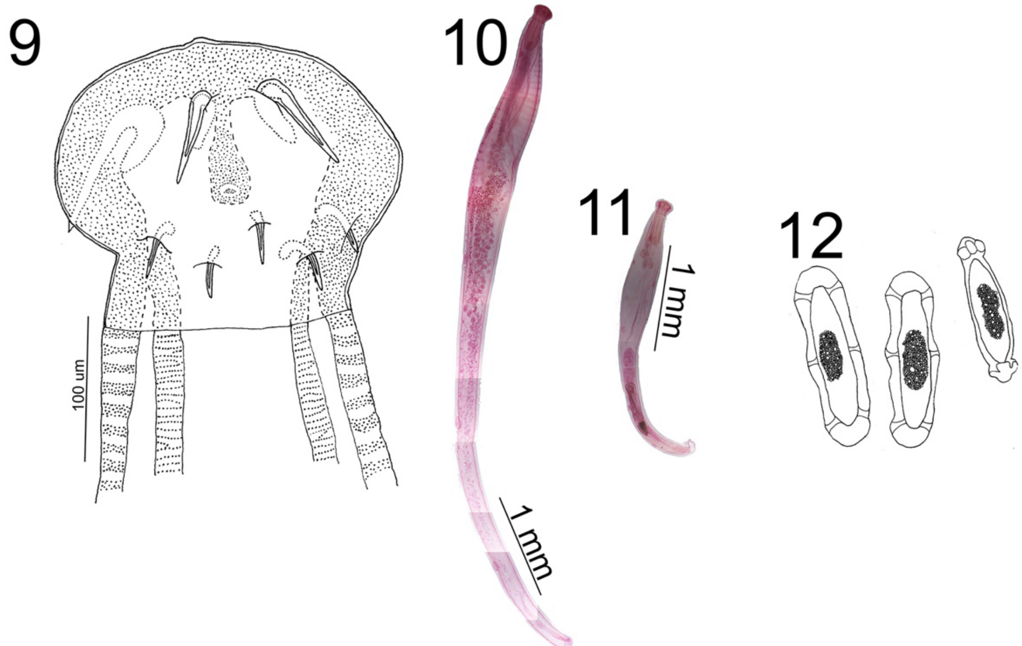
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