Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Cryptic carnivores: Intercontinental sampling reveals extensive novel diversity in a genus of freshwater annelids

Joseph M. Mack [a,*], Mårten Klinth [b], Svante Martinsson [b], Robert Lu [c], Hannah Stormer [c], Patrick Hanington [c], Heather C. Proctor [c], Christer Erséus [b], Alexandra E. Bely [a]

[a] *Department of Biology, University of Maryland, MD 20742, USA*
[b] *Department of Biological and Environmental Sciences, University of Gothenburg, Göteborg, SE-405 30, Sweden*
[c] *Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9, Canada*

ARTICLE INFO

ABSTRACT

Freshwater annelids are globally widespread in aquatic ecosystems, but their diversity is severely underestimated. Obvious morphological features to define taxa are sparse, and molecular phylogenetic analyses regularly discover cryptic diversity within taxa. Despite considerable phylogenetic work on certain clades, many groups of freshwater annelids remain poorly understood. Included among these are water nymph worms of the genus *Chaetogaster* (Clitellata: Tubificida: Naididae: Naidinae). These worms have diverged from the detritivorous diet of most oligochaetes to become more predatory and exist as omnivores, generalist predators, parasites, or symbionts on other invertebrates. Despite their unusual trophic ecology, the true diversity of *Chaetogaster* and the phylogenetic relationships within the genus are uncertain. Only three species are commonly referenced in the literature (*Chaetogaster diaphanus, Chaetogaster limnaei,* and *Chaetogaster diastrophus*), but additional species have been described and prior molecular data suggests that there is cryptic diversity within named species. To clarify the phylogenetic diversity of *Chaetogaster,* we generated the first molecular phylogeny of the genus using mitochondrial and nuclear sequence data from 128 worms collected primarily across North America and Europe. Our phylogenetic analyses suggest that the three commonly referenced species are a complex of 24 mostly cryptic species. In our dataset, *Chaetogaster "diaphanus"* is represented by two species, *C. "limnaei"* is represented by three species, and *C. "diastrophus"* is represented by 19 species. North American and European sequences are largely interspersed across the phylogeny, with four pairs of clades involving distinct North American and European sister groupings. Overall, our study demonstrates that the species diversity of *Chaetogaster* has been underestimated and that carnivory has evolved at least twice in the genus. *Chaetogaster* is being used as a model for symbiotic evolution and the loss of regenerative ability, and our study indicates that researchers must be careful to identify which species of *Chaetogaster* they are working with in future studies.

1. Introduction

Small benthic invertebrates are abundant in freshwater ecosystems, contribute significantly to benthic production, and are critical food sources for larger organisms (Poff et al., 1993; Ptatscheck et al., 2020; Schmid-Araya et al., 2020). Nonetheless, freshwater groups with meiofaunal species, like annelids, flatworms, rotifers, crustaceans, and nematodes, are greatly underrepresented in the molecular phylogenetic and metabarcoding literature (Schenk and Fontaneto, 2020). Many freshwater annelids are particularly difficult to collect and identify, due to small body sizes, an infaunal habitat, and a paucity of apparent morphological differences. Because morphology often fails to capture the full diversity of freshwater annelids, molecular phylogenetic studies on the group frequently discover new and cryptic species (Bely and Weisblat, 2006; Liu et al., 2017a; Martinsson and Erséus, 2021).

Cryptic diversity is prominent within the subfamily Naidinae (Annelida: Clitellata: Tubificida: Naididae) (Bely and Wray, 2004; Envall et al., 2012; Erséus et al., 2017), a group of small annelids that primarily reproduce asexually by fission. Ranging in length between 1 and 20 millimeters (mm), these delicate worms can be found worldwide

---

buried in freshwater sediments or clinging to rocks, submerged aquatic vegetation, and larger organisms. Despite their ubiquity (and charm), only one of the estimated 24 genera in the subfamily (*Nais*) has been the subject of a detailed molecular phylogeny (Envall et al., 2012). Our understanding of evolutionary relationships within the other genera is limited to the handful of representatives that are included in broader scale phylogenies of the Naidinae (Bely and Wray, 2004; Erséus et al., 2017).

The genus *Chaetogaster* is a remarkable group of naidines. Though they exhibit many traits common to the Naidinae, such as asexual reproduction by fission, a cosmopolitan distribution, and a small body size, *Chaetogaster* worms diverge strongly from their relatives in trophic strategy and morphology (Fig. 1A and 1B). Notably, *Chaetogaster* has abandoned the herbivorous and detritivorous diets that are typical of the Naidinae and most other oligochaetes. Instead, a number of *Chaetogaster* species have adapted to carnivorous diets, either as free-living worms or symbionts with other invertebrates (Green, 1954; Gruffydd, 1965). Accompanying such striking dietary shifts are an array of unique morphological adaptations in *Chaetogaster*. These include a heavily muscularized suctorial pharynx, a statocyst near the brain, a well-defined stomach, the absence of dorsal chaetae, and a reduced prostomium (Sperber, 1948). *Chaetogaster* is also conspicuously unable to regrow anteriorly amputated segments, while most of its relatives are capable head regenerators (Bely and Sikes, 2010). Interestingly, many of the traits that distinguish *Chaetogaster* from other naidines are also found in the leeches, a 150 – 200 million year old clitellate clade of muscular, non-regenerative predators and parasites (Erséus et al., 2020). Despite *Chaetogaster*'s position as an ecological, morphological, and developmental outlier in the Naidinae, and its potential as a model for understanding major transitions in the Annelida, the true diversity of the genus and evolutionary relationships between known *Chaetogaster* species is uncertain.

Three species of *Chaetogaster* were originally recognized based on size, ecology, and chaetal morphology: *Chaetogaster limnaei* von Baer 1827 (the type species of the genus)*, Chaetogaster diaphanus* (Gruithuisen 1828)*,* and *Chaetogaster diastrophus* (Gruithuisen 1828).

Chaetogaster limnaei is a small (1 – 2 mm long) ectosymbiont and/or endoparasite on snails and other molluscs; *Chaetogaster diaphanus* is a large (0.5 – 2 cm long) generalist predator of other invertebrates (Monakov, 1972); and *Chaetogaster diastrophus* is a small (1 – 2 mm long) putative omnivore (Streit, 1977). Most *Chaetogaster* research has focused on interactions between *C. limnaei* and its molluscan partners. Early researchers distinguished between two forms of this species. One exists as a potentially mutualistic ectosymbiont that protects its host by consuming harmful parasites like trematodes (Hobart et al., 2022; Michelson, 1964). The other form is endosymbiotic and inhabits mollusc kidneys, ovaries, or gills (Conn et al., 1996; Gruffydd, 1965). In some cases, the endosymbiotic form of *C. limnaei* can negatively impact host fitness as it consumes gill, kidney, or ovarian tissue (Liquin et al., 2021). Vaghin (1946) and Gruffydd (1965) suggested that these two strategies were representative of distinct subspecies, but a recent cytochrome *c* oxidase subunit I (COI) phylogeny of *C. limnaei* rejected this distinction. Instead, a mixed ectosymbiotic/endosymbiotic *C. limnaei* clade was recovered as sister to two exclusively ectosymbiotic *C. limnaei* clades (Smythe et al., 2015). It is uncertain if these three groups represent distinct species. Recent research focused on the large carnivore of the genus, *C. diaphanus,* is more limited. Prior work has primarily been descriptive, with papers highlighting general anatomy (Brinkhurst and Gelder, 1989; DeHorne, 1916; Zattara and Bely, 2015), dietary preferences (Green, 1954; Monakov, 1972), reproductive strategies (Meewis, 1934; Poddubnaya, 1968), and regenerative ability (Bely and Sikes, 2010). Existing research on *C. diastrophus* is even sparser, with only a few studies exploring its diet and population dynamics (McElhone, 1980; Schonborn, 1984; Streit, 1977).

Since the description of *Chaetogaster* in the 1820s, various researchers have relied on often subtle morphological features to describe additional species beyond *C. limnaei, C. diaphanus,* and *C. diastrophus*. In her treatise on the Naidinae (formerly Naididae; Erséus et al. (2008)), Sperber (1948) recognized nine *Chaetogaster* species based on body size and variation in the number of segmental chaetae, in addition to four subspecies of *C. limnaei*. Later dichotomous keys of aquatic oligochaetes only recognized six species in the genus and relied on a mixture of body
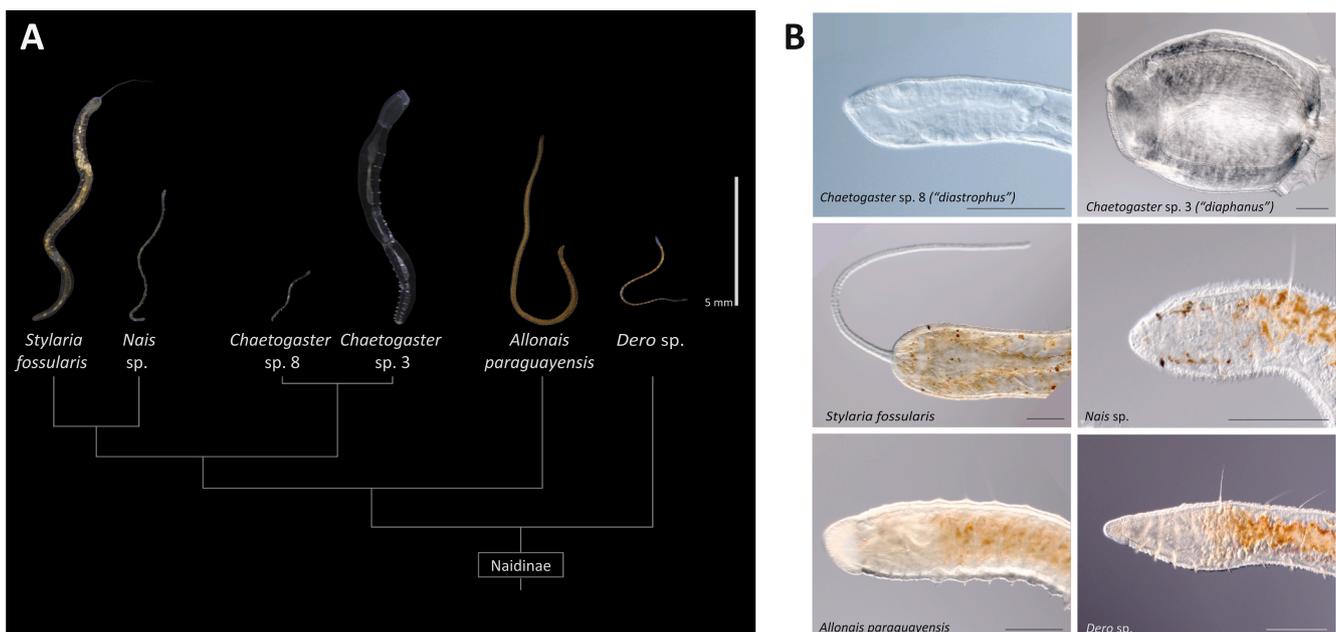

Fig. 1. Overview of evolution and morphology among some common water nymph worm genera (Annelida: Clitellata: Tubificida: Naididae: Naidinae). **A:** Relationships and morphological diversity. Anterior ends are pointing up. Note that the *Chaetogaster "diastrophus"* morphotype (represented here by species 8) is among the smallest of the naidines, while the *C. "diaphanus"* morphotype (represented here by species 3) is among the largest. Images are to scale. Scale bar represents 5 mm. **B:** Diversity of anterior morphology among some common water nymph worm genera. Anterior is left. Bottom and top row images are lateral views. Middle row images are dorsal views. Note that *Chaetogaster* worms are unique in having large, rounded, and muscular heads. Scale bars represent 0.2 mm.

size, chaetal shape, prostomial morphology, and trophic ecology to delimit species (Brinkhurst and Jamieson, 1971). One investigation in Lake Baikal even reported up to ten species in the genus, nine of which were described as endemic to the lake (Semernoi, 1985). To date, the World Register of Marine Species (WoRMs) lists 17 total species of *Chaetogaster* worldwide (WoRMS Editorial Board, 2022). However, it is difficult to assess species diversity without molecular data as many characters can be unreliable in *Chaetogaster*. For example, the range of chaetal counts per segment, an important character in many *Chaetogaster* descriptions, often overlap between presumptive species. Other characters, such as the prostomial incision separating *C. diaphanus* and the putative *Chaetogaster cristallinus* Vejdovsky 1884, can be difficult to detect and may even be found in multiple species (Sperber, 1948). Despite this body of literature on *Chaetogaster*, it is still uncertain how many species exist in the genus beyond *C. diaphanus, C. limnaei,* and *C. diastrophus.*

Morphological descriptions of freshwater meiofauna are often inadequate to capture all the diversity in a particular group and *Chaetogaster* is unlikely to be an exception. Indeed, a recent phylogeny of the entire subfamily Naidinae revealed that two specimens of *C. "diastrophus"* were non-monophyletic. Meanwhile, rather large genetic distances (>5%) separated *C. diaphanus* individuals collected in North America and Europe (Erséus et al., 2017). This prior work, taken in the context of unreliable morphological descriptions, suggests that *Chaetogaster* might be more diverse than previously described.

Here, we evaluate the extent of *Chaetogaster* diversity and investigate intra-generic relationships by leveraging molecular phylogenetic analyses of 128 individuals collected primarily across Europe and North America. We employ multiple sources of evidence to delimit species, including two mitochondrial loci, two nuclear loci, and three statistical species delimitation tools. Generating a robust phylogeny of *Chaetogaster* represents a necessary step toward approximating the true diversity of the Naidinae, and freshwater meiofauna more generally. Furthermore, it provides a needed phylogenetic framework to help develop this genus as a model for challenging questions in evolutionary biology, such as the evolution of carnivory, the maintenance of host-symbiont interactions (Hobart et al., 2022), and the loss of regenerative ability (Bely and Sikes, 2010).

2. Materials and methods

2.1. Specimen collections

We collected 128 *Chaetogaster* individuals from ponds, lake shores, and creeks primarily in North America (mostly within the state of Maryland) and Europe (mostly within Scandinavian countries) (Table 1). Worms were identified as *C. diaphanus, C. diastrophus,* or *C. limnaei* based on body size, habitat, and similarity to descriptions in Kathman and Brinkhurst (1998). *Chaetogaster "diaphanus"* species were typically found clinging to submerged aquatic vegetation in lentic habitats and were identified by their large body size. Meanwhile, most *Chaetogaster "diastrophus"* species were collected by sifting through shallow lotic sediment and were identified by their small body size and free-living ecology. In both lentic and lotic habitats, *Chaetogaster "limnaei"* species were found living on or within freshwater snails and were identified by their sharply hooked chaetae. Worms were preserved in 75 – 95% ethanol. Most European worms and some North American worms were cut in half to preserve the anterior end in formalin or 80 – 95% ethanol as a partial morphological voucher. Because *Chaetogaster* individuals are so small, in some cases the whole animal was used for DNA extractions to ensure adequate DNA yields. Five specimens of worms from the genus *Amphichaeta* were also newly collected for this study as an outgroup to *Chaetogaster*, as these two genera are known to be sister clades (Erséus et al., 2017).

2.2. DNA extraction and sequencing

We extracted DNA from our *Chaetogaster* samples and PCR amplified and Sanger sequenced regions of two mitochondrial loci (COI and 16S) and two nuclear loci (H3 and ITS2). Our collaboration spanned three lab groups (lab group A: JMM, AEB; lab group B: HCP, PH, RL, HS; and lab group C: CE, SM, MK) and specific methodologies differed slightly across groups. Table 1 indicates the sequences contributed by each group and the details of DNA extraction, PCR, and sequencing can be found in the Supplementary methods. Lab group A generated sequences from all worms collected and processed by this group, as well as the 16S, H3, and ITS2 sequences from worms collected and processed by lab group B; lab group B generated all COI sequences from worms collected and processed by this group; and lab group C generated all sequences from worms collected and processed by this group. Table 2 lists the primers used for PCR. New sequences were submitted to GenBank under the numbers listed in Table 1.

2.3. Phylogenetic analyses

Sequences from each locus were aligned in the online version of MAFFT ver. 7 (Katoh et al., 2019) according to default settings. For the ITS gene, only the ITS2 portion was used for both the individual gene trees and the concatenated dataset. The alignments for each locus were concatenated in Mesquite ver. 3.70 (Maddison and Maddison, 2021) to produce a combined dataset of 2,325 characters (COI: 720 bp; 16S: 500 bp; ITS2: 776 bp; H3: 329 bp).

We assessed phylogenetic relationships using both maximum likelihood (ML) and Bayesian Inference (BI) optimality criteria. In all trees, we included sequences from *Nais alpina* and up to three *Amphichaeta* species as outgroups (Envall et al., 2006; Erséus et al., 2010; Liu et al., 2017b).

For ML analyses, we used IQTREE ver. 2.1.2 on the CIPRES science gateway to estimate the best-fitting models of nucleotide evolution for each locus and construct a phylogeny from the concatenated dataset and gene trees for each locus (Kalyaanamoorthy et al., 2017; Miller et al., 2010; Minh et al., 2020). IQTREE assigned five separate partitions to the combined four gene dataset. Each followed a unique model of nucleotide evolution. Most were estimated to follow a GAMMA distribution of nucleotide rates, with empirically calculated base frequencies and an estimated proportion of invariable sites. The exception is the final codon position of H3, which did not include a proportion of invariable sites. IQTREE subsequently constructed the most likely tree topology for the concatenated alignment. Node support values were estimated from 1000 bootstrap replicates. We also used IQTREE to generate gene trees for each individual locus. For the COI gene tree, additional sequences were included. To assess which of our *C. "limnaei"* clades match those recovered in the previous study by Smythe et al. (2015), we added eleven sequences from GenBank to our dataset (Table 1). For the BI analysis, we used MrBayes ver. 3.2.7 on the CIPRES science gateway (Huelsenbeck and Ronquist, 2001; Miller et al., 2010) to generate a phylogeny from the concatenated dataset. The analysis was run for 2 million generations with trees sampled every 1,000 generations. The same partitioning scheme as the ML analysis was used. Burn-in was set to 25% and Tracer ver. 1.7.2 (Rambaut et al., 2018) was used to confirm that the MCMC had reached convergence. Tree files were visualized in FigTree ver. 1.4.4 (Rambaut, 2017) and annotated in Adobe Illustrator.

2.4. Species delimitation

Four statistical approaches were employed to assess the number of species more objectively in *Chaetogaster*: an assessment of genetic distances, two species delimitation analyses of single locus data, and a species delimitation analysis of multi-locus data.

First, to determine the range of inter- and intra-specific distance, uncorrected p - distances were calculated within and between COI clades

Table 1
Specimens and sequences represented in this study. GenBank accession numbers of new sequences generated for this study are indicated in bold.

Collection locality	Coordinates	Collection date	*Chaetogaster* morphotype	Putative species I.D.	Reference number	COI	ITS2	H3	16S	Voucher
Ingroup										
University Hills Duck Pond, MD, USA	38.984, −76.960	Jul. 2020	"diastrophus"	12	JMga001	**OQ281725** [a]	**OQ316538** [a]	**OQ286194** [a]	**OQ316567** [a]	x
Guilford Run, MD, USA	38.978, −76.939	Jul. 2020	"limnaei"	22	JMga010	**OQ281726** [a]	**OQ316539** [a]	**OQ286195** [a]	**OQ316568** [a]	x
Guilford Run, MD, USA	38.978, −76.940	Jul. 2020	"diastrophus"	10	JMga012	**OQ281727** [a]	**OQ316540** [a]	**OQ286196** [a]	**OQ316569** [a]	x
Paint Branch, MD, USA	38.995, −76.933	Jul. 2020	"diastrophus"	8	JMga018	**OQ281728** [a]	**OQ316541** [a]	**OQ286197** [a]	**OQ316570** [a]	x
Gillis Falls, MD, USA	39.390, −77.080	Jul. 2020	"limnaei"	22	JMga025	**OQ281729** [a]	**OQ316542** [a]	**OQ286198** [a]	**OQ316571** [a]	x
Gillis Falls, MD, USA	39.390, −77.080	Jul. 2020	"diastrophus"	8	JMga027	**OQ281730** [a]	**OQ316543** [a]	**OQ286199** [a]	**OQ316572** [a]	x
Alter Pond, MD, USA	39.013, −76.849	May. 2021	"limnaei"	24	JMga099	**OQ281731** [a]	**OQ316544** [a]	**OQ286200** [a]	**OQ316573** [a]	x
Cheltinham Wetland Park, MD, USA	38.752, −76.847	May. 2021	"diastrophus"	18	JMga100	**OQ281732** [a]	**OQ316545** [a]	**OQ286201** [a]	**OQ316574** [a]	x
Brackenridge Field Laboratory, TX, USA	30.283, −97.778	Jan. 2020	"diastrophus"	17	JM005	**OQ281714** [a]	**OQ316521** [a]	**OQ286178** [a]	**OQ316551** [a]	x
Colorado River, TX, USA	30.263, −97.746	Jan. 2020	"diaphanus"	3	JM010	**OQ281715** [a]	**OQ316522** [a]	**OQ286179** [a]	**OQ316552** [a]	x
Paint Branch Creek, MD, USA	38.995, −76.933	May. 2018	"diaphanus"	3	JM015	**OQ281734** [a]	**OQ316523** [a]	**OQ286180** [a]	**OQ316553** [a]	x
Paint Branch Creek, MD, USA	38.995, −76.933	Oct. 2018	"diastrophus"	6	JM016	**OQ281735** [a]	**OQ316524** [a]	x	**OQ316554** [a]	x
Rose Hill Swamp, MD, USA	38.514, −77.023	May. 2018	"diaphanus"	3	JM017	**OQ281736** [a]	**OQ316525** [a]	**OQ286181** [a]	**OQ316555** [a]	x
Campus Creek, MD, USA	38.993, −76.938	Apr. 2018	"diastrophus"	12	JM018	**OQ281713** [a]	**OQ316526** [a]	**OQ286182** [a]	**OQ316556** [a]	x
Paint Branch Creek, MD, USA	38.995, −76.933	Oct. 2018	"diastrophus"	6	JM021	**OQ281716** [a]	**OQ316527** [a]	**OQ286183** [a]	**OQ316557** [a]	x
Watersville Road Run, MD, USA	39.372, −77.108	Jun. 2018	"diaphanus"	3	JM024	**OQ281717** [a]	**OQ316528** [a]	**OQ286184** [a]	**OQ316558** [a]	x
Gillis Falls, MD, USA	39.390, −77.080	Nov. 2018	"diastrophus"	10	JM026	**OQ281718** [a]	**OQ316529** [a]	**OQ286185** [a]	**OQ316559** [a]	x
Carrol Creek, MD, USA	39.423, −77.386	Aug. 2018	"diastrophus"	8	JM028	**OQ281719** [a]	**OQ316530** [a]	**OQ286186** [a]	**OQ316560** [a]	x
Evitts Creek, MD, USA	39.672, −78.7230	Dec. 2018	"diastrophus"	10	JM029	**OQ281720** [a]	**OQ316531** [a]	**OQ286187** [a]	**OQ316561** [a]	x
Evitts Creek, MD, USA	39.672, −78.7230	May. 2018	"diastrophus"	8	JM030	**OQ281721** [a]	**OQ316532** [a]	**OQ286188** [a]	**OQ316562** [a]	x
Cresap Mill Creek, MD, USA	39.619, −78.651	Dec. 2018	"diastrophus"	20	JM031	**OQ281722** [a]	**OQ316533** [a]	**OQ286189** [a]	**OQ316563** [a]	x
Starlight Drive Creek, MD, USA	39.626, −78.734	May. 2019	"diastrophus"	12	JM032	**OQ281723** [a]	**OQ316534** [a]	**OQ286190** [a]	**OQ316564** [a]	x
University Hills Duck Pond, MD, USA	38.984, −76.960	Oct. 2019	"diaphanus"	3	JM034	**OQ281737** [a]	**OQ316535** [a]	**OQ286191** [a]	x	x
University Hills Duck Pond, MD, USA	38.984, −76.960	Oct. 2019	"diastrophus"	20	JM035	**OQ281724** [a]	**OQ316536** [a]	**OQ286192** [a]	**OQ316565** [a]	x
Guilford Run, MD, USA	38.978, −76.939	Sep. 2019	"diastrophus"	8	JM040	**OQ281733** [a]	**OQ316537** [a]	**OQ286193** [a]	**OQ316566** [a]	x
St. John River, NB, Canada	45.963, −66.639	Aug. 2019	"limnaei"	22	HP15-1	**OQ281711** [b]	**OQ316519** [a]	**OQ286176** [a]	**OQ316549** [a]	x
MacGregor Lake, AB, Canada	50.564, −112.915	Aug. 2019	"limnaei"	22	HP14-14	**OQ281710** [b]	**OQ316518** [a]	**OQ286175** [a]	**OQ316548** [a]	x
Lesser Slave Lake, AB, Canada	55.479, −114.902	Aug. 2019	"limnaei"	22	HP15-7	**OQ281712** [b]	**OQ316520** [a]	**OQ286177** [a]	**OQ316550** [a]	x
MacGregor Lake, AB, Canada	50.564, −112.915	Aug. 2019	"limnaei"	24	HP3-12	**OQ281708** [b]	**OQ316516** [a]	**OQ286173** [a]	**OQ316546** [a]	x
Narrow Lake, AB, Canada	54.615, −113.614	Jul. 2019	"limnaei"	24	HP4-24	**OQ281709** [b]	**OQ316517** [a]	**OQ286174** [a]	**OQ316547** [a]	x
Hamilton College Reservoir, NY, USA	43.051, −75.435		"limnaei"	22	Smythe et al. 2015	KF952327 [d]	x	x	x	
Yahnundasis Lake, NY, USA	43.085, −75.321		"limnaei"	22	Smythe et al. 2015	KF952321 [d]	x	x	x	
South Hill Road, NY, USA	43.306, −75.301		"limnaei"	22	Smythe et al. 2015	KF952353 [d]	x	x	x	
Portlandville Swamp, NY, USA	42.551, −75.954		"limnaei"	22	Smythe et al. 2015	KF952342 [d]	x	x	x	
Soule Road, NY, USA	43.304, −75.290		"limnaei"	22	Smythe et al. 2015	KF952341 [d]	x	x	x	
South Hill Road, NY, USA	43.306, −75.301		"limnaei"	22	Smythe et al. 2015	KF952343 [d]	x	x	x	
Gillett Road, NY, USA	43.320, −75.321		"limnaei"	22	Smythe et al. 2015	KF952336 [d]	x	x	x	
Mascuppic Lake, MA, USA	42.678, −71.400		"limnaei"	24	Smythe et al. 2015	KF952295 [d]	x	x	x	
Portlandville Swamp, NY, USA	42.551, −75.954		"limnaei"	22	Smythe et al. 2015	KF952308 [d]	x	x	x	
Yahnundasis Lake, NY, USA	43.085, −75.308		"limnaei",	22	Smythe et al. 2015	KF952333 [d]	x	x	x	
Portlandville Swamp, NY, USA	42.551, −75.954		"limnaei",	22	Smythe et al. 2015	KF952350 [d]	x	x	x	
Lake Lången, Västergötland, Sweden	57.997, 12.586	Oct. 2000	"diastrophus"	8	CE205	x	**OQ272539** [c]	**OQ272641** [c]	AY885586 [j]	x
Lången Lake, Västergötland, Sweden	57.997, 12.587	Jul. 2002	"diaphanus"	4	CE439	JQ519897 [e]	KY633380 [f]	x	DQ459956 [g]	x
Hällekis, Västergötland, Sweden	58.608, 13.393	Sep. 2005	"diastrophus"	9	CE1089	**OQ309289** [c]	**OQ272548** [c]	**OQ272650** [c]	**OQ272743**	x
Hällekis, Västergötland, Sweden	58.608, 13.393	Sep. 2005	"diastrophus"	9	CE1090	JQ519820 [e]	**OQ272498** [c]	**OQ272599** [c]	JQ424952 [e]	x
Ship Creek, Anchorage, AK, USA	61.224, −149.889	Aug. 2005	"diaphanus"	3	CE1162	**OQ309256** [c]	**OQ272515** [c]	**OQ272616** [c]	**OQ272711** [c]	SMNH 210128
Ship Creek, Anchorage, AK, USA	61.224, −149.889	Aug. 2005	"diaphanus"	3	CE1163	**OQ309291** [c]	**OQ272550** [c]	**OQ272652** [c]	**OQ272745** [c]	SMNH 210129
Ship Creek, Anchorage, AK, USA	61.224, −149.889	Aug. 2005	"diaphanus"	3	CE1164	**OQ309239** [c]	**OQ272496** [c]	**OQ272597** [c]	**OQ272694** [c]	SMNH 210130
Lake Lången, Västergötland, Sweden	57.997, 12.586	Jun. 2006	"diaphanus"	4	CE2010	**OQ309228** [c]	**OQ272485** [c]	**OQ272588** [c]	x	SMNH 210131
Guam, USA	13.426, 144.782	Jan. 2007	"diastrophus"	14	CE2303	**OQ309250** [c]	**OQ272509** [c]	**OQ272610** [c]	x	x
Guam, USA	13.426, 144.782	Jan. 2007	"diastrophus"	14	CE2305	**OQ309285** [c]	**OQ272544** [c]	**OQ272646** [c]	**OQ272739** [c]	SMNH 210132
Davyhulme Wastewater Treatment Works, Great Manchester, UK	53.463, −2.374	Feb. 2008	"diastrophus"	12	CE3496	**OQ309292** [c]	**OQ272551** [c]	**OQ272653** [c]	**OQ272746** [c]	SMNH 210069

Table 1 (*continued*)

Collection locality	Coordinates	Collection date	*Chaetogaster* morphotype	Putative species I.D.	Reference number	COI	ITS2	H3	16S	Voucher
Lake Ontario, ON, Canada	43.486, −79.612	Jul. 2007	"diastrophus"	12	CE3537	OQ309278 [c]	OQ272536 [c]	OQ272638 [c]	OQ272732 [c]	SMNH 210070
Seatons, Västergötland, Sweden	57.776, 12.241	Apr. 2008	"diastrophus"	12	CE3904	OQ309210 [c]	OQ272467 [c]	OQ272570 [c]	OQ272667 [c]	SMNH 210071
Åkers Kanal, Uppland, Sweden	59.496, 18.274	Jun. 2009	"diaphanus"	4	CE6553	OQ309199 [c]	OQ272455 [c]	OQ272558 [c]	OQ272656 [c]	SMNH 210072
Bönhamn, Ångermanland, Sweden	62.879, 18.431	Jun. 2010	"diastrophus"	11	CE7924	OQ309218 [c]	OQ272475 [c]	OQ272578 [c]	OQ272674 [c]	SMNH 210073
Bönhamn, Ångermanland, Sweden	62.879, 18.431	Jun. 2010	"diastrophus"	12	CE7926	OQ309270 [c]	x	OQ272630 [c]	OQ272724 [c]	x
Bönhamn, Ångermanland, Sweden	62.879, 18.431	Jun. 2010	"diastrophus"	12	CE7928	OQ309216 [c]	OQ272473 [c]	OQ272576 [c]	OQ272672 [c]	SMNH 210074
Abisko, Lappland, Sweden	68.355, 18.822	Jun. 2010	"diastrophus"	7	CE8493	OQ309198 [c]	OQ272454 [c]	OQ272557 [c]	OQ272655 [c]	SMNH 210075
Björkliden, Lappland, Sweden	68.408, 18.679	Jul. 2010	"diastrophus"	12	CE8497	OQ309207 [c]	OQ272464 [c]	OQ272567 [c]	OQ272664 [c]	SMNH 210133
Björkliden, Lappland, Sweden	68.408, 18.679	Jul. 2010	"diastrophus"	12	CE8498	OQ309255 [c]	OQ272514 [c]	OQ272615 [c]	OQ272710 [c]	SMNH 210076
Björkliden, Lappland, Sweden	68.408, 18.679	Jul. 2010	"diastrophus"	12	CE8499	OQ309275 [c]	OQ272533 [c]	OQ272635 [c]	OQ272729 [c]	SMNH 210134
Strömsvattnet Lake, Bohuslän, Sweden	58.945, 11.193	Sep. 2010	"diaphanus"	4	CE9800	OQ309264 [c]	OQ272523 [c]	OQ272624 [c]	OQ272718 [c]	SMNH 210077
Strömsvattnet Lake, Bohuslän, Sweden	58.945, 11.193	Sep. 2010	"diaphanus"	4	CE9801	OQ309290 [c]	OQ272549 [c]	OQ272651 [c]	OQ272744 [c]	SMNH 210135
Caprera Island, Sardinia, Italy	41.221, 9.463	Sep. 2010	"diastrophus"	12	CE10252	OQ309244 [c]	OQ272502 [c]	OQ272603 [c]	OQ272699 [c]	SMNH 210078
Paraside Coast, Sardinia, Italy	41.042, 8.933	Sep. 2010	"diastrophus"	15	CE10266	OQ309243 [c]	OQ272501 [c]	OQ272602 [c]	OQ272698 [c]	x
Paraside Coast, Sardinia, Italy	41.042, 8.933	Sep. 2010	"diastrophus"	15	CE10267	OQ309281 [c]	OQ272540 [c]	OQ272642 [c]	OQ272735 [c]	x
Paraside Coast, Sardinia, Italy	41.042, 8.933	Sep. 2010	"diastrophus"	15	CE10268	OQ309242 [c]	OQ272500 [c]	OQ272601 [c]	OQ272697 [c]	x
Guldhedstorget, Göteborg, Sweden	57.689, 11.966	May. 2010	"diastrophus"	20	CE10519	OQ309249 [c]	OQ272508 [c]	OQ272609 [c]	OQ272705 [c]	SMNH 210079
Dunsborough, Western Australia, Australia	−33.794, 115.031	Sep. 2012	"diaphanus"	3	CE17416	OQ309236 [c]	OQ272493 [c]	x	OQ272691 [c]	SMNH 210136
Örtedalsåna, Buskerud, Norway	60.486, 7.855	Aug. 2013	"diaphanus"	7	CE19073	OQ309268 [c]	OQ272527 [c]	OQ272628 [c]	OQ272722 [c]	ZMBN 128588
Lake Aspen, Västergötland, Sweden	57.765, 12.252	Oct. 2013	"diastrophus"	19	CE19923	OQ309276 [c]	OQ272534 [c]	OQ272636 [c]	OQ272730 [c]	SMNH 210081
Lake Aspen, Västergötland, Sweden	57.765, 12.252	Oct. 2013	"diastrophus"	1	CE19924	OQ309226 [c]	OQ272483 [c]	OQ272586 [c]	OQ272682 [c]	SMNH 210082
Lake Aspen, Västergötland, Sweden	57.765, 12.252	Oct. 2013	"diastrophus"	8	CE19925	OQ309288 [c]	OQ272547 [c]	OQ272649 [c]	OQ272742 [c]	SMNH 210083
Halden, Ostfold, Norway	58.979, 11.512	Oct. 2013	"diastrophus"	18	CE20273	OQ309287 [c]	OQ272546 [c]	OQ272648 [c]	OQ272741 [c]	x
Randsfjorden, Oppland, Norway	60.239, 10.398	Aug. 2015	"diastrophus"	8	CE26665	OQ309224 [c]	OQ272481 [c]	OQ272584 [c]	OQ272680 [c]	ZMBN 128746
Jölstravatn Lake, Sogn og Fjordane, Norway	61.510, 6.155	Aug. 2015	"diastrophus"	5	CE26867	OQ309215 [c]	OQ272472 [c]	OQ272575 [c]	OQ272671 [c]	ZMBN 152694
Jölstravatn Lake, Sogn og Fjordane, Norway	61.510, 6.155	Aug. 2015	"diastrophus"	5	CE26868	OQ309241 [c]	OQ272499 [c]	OQ272600 [c]	OQ272696 [c]	ZMBN 152695
Ögonakällan, Närke, Sweden	59.04, 15.018	Jul. 2015	"diastrophus"	15	CE27973	OQ309293 [c]	OQ272552 [c]	OQ272654 [c]	OQ272747 [c]	SMNH 210084
Mjösa Lake, Oppland, Norway	60.954, 10.627	Jul. 2016	"limnaei"	23	CE28176	OQ309217 [c]	OQ272474 [c]	OQ272577 [c]	OQ272673 [c]	ZMBN 128853
Florö, Sogn og Fjordane, Norway	61.59, 5.010	Jul. 2016	"diastrophus"	15	CE28551	OQ309211 [c]	OQ272468 [c]	OQ272571 [c]	OQ272668 [c]	x
Masfjorden Storevatn Lake, Hordaland, Norway	60.837, 5.587	Jul. 2016	"diastrophus"	5	CE28644	OQ309229 [c]	OQ272486 [c]	OQ272589 [c]	OQ272684 [c]	ZMBN 128921
Halsatjönnet Lake, Oppland, Norway	61.659, 8.147	Jul. 2016	"diastrophus"	2	CE28741	OQ309261 [c]	OQ272520 [c]	OQ272621 [c]	OQ272715 [c]	ZMBN 128933
Halsatjönnet Lake, Oppland, Norway	61.659, 8.147	Jul. 2016	"diastrophus"	2	CE28742	OQ309257 [c]	OQ272516 [c]	OQ272617 [c]	OQ272712 [c]	ZMBN 152696
Halsatjönnet Lake, Oppland, Norway	61.659, 8.147	Jul. 2016	"diastrophus"	1	CE28745	OQ309234 [c]	OQ272491 [c]	x	OQ272689 [c]	ZMBN 128935
Hjälmaren Lake, Närke, Sweden	59.236, 15.567	Sep. 2016	"diastrophus"	11	CE29683	OQ309246 [c]	OQ272504 [c]	OQ272605 [c]	OQ272701 [c]	SMNH 210085
Hjälmaren Lake, Närke, Sweden	59.236, 15.567	Sep. 2016	"diastrophus"	19	CE29684	OQ309201 [c]	OQ272457 [c]	OQ272560 [c]	OQ272658 [c]	SMNH 210086
Femsjöen Lake, Ostfold Norway	59.131, 11.488	Oct. 2016	"diastrophus"	12	CE29834	OQ309233 [c]	OQ272490 [c]	OQ272593 [c]	OQ272688 [c]	ZMBN 128980
Glomma River, Åkershus, Norway	60.125, 11.464	Oct. 2016	"diastrophus"	11	CE30132	OQ309263 [c]	OQ272522 [c]	OQ272623 [c]	OQ272717 [c]	ZMBN 129034
Baereia Lake, Hedmark, Norway	60.168, 11.966	Oct. 2016	"diastrophus"	1	CE30202	OQ309222 [c]	OQ272479 [c]	OQ272582 [c]	OQ272678 [c]	x
Lomba da Maia, Azores, Portugal	37.839, −25.364	May. 2017	"diastrophus"	10	CE31775	x	OQ272507 [c]	OQ272608 [c]	OQ272704 [c]	SMNH 210137
Trangsdola River, Nord-Trondelag, Norway	63.730, 11.653	May. 2017	"diastrophus"	18	CE31982	OQ309220 [c]	OQ272477 [c]	OQ272580 [c]	OQ272676 [c]	ZMBN 129162
Tingvoll, More og Romsdal, Norway	63.030, 8.025	May. 2017	"diastrophus"	12	CE32094	OQ309232 [c]	OQ272489 [c]	OQ272592 [c]	OQ272687 [c]	ZMBN 129169
Tingvoll, More og Romsdal, Norway	63.030, 8.025	May. 2017	"diastrophus"	15	CE32125	OQ309282 [c]	OQ272541 [c]	OQ272643 [c]	OQ272736 [c]	ZMBN 129178
Laerdalselva River, Sogn of Fjordane, Norway	61.842, 7.842	Jun. 2017	"diastrophus"	2	CE32668	OQ309242 [c]	OQ272521 [c]	OQ272622 [c]	OQ272716 [c]	ZMBN 129253
Sandbu, Buskerud, Norway	60.181, 8.582	Jul. 2017	"diastrophus"	12	CE32809	OQ309235 [c]	OQ272492 [c]	OQ272594 [c]	OQ272690 [c]	ZMBN 152698
Sudndalsfjorden Lake, Buskerud, Norway	60.631, 8.0722	Jul. 2017	"diastrophus"	12	CE32880	OQ309240 [c]	OQ272497 [c]	OQ272598 [c]	OQ272695 [c]	ZMBN 152699
Lesjaskogsvatnet Lake, Oppland, Norway	62.229, 8.405	Jul. 2017	"diastrophus"	11	CE33168	OQ309209 [c]	OQ272466 [c]	OQ272569 [c]	OQ272666 [c]	ZMBN 129323
Torneträsk Lake, Lappland, Sweden	68.290, 19.302	Sep. 2017	"Chaetophus"	12	CE33337	OQ309230 [c]	OQ272487 [c]	OQ272590 [c]	OQ272685 [c]	SMNH 210090
Torneträsk Lake, Lappland, Sweden	68.302, 19.248	Sep. 2017	"diastrophus"	16	CE33363	OQ309247 [c]	OQ272505 [c]	OQ272606 [c]	OQ272702 [c]	SMNH 210091
Torneträsk Lake, Lappland, Sweden	68.302, 19.248	Sep. 2017	"diastrophus"	16	CE33364	OQ309200 [c]	OQ272456 [c]	OQ272559 [c]	OQ272657 [c]	SMNH 210092
Torneträsk Lake, Lappland, Sweden	68.302, 19.248	Sep. 2017	"diastrophus"	7	CE33365	OQ309269 [c]	OQ272528 [c]	OQ272629 [c]	OQ272723 [c]	SMNH 210093
Torneträsk Lake, Lappland, Sweden	68.302, 19.248	Sep. 2017	"diaphanus"	4	CE33366	OQ309208 [c]	OQ272465 [c]	OQ272568 [c]	OQ272665 [c]	SMNH 210094
Urdalsvatnet Lake, Nordland, Norway	68.474, 17.995	Sep. 2017	"diaphanus"	7	CE33406	OQ309205 [c]	OQ272462 [c]	OQ272565 [c]	OQ272662 [c]	ZMBN 152702
Forfjordelva, Nordland, Norway	68.817, 15.684	Sep. 2017	"diastrophus"	20	CE33537	OQ309203 [c]	OQ272459 [c]	OQ272562 [c]	OQ272660 [c]	ZMBN 129346
Övre Aeråsvatnet Lake, Nordland, Norway	69.253, 16.039	Sep. 2017	"diastrophus"	5	CE33672	OQ309277 [c]	OQ272535 [c]	OQ272637 [c]	OQ272731 [c]	ZMBN 129364

Table 1 (*continued*)

Collection locality	Coordinates	Collection date	*Chaetogaster* morphotype	Putative species I.D.	Reference number	COI	ITS2	H3	16S	Voucher
Övre Aeråsvatnet Lake, Nordland, Norway	69.253, 16.039	Sep. 2017	*"diastrophus"*	1	CE33683	OQ309219 [c]	OQ272476 [c]	OQ272579 [c]	OQ272675 [c]	ZMBN 152703
Rambergselva River, Nordland, Norway	69.298, 16.049	Sep. 2017	*"diastrophus"*	19	CE33831	OQ309221 [c]	OQ272478 [c]	OQ272581 [c]	OQ272677 [c]	ZMBN 152704
Rörvikelva River, Nordland, Norway	68.203, 14.236	Sep. 2017	*"diastrophus"*	20	CE33932	OQ309245 [c]	OQ272503 [c]	OQ272604 [c]	OQ272700 [c]	ZMBN 129397
Storvatnet Lake, Nordland, Norway	68.122, 13.367	Sep. 2017	*"diastrophus"*	5	CE34041	OQ309266 [c]	OQ272525 [c]	OQ272626 [c]	OQ272720 [c]	ZMBN 152707
Lavangsvatnet Lake, Nordland, Norway	68.509, 16.653	Sep. 2017	*"diastrophus"*	2	CE34390	OQ309260 [c]	OQ272519 [c]	OQ272620 [c]	x	ZMBN 129467
Lången Lake, Västergötland, Sweden	57.997, 12.587	Aug. 2018	*diastrophus*	11	CE35366	OQ309225 [c]	OQ272482 [c]	OQ272585 [c]	OQ272681 [c]	SMNH 210095
Säveån River, Västergötland, Sweden	57.791, 12.319	Jun. 2019	*diastrophus*	11	CE35678	OQ309202 [c]	OQ272458 [c]	OQ272561 [c]	OQ272659 [c]	SMNH 210096
Säveån River, Västergötland, Sweden	57.791, 12.319	Jun. 2019	*diastrophus*	5	CE35679	OQ309204 [c]	OQ272460 [c]	OQ272563 [c]	OQ272661 [c]	SMNH 210097
Säveån River, Västergötland, Sweden	57.804, 12.351	Jun. 2019	*diastrophus*	18	CE35710	OQ309280 [c]	OQ272538 [c]	OQ272640 [c]	OQ272734 [c]	SMNH 210098
Bälingesjön Lake, Skåne, Sweden	56.251, 13.373	Sep. 2020	*"limnaei"*	23	CE36252	OQ309271 [c]	OQ272529 [c]	OQ272631 [c]	OQ272725 [c]	SMNH 210099
Julebodaån River, Skåne, Sweden	55.763, 14.155	Sep. 2020	*"diastrophus"*	8	CE36534	OQ309267 [c]	OQ272526 [c]	OQ272627 [c]	OQ272721 [c]	SMNH 210100
Julebodaån River, Skåne, Sweden	55.763, 14.155	Sep. 2020	*"diastrophus"*	9	CE36535	OQ309227 [c]	OQ272484 [c]	OQ272587 [c]	OQ272683 [c]	SMNH 210101
Norrlia, Skåne, Sweden	55.821, 14.102	Sep. 2020	*"diaphanus"*	7	CE36751	OQ309259 [c]	OQ272518 [c]	OQ272619 [c]	OQ272714 [c]	SMNH 210102
Norrlia, Skåne, Sweden	55.821, 14.102	Sep. 2020	*"diaphanus"*	7	CE36753	OQ309253 [c]	OQ272512 [c]	OQ272613 [c]	OQ272708 [c]	SMNH 210103
Segesholmsån River, Skåne, River	55.821, 14.08	Sep. 2020	*"diastrophus"*	8	CE37140	OQ309272 [c]	OQ272530 [c]	OQ272632 [c]	OQ272726 [c]	SMNH 210104
Segesholmsån River, Skåne, River	55.821, 14.08	Sep. 2020	*"diaphanus"*	7	CE37141	OQ309238 [c]	OQ272495 [c]	OQ272596 [c]	OQ272693 [c]	SMNH 210105
Haväng, Skåne, Sweden	55.724, 14.195	Sep. 2020	*"diastrophus"*	19	CE37561	OQ309270 [c]	OQ272532 [c]	OQ272634 [c]	OQ272728 [c]	SMNH 210106
Haväng, Skåne, Sweden	55.724, 14.195	Sep. 2020	*"diastrophus"*	1	CE37564	OQ309258 [c]	OQ272517 [c]	OQ272618 [c]	OQ272713 [c]	SMNH 210107
Haväng, Skåne, Sweden	55.724, 14.195	Sep. 2020	*"diaphanus"*	4	CE37567	OQ309223 [c]	OQ272480 [c]	OQ272583 [c]	OQ272679 [c]	SMNH 210108
Haväng, Skåne, Sweden	55.724, 14.195	Sep. 2020	*"diastrophus"*	13	CE37678	OQ309248 [c]	OQ272506 [c]	OQ272607 [c]	OQ272703 [c]	SMNH 210109
Haväng, Skåne, Sweden	55.724, 14.195	Sep. 2020	*"diastrophus"*	11	CE37679	OQ309286 [c]	OQ272545 [c]	OQ272647 [c]	OQ272740 [c]	SMNH 210110
Verkasjön Lake, Skåne, Sweden	55.717, 13.985	Sep. 2020	*"limnaei"*	23	CE37773	OQ309254 [c]	OQ272513 [c]	OQ272614 [c]	OQ272709 [c]	SMNH 210111
Verkasjön Lake, Skåne, Sweden	55.717, 13.985	Sep. 2020	*"limnaei"*	23	CE37774	OQ309214 [c]	OQ272471 [c]	OQ272574 [c]	OQ272670 [c]	SMNH 210112
Verkasjön Lake, Skåne, Sweden	55.717, 13.985	Sep. 2020	*"limnaei"*	23	CE37775	OQ309231 [c]	OQ272488 [c]	OQ272591 [c]	OQ272686 [c]	SMNH 210113
Verkaån River, Skåne, Sweden	55.727, 14.119	Sep. 2020	*"diaphanus"*	4	CE37832	OQ309206 [c]	OQ272463 [c]	OQ272566 [c]	OQ272663 [c]	SMNH 210114
Verkasjön Lake, Skåne, Sweden	55.717, 13.985	Sep. 2020	*"diastrophus"*	8	CE37906	OQ309212 [c]	OQ272469 [c]	OQ272572 [c]	OQ272669 [c]	SMNH 210115
Verkasjön Lake, Skåne, Sweden	55.717, 13.985	Sep. 2020	*"diaphanus"*	4	CE37909	OQ309273 [c]	OQ272531 [c]	OQ272633 [c]	OQ272727 [c]	SMNH 210116
Verkasjön Lake, Skåne, Sweden	55.717, 13.985	Sep. 2020	*"diaphanus"*	4	CE37955	OQ309283 [c]	OQ272542 [c]	OQ272644 [c]	OQ272737 [c]	SMNH 210117
Verkasjön Lake, Skåne, Sweden	55.717, 13.985	Sep. 2020	*"diastrophus"*	21	CE37961	OQ309252 [c]	OQ272511 [c]	OQ272612 [c]	OQ272707 [c]	SMNH 210118
Verkasjön Lake, Skåne, Sweden	55.717, 13.985	Sep. 2020	*"diastrophus"*	21	CE37964	OQ309284 [c]	OQ272543 [c]	OQ272645 [c]	OQ272738 [c]	SMNH 210119
Verkasjön Lake, Skåne, Sweden	55.717, 13.985	Sep. 2020	*"diastrophus"*	21	CE38010	OQ309213 [c]	OQ272470 [c]	OQ272573 [c]	x	SMNH 210120
Julebodaån River, Skåne, Sweden	55.761, 14.136	Sep. 2020	*"diastrophus"*	18	CE38200	OQ309237 [c]	OQ272494 [c]	OQ272595 [c]	OQ272692 [c]	SMNH 210121
Julebodaån River, Skåne, Sweden	55.761, 14.136	Sep. 2020	*"diastrophus"*	18	CE38201	OQ309265 [c]	OQ272524 [c]	OQ272625 [c]	OQ272719 [c]	SMNH 210122
Outgroup										
Gillis Falls, MD, USA	39.39, −77.080	Jul. 2020		*Amphichaeta* sp.	JMga022	OQ281738 [a]	x	x	x	x
Cheltingham Wetlands Park, MD, USA	38.752, −76.847	May. 2021		*Amphichaeta* sp.	JMga101	OQ281739 [a]	x	x	x	x
Tjärnö Marine Station, Bohuslän, Sweden	58.876, 11.146	Sep. 2000		*Amphichaeta sannio*	CE185	KY633392 [f]	OQ272461 [c]	OQ272564 [c]	DQ459955 [g]	x
Glomma River, Hedmark, Norway	60.193, 12.028	Oct. 2016		*Amphichaeta leydigi*	CE30245	OQ309251 [c]	OQ272510 [c]	OQ272611 [c]	OQ272706 [c]	ZMBN 153051
Rombaken Fjord, Nordland, Norway	68.454, 17.703	Sep. 2017		*Amphichaeta sannio*	CE33454	OQ309279 [c]	OQ272537 [c]	OQ272639 [c]	OQ272733 [c]	ZMBN 153061
Igelbäcken stream, Uppland, Sweden	59.389, 18.010	Oct. 2002		*Nais alpina*	CE529	GU902104 [h]	KY633365 [f]	KY637001 [i]	DQ459943 [g]	x

[a] sequenced by lab group A.
[b] sequenced by lab group B.
[c] sequenced by lab group C.
[d] Smythe et al., 2015.
[e] Envall et al., 2012.
[f] Erséus et al., 2017.
[g] Envall et al., 2006.
[h] Erséus et al., 2010.
[i] Liu et al., 2017b
[j] Sjölin et al. 2005.

Table 2
The primers used for amplification and sequencing of genes included in our study.

Gene	Primer Name	Sequence (5′ – 3′)	Citation
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994
	CHCr	TTATGWGCIACAATATGAAATTGC	This study.
	COI-E-	TATACTTCTGGGTGTCCGAAGAATCA	Bely and Wray 2004
16S	16SAR-L	CGCCTGTTTATCAAAAACAT	Palumbi et al. 1991
	16SBRH	CCGGTCTGAACTCAGATCACGT	Palumbi et al. 1991
H3	H3F	ATGGCTCGTACCAAGCAGACVGC	Colgan et al. 1998
	H3R	ATATCCTTRGGCATRATRGTGAC	Colgan et al. 1998
ITS	CH18sF	CGAGTCATAAGCTCGCGTTGATTACG	This study.
	CH28sR	CCTAAACACCACAGTTCGCGACGTCC	This study.
	606F	GTCGATGAAGAGCGCAGCCA	Liu & Erséus 2017
	1082R	TTAGTTTCTTTTCCTCCGCTT	Liu & Erséus 2017
M13	M13F	GTAAAACGACGGCCAGT	Messing et al. 1981; Vieira and Messing 1982
	M13R	GGAAACAGCTATGACCATG	Messing et al. 1981; Vieira and Messing 1982

in MEGA11 (Tamura et al., 2021). Standard errors for the distances were estimated from 1000 bootstrap replicates.

Second, we delimited species based on single-locus data using two methods: Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al., 2021) and General Mixed Yule Coalescence (GMYC) (Pons et al., 2006). ASAP takes sequence data from a single locus and searches for a natural "barcoding gap," inferred as the threshold between intraspecific distances and interspecific distances. Based on this limit, the software assigns taxa in the dataset to distinct species partitions. Both COI and 16S ingroup alignments were separately input into ASAP for delimitation analysis. Meanwhile, GMYC delimits species from an ultrametric tree by finding the most likely combination of nodes that define transitions between interspecific diversification and intraspecific coalescence. For the GMYC analysis, ingroup ultrametric trees were constructed using BEAST implemented on the CIPRES platform (Suchard et al., 2018). The following settings were used to generate the trees for each locus: the HKY + F + I + G4 model of nucleotide evolution; base frequencies 'estimated'; clock model 'lognormal relaxed clock (uncorrelated)'; tree prior 'coalescent/constant size'; UPGMA starting tree; constant.popsize 'lognormal: Log(Mean) = 0.0, Log(Stdev) = 1.0, offset = 0.0'. For the COI ultrametric tree, the Ucld.stdev was set to "normal'' with a mean = 1.0, Stdev = 1.0. Default settings were retained for the remaining priors. Tree searches were run for 100 million generations with sampling every 10,000 generations. Burn-in was set to 10% and Tracer ver. 1.7.2 was used to confirm MCMC convergence. Trees were summarized with TreeAnnotator ver 1.10.4 included in the BEAST package. The GMYC analysis was separately run on the ultrametric tree for each of the four loci in R ver. 4.1.2 (R Core Team, 2021) using the "splits" package (Ezard et al., 2021).

Finally, to further evaluate the species delimitations resulting from the ASAP and GMYC analyses, we performed multi-locus species delimitation using Bayesian Phylogenetics and Phylogeography (BPP) ver. 4.3.8 (Yang, 2015). BPP uses a multi-species coalescent model to compare the species groupings based on one locus with sequence data from other loci. For each species-grouping, it outputs a posterior probability that can be interpreted as statistical support across loci for each species hypothesis. In this study, joint Bayesian species delimitations (Rannala and Yang, 2013; Yang and Rannala, 2010) and species tree

estimations (Rannala and Yang, 2017; Yang and Rannala, 2014) were conducted to assess the support of COI delimitations against a concatenated 16S, H3, and ITS2 alignment. Three analyses with different population size (θ) and divergence time (τ0) inverse-gamma priors were conducted (A: θ = 3, 0.01, τ0 = 3, 0.02; B: θ = 3, 0.004, τ0 = 3, 0.02; C: θ = 3, 0.002, τ0 = 3, 0.02). These were chosen to match the gamma prior means used in similar studies and correspond to large, intermediate, and small estimates of genetic distances (Martinsson and Erséus, 2018). Each analysis was run thrice for 300,000–400,000 generations to ensure consistency between runs.

2.5. Morphological comparisons

To compare body dimensions among *Chaetogaster* species, live worms from six putative species (species 3, 6, 8, 10, 12, and 22) spanning the genus were collected from North American localities, identified to putative species with COI, and imaged at 10X magnification on a Leica MZ16 stereomicroscope (for species 3) or 100X magnification on a Zeiss Axioplan2 microscope (for all other species). Body length and width at the widest point of the pharynx were measured for 1 – 10 specimens of each species using ImageJ and averaged (Abràmoff et al., 2004).

3. Results

3.1. Extensive phylogenetic diversity within Chaetogaster

Phylogenetic analyses of our concatenated, four-locus dataset recovered largely congruent topologies using ML (Fig. 2) and BI (Fig. 3) criteria. Each tree yielded 24 highly supported (>75% likelihood bootstrap support [LBS] and > 0.9 posterior probability [PP]) terminal clades within *Chaetogaster*. From this point onwards, these 24 terminal clades are interpreted to be putative species based on multiple lines of evidence reported here (3.3 and 3.4). These 24 clades include two terminal clades of large-bodied *Chaetogaster "diaphanus"* species (species 3 and 4), three terminal clades of the mollusc-associating *Chaetogaster "limnaei"* species (species 22, 23, and 24), and 19 terminal clades of small-bodied *Chaetogaster "diastrophus"* species (species 1, 2, and 5–21). Both ML and BI analyses recover a clade of *Amphichaeta* sequences as sister to *Chaetogaster*. However, only the BI analysis highly supports this node (PP = 1). The relationships among ingroup terminal clades are also consistent between ML and BI trees. Both analyses recover putative *Chaetogaster* species 1 and 2 as sister to the remainder of the genus with high support (LBS = 100%; PP = 1). Other highly supported internal relationships include a clade formed by putative species 3 and 4 (the *C. "diaphanus"* species) along with species 5, 6, 7, 8, and 9 (LBS = 80%; PP = 0.98); a clade formed by species 10, 11, 12, and 13 (LBS = 87%; PP = 1); a clade formed by species 14, 15, 16, and 17 (LBS = 100%; PP = 1); and a clade formed by species 19, 20, 21, 22, 23, and 24 (the latter 3 are the *C. "limnaei"* species) (LBS = 100%; PP = 1).

Individual gene trees are largely congruent with results from the concatenated analyses. ML gene trees of COI (Supplementary Fig. 1) and ITS2 (Supplementary Fig. 2) each recover the same 24 terminal clades with high support (LBS > 75%). However, support at deeper nodes is generally poor and neither gene tree recovers the clade containing putative *Chaetogaster* species 1 and 2 as sister to the remainder of the genus, which was recovered in the concatenated dataset. Both gene trees agree in placing putative species 5 as sister to the *C. "diaphanus"* species (3 and 4) (COI LBS = 100%; ITS2 LBS = 98%) and in grouping the three putative *C. "limnaei"* species (22 – 24) together with putative species 19, 20, and 21, albeit with poor support in the COI tree (COI LBS = 44%; ITS2 LBS = 98%). Many of the same terminal clades are recovered in the 16S and H3 gene trees, with a few exceptions. In the former, putative species 18 is paraphyletic (Supplementary Fig. 3). In the latter, two of the putative *C. "limnaei"* species (22 and 23) group together in a single clade alongside sequences collected by Smythe et al. (2015)
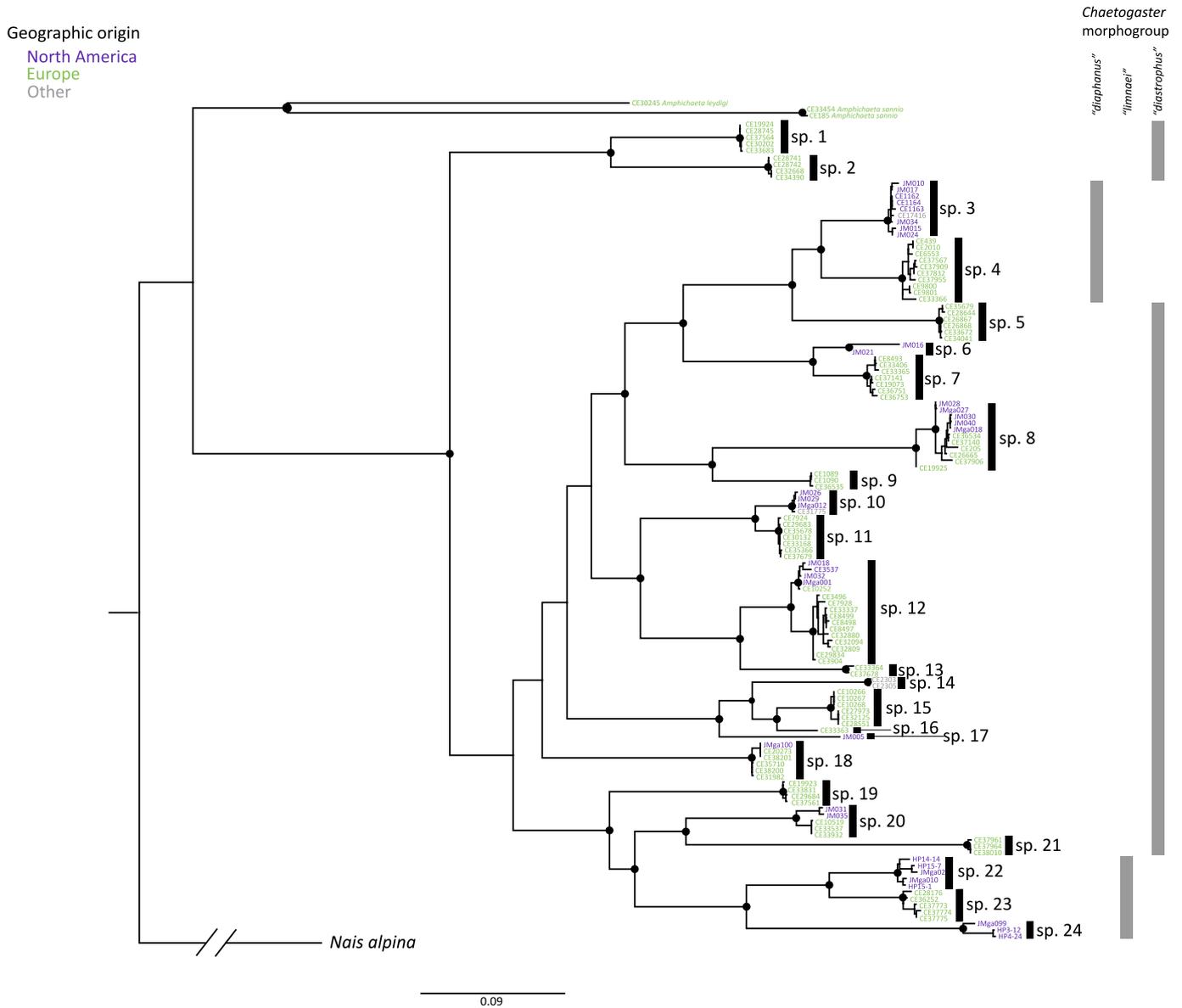
Fig. 2. Maximum likelihood phylogeny (*lnL* = -19440.5590 ± 523.5033) based on a concatenated alignment of four loci (COI, 16S, ITS2, and H3). Nodes with bootstrap support>75% are indicated with circles. Sequences are color coded according to sampling locality. *Chaetogaster* morphogroup labels indicate the preliminary identifications of each specimen prior to our analyses. The numbered clades are interpreted as putative species.

([Supplementary Fig. 4](#)).

3.2. Mixed phylogeographic patterns in Chaetogaster

Both ML and BI phylogenies include four instances of North American and European sister lineages that are highly supported. Some of these sister lineages are deeply separated, such as the division between putative species 3 and 4, which represent *C. "diaphanus"* worms largely collected in North America and Europe, respectively (LBS = 96%; PP = 0.99). Other divisions are much shallower, such as the split between North American and European sequences of putative species 20 (LBS = 100%, PP = 1). A few clades do not show deep divergence between continents. For example, in putative species 12 an Italian sequence (CE10252) falls with North American sequences (LBS = 87%; PP = 0.84), while the sole North American sequence of putative species 18 forms a clade with Scandinavian sequences (LBS = 100%; PP = 1). Interestingly, the well-supported grouping of putative species 14, 15, 16, and 17 shows a particularly broad geographic range, including specimens from Guam (putative species 14), Sardinia (putative species 15),

Sweden (putative species 15 and 16), and Texas (putative species 17). Some terminal clades do not have a counterpart in North America (*i.e.,* putative species 1, 2, 5, 9, 13, 15, 16, 19, and 21) or Europe (*i.e.,* putative species 17 and 24).

3.3. Species delimitation methods recover at least 24 species in Chaetogaster

The ASAP analysis to delimit species based on genetic distances recovered a large barcoding gap in the COI dataset between 3.4 and 10% pairwise distance. The most likely delimitation scheme based on this threshold includes 24 potential species, which correspond to the 24 terminal clades identified in the ML and BI phylogenies. A large barcoding gap was not found for the 16S alignment, but 24 delimitations are still recovered based on a 0.8–1% threshold separating intraspecific and interspecific distances. GMYC analyses for COI, 16S, H3, and ITS2 arrived at a similar species count to ASAP. Individual gene BEAST trees input into GMYC delimited 24 species based on either COI or ITS2, 21 species based on 16S, and 19 species based on H3. The modest
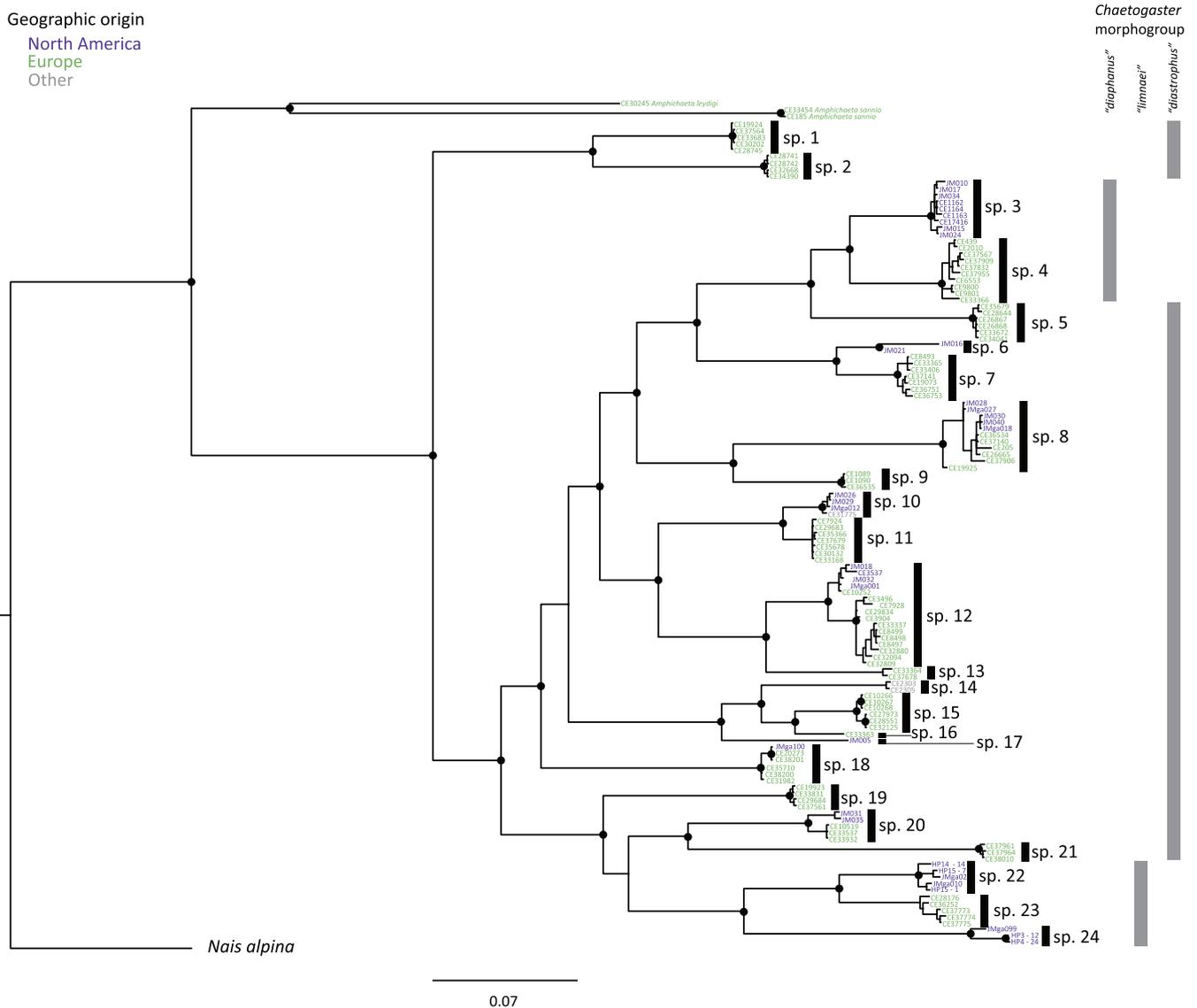
Fig. 3. Bayesian inference phylogeny based on a concatenated alignment of four loci (COI, 16S, ITS2, and H3). Nodes with posterior probabilities>0.9 are indicated with circles. Sequences are color coded according to sampling locality. *Chaetogaster* morphogroup labels indicate the preliminary identifications of each specimen prior to our analyses. The numbered clades are interpreted as putative species.

differences between analyses are not surprising, as distinct molecular markers can often produce somewhat conflicting GMYC results (Ritchie et al., 2016).

To further evaluate these species delimitations, we chose to test the ASAP and GMYC COI results using BPP. Most groups delimited according to ASAP and GMYC were highly supported in the BPP analysis (PP > 0.95) across large, intermediate, and small priors of genetic distance (Supplementary table 1). Species 6, 8, 9, and 15 had poorer support for one of the three runs (run C) in the BPP analysis, perhaps because the small prior does not reflect the large range of genetic distances in our dataset.

3.4. Large pairwise COI distances separate delimited species

We calculated uncorrected p - distances for the 24 delimited species of *Chaetogaster* to assess the extent of intra- and interspecific divergence (Supplementary Tables 2 and 3). In summary, the average interspecific pairwise distance is 15% ± 1.3 (range 5 – 18.5%), while the average intraspecific pairwise distance is 0.7% ± 0.2 (range 0 – 2.3%). Pairwise

distances between North American and European sequences range widely. For the four pairs of species comprising North American and European sister species (species 3 and 4; 6 and 7; 10 and 11, and 22 and 23), the average between species distance is 8.2% ± 1 (range 5.7 – 10.4%). Meanwhile, the average between group distance for the four terminal clades where North American and European sequences are not split into separate species (species 8, 12, 18, and 20) is 1.1% ± 0.3 (range 0.6 – 2.4%).

3.5. Most Chaetogaster species share similar chaetal morphology and body size

Despite efforts to identify morphological features that can distinguish the different *Chaetogaster* species, we found that most worms were extremely similar in external morphology. Our morphological assessments of North American *Chaetogaster* worms (Fig. 4) indicate that several species spanning the genus share a similar body size range. Species 8, 10, 12, 20, and 22 (which collectively span much of the phylogenetic diversity in the genus, including worms originally
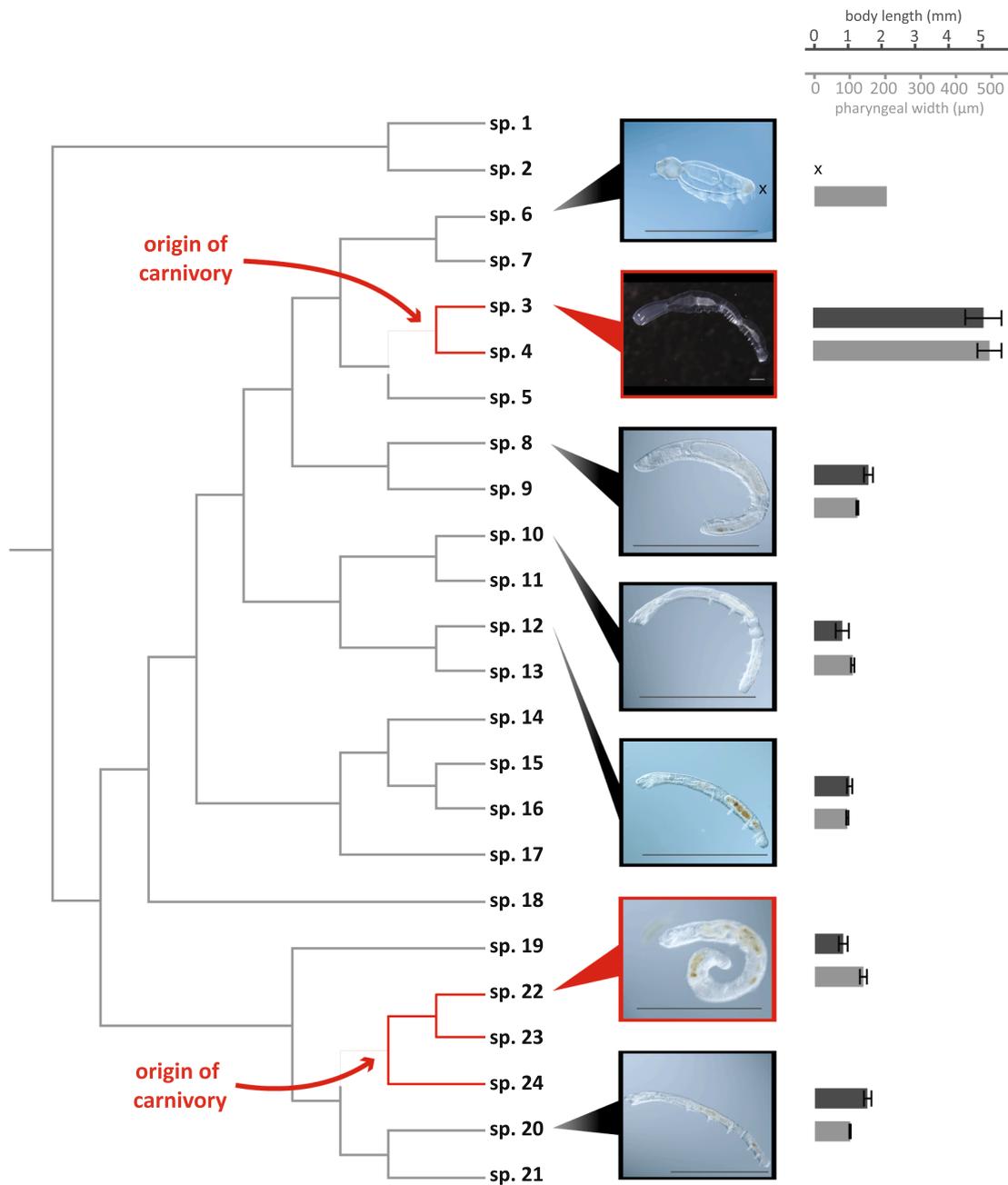
Fig. 4. Overview of phylogenetic relationships and morphological diversity in *Chaetogaster*. Carnivory is inferred to have evolved twice within the genus. Body sizes (average body length and average pharyngeal width) are shown for a subset of North American species. Error bars represent standard error where more than one individual was available for measurement. The measurements represent averages from 10 individuals for species 3, 8, 10, 12, and 20; from 8 individuals for species 22, and from the single available individual of species 6.. Scale bars represent 1 mm. Most specimens were photographed alive and are not represented in the phylogenetic dataset. An exception is the representative of species 6, which is the formalin-preserved anterior end of JM016. The x indicates the posterior cut site, which precluded obtaining a body length measurement.

identified by us as *C. "diastrophus"* and *C. "limnaei"*) tend to be 1 – 2 mm long, with pharynxes that are around 100–200 μm wide. Species 3 (a *C. "diaphanus"* species) is consistently much larger, with an average body length of 5 mm and an average pharyngeal width of 500 μm. Interestingly, the one specimen of species 6 available for morphological assessment appears to have a pharyngeal width in between the range of widths for the *C. "diaphanus"* species (species 3) and the other *C. "diastrophus"* species (species 8, 10, 12, and 20).

Across the *Chaetogaster* species in our dataset, chaetae are bifid with curved teeth. The only notable exception are the *C. "limnaei"* group species, which have bifid chaetae that are longer and more strongly

hooked than the other *Chaetogaster* species (Kathman and Brinkhurst, 1998).

4. Discussion

4.1. Extensive novel diversity within the genus Chaetogaster

Here we present the first in-depth molecular phylogenetic analysis of *Chaetogaster*, a widespread and unusual genus of freshwater annelids that includes large predators, small omnivores, and mollusc symbionts. Our data from two mitochondrial and two nuclear loci strongly supports

the existence of at least 24 species in the genus. This is far more than the three species commonly recognized and even greater than the maximum number of species proposed from morphological descriptions. In our dataset, the large-bodied *C. "diaphanus"* and mollusc symbiont *C. "limnaei"* are represented by two and three species, respectively. Meanwhile, the name *C. "diastrophus"* encompasses at least 19 species. Most of these *C. "diastrophus"* species are extremely similar in overall body shape, body dimensions, and chaetal morphology. We were unable to distinguish these *C. "diastrophus"* species using morphology alone. Although surprising, such extensive cryptic diversity is not unprecedented as the biodiversity of small freshwater invertebrates has long been underestimated. For example, comparable or greater levels of crypticity are common in other freshwater annelids (Liu et al., 2017a), rotifers (Obertegger et al., 2014), crustaceans (Schön et al., 2017), and flatworms (Atherton and Jondelius, 2018).

While molecular analyses have proven to be effective tools for the identification and eventual description of new species (*e.g.,* Knutson and Gosliner, 2022; Lawley et al., 2021), further morphological analyses of *Chaetogaster* are necessary to formally document the diversity reported in this study. Our phylogenetic analyses and proposal of putative species merely represent the first step toward a deeper understanding of the diversity within *Chaetogaster*.

4.1.1. The large-bodied predator Chaetogaster "diaphanus" is at least two distinct species

Under the microscope, *C. "diaphanus"* worms stand-out amid the bustle of a freshwater community. They can be found prowling in search of prey or wrapped around an algal frond, waiting to ambush passing crustaceans. Given their intriguing lifestyle, it is not surprising that images and videos of *C. "diaphanus"* are common online and even place highly in microscopy competitions (Supplementary links). Nonetheless, despite its popular appeal, *C. "diaphanus"* as a species has received little attention in the scientific literature.

In this study, we show that the charismatic worms often identified as *C. "diaphanus"* are unlikely to be a single species. In all our analyses, *C. "diaphanus"* is clearly split between a North American lineage (species 3) and a European lineage (species 4), with an average between group COI divergence of 10.4% ± 1.1. These two lineages are highly supported as distinct delimitations in the BPP analysis, strongly suggesting that the name *Chaetogaster "diaphanus"* refers to two species. One might be largely confined to North America and the other to continental Europe, but broader sampling is necessary to confirm this. Interestingly, a sequence obtained from Australia (CE17416) falls within an otherwise North American clade of species 3. This may represent an example of human-mediated dispersal and it does not rule out the possibility of a yet undiscovered Australian lineage of *C. "diaphanus"*. Nonetheless, it is apparent from our dataset that large-bodied and predatory *Chaetogaster* worms diversified into at least two species (3 and 4) from an ancestral assemblage of small-bodied, possibly omnivorous *Chaetogaster* worms.

It is unlikely that the North America – Europe separation seen in species 3 and 4 corresponds to *C. "diaphanus"* and *C. "cristallinus"*. Both species have been reported from Europe and North America and the latter purportedly differs from *C. "diaphanus"* in possessing a prostomial incision, a shorter overall body length, and shorter chaetae in segment II (Brinkhurst and Jamieson, 1971; Sperber, 1948). However, the absence of significant phylogenetic structure within species 3 and within species 4, despite the former including sequences from widely separated American localities, supports the presence of one large-sized and predatory *Chaetogaster* species in North America and another in Europe. Median incisions are common among North American *C. "diaphanus"* worms, but it is uncertain if all large, predatory *Chaetogaster* worms on the continent have the trait. It could also be a plastic trait determined by environmental conditions, like the intraspecific variability observed in chaetal morphology for other naidids (Chapman and Brinkhurst, 1987; Smith, 1985). The closest relatives to the *C. "diaphanus"* species in our

dataset are species 5, 6, or 7 and it is more likely that one of these represents *C. "cristallinus"*. In support of this, the single preserved specimen of species 6 (JM016) has a pharyngeal width between that of species 3 and the other *Chaetogaster* worms, suggesting that it may represent an intermediate form that has been identified as *C. "cristallinus"* in the past (this specimen's body length could not be assessed because the posterior end was removed for DNA analysis). Further sampling and analysis of species 5, 6, and 7, in addition to comparisons with species 3 and 4, will be necessary to assess whether one of those three species may be assigned to *C. "cristallinus"*.

4.1.2. The small-bodied mollusc symbiont Chaetogaster "limnaei" is at least three distinct species

Chaetogaster "limnaei" worms are notable for their ectosymbiotic and endosymbiotic relationships with molluscs. As a result, they are a productive study system for research on the evolution of host-symbiont interactions (Hobart et al., 2022; Hopkins et al., 2022; Stoll et al., 2013). However, this body of work usually refers to *C. "limnaei"* as a single species, regardless of sampling locality. We have shown that this underlying assumption is incorrect. Our analyses divide *Chaetogaster "limnaei"* into three clades: *C. "limnaei"* worms collected from North America (species 22), *C. "limnaei"* worms collected from Europe (species 23), and a third species distantly related to the other two only recovered from North America (species 24). Two distinct North American clades were also recovered in a prior COI analysis of *C. "limnaei"* (Smythe et al. 2015). These clades likely correspond to species 22 and 24, as representative sequences from the prior study fall alongside sequences from either species 22 or 24 in our COI gene tree.

It is important that future research on *C. "limnaei"* considers the new diversity we report here. North American and European *C. "limnaei"* cannot be treated as a single species, nor can researchers assume that all *C. "limnaei"* worms collected in North America are one species. The two North American species recovered in this study may even overlap in range, as some specimens of species 22 and 24 were collected from localities less than 10 km apart in Maryland. We cannot say if the three *C. "limnaei"* species recovered in our dataset show distinct host preferences or clear morphological synapomorphies. Each species was collected from at least two families of host snail (Physidae and Lymnaeidae), and all display the distinctive hooked chaetal morphology that characterizes *C. "limnaei"*. Further investigation with larger sample sizes is needed to determine if the new *C. "limnaei"* species reported here show strong differences in host preferences, symbiotic behavior, and/or morphology. In the meantime, DNA barcoding using the COI locus is a reliable means of distinguishing between the three lineages. A barcoding approach for species identification has been similarly recommended when working on at least three other freshwater annelid models in which cryptic species have been recognized (*Helobdella*: Bely and Weisblat 2006; *Lumbriculus:* Gustafsson et al., 2009; and *Tubifex:* Beauchamp et al., 2001).

4.1.3. The small-bodied free-living Chaetogaster "diastrophus" is many distinct species

Most of the diversity in *Chaetogaster* appears to be represented by worms resembling the small, free-living, and potentially omnivorous *C. "diastrophus"* species. Historically, keys of freshwater annelids have included three names for these *Chaetogaster* worms: *Chaetogaster "diastrophus", Chaetogaster "langi"* Bretscher, 1896, and *Chaetogaster "setosus"* Svetlov 1925. Of these, *C. "diastrophus"* and *C. "langi"* (both originally described from Europe) could refer to any number of the 19 species recovered in our phylogenies, as they resemble all of the "*C. diastrophus"* specimens that we collected and there are few morphological features to distinguish the two (Brinkhurst and Jamieson, 1971). For this reason, authors have synonymized *C. "diastrophus"* and *C. "langi"* (Brinkhurst and Wetzel, 1984; Kathman and Brinkhurst, 1998). Because the name *C. "diastrophus"* likely encompasses many morphologically similar species, *C. "langi"* might be retained in formal

descriptions for one of them, but the others will likely need new names.

It is unlikely that *C. "setosus"* is present in our dataset. Originally described from Russia, *C. "setosus"* is notable among *Chaetogaster* worms in having simple pointed chaetae, rather than the curved bifid chaetae present in all other known species. None of the European or North American specimens in our dataset fit this description, despite keys indicating that *C. "setosus"* can be found on both continents (Brinkhurst and Jamieson, 1971). It is possible that more intensive phylogenetic sampling in North America and Europe may recover *C. "setosus"* as one or more species separate from the new taxa discovered in this study.

4.2. At least two origins of carnivory in Chaetogaster

Carnivory is a rare feeding strategy among the ∼ 8,000 species of clitellate annelids and represents a highly derived condition. Most extant clitellate lineages are detritivorous and/or herbivorous, subsisting on organic debris, algae, and biofilms. Of the clitellate predators and parasites, most are confined to the leeches, which likely represent a single 150-million-year-old acquisition of blood-feeding followed by adaptation to carnivory in some lineages (Borda and Siddall, 2004; Phillips and Siddall, 2009; Siddall et al., 2016). *Chaetogaster* is highly unusual among clitellates in having evolved carnivory. Our phylogenetic analyses strongly suggest that within *Chaetogaster* there have been at least two origins of carnivorous diets (Fig. 4). In one lineage, the *Chaetogaster "diaphanus"* clade (formed by species 3 and 4) has evolved a generalist predatory strategy, favoring crustaceans, other annelids, and miscellaneous small invertebrates (Green, 1954; Monakov, 1972). In another lineage, the *Chaetogaster "limnaei"* clade (formed by species 22, 23, and 24) has adapted to a mixed lifestyle of ectosymbiosis and endosymbiosis on and within molluscs. While the ectosymbionts show a mixed diet of invertebrates, ciliates, and diatoms, the endosymbionts exclusively subsist on host cells (Conn et al., 1996; Gruffydd, 1965). It is uncertain what the remaining 19 *Chaetogaster* species eat, as dietary reports on *Chaetogaster "diastrophus"* are contradictory and rely on a now outdated taxonomy of the genus. However, it is probable that they rely on a mixed omnivorous diet of ciliates, diatoms, and rotifers (McElhone, 1980; Schonborn, 1984; Taylor, 1980). Thus, two separate origins of carnivory derived from likely non-predatory relatives in *Chaetogaster*, coupled with the diatom-feeding sister genus *Amphichaeta* (Mastrantuono, 1988), make *Chaetogaster* an excellent system for the study of trophic evolution. The novel phylogeny presented in this study deepens our understanding of the diversity and relationships across *Chaetogaster*, providing an important foundation for future comparative work that demystifies how and why these worms evolved such distinct trophic strategies.

4.3. A complex biogeographic history in Chaetogaster

Chaetogaster lineages from North America and Europe are broadly interspersed across our phylogeny. Most species appear confined to one continent or the other, but we did not recover large continent-specific clades of species. Eight subclades in our phylogeny contain both North American and European sequences. Four of these represent sets of sister species each with a North American and a European species (species 3 and 4; species 6 and 7; species 10 and 11; and species 22 and 23), while another four are likely intercontinental species with representatives on both North America and Europe (species 8, 12, 18, and 20). This pattern suggests significant intercontinental migration during the diversification of the genus. For the latter four species, intercontinental migration is inferred to have been recent, such that lineages on each continent have not yet completely speciated or that there have been repeated reintroductions through dispersal. As *Chaetogaster* worms are small, soft-bodied freshwater invertebrates with no known adaptations to resist desiccation, successful migration between continents is likely to be rare. However, because *Chaetogaster* can reproduce clonally, even a single

individual migrating to a new continent could rapidly establish a population, likely facilitating successful intercontinental transfers.

Because freshwater annelids have a poor fossil record, it is challenging to infer the role and timing of biogeographic events that contributed to the diversification of the genus. However, based on molecular divergences, we propose that the diversification of *Chaetogaster* lineages in our dataset occurred both prior to and following the breakup of Laurasia into North America and Eurasia. A recent fossil-calibrated molecular clock phylogeny of clitellate annelids indicates that *Chaetogaster* diverged from the other naidines between 230 and 80 million years ago (Erséus et al., 2020), well before the break up of Laurasia, approximately 80–40 million years ago (Seton et al., 2012). The four instances of North American and European sister species in our dataset thus likely represent not vicariance from continental drift but speciation events that occurred well after North America and Eurasia were separated. This hypothesis is supported by the relatively shallow genetic divergences between the North American and European sister species in our COI dataset. If we assume that mitochondrial DNA in annelids evolves at a rate similar to that of other animals, then the average 8.2% (range 5.7 – 10.4%) COI divergence between North American and European sister species of *Chaetogaster* would have been generated only in the past 1 – 5 million years (DeSalle et al., 1987; Fleischer et al., 1998).

Clearly, the biogeographic history of *Chaetogaster* is complex and there have likely been a range of factors responsible for the diversification of the genus. *Chaetogaster* species are reported from most continents yet molecular data are thus far limited primarily to North America and Europe. With morphological data being of such limited utility for inferring patterns of diversification in this group, expanded molecular sampling of this genus is needed.

4.4. A note on future Chaetogaster sampling

We discovered extensive cryptic diversity in the genus *Chaetogaster,* but sampling of the genus could and should be expanded. Most of our North American sequences were gathered from worms collected in Maryland, while our European sequences were largely sourced from worms collected in Scandinavian countries. Given the diversity observed in our dataset, it is probable that sampling from additional localities will reveal more species in the genus. Various *Chaetogaster* species have been reported from localities in Eurasia (Park et al., 2013; Semernoi, 1985; Zalozny and Vorobiev, 2017), South America (Collado et al., 2019), Africa (Bayer and Matthews, 1955), Australia (Mitchell and Leung, 2016), and India (Annandale, 1905). It remains to be determined whether any of these populations correspond to one or more of the 24 species in our dataset or if they represent additional *Chaetogaster* species beyond those recognized here. Lake Baikal is a particularly promising locality for new *Chaetogaster* diversity, as nine species endemic to the lake have been morphologically described in the past (Semernoi, 1985).

Future phylogenetic studies that leverage more global sampling will be necessary to estimate the true diversity and biogeographic history of *Chaetogaster*. Such endeavors are becoming increasingly important for small freshwater invertebrates. As the sixth mass extinction looms over precious freshwater ecosystems (*e.g.,* Burkhead, 2012; Rocha-Ortega et al., 2020), it is essential to document and conserve the incredible diversity of the small invertebrates that dwell beneath the surface of ponds, creeks, lakes, and rivers. Otherwise, we risk losing a myriad of tiny, remarkable, and poorly studied organisms like *Chaetogaster* and the fascinating evolutionary stories that they can tell.

CRediT authorship contribution statement

Joseph M. Mack: Conceptualization, Writing – review & editing, Investigation, Formal analysis. **Mårten Klinth:** Conceptualization, Writing – review & editing, Investigation. **Svante Martinsson:** Conceptualization, Investigation. **Robert Lu:** . **Hannah Stormer:**

Investigation. **Patrick Hanington:** Investigation. **Heather C. Proctor:** Writing – review & editing, Investigation. **Christer Erséus:** Conceptualization, Writing – review & editing, Investigation. **Alexandra E. Bely:** Conceptualization, Writing – review & editing, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2023.107748.

References

Abràmoff, M., Magalhães, P.J., Ram, S.J., 2004. Image Processing with ImageJ. Biophotonics International 7.

Annandale, N., 1905. Notes on an Indian worm of the genus *Chaetogaster*. Journal of the Asiatic Society of Bengal 1, 117–120.

Atherton, S., Jondelius, U., 2018. Wide distributions and cryptic diversity within a *Microstomum* (Platyhelminthes) species complex. Zoologica Scripta 47, 486–498.

Bayer, F., Matthews, H., 1955. Notes on a carnivorous oligochaete commensal on certain fresh-water snails in South Africa. Proceedings of the Zoological Society of London 125, 407–409.

Beauchamp, K.A., Kathman, R.D., McDowell, T.S., Hedrick, R.P., 2001. Molecular Phylogeny of Tubificid Oligochaetes with Special Emphasis on *Tubifex tubifex* (Tubificidae). Molecular Phylogenetics and Evolution 19, 216–224. https://doi.org/10.1006/mpev.2001.0923.

Bely, A.E., Sikes, J.M., 2010. Latent regeneration abilities persist following recent evolutionary loss in asexual annelids. Proceedings of the National Academy of Sciences 107, 1464–1469. https://doi.org/10.1073/pnas.0907931107.

Bely, A.E., Weisblat, D.A., 2006. Lessons from leeches: a call for DNA barcoding in the lab. Evolution & Development 8, 491–501. https://doi.org/10.1111/j.1525-142X.2006.00122.x.

Bely, A.E., Wray, G.A., 2004. Molecular phylogeny of naidid worms (Annelida: Clitellata) based on cytochrome oxidase I. Molecular Phylogenetics and Evolution 30, 50–63. https://doi.org/10.1016/S1055-7903(03)00180-5.

Borda, E., Siddall, M.E., 2004. Arhynchobdellida (Annelida: Oligochaeta: Hirudinida): phylogenetic relationships and evolution. Molecular Phylogenetics and Evolution 30, 213–225. https://doi.org/10.1016/j.ympev.2003.09.002.

Bretscher, K., 1896. Die Oligochaeten von Zürich. Revue suisse de Zoologie 3, 499–352.

Brinkhurst, R., Gelder, S.R., 1989. Did the lumbriculids provide the ancestors of the branchiobdellidans, acanthobdellidans and leeches? Hydrobiologia 180, 7–15.

Brinkhurst, R., Jamieson, B.G.M., 1971. Aquatic Oligochaeta of the World. Oliver and Boyd, Edinburgh.

Brinkhurst, R., Wetzel, M.J., 1984. Aquatic Oligchaeta of the World: supplement to a catalogue of new freshwater species, descriptions, and revisions, 44. Canadian Technical Report of Hydrography and Ocean Sciences.

Burkhead, N.M., 2012. Extinction Rates in North American Freshwater Fishes, 1900–2010. BioScience 62, 798–808. https://doi.org/10.1525/bio.2012.62.9.5.

Chapman, P.M., Brinkhurst, R.O., 1987. Hair today, gone tomorrow: induced chaetal changes in tubificid oligochaetes. Hydrobiologia 45–55.

Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G., Gray, M.R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46, 419–437. https://doi.org/10.1071/ZO98048.

Collado, G.A., Cabrera, F.J., Ballesteros, G.I., Villalobos, N.I., Aguayo, K.P., 2019. First report of *Chaetogaster limnaei* (Annelida: Naididae) in Chile based on samples retrieved from an invasive freshwater snail. Rev. Mex. Biodiv. 90 https://doi.org/10.22201/ib.20078706e.2019.90.2572.

Conn, D.B., Ricciardi, A., Babapulle, M., Klein, K., Rosen, D., 1996. *Chaetogaster limnaei* (Annelida: Oligochaeta) as a parasite of the zebra mussel *Dreissena polymorpha*, and the quagga mussel *Dreissena bugensis* (Mollusca: Bivaivia). Parasitology Research 82, 1–7.

DeHorne, L., 1916. Les Naïdimorphes et Leur Reproduction Asexuée. Archives de Zoologie Experimentale et Générale 56, 25–157.

DeSalle, R., Freedman, T., Prager, E.M., Wilson, A.C., 1987. Tempo and Mode of Sequence Evolution in Mitochondrial DNA of Hawaiian *Drosophila*. J Mol Evol 26, 157–164.

Envall, I., Källersjö, M., Erséus, C., 2006. Molecular evidence for the non-monophyletic status of Naidinae (Annelida, Clitellata, Tubificidae). Molecular Phylogenetics and Evolution 40, 570–584. https://doi.org/10.1016/j.ympev.2006.03.021.

Envall, I., Gustavsson, L.M., Erséus, C., 2012. Genetic and chaetal variation in *Nais* worms (Annelida, Clitellata, Naididae). Zoological Journal of the Linnean Society 165, 495–520. https://doi.org/10.1111/j.1096-3642.2012.00828.x.

Erséus, C., Williams, B.W., Horn, K.M., Halanych, K.M., Santos, S.R., James, S.W., Creuzé des Châtelliers, M., Anderson, F.E., 2020. Phylogenomic analyses reveal a Palaeozoic radiation and support a freshwater origin for clitellate annelids. Zoologica Scripta 49, 614–640. https://doi.org/10.1111/zsc.12426.

Erséus, C., Wetzel, M.J., Gustavsson, L., 2008. ICZN rules-a farewell to Tubificidae (Annelida, Clitellata). Zootaxa 1744, 66–68.

Erséus, C., Rota, E., Matamoros, L., De Wit, P., 2010. Molecular phylogeny of Enchytraeidae (Annelida, Clitellata). Molecular Phylogenetics and Evolution 57, 849–858. https://doi.org/10.1016/j.ympev.2010.07.005.

Erséus, C., Envall, I., De Wit, P., Gustavsson, L.M., 2017. Molecular data reveal a tropical freshwater origin of Naidinae (Annelida, Clitellata, Naididae). Molecular Phylogenetics and Evolution 115, 115–127. https://doi.org/10.1016/j.ympev.2017.07.016.

Ezard, T., Fujisawa, T., Barraclough, T., 2021. splits: SPecies' LImits by Threshold Statistics.

Fleischer, R., McIntosh, C., Tarr, C., 1998. Evolution on a volcanic conveyor belt using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. Molecular Ecology 533–545.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3, 294–299.

Green, J., 1954. CII.—A note on the food of *Chaetogaster diaphanus*. Annals and Magazine of Natural History 7, 842–844. https://doi.org/10.1080/00222935408651798.

Gruffydd, Ll.D., 1965. Evidence for the existence of a new subspecies of *Chaetogaster limnaei* (Oligochaeta), in Britain. Proceedings of the Zoological Society of London 146, 175–196. https://doi.org/10.1111/j.1469-7998.1965.tb05208.x.

Gruithuisen, F.V.P., 1828. Uber die *Nais diaphana* und *Nais diastropha* mit dem Nerven- und Blut-system derselben. Nova Acta Physico-Medica Academiae Caesarae Leopoldino-Carolinae Naturae Curiosorum 14, 407–420.

Gustafsson, D.R., Price, D.A., Erséus, C., 2009. Genetic variation in the popular lab worm *Lumbriculus variegatus* (Annelida: Clitellata: Lumbriculidae) reveals cryptic speciation. Molecular Phylogenetics and Evolution 51, 182–189. https://doi.org/10.1016/j.ympev.2008.12.016.

Hobart, B.K., Moss, W.E., McDevitt-Galles, T., Stewart Merrill, T.E., Johnson, P.T.J., 2022. It's a worm-eat-worm world: Consumption of parasite free-living stages protects hosts and benefits predators. Journal of Animal Ecology 91, 35–45. https://doi.org/10.1111/1365-2656.13591.

Hopkins, S.R., McGregor, C.M., Belden, L.K., Wojdak, J.M., 2022. Host preferences inhibit transmission from potential superspreader host species. Proceedings of the Royal Society B: Biological Sciences 289, 20220084. https://doi.org/10.1098/rspb.2022.0084.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755. https://doi.org/10.1093/bioinformatics/17.8.754.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14, 587–589. https://doi.org/10.1038/nmeth.4285.

Kathman, R.D., Brinkhurst, R.O., 1998. Guide to the Freshwater Oligochaetes of North America.

Katoh, K., Rozewicki, J., Yamada, K.D., 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20, 1160–1166. https://doi.org/10.1093/bib/bbx108.

Knutson, V.L., Gosliner, T.M., 2022. The first phylogenetic and species delimitation study of the nudibranch genus *Gymnodoris* reveals high species diversity (Gastropoda: Nudibranchia). Molecular Phylogenetics and Evolution 171, 107470. https://doi.org/10.1016/j.ympev.2022.107470.

Lawley, J.W., Gamero-Mora, E., Maronna, M.M., Chiaverano, L.M., Stampar, S.N., Hopcroft, R.R., Collins, A.G., Morandini, A.C., 2021. The importance of molecular characters when morphological variability hinders diagnosability: systematics of the moon jellyfish genus *Aurelia* (Cnidaria: Scyphozoa). PeerJ 9, e11954.

Liquin, F., Hünicken, L.A., Arrighetti, F., Davies, D., Paolucci, E.M., Sylvester, F., 2021. Parasitism and fitness of invaders: oligochaete *Chaetogaster limnaei* produces gill damage and increased respiration rates in freshwater Asian clams. Hydrobiologia 848, 2213–2223. https://doi.org/10.1007/s10750-020-04424-6.

Liu, Y., Erséus, C., 2017. New specific primers for amplification of the Internal Transcribed Spacer region in Clitellata (Annelida). Ecology and Evolution 7, 10421–10439. https://doi.org/10.1002/ece3.3212.

Liu, Y., Fend, S.V., Martinsson, S., Erséus, C., 2017a. Extensive cryptic diversity in the cosmopolitan sludge worm *Limnodrilus hoffmeisteri* (Clitellata, Naididae). Org Divers Evol 17, 477–495. https://doi.org/10.1007/s13127-016-0317-z.

Liu, Y., Fend, S.V., Martinsson, S., Luo, X., Ohtaka, A., Erséus, C., 2017b. Multi-locus phylogenetic analysis of the genus *Limnodrilus* (Annelida: Clitellata: Naididae). Molecular Phylogenetics and Evolution 112, 244–257. https://doi.org/10.1016/j.ympev.2017.04.019.

Maddison, W.P., Maddison, D.R., 2021. Mesquite: a modular system for evolutionary analysis.

Martinsson, S., Erséus, C., 2018. Cryptic diversity in supposedly species-poor genera of Enchytraeidae (Annelida: Clitellata). Zoological Journal of the Linnean Society 183, 749–762. https://doi.org/10.1093/zoolinnean/zlx084.

Martinsson, S., Erséus, C., 2021. Cryptic Clitellata: Molecular Species Delimitation of Clitellate Worms (Annelida): An Overview. Diversity 13, 36. https://doi.org/10.3390/d13020036.

Mastrantuono, L., 1988. A note on the feeding of *Amphichaeta leydigii* (Oligochaeta, Naididae) in lacustrine sandy shores. Hydrobiological Bulletin 22, 195–198. https://doi.org/10.1007/BF02256823.

McElhone, M.J., 1980. Some factors influencing the diet of coexisting, benthic, algal grazing Naididae (Oligochaeta). Can. J. Zool. 58, 481–487. https://doi.org/10.1139/z80-066.

Meewis, H., 1934. Eléments régénérateurs dans le bourgeonnement pygidial et dans la zone de scissiparité chez *Chaetogaster diaphanus* Gruith. Annals de la Société royale zoologique de Belgique 65, 9–39.

Messing, J., Crea, R., Seeburg, P., 1981. A system for shotgun DNA sequencing. Nucleic Acids Research 9, 309–321.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, in 2010 Gateway Computing Environments Workshop (GCE). Presented at the 2010 Gateway Computing Environments Workshop (GCE), IEEE, New Orleans, LA, USA, pp. 1–8. https://doi.org/10.1109/GCE.2010.5676129.

Michelson, E.H., 1964. The Protective Action of *Chaetogaster limnaei* on Snails Exposed to *Schistosoma mansoni*. The Journal of Parasitology 50, 441. https://doi.org/10.2307/3275851.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Molecular Biology and Evolution 37, 1530–1534. https://doi.org/10.1093/molbev/msaa015.

Mitchell, D.R., Leung, T.L.F., 2016. Sharing the load: a survey of parasitism in the invasive freshwater pulmonate, *Physa acuta* (Hygrophila: Physidae) and sympatric native snail populations. Hydrobiologia 766, 165–172. https://doi.org/10.1007/s10750-015-2452-5.

Monakov, A.V., 1972. Review of Studies on Feeding of Aquatic Invertebrates Conducted at the Institute of Biology of Inland Waters, Academy of Science, USSR. Journal of the Fisheries Research Board of Canada 29, 363–383.

Obertegger, U., Flaim, G., Fontaneto, D., 2014. Cryptic diversity within the rotifer *Polyarthra dolichoptera* along an altitudinal gradient. Freshwater Biology 59, 2413–2427. https://doi.org/10.1111/fwb.12447.

Palumbi, S., Martin, A., Romano, S., MacMillan, W., Stice, L., Grabowski, G., 1991. The Simple Fool's Guide to PCR. Department of Zoology, Kewalo Marine Laboratory, University of Hawaii, Honolulu. Ver. 2.0.

Park, H.J., Timm, T., Bae, Y.J., 2013. Taxonomy of the Korean Freshwater Oligochaeta (Annelida) with Eight Species New to Korea. Entomological Research Bulletin 29, 180–181.

Phillips, A.J., Siddall, M.E., 2009. Poly-paraphyly of Hirudinidae: many lineages of medicinal leeches. BMC Evol Biol 9, 246. https://doi.org/10.1186/1471-2148-9-246.

Poddubnaya, T., 1968. Biology of reproduction of *Chaetogaster diaphanus*. Trudy Instituta Biologii Vnutrennikh Vod Akademii Nauk SSSR.

Poff, N.L., Palmer, M.A., Angermeier, P.L., Vadas, R.L., Hakenkamp, C.C., Bely, A., Arensburger, P., Martin, A.P., 1993. Size structure of the metazoan community in a Piedmont stream. Oecologia 95, 202–209. https://doi.org/10.1007/BF00323491.

Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D., Vogler, A.P., 2006. Sequence-Based Species Delimitation for the DNA Taxonomy of Undescribed Insects. Systematic Biology 55, 595–609. https://doi.org/10.1080/10635150600852011.

Ptatscheck, C., Brückner-Hüttemann, H., Kreuzinger-Janik, B., Weber, S., Traunspurger, W., 2020. Are meiofauna a standard meal for macroinvertebrates and juvenile fish? Hydrobiologia 847, 2755–2778. https://doi.org/10.1007/s10750-020-04189-y.

Puillandre, N., Brouillet, S., Achaz, G., 2021. ASAP: assemble species by automatic partitioning. Molecular Ecology Resources 21, 609–620. https://doi.org/10.1111/1755-0998.13281.

R Core Team, 2021. R: A language and environment for statistical computing.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic Biology 67, 901–904. https://doi.org/10.1093/sysbio/syy032.

Rambaut, A., 2017. FigTree, a graphical viewer of phylogenetic trees.

Rannala, B., Yang, Z., 2013. Improved Reversible Jump Algorithms for Bayesian Species Delimitation. Genetics 194, 245–253. https://doi.org/10.1534/genetics.112.149039.

Rannala, B., Yang, Z., 2017. Efficient Bayesian Species Tree Inference under the Multispecies Coalescent. Systematic Biology 66, 823–842. https://doi.org/10.1093/sysbio/syw119.

Ritchie, A.M., Lo, N., Ho, S.Y.W., 2016. Examining the sensitivity of molecular species delimitations to the choice of mitochondrial marker. Organisms Diversity & Evolution 16, 467–480. https://doi.org/10.1007/s13127-016-0275-5.

Rocha-Ortega, M., Rodríguez, P., Bried, J., Abbott, J., Córdoba-Aguilar, A., 2020. Why do bugs perish? Range size and local vulnerability traits as surrogates of Odonata extinction risk. Proceedings of the Royal Society B: Biological Sciences 287, 20192645. https://doi.org/10.1098/rspb.2019.2645.

Schenk, J., Fontaneto, D., 2020. Biodiversity analyses in freshwater meiofauna through DNA sequence data. Hydrobiologia 847, 2597–2611. https://doi.org/10.1007/s10750-019-04067-2.

Schmid-Araya, J.M., Schmid, P.E., Majdi, N., Traunspurger, W., 2020. Biomass and production of freshwater meiofauna: a review and a new allometric model. Hydrobiologia 847, 2681–2703. https://doi.org/10.1007/s10750-020-04261-7.

Schön, I., Pieri, V., Sherbakov, D.Y., Martens, K., 2017. Cryptic diversity and speciation in endemic *Cytherissa* (Ostracoda, Crustacea) from Lake Baikal. Hydrobiologia 800, 61–79. https://doi.org/10.1007/s10750-017-3259-3.

Schonborn, W., 1984. The annual energy transfer from the communities of ciliata to the population of *Chaetogaster diastrophus* (Gruithuisen) in the River Saale. Limnologica 16, 15–23.

Semernoi, V.P., 1985. The genus *Chaetogaster* Baer (Oligochaeta, Naididae). Bespozvonochnye i ryby 28–29.

Seton, M., Müller, R.D., Zahirovic, S., Gaina, C., Torsvik, T., Shephard, G., Talsma, A., Gurnis, M., Turner, M., Maus, S., Chandler, M., 2012. Global continental and ocean basin reconstructions since 200Ma. Earth-Science Reviews 113, 212–270. https://doi.org/10.1016/j.earscirev.2012.03.002.

Siddall, M.E., Brugler, M.R., Kvist, S., 2016. Comparative Transcriptomic Analyses of Three Species of *Placobdella* (Rhynchobdellida: Glossiphoniidae) Confirms a Single Origin of Blood Feeding in Leeches. Journal of Parasitology 102, 143–150. https://doi.org/10.1645/15-802.

Sjölin, E., Erséus, C., Källersjö, M., 2005. Phylogeny of Tubificidae (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. Molecular Phylogenetics and Evolution 35, 431–441. https://doi.org/10.1016/j.ympev.2004.12.018.

Smith, M.E., 1985. Setal Morphology and Its Intraspecific Variation in *Dero digitata* and *Dero nivea* (Oligochaeta: Naididae). Transactions of the American Microscopical Society 104, 45–51.

Smythe, A.B., Forgrave, K., Patti, A., Hochberg, R., Litvaitis, M.K., 2015. Untangling the Ecology, Taxonomy, and Evolution of *Chaetogaster limnaei* (Oligochaeta: Naididae) Species Complex. Journal of Parasitology 101, 320. https://doi.org/10.1645/13-465.1.

Sperber, C., 1948. A taxonomical study of the Naididae. Zool. Bidr. Upps., 28.

Stoll, S., Früh, D., Westerwald, B., Hormel, N., Haase, P., 2013. Density-dependent relationship between *Chaetogaster limnaei limnaei* (Oligochaeta) and the freshwater snail *Physa acuta* (Pulmonata). Freshwater Science 32, 642–649. https://doi.org/10.1899/12-072.1.

Streit, B., 1977. Morphometric relatioships and feeding habits of two species of *Chaetogaster, Ch. limnaei* and *Ch. diastrophus* (Oligochaeta). Archive fur Hydrobiologie Supplement 3, 424–437.

Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J., Rambaut, A., 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus. Evolution 4. https://doi.org/10.1093/ve/vey016.

Svetlov, P., 1925. Einige Angaben uber die Oligochaetenfauna des Tscherdynbezirkes (Uralgebeit). Izv. biol. nauchnoiss. led. Inst. biol. Sta. perm. gosud. Univ. 471–475.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 38, 3022–3027. https://doi.org/10.1093/molbev/msab120.

Taylor, W.D., 1980. Observations on the Feeding and Growth of the Predacious Oligochaete *Chaetogaster langi* on Ciliated Protozoa. Transactions of the American Microscopical Society 99, 360–368.

Vaghin, V., 1946. On the biological species of *Chaetogaster limnaei*, k. Baer. Comptes Rendus (Doklady) de l'Académie des Sciences de l'URSS 51, 481–484.

Vejdovsky, F., 1884. Revisio Oligochaetorum Bohemiae. Sber. Bohm. Ges. Naturw. 215–228.

Vieira, J., Messing, J., 1982. The pUC plasmids, an M13mp7derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene 19, 259–268.

von Baer, K., 1827. Beitrage zur Kenntniss der niedern Thiere. III. Nova Acta Physico-Medica Academiae Caesarae Leopoldino-Carolinae Naturae Curiosorum 13, 605–659.

WoRMS Editorial Board, 2022. World Register of Marine Species. URL https://www.marinespecies.org (accessed 12.26.22).

Yang, Z., 2015. The BPP program for species tree estimation and species delimitation. Current Zoology 61, 854–865. https://doi.org/10.1093/czoolo/61.5.854.

Yang, Z., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. Proceedings of the National Academy of Sciences 107, 9264–9269. https://doi.org/10.1073/pnas.0913022107.

Yang, Z., Rannala, B., 2014. Unguided Species Delimitation Using DNA Sequence Data from Multiple Loci. Molecular Biology and Evolution 31, 3125–3135. https://doi.org/10.1093/molbev/msu279.

Zalozny, N.A., Vorobiev, D.S., 2017. Oligochaetes in the water ecosystems of Western Siberia. International Journal of Environmental Studies 74, 811–817. https://doi.org/10.1080/00207233.2017.1283952.

Zattara, E.E., Bely, A.E., 2015. Fine taxonomic sampling of nervous systems within Naididae (Annelida: Clitellata) reveals evolutionary lability and revised homologies of annelid neural components. Frontiers in Zoology 12, 8. https://doi.org/10.1186/s12983-015-0100-6.