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UNTANGLING THE ECOLOGY, TAXONOMY, AND EVOLUTION OF *CHAETOGASTER LIMNAEI* (OLIGOCHAETA: NAIDIDAE) SPECIES COMPLEX

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ABSTRACT: Within Oligochaeta, *Chaetogaster limnaei* is unusual in exhibiting a parasitic relationship with freshwater pulmonate snails. Taxonomic confusion has been caused by differences in what have been considered 2 subspecies of this worm: *Chaetogaster limnaei limnaei* is an ectosymbiont and is present inside the mantle cavity of the snail, whereas *Chaetogaster limnaei vaghini* is parasitic and lives in the kidney of the snail. This study explored the distribution of these annelids in central New York and used mitochondrial DNA sequence data from the COI locus to examine the relationship, evolution, and species status of the ectosymbiotic and parasitic forms of *C. limnaei*. Snails (*Physa gyrina*) were collected from 6 streams and lakes in central New York, with additional specimens collected from a lake in Massachusetts for comparison. One hundred and forty snails were examined, and at least 1 form of *Chaetogaster* was present in 88 specimens, a prevalence of 62.9%. COI sequence data from New York and Massachusetts did not reveal separate ectosymbiotic and parasitic lineages. Instead, all parasitic forms were part of a mixed clade that included both ectosymbiotic and parasitic forms. This mixed clade was nested within clades of ectosymbiotic forms only, suggesting that a plastic lineage of *C. limnaei*, able to be both ectosymbionts and parasites, evolved from ectoparasitic ancestors.

Chaetogaster limnaei von Baer, 1827 (Oligochaeta: Naididae) is a cosmopolitan freshwater oligochaete commonly associated with molluscs (Brinkhurst and Jamieson, 1971). *Chaetogaster limnaei* is the type species of the genus, which includes 5 other species (Brinkhurst and Jamieson, 1971). It was first described by von Baer (1827) from the kidney and mantle cavity of snails in Lymnaeidae (Gruffydd, 1965a) but has since been reported from a wider range of freshwater hosts, primarily snails but also other molluscs, sponges, crayfish, and fish (Supplementary Table I). *Chaetogaster limnaei* has been reported from more than 40 species of freshwater snails from at least 10 families, primarily members of Lymnaeidae, Physidae, and Planorbidae (Buse, 1974; Joy and Welch, 1984; Fried et al., 2008). *Chaetogaster limnaei* has also been reported from other molluscs including zebra mussels (*Dreissena polymorpha*, Piesik, 1983; Conn et al., 1996; Ricciardi et al., 1997), fingernail clams (Sphaeriidae, Gale, 1973; Young, 1974; Barbour, 1977), and basket clams (Corbiculidae, Eng, 1976; Sickel and Lyles, 1981). Reports of non-molluscan hosts for *C. limnaei* include sturgeon (Acipenseridae, Baska and Bona-Puppan, 1992; Cakic et al., 2000), sponges (Spongillidae, Stephenson, 1918, 1923), and an unidentified species of crayfish (Michaelsen, 1926). *Chaetogaster limnaei* has been reported as free-living only by Wolf (1928).

Vaghin (1946) first noted that *C. limnaei* exists in 2 distinct forms, a commensal, ectosymbiotic form present on the outside of the snail or just inside the shell and a parasitic form present in the kidney. With different microhabitats, food choices, and reproductive strategies (with the parasitic form performing sexual reproduction to a much greater degree than the primarily asexual ectosymbiont), Vaghin (1946) suggested that the different forms of *C. limnaei* were separate "biological species." Vaghin (1946) did point out, however, that he could not morphologically distinguish between the parasitic and ectosymbiotic forms. Soon after, Sperber (1948), apparently unaware of Vaghin (1946) or the different forms of the worm (parasitic vs. ectosymbiotic), created the subspecies *Chaetogaster limnaei limnaei* for the European, Asian, and North American representatives of *C. limnaei*. Sperber (1948) also transferred *Chaetogaster bengalensis* Annandale 1905 from India and Burma and *Chaetogaster victoriensis* Davies 1913 and *Chaetogaster australis* Davies 1913, both from Australia, to *C. limnaei* as the subspecies *Chaetogaster limnaei bengalensis*, *Chaetogaster limnaei victoriensis*, and *Chaetogaster limnaei australis,* respectively. Gruffydd (1965a) found that in Britain the parasitic form was unable to survive and feed when transferred to the external surface of the snail. Gruffydd (1965a) also found differences in the number and length of chaetae (fewer and shorter chaetae in the parasite), gut wall thickness, and gut contents (Table I). Gruffydd (1965a) did, however, indicate that several worms matching the parasitic morphology were found externally, but with only kidney cells in their gut, suggesting that these were parasitic forms temporarily living externally. Buse (1974) also found the chaetae of the parasitic form to be shorter but found significant differences in the length of chaetae from different localities. Despite his conflicting observations, Gruffydd (1965a) proposed that the parasitic form be recognized as the subspecies *Chaetogaster limnaei vaghini* Gruffydd, 1965. The subspecies designation *C. l. vaghini* has almost always been maintained in the literature when authors have specified that they are studying the parasitic form from the kidney (Gruffydd, 1965b; Buse, 1971, 1972, 1974; Sankurathri and Holmes, 1976; Golubev et al., 1978; Learner et al., 1978). The exception is Sapaev (1975, 1977), who considered the 2 forms "ecotypes" and not subspecies. To date, the parasitic form of *C. limnaei* from the kidney has been reported from only gastropod hosts in the families Ancylidae, Bythiniidae, Lymnaeidae, Physidae, and Viviperidae (though not always referred to as *C. l. vaghini*). Table I lists all uses of the subspecies name *C. l. vaghini* from the literature, the snail host, and how the authors distinguished *C. l. vaghini* from *C. l. limnaei*.

Since Wagin (1941) reported that *C. l. limnaei* feed on trematode cercariae in addition to planktonic organisms such as diatoms and rotifers, research has focused on the possible protective action of the oligochaete against trematode (e.g., Fernandez et al., 1991; Rodgers et al., 2005; Fried et al., 2008) and later nematode parasitism (Zimmermann et al., 2011). Other

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TABLE I. Features used to distinguish between the 2 subspecies of *Chaetogaster limnaei*, *C. l. limnaei* (ectosymbiont), and *C. l. vaghini* (parasite). Host snail species is in parentheses. "Infection site" indicates that worms were identified not morphologically but only by where on/in the host the worms were found. "Exterior" indicates the exterior surface or the mantle cavity of the snail host. "Unstudied" indicates *C. l. limnaei* was not collected or examined for the study.

Author	*C. l. limnaei*	*C. l. vaghini*
Gruffydd, 1965a	Segment 2 chaetae average 85 μm long; segment 6, 7, 8 chaetae average 50 μm, maximum of 12 chaetae per bundle; thicker gut wall; gut contents planktonic organisms (*Lymnaea pereger*)	Segment 2 chaetae average 60 μm long; segment 6, 7, 8 chaetae average 45 μm; maximum of 7 chaetae per bundle; thinner gut wall; gut contents kidney cells (*Lymnaea pereger*)
Gruffydd, 1965b	Infection site exterior (*L. pereger*)	Infection site kidney (*L. pereger*)
Buse, 1971	Unstudied	Infection site kidney (*L. stagnalis*)
Buse, 1972	Infection site exterior (*L. pereger*)	Infection site kidney (*L. stagnalis*)
Buse, 1974	Infection site exterior (18 species; see Supplementary Table I)	Infection site kidney (*L. stagnalis*, *L. pereger*, *Planorbis carinatus*, *Potamopyrgus jenkensi*)
Sankurathri and Holmes, 1976	Infection site exterior (*Physa gyrina*)	Infection site kidney (*P. gyrina*)
Golubev et al., 1978	Ultrastructure: nerve cells more complex (*Planorbarius corneus*, *Lymnaea stagnalis*)	Ultrastructure: nerve cells less complex (*Radix ovata*)
Learner et al., 1978	Infection site exterior (*L. stagnalis*, *L. pereger*)	Infection site kidney (*L. stagnalis*, *L. pereger*)
McCarthy, 1974	Infection site exterior (*L. pereger, L. stagnalis, Planorbis planorbis, Bithynia tentaculata, Potamopyrgus jenkinsi, Ancylastrum fluviatile, Physa fontinalis*)	Infection site kidney (*L. pereger*)
Present study	Infection site exterior (*P. gyrina*, *Physa* sp.)	Infection site kidney (*P. gyrina*, *Physa* sp.)

studies have examined the host–parasite relationship, such as Buse (1974), who documented the host specificity and host attraction behavior of both *C. l. limnaei* and *C. l. vaghini*. Gamble and Fried (1976) suggested that *C. l. limnaei* consumes host snail mantle tissue and may have a more parasitic relationship with the snail than previously thought. Stoll et al. (2013) showed that *Physa acuta* highly infected with *C. l. limnaei* exhibited lower growth rates but did not examine the snails for *C. l. vaghini* in the kidney, which may have confounded the study. To date, no studies have examined the evolutionary history of *C. limnaei* and the relationship between the ectosymbiotic and parasitic forms. Here we explore the evolutionary relationships among several populations of these 2 forms in the northeastern United States and address the question if parasitism or ectosymbiosis represents the ancestral lifestyle in this group. Using molecular data, we also ask if perhaps the 2 forms are divergent enough to be considered separate species. Finally, we examine the ecology of the ectosymbiotic and parasitic forms of *C. limnaei* in *Physa gyrina* (Say, 1821) snails in central New York.

MATERIALS AND METHODS

Ecological methods

One hundred and forty snails (*Physa gyrina*) were collected by dip net between July and November 2011 from 6 central New York (NY) streams and ponds and examined for *C. limnaei* (Table II). Additional snails (*Physa* sp.) were collected by hand from shallow (1 m depth), submerged vegetation in Mascuppic Lake in Massachusetts (MA) during the summer months in 2008–2009 (Table II). Snails were dissected to reveal the internal reproductive anatomy for species identification (Wethington and Lydeard, 2007). A subset of 68 NY snails was examined for abundance of each subspecies of *Chaetogaster*. Worms were removed from the exterior of the snail, the mantle cavity, or inside the kidney. Confidence intervals (95%) for prevalence and mean intensities were calculated with Quantitative Parasitology on the Web 1.0 (Rozsa et al. 2000). Voucher specimens were preserved in 5% buffered formalin and deposited in the Smithsonian's National Museum of Natural History (USNM 1226917–1226918), and those for molecular analysis were fixed in DMSO and stored at –20 C.

Molecular methods

DNA extractions were performed using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California) according to the manufacturer's instructions. An approximately 710 base pair (bp) fragment of the cytochrome oxidase 1 (COI) mitochondrial DNA (mtDNA) locus was amplified using primers LCO1490 and HCO2198 (Folmer et al., 1994). PCR reactions (25 μl total reaction volume) were performed using 2–3 μl of DNA template, 0.5–1 unit of Finnzyme DNAzyme EXT proofreading polymerase (MJ Research, Waltham, Massachusetts), and final concentrations of 0.8 mM deoxynucleoside triphosphates (dNTPs), 1.5 mM $MgCl_2$, and 0.5 μM of each primer. The PCR thermocycling parameters included denaturation at 94 C for 5 min, followed by 35 cycles of 94 C for 30 sec, 40 C for 1 min, and 72 C for 1.5 min. Prior to direct sequencing, PCR products were enzymatically treated with ExoSAP-IT (Affymetrix Corp., Santa Clara, California) to remove excess primers and dNTPs. Alternatively, amplicons were gel-purified on a 1% SeaKem agarose gel (Lonza, Portsmouth, New Hampshire), and DNA was recovered using a SpinEx column (Fisher Scientific, Waltham, Massachusetts). Purified amplicons were submitted to Genewiz (South Plainfield, New Jersey) or Geneway Research LLC (Hayward, California) with the original PCR primers for bi-directional sequencing. Sequences were deposited in GenBank (accession no. KF952295–KF952355).

Fifty-two *C. limnaei* were sequenced from central NY *P. gyrina* and combined with 9 sequences of *C. limnaei* from *Physa* sp. snails from Mascuppic Lake, MA. Five additional sequences from GenBank were included in the phylogenetic analysis. These included a previously published sequence of *C. limnaei* from Sacramento, California (AF534834, presumed to be the ectosymbiotic form), *C. diaphanus* AF534831 and AF534832, and *C. diastrophus* AF534833 to represent other, free-living, species of *Chaetogaster* not associated with snails. Because Bely and Wray (2004) showed that *Amphichaeta* represents the sister group to *Chaetogaster*, we included *A. raptisea* AF534829 as an outgroup. The sequences were aligned using ClustalX (Thompson et al., 1997) to produce an unambiguous alignment. We used MEGA5 (Tamura et al., 2011) to choose GTR plus gamma as the best-fitting model of nucleotide substitution by the Bayesian Information Criterion (Schwarz, 1978).

To assess haplotype diversity among populations and visualize possible reticulations in our sequence data we used Network 4.6.1 and Network Publisher 2.0 (Fluxus Technology, www.fluxus-engineering.com) to create a median joining network of haplotypes color coded by locality (Bandelt et al., 1999). The outgroup taxa *A. raptisea, C. diaphanus,* and *C. diastrophus*

TABLE II. Georeferences of the 7 collection localities for *Physa gyrina* (New York) and *Physa* sp. (Massachusetts).

Locality name	Coordinates
Beaver Meadow Brook 1 (South Hill Rd.), Holland Patent, NY	43°18′23.7″N, 75°18′3.8″W
Beaver Meadow Brook 2 (Soule Rd.), Holland Patent, NY	43°18′16.2″N, 75°17′22.8″W
Beaver Meadow Brook 3 (Gillett Rd.), Holland Patent, NY	43°19′13.3″N, 75°19′16.2″W
Portlandville Swamp, Portlandville, NY	42°33′3.8″N, 74°57′15.7″W
Hamilton College Reservoir, Clinton, NY	43°03′7.0″N, 75°26′9.4″W
Yahnundasis Lake, New Hartford, NY	43°05′6.8″N, 75°18′28.7″W
Mascuppic Lake, Tyngsboro, MA	42°40′42.2″N, 71°24′1.5″W

were excluded (leaving 62 sequences) from the network analysis due to the high number of mutation steps from members of the ingroup and to simplify the visualization. We then used MEGA5 to conduct a maximum likelihood (ML) bootstrap (BS) analysis with 1,000 replications. As a measure of raw sequence divergence, uncorrected pairwise p-distances were also computed with MEGA5 for all *C. limnaei* sequences. We used MrBayes version 3.2 (Ronquist et al., 2012) in a Bayesian inference (BI) analysis to approximate posterior probabilities (PP) using the GTR plus gamma model, default priors, 4 chains for 2×10^6 generations, discarding the first 25% of samples as "burn in," and sampling Monte Carlo Markov chains every 500 generations.

RESULTS

Ecological results

Chaetogaster limnaei from both the mantle and kidney combined was present in 88/140 *P. gyrina* in NY, an overall prevalence of 62.9%. Ectosymbionts were present at all collecting sites, and parasites were present at all but 1 locality, Beaver Meadow Brook 3 (BMB3), but only a single snail was examined from this locality. In the subset of 68 snails examined for abundance of *C. limnaei* from each locality (ectosymbiont vs. parasite), 44.5% of the worms present were ectosymbionts and 55.5% were parasites, with 14 snails (20.6%) harboring both forms. We found a mean abundance (Bush et al., 1997) of 1.4 (±0.30 SE) ectosymbionts per snail and 1.8 (±0.32 SE) parasites per snail. Table III compares the prevalence and mean intensity of parasites and ectosymbionts at each locality.

Molecular results

Sequence divergence for COI (uncorrected p-distances) ranged from 0% to 18.2%, and parasites and ectosymbionts often showed no sequence divergence from one another. The median joining network analysis showed an overall treelike pattern and strong separation of most *C. limnaei* sequences from 3 Mascuppic Lake (MaL) sequences and 1 from Beaver Meadow Brook 2 (BMB2) (Fig. 1). There were 10 total haplotypes, of which 4 were present as singletons. Sequences from MaL showed the most haplotype diversity, with several divergent sequences distant from all others and 1 sequence that matched a geographically widespread haplotype from NY (Hamilton College Reservoir [HR], Yahnundasis Lake [YL], Portlandville Swamp [PS] and Beaver Meadow Brook [BMB1]). The network produced alternative connections for the relationship between 3 of the MaL sequences, the single BMB2 sequence, and the rest of the ingroup sequences. Alternative connections were also provided for haplotypes represented by 4 YL sequences and 4 sequences from YL, PS, and CA.

Phylogenetic analyses (ML and BI) resulted in largely congruent trees, containing several well-defined clades (Fig. 2). With the exception of 1 specimen (*C. limnaei* 1025), a well-supported clade (ML: 97% [BS], BI: 100% [PP]), separate from other free-living species of *Chaetogaster* and from the outgroup, was recovered. Strong support (100% ML, BI) for a basal clade consisting of only MaL ectosymbionts, was found (Fig. 2). Furthermore, some geographic structuring associated with the clades was evident. Specifically, 3 individuals from MaL clustered separately from a clade of HR specimens, and from a clade of mostly YL and PS individuals (Fig. 2). A fourth clade of ectosymbionts consisted of individuals from YL, PS, MaL, and CA. Because of its singleton status, a sole individual from BMB3 (*C. limnaei* 998) did not reveal any geographic affinities with other populations; however, it correctly grouped within ectosymbionts (Fig. 2).

The remaining large clade consisted of mostly parasites and a few ectosymbionts and was supported by 100% Bayesian PP and

TABLE III. Number of *Physa gyrina* snails examined (n), prevalence (%), and mean intensity of parasites (kidney) and ectosymbionts (exterior of snail or mantle cavity) of *Chaetogaster limnaei* from 7 collection localities in central New York. Confidence intervals (95%) for prevalence and mean intensities were calculated with Quantitative Parasitology on the Web 1.0 (Rozsa et al., 2000) and are shown in parentheses.

		Prevalence (%)		Mean Intensity	
Locality Name	n	Parasites	Ectosymbionts	Parasites	Ectosymbionts
Beaver Meadow Brook 1	14	78.6 (49.2–95.3)	42.9 (17.7–71.1)	5.6 (4.2–7.0)	3.3 (1.8-4.2)
Beaver Meadow Brook 2	4	25.0 (0.6–80.6)	75.0 (19.4–99.4)	9.0 (na)	4.7 (3.0–6.3)
Beaver Meadow Brook 3	1	100.0 (na)	0.0 (na)	0.0 (na)	6 (na)
Portlandville Swamp	5	40.0 (5.3–85.3)	80.0 (28.4–99.5)	5.0 (na)	4.5 (1.0–6.0)
Hamilton College Reservoir	6	50.0 (11.8–88.2)	66.7 (22.3–95.7)	5.0 (2.0–6.7)	7.0 (3.1–8.8)
Yahnundasis Lake	38	44.7 (28.6–61.7)	18.4 (7.7–34.3)	1.6 (1.3-1.9)	1.6 (1.1–2.1)

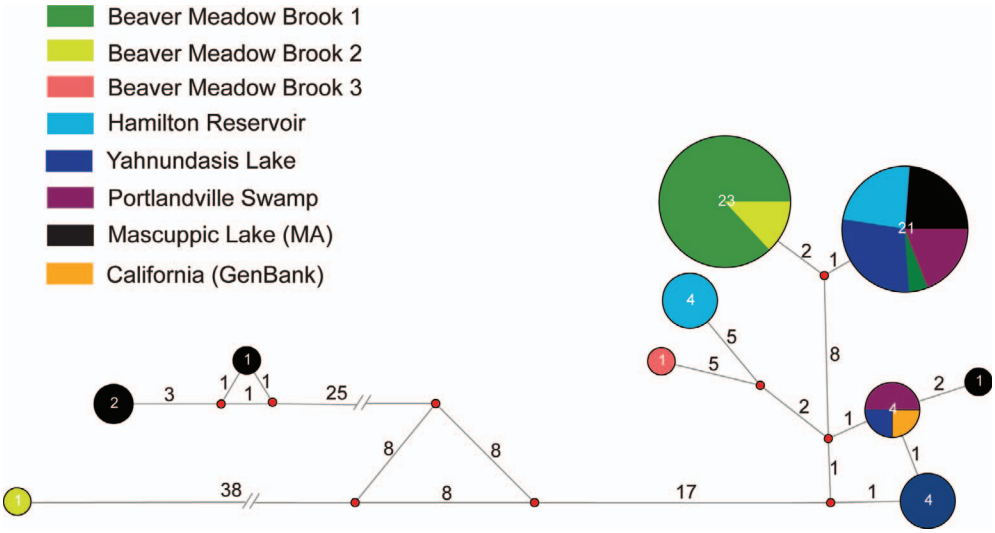

FIGURE 1. Median joining network of 62 COI sequences of *Chaetogaster limnaei*. The numbers within the circles represent the number of sequences of that haplotype and the numbers between circles represent the number of mutation steps (base pair changes) between haplotypes. The size of the circles is proportional to the number of individuals sampled with that haplotype. Small red circles represent median vectors, inferred haplotypes unseen in the data. Horizontal line breaks indicate lines that have been shortened and are not proportional to the number of mutation steps.

97% ML bootstrap values (Fig. 2). Again, 3 major geographic clusters were present: (a) a clade of BMB1 and BMB2, (b) a second clade of specimens from YL, HR, and MaL, and (c) a third clade consisting of mostly PS individuals. Of these 3, the BMB1-BMB2 clade comprised the largest one and consisted of mostly parasitic specimens; it did, however, contain a few ectosymbionts. However, except for *C. limnaei* 1025, which grouped with the free-living *Chaetogaster* species (see above), the BMB1-BMB2 localities had no ectosymbiotic representatives at the base of the tree (Fig. 2). A second geographic cluster (YL-HR-MaL clade) also contained mostly parasitic specimens with the exception of 2 individuals. Specifically, the YL population in that clade consisted of parasitic specimens only, with its ectosymbionts clustering in another well-supported clade with PS and MaL ectosymbionts. The HR and MaL populations, which also were part of this clade, each had a single ectosymbiont (*C. limnaei* 1002, *C. limnaei* 05) group within this cluster (Fig. 2), but the majority of the HR ectosymbionts were contained in a well-supported clade near the base of the ingroup. Four PS and 1 BMB1 sequences were unresolved with respect to the YL, HR, and MaL clade.

When considering *C. limnaei* within individual snails, most specimens belonged to the same clade and showed little to no sequence divergence (e.g., parasites *C. limnaei* 995 and 996 and ectosymbiont *C. limnaei* 997, all isolated from *P. gyrina* 40 collected from PS) (Fig. 2). This example also highlights that some individual snails hosted ectosymbionts and parasites that all share the same haplotype, such as *P. gyrina* 34 from BMB1 that hosted ectosymbionts (*C. limnaei* 988 and 989) and parasites (*C. limnaei* 990 and 991), all of which share the same haplotype. However, some snails were host to *C. limnaei* belonging to different clades. For example, *P. gyrina* 33 simultaneously hosted an ectosymbiont that was part of the well-supported basal ectosymbiont clade and parasitic forms that were part of the mixed YL-HR-MaL clade (Fig. 2).

DISCUSSION

Though parasitic and ectosymbiotic forms of *C. limnaei* are present worldwide (see Supplementary Table I), their evolutionary history and taxonomic status have never been examined. Our COI data suggest that rather than a distinct species or subspecies, the parasitic form of *C. limnaei*, known generally as *C. l. vaghini*, is part of a large, plastic, lineage of worms that are able to exploit both the internal kidney habitat and the external habitat in the host's pallial cavity or just under the shell. This "mixed" lineage was shown to be sister to 2 clades of ectosymbionts exclusively and nested within 2 lineages of ectosymbionts, suggesting an ectosymbiotic ancestral lifestyle. However, our results are based on a single locus; hence, to distinguish if parasites and ectosymbionts are from a single phenotypically plastic species or if they represent distinct lineages, data from additional loci or infection experiments with offspring from both forms are needed. Because 3 ectosymbionts from MA represented a distinct basal lineage giving rise to the second purely ectosymbiotic clade and a clade of parasites plus ectosymbionts, they may actually represent a separate species. Further exploration with other loci and morphological features may confirm the separate species status of this lineage.

Our study revealed a number of different lineages within the *C. limnaei* complex, and based on historical studies of host–symbiont interactions, there may be lineage-specific effects on the snail hosts. Stoll et al. (2013) found that at high densities *C. l. limnaei* had a negative impact on the growth of the snail *Physa acuta*. Other studies have shown a positive effect of *C. l. limnaei* in reducing trematode infection in snails (Fernandez et al., 1991; Rodgers et al., 2005; Fried et al., 2008). Worms from different lineages could have different effects on their snail hosts, especially a more divergent lineage such as the one from MA that may represent an undescribed species. The incorporation of a molecular phylogenetic perspective into future ecological studies could help to clarify the impact *C. limnaei* has on its snail hosts and aquatic communities.

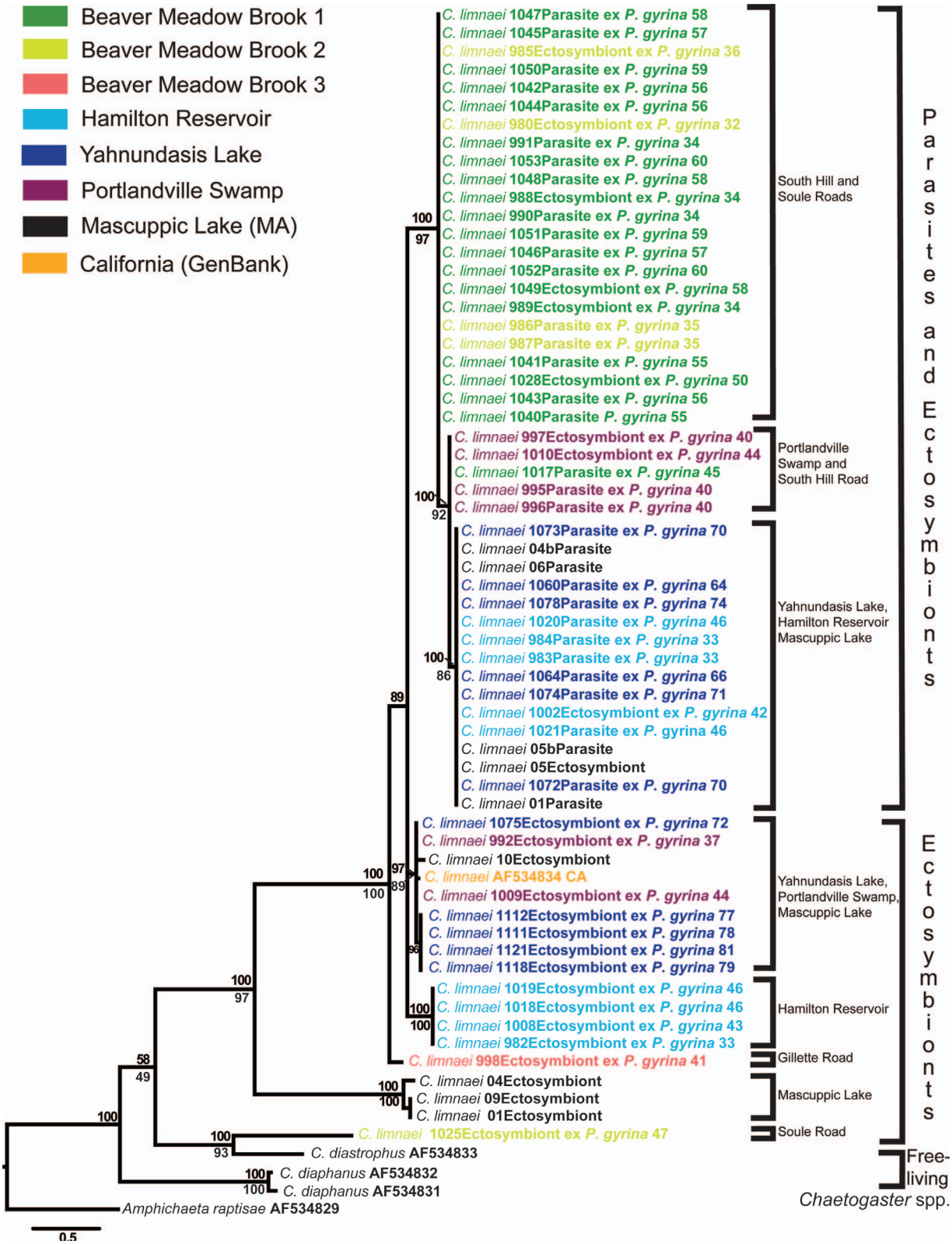

FIGURE 2. Bayesian inference (BI) tree of 62 COI sequences of *Chaetogaster limnaei*, 2 sequences of *C. diaphanus*, 1 sequence of *C. diastrophus*, and the outgroup *Amphichaeta raptisae*. Taxon labels include *C. limnaei* specimen number, whether it was an ectosymbiont or a parasite, and the specimen number for the snail (*Physa gyrina, P.g.*) from which it was collected. Numbers above nodes represent Baysian posterior probabilities, numbers below nodes are ML bootstrap values. The scale bar represents the number of substitutions per site. The ML tree was largely congruent with the BI tree.

One possible source of error in our study is that the worms we considered ectosymbionts based on their position within the host were actually the parasitic forms migrating toward the kidney (i.e., a select number of ectosymbionts were caught "in the act" of establishing a parasitic infection). We consider this unlikely as the parasitic form probably moves quickly to its preferred tissue (Gruffydd, 1965a). As demonstrated by Gruffydd (1965a), while in the kidney the parasitic form feeds on kidney cells. In contrast, the ectosymbiotic form feeds on planktonic organisms such as diatoms, ciliates, rotifers (Gruffydd, 1965a; Shigina, 1970) and trematode larvae (Wagin, 1941; Shigina, 1970). Thus, it is unlikely that the parasitic form would become established for any period of time in the mantle cavity prior to infecting the kidney.

Our results also suggest that other species of *Chaetogaster*, previously thought to be free-living, may have symbiotic associations with snails. Our phylogenetic results placed a specimen that we identified as *C. limnaei* (1025), present ectosymbiotically on a snail in NY, as the sister taxon to *C. diastrophus*. Our molecular phylogenetic results suggest that this specimen may instead represent an individual of *C. diastrophus*. Smith and Kaster (1986) found that *C. diastrophus* consumes primarily diatoms and reported no association with snails. Further exploration of snails and their oligochaete associates is warranted to determine if perhaps species beyond *C. limnaei* interact closely with snails.

Our ecological results suggest that *C. limnaei* is widespread in central NY, as it was present in *P. gyrina* at all collection localities. We found parasites to be more common and abundant than ectosymbionts. More than 20% of the examined snails hosted both ectosymbionts and parasites, so neither form of *C. limnaei* directly inhibits the presence of the other. In a survey of 21 snail species, Buse (1974) found the ectosymbiont in 86% of snail species but the parasite in only 19%, suggesting some degree of host selectivity. Future studies incorporating other snail species could reveal additional lineages of ectosymbionts and parasites, perhaps accounting for this apparent host specificity. For example, 2 of the authors (A.P., R.H.) have found both forms of *C. limnaei* in other species of *Physa,* as well as species of *Menetus* and including an undetermined species of freshwater limpet (pers. obs.). *Chaetogaster limnaei* has been reported worldwide from a variety of molluscs, and our research suggests that further exploration of additional hosts is warranted. Additional research on the morphology of the symbionts is also needed, since Gruffydd (1965a) found differences in the number and size of the chaetae between the subspecies (e.g., more chaetae, longer chaetae in the ectosymbiont) and also noted that the thickness of the gut epithelium differs between the ectosymbionts and the parasites. Ultrastructural analysis has also shown simplifications in the nervous system (Golubev et al., 1978) and musculature (Sapaev, 1977) of the parasitic form of *C. limnaei*. These observations should be further explored in light of our understanding of the evolutionary history of the ectosymbiotic and mixed lineages of *C. limnaei*.

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