Phylogenetic Position of Neogogatea (Diplostomoidea; Cyathocotylidae)

Authors: Achatz, Tyler J., Holten, Zoe S. Von, Calhoun, Dana M., Johnson, Pieter T. J., Pritchard, Jonathon, et al.

Source: Journal of Parasitology, 111(6) : 703-708

Published By: American Society of Parasitologists

URL: https://doi.org/10.1645/24-152

The BioOne Digital Library (https://bioone.org/) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (https://bioone.org/subscribe), the BioOne Complete Archive (https://bioone.org/archive), and the BioOne eBooks program offerings ESA eBook Collection (https://bioone.org/esa-ebooks) and CSIRO Publishing BioSelect Collection (https://bioone.org/csiro-ebooks).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commmercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Journal-of-Parasitology on 25 May 2026
Terms of Use: https://bioone.org/terms-of-use Access provided by University of Colorado Boulder Libraries - University of Colorado System

Journal of Parasitology 2025 111(6) 703–708

Contents and archives available through www.bioone.org or www.jstor.org

Journal of Parasitology

journal homepage: www.journalofparasitology.org

DOI: 10.1645/24-152

PHYLOGENETIC POSITION OF *NEOGOGATEA* (DIPLOSTOMOIDEA; CYATHOCOTYLIDAE)

Tyler J. Achatz[1], Zoe S. Von Holten[1,2], Dana M. Calhoun[3], Pieter T. J. Johnson[3], Jonathon Pritchard[4], Matthew Keel[4], and Vasyl V. Tkach[5]

[1] Department of Natural Sciences, Middle Georgia State University, 100 University Parkway, Macon, Georgia 31206.
[2] Department of Biology, Georgia Southern University, 4324 Old Register Road, Statesboro, Georgia 30460.
[3] Ecology and Evolutionary Biology, University of Colorado, 1900 Pleasant Street, Boulder, Colorado 80309.
[4] Georgia Department of Natural Resources, 2067 US-278, Social Circle, Georgia 30025.
[5] Department of Biology, University of North Dakota, 10 Cornell Street Stop 9019, Grand Forks, North Dakota 58202.
Correspondence should be sent to Vasyl V. Tkach (https://orcid.org/0000-0001-5084-7566) at: *vasyl.tkach@und.edu*

KEY WORDS

Diplostomoidea
Cyathocotylidae
Neogogatea
Pandion
Osprey
Molecular phylogeny

ABSTRACT

Neogogatea Chandler and Rausch, 1947, is a small genus of cyathocotylid digeneans parasitic in the intestines of birds of prey. The genus contains only 3 species: 2 from the Nearctic and 1 from the Palearctic. No sequence data from mature, properly identified representatives of *Neogogatea* were available until now. In the present study, we collected mature adult *Neogogatea kentuckiensis* (Cable, 1935) Hoffman and Dunbar, 1963, from osprey in Georgia, Florida, and Delaware, and sequenced fragments of the nuclear large ribosomal subunit (*28S*) gene and cytochrome *c* oxidase subunit I (*COI*) mtDNA gene. We used *28S* sequence data for phylogenetic inference and *COI* data for comparisons at the species level. In the phylogenetic tree, *N. kentuckiensis* was situated in a clade with representatives of the genera *Mesostephanus* Lutz, 1935, *Braunina* Wolf, 1903, and an unidentified cyathocotylid cercaria from Australia. The observed variability in the *COI* gene between our isolates suggests the need for collecting fresh, well-fixed, and mature specimens from a broader geographic range to test for the presence of more than 1 *Neogogatea* species in New World osprey.

Neogogatea Chandler and Rausch, 1947, is a small genus of cyathocotylids parasitic as adults in the intestines of birds of prey in the Nearctic and Palearctic (Chandler and Rausch, 1947, 1948; Hoffman and Dunbar, 1963; Zazornova, 1995). Chandler and Rausch (1947) erected the genus for *Neogogatea bubonis* Chandler and Rausch, 1947, from great horned owl *Bubo virginianus* (Gmelin) collected from Wisconsin. Dwivedi (1967) considered the genus to be synonymous with *Gogatea* Lutz, 1935, based on morphology. Dubois (1975) maintained *Neogogatea* and *Gogatea* as separate genera. Only 3 species are currently recognized: (1) *N. bubonis* (type species); (2) *Neogogatea kentuckiensis* (Cable, 1935) Hoffman and Dunbar, 1963 (syn. *Neogogatea pandionis* Chandler and Rausch, 1948) from osprey *Pandion haliaetus* in the United States (Chandler and Rausch, 1948; Hoffman and Dunbar, 1963); and (3) *Neogogatea rauschi* Zazornova, 1995, from white-tailed eagle *Heliaetus albicilla* (L.) in Russia (Zazornova, 1995).

Achatz et al. (2019) generated the first DNA sequence attributed to a species of *Neogogatea*; however, it originated from an immature specimen collected from a hooded merganser *Lophodytes cucullatus* (L.) in Mississippi. More recently, Sokolov et al. (2024) demonstrated a close phylogenetic affinity between *Neogogatea* sp. of Achatz et al. (2019) and *Paracoenogonimus ovatus* Katsurada, 1914, which suggests that *Neogogatea* sp. may have been misidentified. In the present study, we collected and sequenced fragments of the nuclear large ribosomal subunit (*28S*) gene, internal transcribed spacer 2 (*ITS2*) region, and cytochrome *c* oxidase subunit I (*COI*) mtDNA gene of mature adult *N. kentuckiensis* from osprey in the United States. Molecular data combined with reevaluation of morphological characters, such as the presence or absence of ventral sucker, relative size of pharynx to oral sucker, and the presence or absence of the vaginal sphincter, allowed us to confirm the status of *Neogogatea* and *Gogatea* as separate genera as well as analyze relationships of *Neogogatea* with other cyathocotylid taxa.

MATERIALS AND METHODS

Neogogatea kentuckiensis specimens were obtained from 2 ospreys collected from Lake Seminole, Silver Lake Wildlife Management Area, Decatur County, Georgia (30°48′55.6″N, 84°45′28.9″W). Live digeneans were rinsed in saline, killed in hot water, and immediately fixed in 80% ethanol. Additional specimens were obtained from frozen carcasses of ospreys in Sussex County, Delaware (38°35′29.4″N, 75°17′28.7″W) and Sumter County, Florida (28°55′37.2″N, 82°00′13.7″W), donated by the Tri-State Bird

Table I. Geographical origin (U.S. state), GenBank accession numbers of sequences, and Museum of Southwestern Biology Division of Parasites (MSBP) accession numbers of *Neogogatea kentuckiensis* collected from *Pandion haliaetus* in the present study.

Geographic origin	Museum number	GenBank accession numbers		
		ITS2	*28S*	*COI*
Delaware	—*	PV755389	PV755389	PV755400
Florida	—	PV755390	—	PV755401
Georgia	MSB:Para:51937	PV755391	PV755391, PV755392	PV755402– PV755404

* Dash (—) indicates no slides and/or sequence is deposited.

Rescue and Rehabilitation, the Clinic of the Rehabilitation of Wildlife (CROW), and the Audubon Center of Birds of Prey. After thawing, dead digeneans were removed from the intestine and immediately fixed in 80% ethanol. Freshly fixed, high-quality specimens from Georgia were used in the morphological and molecular study, while those from Delaware and Florida were used for only the molecular study.

Specimens used for light microscopy were stained with aqueous alum carmine according to the protocol of Lutz et al. (2017) and studied using a DIC-equipped Olympus BX53 microscope (Olympus Corp., Tokyo, Japan). Voucher specimens are deposited in the Museum of Southwestern Biology Division of Parasites (Table I).

DNA was extracted from entire specimens according to the protocol provided by Tkach and Pawlowski (1999). The fragments of *28S* and *ITS2* rDNA, as well as *COI* mtDNA, were amplified by polymerase chain reactions (PCR). The PCR amplifications of *28S* were performed using the forward primer digL2 (5′-AAG CAT ATC ACT AAG CGG-3′) and reverse primer 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) (Tkach et al., 2003); the *ITS2* region was amplified using forward primer d58f (5′-GCG GTG GAT CAC TCG GCT CGT G-3′) and reverse primer 300R (5′-CAA CTT TCC CTC ACG GTA CTT G-3′) (Snyder and Tkach, 2007; Kudlai et al., 2015); the *COI* fragment was amplified using forward primer Dipl_Cox_5′ (5′-ACK TTR GAW CAT AAG CG-3′) and 1 of the following reverse primers Dipl650R (5′-CCA AAR AAY CAR AAY AWR TGY TG-3′) or Dipl_Cox_3′ (5′-WAR TGC ATN GGA AAA AAA CA-3′) (Achatz et al., 2021). PCRs were carried out in a total volume of 25 µl using One-Taq quick load PCR mix from New England Biolabs (Ipswich, Massachusetts) with annealing temperatures of 53 C (*28S*) and 45 C (*COI*).

The PCR amplicons were purified using an Illustra ExoProStar PCR clean-up enzymatic kit (Cytiva, Marlborough, Massachusetts) and cycle sequenced using a BrightDye Terminator Cycle Sequencing Kit (MCLAB, South San Francisco, California). Sequencing reactions were purified using a BigDye Sequencing Clean Up Kit from MCLAB, then run on an ABI 3130 automated capillary sequencer (Thermo Fisher Scientific, Waltham, Massachusetts). The PCR primers were used for sequencing reactions. Contiguous sequences were assembled using Sequencher 4.2 software (GeneCodes Corp., Ann Arbor, Michigan) and deposited in GenBank (Table I).

The *28S* sequence of *N. kentuckiensis* was aligned with 24 previously published sequences of cyathocotylids using ClustalW as implemented in MEGA7 software (Kumar et al., 2016). The alignment was trimmed to the length of the shortest sequence included in the analysis (1,085 bp); 38 nucleotide positions with ambiguous homology were excluded from the analyses. *Harmotrema laticaudae* Yamaguti, 1933, was used as the outgroup in the analysis, based on the tree topology published by Pérez-Ponce de León and Hernández-Mena (2019). The general time-reversible model with estimates of invariant sites and gamma-distributed among-site variation (GTR + G + I) model was determined to be the best-fitting nucleotide substitution model using MEGA7. The phylogenetic analysis was conducted using Bayesian Inference as implemented in MrBayes v3.2.6 software (Ronquist and Huelsenbeck, 2003) with Markov chain Monte Carlo (MCMC) chains run for 3,000,000 generations with a sample frequency set at 1,000. The length of analysis was considered sufficient because the standard deviation stabilized below 0.01. Log-likelihood scores were plotted, and only the final 75% of trees were retained to produce the consensus trees.

RESULTS

Molecular phylogeny

The topology and supports of the phylogeny resulting from the analysis of *28S* were essentially identical to those published in recent studies (Fig. 1; Achatz et al., 2024; Sokolov et al., 2024). *Neogogatea kentuckiensis* was situated in a weakly supported clade as a sister species to an unknown cyathocotylid cercaria from Australia. These 2 taxa were positioned as a clade in a 99% supported polytomy that also included the clades of *Braunina* spp. (100% supported) and *Mesostephanus* spp. (100% supported).

Similar to the results of Sokolov et al. (2024), in our phylogenetic tree, the *Neogogatea* sp. of Achatz et al. (2019) was positioned away from *N. kentuckiensis* in a 100% supported clade together with *P. ovatus*. These 2 species were situated in a 99% supported larger clade that also included *Holostephanoides ictaluri* (Vernberg, 1952) and *Gogatea* spp. (Fig. 1).

DISCUSSION

Neogogatea kentuckiensis was originally described as *Cercaria kentuckiensis* Cable, 1935, based on cercariae from *Pleurocera semicarinata* (Say) (referred to as *Goniobasis semicarinata*) in Kentucky (Cable, 1935). *Cercaria kentuckiensis* was later considered *N. kentuckiensis*. Several subsequent authors (Anderson, 1944; Vernberg, 1952; Hoffman, 1959; Myer, 1960; Hoffman and Dunbar, 1963) have collected and described all life stages of *N. kentuckiensis* from various hosts (first intermediate host: pleurocerid snails *Pleurocera* and *Leptoxis* spp.; second intermediate host: centrarchid, cyprinid, and salmonid fishes; definitive host: osprey). Hoffman and Dunbar (1963) reported moderate to severe pathogenicity as well as mortality caused by *N. kentuckiensis* in centrarchid and salmonid fishes, particularly young individuals.

We would like to address some of the controversies related to certain morphological features of *Neogogatea* spp. and their taxonomic value. Chandler and Rausch (1947, 1948) stated that *Neogogatea* spp. lack a ventral sucker. In these digeneans, the holdfast organ often partially or entirely overlaps the ventral sucker, which could lead researchers to mistakenly assume the ventral sucker is missing. Hoffman and Dunbar (1963) provided descriptions of cercarial, metacercarial, and adult *N. kentuckiensis* and noted that the ventral sucker was apparent in metacercariae, but only feebly developed (but present) in adults. Similarly, our

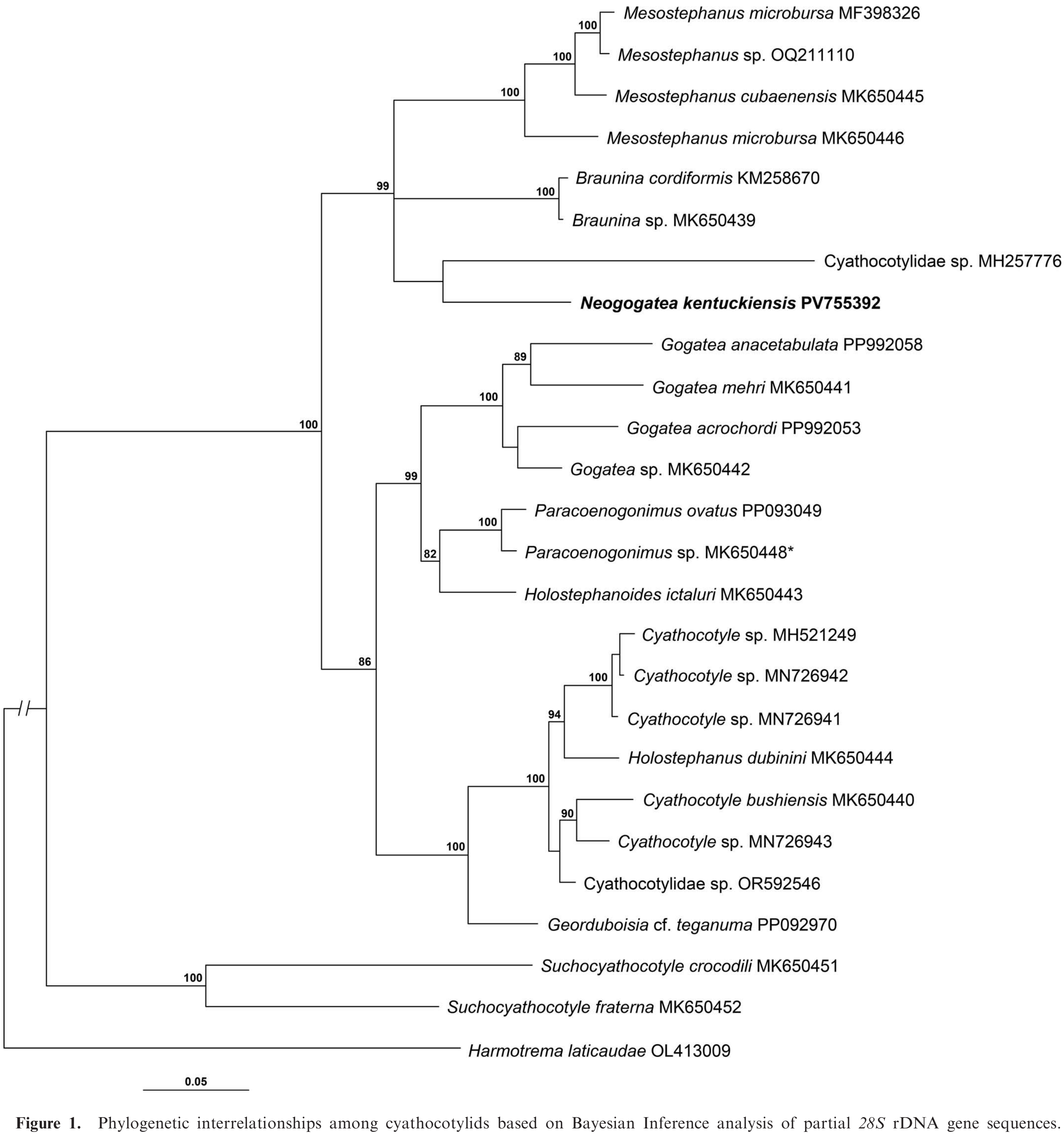

Figure 1. Phylogenetic interrelationships among cyathocotylids based on Bayesian Inference analysis of partial *28S* rDNA gene sequences. Posterior probabilities are provided above internodes; values below 80% are not shown. The new *28S* sequence generated in this study is in bold. The scale bar indicates the number of substitutions per site. GenBank accession numbers are provided after the names of taxa. *Formerly identified as *Neogogatea* sp. of Achatz et al. (2019).

specimens have a feebly developed, or essentially absent, ventral sucker (Fig. 2a, b, d). This situation is reminiscent of that in the closely related genus *Gogatea*. Although *Gogatea* spp. typically have a ventral sucker, at least 1 species, *Gogatea anacetabulata* Achatz, Von Holten, Binh and Tkach, 2024, does not have it (Achatz et al., 2024). Hence, the presence or absence of the ventral sucker cannot be considered among the features that can be used to reliably distinguish between these genera.

Dubois (1975) noted that *Gogatea* spp. tend to have a pharynx similar in size or smaller than the oral sucker, while the pharynx in *Neogogatea* spp. is larger than the oral sucker. However, in the

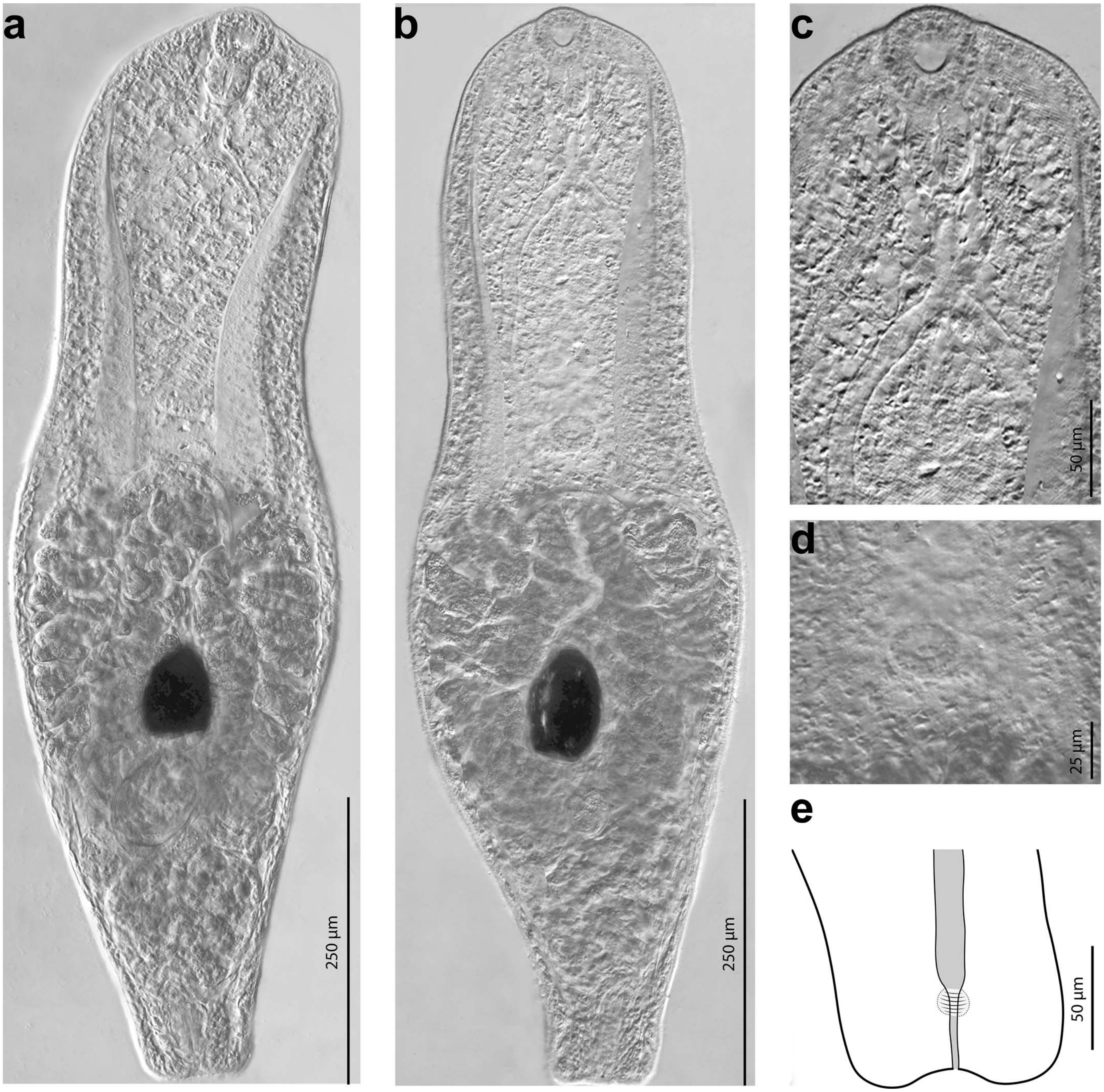

Figure 2. *Neogogatea kentuckiensis* collected in the present study. (**a, b**) Photographs of entire adult specimens, ventral view. (**c**) Anterior part of prosoma. (**d**) Weakly developed ventral sucker. (**e**) Posterior end of opisthosoma with the terminal part of the female reproductive tract shown. Note the weakly developed vaginal sphincter.

drawings by Hoffman and Dunbar (1963), 1 of the specimens (shown laterally) has pharynx larger than the oral sucker, while in the other specimen (positioned dorso-ventrally) these structures are similar in size. Likewise, the oral sucker and pharynx of our specimens of *N. kentuckiensis* are similar in size (Fig. 2a–c). Thus, similar to the presence or absence of the ventral sucker, this character also cannot the used to clearly differentiate between *Gogatea* and *Neogogatea*.

Furthermore, Dubois (1975) wrote that *Gogatea* spp. lack a vaginal sphincter (see Achatz et al., 2024), while *Neogogatea* spp. have a weakly developed but visible vaginal sphincter (our Fig. 2f). Although this structure is difficult to observe unless specimens are of high quality, it seems to be the main morphological feature that allows for reliable differentiation between the 2 genera. Otherwise, the adults of *Gogatea* and *Neogogatea* are morphologically similar. Despite their morphological similarity,

Table II. Pairwise comparisons of partial cytochrome *c* oxidase subunit I (*COI*) mtDNA gene sequences of *Neogogatea kentuckiensis* based on a 461-bp-long alignment. The percentage differences are given above the diagonal, and the number of base pair differences are given below the diagonal. GenBank numbers provided after species name.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	*Neogogatea kentuckiensis* lineage 1 HM064654*	—	0.2%	0.4%	0.4%	0.7%	0.9%	1.1%	1.5%	0.9%	0.9%	0.9%	3.0%	3.0%
2	*N. kentuckiensis* lineage 1 HM064657*	1	—	0.2%	0.2%	0.4%	0.7%	0.9%	1.3%	1.1%	0.7%	1.1%	3.3%	3.3%
3	*N. kentuckiensis* lineage 1 PV755400 DE	2	1	—	0.4%	0.7%	0.9%	1.1%	1.1%	1.3%	0.9%	0.9%	3.0%	3.0%
4	*N. kentuckiensis* lineage 1 PV755404 GA	2	1	2	—	0.2%	0.4%	0.7%	1.1%	0.9%	0.4%	0.9%	3.0%	3.0%
5	*N. kentuckiensis* lineage 1 HM064658*	3	2	3	1	—	0.7%	0.9%	1.3%	1.1%	0.7%	1.1%	2.8%	2.8%
6	*N. kentuckiensis* lineage 1 HM064659*	4	3	4	2	3	—	0.7%	1.5%	1.3%	0.9%	1.3%	3.0%	2.6%
7	*N. kentuckiensis* lineage 1 PV755402 GA	5	4	5	3	4	3	—	1.7%	1.1%	1.1%	1.1%	2.8%	2.8%
8	*N. kentuckiensis* lineage 1 HM064653*	7	6	5	5	6	7	8	—	1.1%	0.7%	1.5%	3.7%	3.7%
9	*N. kentuckiensis* lineage 1 HM064655*	4	5	6	4	5	6	5	5	—	0.4%	0.9%	3.0%	3.0%
10	*N. kentuckiensis* lineage 1 HM064656*	4	3	4	2	3	4	5	3	2	—	1.3%	3.5%	3.5%
11	*N. kentuckiensis* lineage 1 PV755403 GA	4	5	4	4	5	6	5	7	4	6	—	2.4%	2.4%
12	*N. kentuckiensis* lineage 2 HM064651*	14	15	14	14	13	14	13	17	14	16	11	—	1.1%
13	*N. kentuckiensis* lineage 2 PV755401 FL	14	15	14	14	13	12	13	17	14	16	11	5	—

* Metacercariae previously identified as *Mesostephanus* (Locke et al., 2010).

Neogogatea spp. are known to parasitize only birds as definitive hosts, while all adult *Gogatea* spp. are parasitic in snakes (Chandler and Rausch, 1947, 1948; Dubois, 1975; Achatz et al., 2024).

Myer (1960) considered *N. kentuckiensis* to belong to *Mesostephanus* Lutz, 1935, based on adult morphology, notably the presence of a vaginal sphincter. However, other authors (e.g., Hoffman and Dunbar, 1963; Dubois, 1989) rejected this transfer. In our phylogenetic analysis, *Neogogatea* is well separated from both *Gogatea* and *Mesostephanus* (Fig. 1). *Neogogatea* appeared in a polytomy that otherwise consisted of marine cyathocotylids (i.e., *Braunina* and *Mesostephanus* spp.; Fig. 1); the unknown cercariae positioned in the polytomy were collected from the channeled cerith, *Clypeomorus batillariaeformis* Habe and Kosuge, 1966, a marine mollusk (Huston et al., 2018). The topology in this clade suggests either (1) a single transition from marine to freshwater habitats in the case of *Neogogatea*, or, less likely, (2) 3 independent transitions to marine habitats in the case of *Braunina* and *Mesotephanus* spp., as well as the unknown cercaria.

In our molecular phylogeny (Fig. 1), *N. kentuckiensis* appeared to be well separated from *Neogogatea* sp. of Achatz et al. (2019), which strongly suggests that these species represent separate genera. Recently, Sokolov et al. (2024) redescribed and sequenced *P. ovatus* (the type species of the genus) from Russia. Based on their data, *Neogogatea* sp. of Achatz et al. (2019) and *P. ovatus* differ by only 0.8% in partial *28S* sequences, similar to the intrageneric variation exhibited by other cyathocotylid genera, e.g., *Braunina* Wolf, 1903 (up to 0.2%), *Mesostephanus* (up to 2.7%), or *Gogatea* (up to 3.7%) (Achatz et al., 2019, 2024). Both *P. ovatus* and *Neogogatea* sp. of Achatz et al. (2019) have overall similar anatomy; however, *P. ovatus* possesses a ventral sucker, while *Neogogatea* sp. of Achatz et al. (2019) lacks a ventral sucker. Our phylogeny and pairwise comparison suggest that *Neogogatea* sp. of Achatz et al. (2019) is likely a member of *Paracoenogonimus*. It is almost certainly a new species because no *Paracoenogonimus* has been previously reported in the New World, nor do any known *Paracoenogonimus* lack a ventral sucker. Mature adults need to be collected to formally describe the species.

Based on comparison of partial *COI* sequences (Table II), our adult specimens are certainly conspecific with metacercariae from pumpkinseed *Lepomis gibbosus* (L.) and black crappie *Pomoxis nigromaculatus* (Lesueur) in Canada identified as *Mesostephanus* sp. by Locke et al. (2010). We conclude that the metacercariae collected and sequenced by Locke et al. (2010) represent *N. kentuckiensis*.

No variation was detected among our *28S* or *ITS2* sequences. However, we were unable to amplify *28S* fragment from specimens of *N. kentuckiensis* collected in Florida and obtained only a shorter *28S* sequence from specimens collected in Delaware; at the same time, we were able to obtain *COI* sequences from these isolates. Among 13 sequences of the barcoding *COI* region available for our comparison (Table II), our sequence of adult digeneans from Florida (PV755401) and a sequence of a metacercaria from Canada (HM064651) appear to represent a genetic lineage distinct from the remaining sequences. The 2 lineages identified in the present study exhibited 0.2 – 1.7% intra-lineage variation (lineage 1) and 1.1% intra-lineage variation (lineage 2) in the standard 'barcoding' region and 2.4–3.7% difference in the same DNA region between the lineages (Table II). At the same time, the longer *COI* fragment (1,089 bp) from the Delaware and Georgia (lineage 1) and Florida (lineage 2) isolates differed by only 2.0 – 2.1% of nucleotide positions. A collection of fresh, high-quality specimens of *N. kentuckiensis* lineage 2 is necessary for morphological and additional molecular study. DNA sequences of *N. bubonis* and *N. rauschi* are needed to explore the interrelationships among members of the genus.

ACKNOWLEDGMENTS

We are grateful to Andrea Howey (Tri-State Bird Rescue and Rehabilitation), Robin Bast and Becca Wehmeier (CROW), and Samantha Little (Audubon Center of Birds of Prey) for providing osprey carcasses and to Jasmine Groves for helping to process carcasses for parasites. We also thank the Middle Georgia State University parasitology research group (Caley Chun, Jaipreet Raina, and Cicero De Guzman) and the Georgia Department of Natural Resources for their assistance in collecting specimens. This work was financially supported by the University System of Georgia Stem Initiative IV (Middle Georgia State University), Center for Middle Georgia Studies, and the Eppley Foundation (University of Colorado).

LITERATURE CITED

Achatz, T. J., E. S. Brito, A. Fecchio, and V. V. Tkach. 2021. Description and phylogenetic position of a new species of *Herpetodiplostomum* from *Phrynops geoffroanus* in Brazil and a re-evaluation of *Cheloniodiplostomum*. Journal of Parasitology 107: 455–462.

Achatz, T. J., E. E. Pulis, K. Junker, T. T. Binh, S. D. Snyder, and V. V. Tkach. 2019. Molecular phylogeny of the Cyathocotylidae (Digenea, Diplostomoidea) necessitates systematic changes and reveals a history of host and environment switches. Zoologica Scripta 48: 545–556.

Achatz, T. J., Z. S. Von Holten, T. T. Binh, and V. V. Tkach. 2024. Phylogeny and systematics of cyathocotylid digeneans (Digenea: Diplostomoidea) parasitizing snakes with description of three new species of *Gogatea* from Australia and Vietnam. Journal of Parasitology 110: 590–606.

Anderson, D. J. 1944. Studies on *C. szidati* sp. nov., a new furcocercous cercaria of the Vivax type. Journal of Parasitology 30: 264–268.

Cable, R. M. 1935. *Cercaria kentuckiensis* n. sp., first representative of the Vivax group known to occur in the United States. Journal of Parasitology 21: 411.

Chandler, A. C., and R. Rausch. 1947. A study of strigeids from owls in north central United States. Transactions of the American Microscopical Society 66: 283–292.

Chandler, A. C., and R. Rausch. 1948. A contribution to the study of certain avian strigeids (Trematoda). Journal of Parasitology 34: 207–210.

Dubois, G. 1975. Comparaison des genres *Gogatea* Lutz, 1935 et *Neogogatea* Chandler et Rausch, 1947 (Strigeata: Cyathocotyloidea: Prohemistomidae). Bulletin de la Société Neuchâteloise des Sciences Naturelles 98: 39–41.

Dubois, G. 1989. Repertoire des synonymes des Cyathocotyloidea (Trematoda: Strigeata). Bulletin de la Société Neuchâteloise des Sciences Naturelles 112: 39–46.

Dwivedi, M. P. 1967. Record of the genus *Neodiplostomum* (*Fibricola*) (Diplostomidae: Trematoda) from Tamia Ghat, Chhindwara, M. P., India. Proceedings of the Indian Academy of Sciences 66B: 10–18.

Hoffman, G. L. 1959. Studies on the life cycle of *Neogogatea pandionis* Chandler and Rausch, 1948 (Trematoda: Strigeoidea-Cyathocotylidae). Journal of Parasitology 45: 19.

Hoffman, G. L., and C. E. Dunbar. 1963. Studies on *Neogogatea kentuckiensis* (Cable, 1935) n. comb. (Trematoda: Strigeoidea: Cyathocotylidae). Journal of Parasitology 49: 737–744.

Huston, D. C., S. C. Cutmore, and T. H. Cribb. 2018. Molecular systematics of the digenean community parasitising the cerithiid gastropod *Clypeomorus batillariaeformis* Habe & Kusage on the Great Barrier Reef. Parasitology International 67: 722–735.

Kudlai, O., A. Kostadinova, E. E. Pulis, and V. V. Tkach. 2015. A new species of *Drepanocephalus* Dietz, 1909 (Digenea: Echinostomatidae) from the double-crested cormorant *Phalacrocorax auritus* (Lesson) (Aves: Phalacrocoracidae) in North America. Systematic Parasitology 90: 221–230.

Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874.

Locke, S. A., J. D. McLaughlin, and D. J. Marcogliese. 2010. DNA barcodes show cryptic diversity and a potential physiological basis for host specificity among Diplostomoidea (Platyhelminthes: Digenea) parasitizing freshwater fishes in the St. Lawrence River, Canada. Molecular Ecology 19: 2813–2827.

Lutz, H. L., V. V. Tkach, and J. D. Weckstein. 2017. Methods for specimen-based studies of avian symbionts. *In* The Role of Collections in Ornithology: The Extended Specimen. Studies in Avian Biology, M. Webster (ed.). CRC Press, Boca Raton, Florida, p. 127–183.

Myer, D. 1960. On the life history of *Mesostephanus kentuckiensis* (Cable, 1935) n. comb. (Trematoda: Cyathocotylidae). Journal of Parasitology 46: 819–832.

Pérez-Ponce de León, G., and D. I. Hernández-Mena. 2019. Testing the higher-level phylogenetic classification of Digenea (Platyhelminthes, Trematoda) based on nuclear rDNA sequences before entering the age of the 'next-generation' Tree of Life. Journal of Helminthology 93: 260–276.

Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Snyder, S. D., and V. V. Tkach. 2007. *Neosychnocotyle maggiae*, n. gen., n. sp. (Platyhelminthes: Aspidogastrea) from freshwater turtles in northern Australia. Journal of Parasitology 93: 399–403.

Sokolov, S. G., S. A. Vlasenkov, S. V. Bugmyrin, A. P. Kalmykov, and D. I. Lebedeva. 2024. Phylogeny and morphology of some European cyathocotylid digeneans (Trematoda: Diplostomoidea). Journal of Helminthology 98: e44. doi:10.1017/S0022149X24000328.

Tkach, V. V., D. T. J. Littlewood, P. D. Olson, J. M. Kinsella, and Z. Swiderski. 2003. Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). Systematic Parasitology 56: 1–15.

Tkach, V. V., and J. Pawlowski. 1999. A new method of DNA extraction from the ethanol-fixed parasitic worms. Acta Parasitologica 44: 147–148.

Vernberg, W. B. 1952. Studies on the trematode family Cyathocotylidae Poche, 1926, with a description of a new species from *Holostephanus* from fish and the life history of *Prohemistomum chandleri* n. sp. Journal of Parasitology 38: 327–340.

Zazornova, O. P. 1995. *Neogogatea rauschi* n. sp. (Prohemistomatidae)—A new trematode from Russia. Parazitologiya 29: 222–224. (In Russian.)