ORIGINAL ARTICLE

Is 'everything everywhere'? Unprecedented cryptic diversity in the cosmopolitan flatworm *Gyratrix hermaphroditus*

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Abstract

Many nominal species of microscopic animals traditionally fitting the 'everything is everywhere' paradigm have been revealed to be complexes of cryptic species. Here, we explore species diversity within the micrometazoan flatworm *Gyratrix hermaphroditus*—unique among meiofauna because of its global occurrence in a wide variety of brackish, freshwater and marine environments. With maximum likelihood and Bayesian approaches, we analysed 18S, 28S, 5.8S and ITS2 rDNA sequences from 401 specimens across the global distribution of *G. hermaphroditus*. Generalized Mixed Yule Coalescent (GMYC) and Automatic Barcode Gap Discovery (ABGD) methods delineated 78 and 62 putative species, respectively. This renders *G. hermaphroditus* one of the most species-rich complexes known to date. Based on shape variations of the male copulatory organ, 14 morphotypes corresponding with molecular clades were identified within the species complex. Within morphotypes, morphometric measurements were able to further discriminate between GMYC species using discriminant analyses. While most putative species occur on local or regional scales, over 10% are distributed over vast distances (>500 km apart) and two GMYC and six ABGD species have colonised multiple continents. This suggests that the cosmopolitanism of the *G. hermaphroditus* species complex is not just caused by mixing cryptic species with a more limited geographic distribution, but is due to the presence of previously unrecognised cosmopolitan taxa. The wide variation in distribution patterns between putative species indicates that meiofaunal biogeography should not be simplified into 'everything is everywhere', but rather entails every ecological state, extending from local endemism to true cosmopolitanism.

KEYWORDS
cosmopolitan species complexes, Platyhelminthes, Rhabdocoela, species delineation

1 | INTRODUCTION

'Everything is everywhere, but the environment selects', the widely cited tenet of Baas Becking (1934), captures the notion that species of microorganisms (<2 mm) such as bacteria, protists and small metazoans occur everywhere as long as environmental conditions are suitable (Girguis, 2016). This ubiquitousness is attributed to large effective population sizes,

dormancy capabilities and short generation times. However, this paradigm is contradicted by accumulating evidence of biogeographic provincialism in microscopic organisms (e.g. Derycke et al., 2013; Ribeiro et al., 2018; Spatharis et al., 2019).

Biogeographic patterns of microscopic metazoans are still poorly documented. For example, many freshwater zooplankton species are thought to comply with the 'everything is everywhere' paradigm because of their high dispersal potential through passive transport of resting stages (reviewed by Fontaneto, 2019). Conversely, cosmopolitan distributions are considered paradoxical in marine meiobenthic species—a proposition known as the meiofauna paradox—because many of these organisms lack dispersal stages such as planktonic larvae or resting stages (Giere, 2009, 2019). In several cases, this paradox was resolved by the discovery that these cosmopolitan species consist of several cryptic species with a more limited distribution (Cerca et al., 2018 and references therein). Nevertheless, evidence for transoceanic dispersal has been found for some meiobenthic taxa, suggesting that some species do have vast distributions (Derycke et al., 2008; George, 2013; Packmor & Riedl, 2016).

Understanding the biogeography of an organism requires a solid taxonomic framework, a prerequisite often lacking in microscopic metazoans. Recent studies based on molecular analyses revealed a high degree of cryptic diversity in micrometazoan morphospecies as diverse as copepods (e.g. Cornils & Held, 2014), nematodes (e.g. Janssen et al., 2017), nemerteans (e.g. Leasi & Norenburg, 2014), polychaetes (e.g. Cerca et al., 2020) and rotifers (e.g. Mills et al., 2017). Some of these morphospecies seem to exhibit cosmopolitan distributions, but this may be caused by lumping together cryptic species with more confined geographic distributions. However, distribution ranges of cryptic species are often difficult to pinpoint, because many studies cover only a small part of the complete range of a species complex (e.g. Jiu et al., 2017; Kordbacheh et al., 2017; Tomioka et al., 2016). The few studies assessing cryptic diversity on a comprehensive scale suggest that some cryptic species within 'cosmopolitan' morphospecies have a very wide distribution and even occur on several continents (e.g. Liu et al., 2017; Mills et al., 2017; Worsaae et al., 2019). Hence, the presence of abundant cryptic diversity in cosmopolitan micrometazoans is in itself insufficient to reject the 'everything is everywhere' hypothesis, while it is also not clear to what extent individual cryptic species do show a wide geographic distribution and thus are in line with this hypothesis. As such, there is a need for more in-depth taxonomic and biogeographic studies of cosmopolitan micrometazoans on a global scale.

One of the most striking examples of a cosmopolitan micrometazoan morphospecies is the flatworm *Gyratrix hermaphroditus* Ehrenberg, 1831 (Rhabdocoela, Polycystididae). Unlike any other microscopic animal, this microturbellarian combines a global distribution and an exceptional ecological diversity, with some populations occurring exclusively in

marine environments, while others are confined to brackish or freshwater habitats (Tessens, 2012). Marine populations live interstitially in sand and on algae, from the eulittoral zone up to depths of >400 m (Artois et al., 2000), while freshwater populations live on aquatic macrophytes and even mosses in wet terrestrial habitats. These features, together with cytogenetic and subtle morphological evidence, suggest that *G. hermaphroditus* may be prone to a high degree of cryptic diversity (Artois & Tessens, 2008; Curini-Galletti & Puccinelli, 1989, 1990, 1994, 1998; Hallez, 1873; Heitkamp, 1978; l'Hardy, 1986; Puccinelli & Curini-Galletti, 1987; Puccinelli et al., 1990; Reuter, 1961; Timoshkin et al., 2004).

In this study, we explore cryptic diversity in *G. hermaphroditus* throughout its global range. In a multiple evidence approach, we combine species delimitation analyses based on molecular data and morphometric measurements. Based on this comprehensive analysis, we assess to what extent this species complex complies with the 'everything is everywhere' hypothesis.

2 | MATERIAL AND METHODS

2.1 | Sampling

Individuals of *Gyratrix hermaphroditus* were collected from 70 aquatic sites in various freshwater, marine and brackish water habitats across the global distribution of the morphospecies (Table S1). Several specimens of the congeners *G. proavus* Meixner, 1938, and *G. proaviformis* Karling & Schockaert, 1977, were also collected to be included as an outgroup in the phylogenetic analyses. Worms were extracted from sediments and aquatic plants following Schockaert (1996) and identified with the aid of a stereomicroscope and a compound microscope. From each location, a number of specimens were stored in absolute ethanol for DNA analysis, the remainder being whole-mounted with lactophenol for morphological study. Micrographs of live specimens selected for molecular work are stored as vouchers in the collection of the Centre for Environmental Sciences at Hasselt University and are available upon request.

2.2 | DNA extraction, amplification and sequencing

DNA was extracted from whole or partial (see below) specimens using the QIAamp DNA micro kit (QIAGEN) according to manufacturer's instructions. Extracts were stored in duplicates (40 and 20 μl) for each specimen. Four partitions of the nuclear ribosomal gene complex (18S, 5.8S, ITS2 and partial 28S) were amplified by PCR (primers and protocols in Table 1). Species-specific primers for ITS2 and 5.8S were

TABLE 1 Primers and PCR protocols

Primers	Sequence (5'→3')	Usage	Reference
18S			
TimA	AMCTGGTTGATCCTGCCAG	PCR/Seq	Norén and Jondelius (1999)
TimB	TGATCCATCTGCAGGTTCACCT	PCR/Seq	Norén and Jondelius (1999)
TimA/TimB PCR regime:			
5 min 10 s at 95°C, 30x (30 s at 94°C , 30 s at 55°C , 90 s at 72°C), 5 min at 72°C			
600F	GGTGCCAGCAGCCGCGGT	Seq	Willems et al. (2006)
600R	ACCGCGGCTGCTGGCACC	Seq	Willems et al. (2006)
1100F	CAGAGGTTCGAAGACGATC	Seq	Norén and Jondelius (1999)
1100R	GATCGTCTTCGAACCTCTG	Seq	Norén and Jondelius (1999)
18S7F	GCAATAACAGGTCTGTGATGC	Seq	Norén and Jondelius (1999)
18S7FK	GCATCACAGACCTGTTATTGC	Seq	Norén and Jondelius (1999)
TimB	TGATCCATCTGCAGGTTCACCT	Seq	Norén and Jondelius (1999)
28S			
LSU5	TAGGTCGACCCGCTGAAYTTA	PCR/Seq	Littlewood et al. (2000)
LSUD6-3	GGAACCCTTCTCCACTTCAGTC	PCR/Seq	Littlewood et al. (2000)
LSU5/LSUD6-3 PCR regime:			
5 min at 95°C , 30x (60 s at 94°C , 60 s at 50°C , 90 s at 72°C), 5 min at 72°C			
L300F	CAAGTACCGTGAGGGAAAGTTG	Seq	Littlewood et al. (2000)
L300R	CAACTTTCCCTCACGGTACTTG	Seq	Littlewood et al. (2000)
L1600F	GCAGGACGGTGGCCATGGAAG	Seq	Littlewood et al. (2000)
L1600R	CTTCCATGGCCACCGTCCTGC	Seq	Littlewood et al. (2000)
5.8S+ITS2			
58SITS2F1	GCGGTGGATCACTCGGCTCG	PCR/Seq	This study
58SITS2R1	TCGCTCGCCGCTACTRRGGGA	PCR/Seq	This study
58ITS2F/58ITS2R PCR regime:			
5 min at 95°C , 35x (60 s at 94°C , 60 s at 55°C , 72 s at 72°C), 5 min at 72°C			

designed *in silico*. Illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare) were used to prepare reactions containing 3 μl DNA extract, 0.2 μM of each primer and water for a final volume of 25 μl. PCR products were checked on 1.4% agarose gels stained with Gelred (Biotum Inc.), cleaned in NucleoFast 96 PCR plates (MN Düren) and bidirectionally sequenced (ABI3730XL, Macrogen). Trace files were visually inspected and assembled into full sequences in SeqScape v2.5 (Life Technologies) or Geneious Pro v5.7.5 (Biomatters Ltd). All sequences are deposited in Genbank under accession numbers MZ575164–MZ575591 and MZ598667–MZ599582.

2.3 | DNA sequence analysis

2.3.1 | Sequence alignment

Sequences of each gene partition were aligned separately using the Q-INS-i option in MAFFT v7.012 (Katoh & Standley, 2013), which accounts for secondary structures. Alignment ambiguities were identified with ALISCORE

v2.0 (Kück et al., 2010; Misof & Misof, 2009) and removed from the alignments with ALICUT (Kück, 2009), treating gaps as ambiguous characters and using a sliding window size $w = 6$. Substitution saturation was checked for each gene partition by plotting transitions and transversions against genetic distance in DAMBE 5.3.00 (Xia & Xie, 2001), yet no sign of saturation was detected. Alignments of the four gene partitions were concatenated and only specimens with sequence information for all four markers were included in subsequent analyses. Identical sequences were removed from the alignment.

2.3.2 | Phylogenetic analyses

Heterozygotic or ambiguous sites were coded using IUPAC codes and considered as different characters. Mean/maximum uncorrected p-distances among haplotypes were calculated in MEGA5 (Tamura et al., 2011). Bayesian (BI) trees were inferred in MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Two parallel runs with four

independent chains were run for 10,000,000 generations using default priors and Markov Chain Monte Carlo (MCMC) settings. Convergence was evaluated based on the logL values and the average standard deviation of split frequencies. Trees were sampled every 100th generation after a burn-in of 25%. Three partitioning schemes were applied as follows: unpartitioned, partitioned by 'function' (i.e. 18S + 5.8S + 28S versus ITS2) and partitioned by rDNA segment (i.e. 18S, 5.8S, 28S, ITS2). Evolutionary models of the different partitions were selected using the Akaike (AIC) and Bayesian (BIC) information criteria implemented in jModelTest v0.1.1 (Posada, 2008). When AIC and BIC selected different models, both were tried. All combinations of partitioning schemes and evolutionary models were specified in different runs. Based on calculated Bayes factors, final analyses were partitioned by segment and run under the GTR + G + I model for 18S, ITS2, 28S and SYM + G + I for 5.8S. The resulting 75,000 trees were summarised in a 50% majority-rule consensus tree.

Maximum likelihood (ML) analyses were conducted for the concatenated datasets in RAxML v7.2.8 (Stamatakis, 2006) using the same partitioning scheme, but under the GTR + G model following the recommendation of the developer. Hundred independent runs of thorough searches and 1,000 standard non-parametric bootstrap (bs) replicates were performed.

Trees were rooted with the outgroup taxa *G. proavus* and *G. proaviformis*.

2.3.3 | Species delineation

Based on the molecular data, putative species were delineated using the following two methods: Generalized Mixed Yule Coalescent (GMYC) (Pons et al., 2006) and Automatic Barcode Gap Discovery (ABGD; Puillandre et al., 2012).

The GMYC method requires an ultrametric tree, which was constructed in BEAST v1.6.2 (Drummond & Rambaut, 2007) under an uncorrelated lognormal relaxed clock with the mean substitution rate set to 1. Evolutionary models, partitioning scheme and tree topology were fixed to those resulting from the final MrBayes analysis. A constant size coalescent prior was used because a single coalescent cluster constitutes the GMYC null model and is considered more conservative than a Yule prior for species

delimitation (Monaghan et al., 2009). The improper prior on the ucld.mean parameter was changed into a uniform prior with an initial value of 1, a lower bound of 0 and an upper bound of 100. All other prior distributions and operators were left at default settings. Five independent MCMC chains were run for 10,000,000 generations and sampled every 1,000[th] generation. Independent runs were combined using LogCombiner v1.6.2 (implemented in BEAST) with a 50% burn-in and thinned by a factor 2.5, resulting in 10,000 trees. The estimated sample size of all model parameters in the combined log file was checked in Tracer v1.4 (Rambaut & Drummond, 2007) and was well above 200. An ultrametric maximum clade credibility tree was inferred using TreeAnnotator v1.6.2 (Drummond & Rambaut, 2007) with default options. Both a single- and multiple-threshold GMYC model were optimised on this tree after removing the outgroup, using the script available in the SPLITS package for R (Ezard et al., 2009; Fujisawa & Barraclough, 2013; R Core Team, 2017). The single-threshold and multiple-threshold models were compared using the likelihood ratio test implemented in the spiderDev package (Brown et al., 2012, 2018).

The ABGD method was implemented using the online version of the program (http://www.abi.snv.jussieu.fr/public/abgd/), using the concatenated dataset and default parameters.

2.3.4 | Rarefaction

To investigate the effect of the sample size on the number of suggested GMYC and ABGD species, individual-based rarefaction curves were produced in EstimateS v.8.2.0 (Colwell, 2005).

2.3.5 | Genetic diversity

To explore relationships among haplotypes (i.e. clusters of identical ribosomal gene complexes) within the most widespread (multicontinental) putative species resulting from the most conservative species delineation analyses (ABGD), haplotype networks were constructed from the concatenated dataset using the statistical parsimony procedure in TCS v.1.21 (Clement et al., 2001). Haplotypes were connected at the 95% confidence level.

FIGURE 1 (a) Bayesian majority-rule consensus tree of the concatenated dataset obtained using BEAST v1.6.2 (Drummond & Rambaut, 2007) and made ultrametric under an uncorrelated lognormal relaxed clock with the mean substitution rate set to 1. Clades with a bootstrap support (bs) < 75 are indicated with an asterisk (*), and all branches with a posterior probability (pp) < 0.95 have been collapsed, except for node 4 (pp = 0.92, bs = 57) and GMYC-G1 (pp = 0.65, bs = 95). Dots behind the terminals represent the habitat where the genotype was found (red = marine, yellow = brackish and blue = freshwater). Bars indicate putative species inferred by GMYC and ABGD and are numbered per morphotype. (b) Morphology of the sclerotised parts in the male copulatory organ of clades A to O. Measurements taken are indicated for clade A. Morphotypes F* and G are identical, but the copulatory organ of specimens in F* is almost twice the size of that in G (F* not drawn to scale).

(a)

(b)
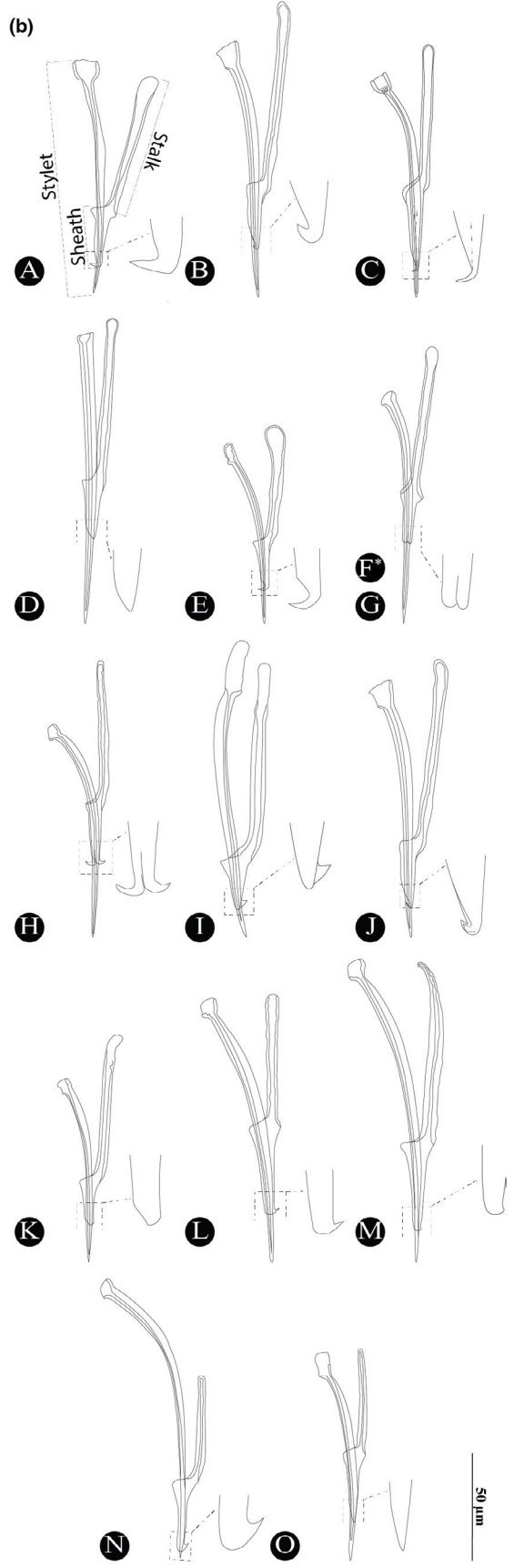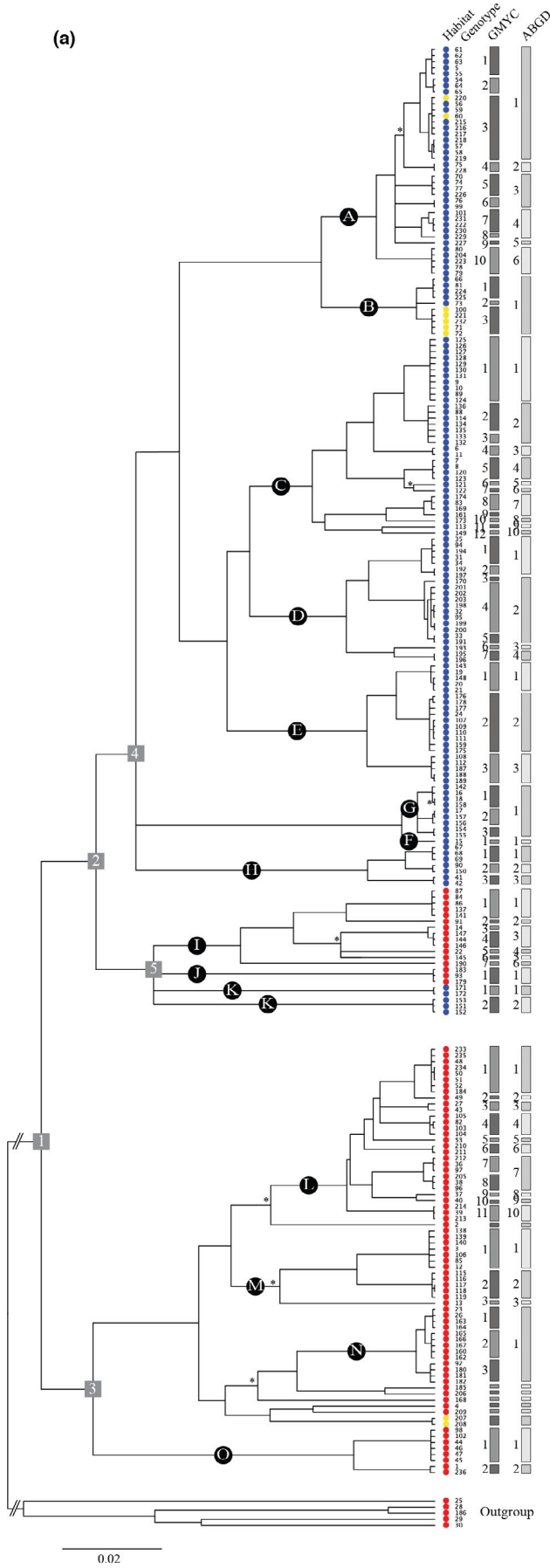
2.4 | Morphology and morphometrics

Based on the results of the molecular phylogeny, we explored to what extent clades could be morphologically differentiated by the sclerotised parts of the male copulatory organ. These structures are the standard diagnostic characters for species identification in polycystidid flatworms. In *G. hermaphroditus,* they consist of a stylet, which slides through a sheath with a proximal stalk (Figure 1b). We examined and imaged the stylet, sheath and stalk of 405 specimens from the same localities as specimens collected for DNA analysis using differential interference contrast on Polyvar and Nikon Eclipse 80i microscopes equipped with a Nikon DS-Ri2 digital camera. When more than one morphotype was detected in the same location, a direct link between each morphotype and sequence data was established by cutting individual worms in half with a scalpel, using the frontal half for DNA extraction and whole-mounting the stylet-containing posterior part as a voucher.

The morphological variation of the stylet, sheath and stalk (annotated on copulatory organ A in Figure 1b) within and between morphotypes was quantified by taking measurements in the digital images using the program ImageJ (Abramoff et al., 2004). Measurements were taken along the longest axis of each structure. Raw data are available in Table S2. Correlations between the three measurements were tested using Pearson's correlation coefficient.

All morphotypes with sufficient measurements were included in discriminant analyses (DA) in SPSS 20 (IBM Corp., 2011). Specifically, this included all morphotypes of which measurements from at least three different specimens of at least two GMYC species were obtained, i.e. clades A, C, E, H, I, L and N. These data were selected to explore whether morphometric data can discriminate between putative GMYC species within a single morphotype. A measure of error was expressed in a misclassification matrix. As some putative GMYC species were combined in single putative ABGD species, we checked whether misclassifications were more common among GMYC clusters than putative ABGD species.

3 | RESULTS

3.1 | Phylogeny and species delimitation

The 3,906 bp long concatenated alignment (18S: 1,781 bp; 5.8S: 105 bp; ITS2: 354 bp; 28S: 1,666 bp) contains 1,346 (34.5%) variable sites (18S: 316; 5.8S: 34; ITS2: 257; 28S: 751). As such, the concatenated sequences of 401 specimens of *G. hermaphroditus* included here yield 229 different haplotypes. The mean/maximum uncorrected p-distances among haplotypes are very high (18S: 0.028/0.053; 5.8S:

0.045/0.126; ITS2: 0.159/0.306; 28S: 0.065/0.107) for what are generally considered slow-evolving genes (18S, 5.8S) (Hillis & Dixon, 1991). BI and ML tree topologies are nearly identical (Figure 1a) and reveal deep divergences and many well-supported clades.

The GMYC model supports the presence of cryptic species in *G. hermaphroditus* (likelihood ratio test, $p < 0.001$). A multiple-threshold model did not fit the data significantly better than the single-threshold model ($\chi^2 = 8.83$, $p = 0.453$). A total of 78 putative species consisting of 50 clusters and 28 singletons were identified with the GMYC method (confidence interval 72–82, Figure 1a, Table S1). All GMYC clusters are well supported (posterior probability/pp > 0.95, bs > 75), except for clusters C3 (not retrieved by BI, bs = 78), D4 (pp = 0.98, bs = 67), G1 (pp = 0.65, bs = 95) and I4 (pp = 0.99, bs = 50).

ABGD results suggest 62 putative species with 38 clusters and 24 singletons (Figure 1a, Table S1). All ABGD clusters are well supported (pp > 0.95, bs > 75), except for cluster A3 (pp = 0.65, not retrieved in ML analysis). All GMYC species correspond to or are nested within the ABGD species.

3.2 | Morphology and morphometrics

Based on subtle variations in the shape and relative length of the sclerotised structures of the male copulatory organ, 14 morphotypes were distinguished (Figure 1b) and related to the different clades in the phylogeny (clades A–O, Figure 1a).

The lengths of the stylet, sheath and stalk were significantly correlated (Table S3). Therefore, measurements of the sheath and stalk relative to the length of the stylet were used in subsequent analyses (Table S3). For morphotypes A, C, E, H, I, L and N, we were able to test whether morphometric data discriminate between putative GMYC species within each morphotype. DA clearly separated the GMYC clusters within each morphotype (Figure S1), and there was a low misclassification proportion (0 to 10.4%, Table S4). Even shallow GMYC clades (e.g. A1, A2, A3 and N1, N2, N3) were successfully discriminated by the morphometric data (Figure S1, Table S4).

3.3 | Biogeography

A total of 35 GMYC species (45%; 17 freshwater, 17 marine, 1 brackish) and 22 ABGD species (36%; 11 freshwater, 10 marine, 1 brackish), excluding those represented by a single specimen, were found at single sites. An additional 20 GMYC species (26%; 13 freshwater, 5 marine, 1 freshwater + marine, 1 freshwater + brackish) and 18 ABGD species (30%; 12 freshwater, 5 marine, 1 freshwater + marine) were

found at locations separated by <100 km. Conversely, eight GMYC species (3 freshwater, 5 marine) have wide distributions within a single continent (>500 km apart) and three GMYC species (1 freshwater, 2 marine) were found on at least two continents. ABGD revealed even more putative species with very wide distributions: eight ABGD species (3 freshwater, 5 marine) separated by >500 km within a single continent and six ABGD species (4 freshwater, 2 marine) on more than one continent. Each of the six ABGD species with the widest distributions formed interconnected statistical parsimony networks at the 95% confidence level (Figure 2). In these networks, haplotypes were never shared between different geographic regions.

Many putative species occur sympatrically, i.e. at exactly the same site: 34/37% of freshwater and 59/57% of marine sites contain more than one GMYC/ABGD species. While most instances of sympatry pertain to species with differing copulatory organs, six (GMYC & ABGD) cases of identical morphotypes co-occurring were also observed. There were on average 1.7/1.8 GMYC/ABGD species per site, with a maximum number of 7/9 GMYC/ABGD species found at a single site (a beach near Alghero, Sardinia).

3.4 | Habitat

All putative species occur either exclusively in marine, brackish or freshwater habitats, except clade A3/A1 GMYC/ABGD, which contains both freshwater and brackish water (= Baltic Sea) populations (Figures 1 and 3). No habitat type conforms to a single monophyletic group. Indeed, the brackish water taxa are distributed over three unrelated clades deeply nested in otherwise freshwater (node 4, Figure 1a) or marine (node 3, Figure 1a) clades.
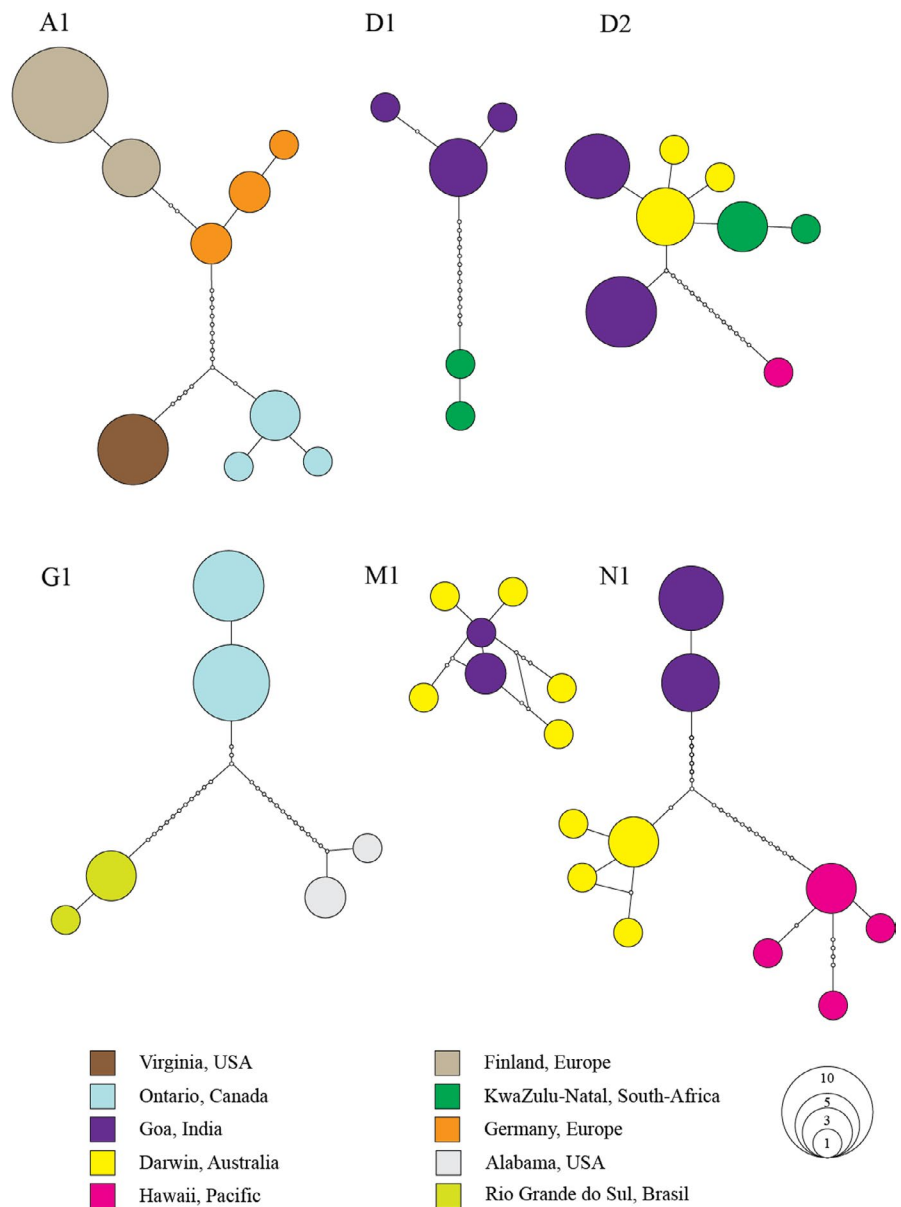
FIGURE 2 TCS networks of the six ABGD species occurring on more than one continent. Colours represent geographic regions, and the size of circles is proportional to the number of specimens with a particular haplotype.
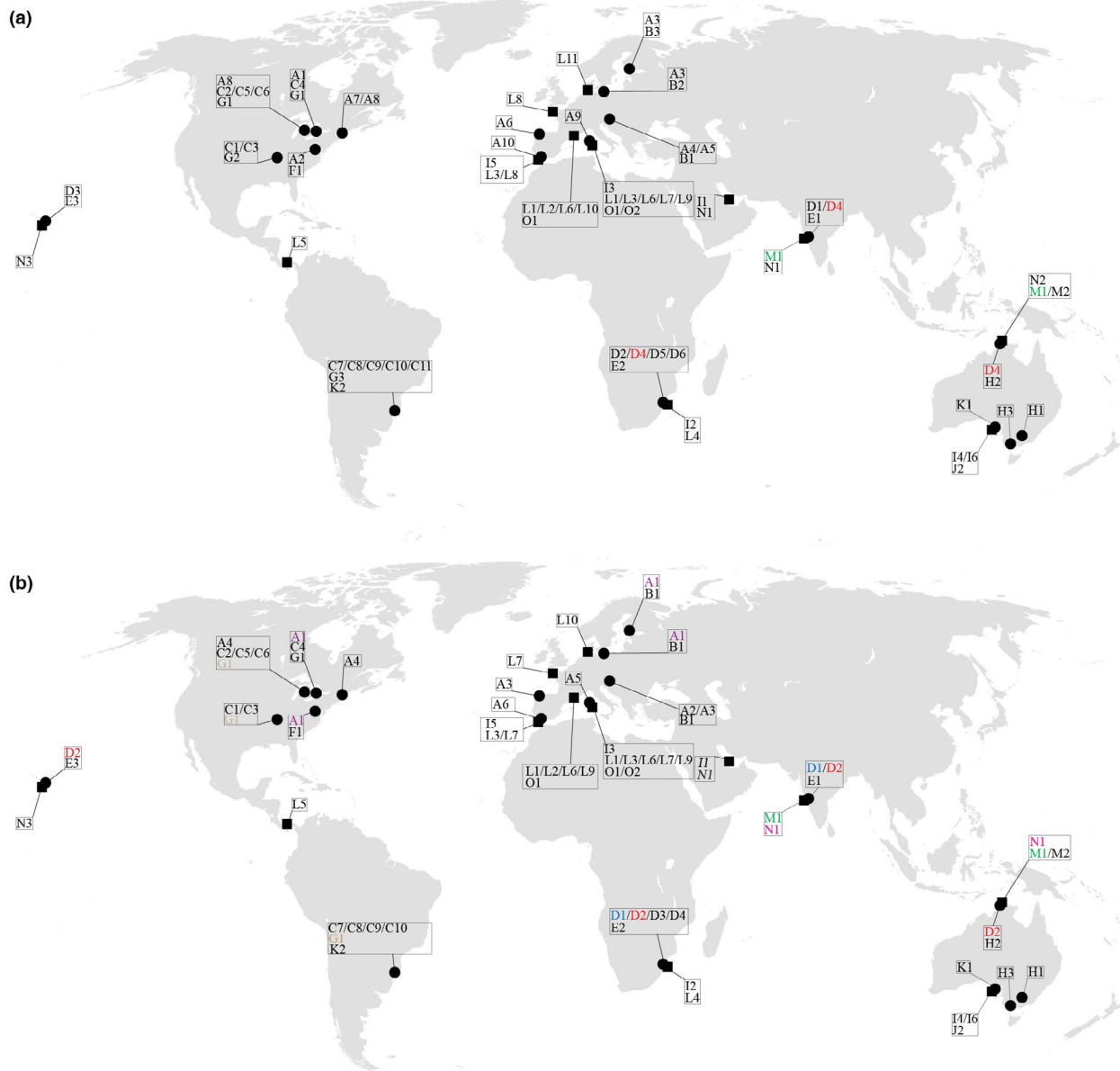
FIGURE 3 Global distribution of putative species according to (a) GMYC and (b) ABGD. Circles represent freshwater and rectangles marine sampling sites. Putative species occurring in more than one continent are highlighted in colour.

4 | DISCUSSION

4.1 | Cryptic species

The amount of cryptic diversity uncovered here renders *G. hermaphroditus* one of the largest species complexes known to date (78 GMYC and 62 ABGD putative species). To the best of our knowledge, the only species complex of comparable size is the bdelloid rotifer *Rotaria rotatoria* (Pallas, 1766) Scopoli, 1777 (Fontaneto et al., 2013 and references therein). Formally recognising the new entities delineated in *G. hermaphroditus* would increase the total number of polycystidid species by >33%, and rarefaction curves (Figure 4)

indicate that still more cryptic diversity remains undetected in this complex.

Results from single-gene (or gene cluster) species delineations may be misleading as they may be affected by introgression (Cong et al., 2017) and incomplete lineage sorting (Mallo & Posada, 2016). Moreover, population sampling effects might cause the GMYC model to overestimate species numbers, because geographic structuring may be mistaken for species limits (but see Talavera et al., 2013). These delineations should therefore be viewed as *prima facie* estimates of species boundaries to be re-evaluated and refined when new lines of evidence become available (Yeates et al., 2011). Our putative species are consistent with a phylogenetic (lineage)

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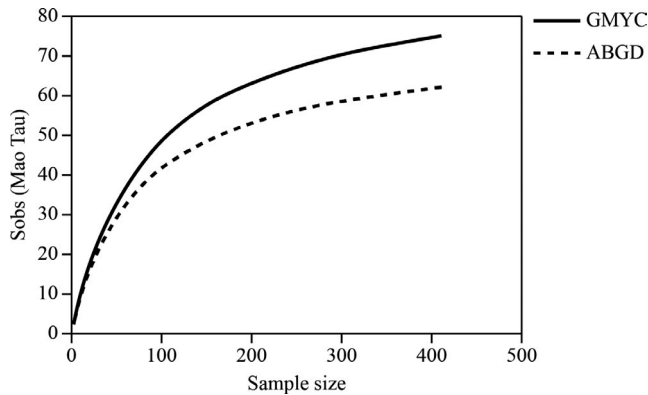
FIGURE 4 Individual-based rarefaction curve of the number of GMYC and ABGD clusters.

species concept, and several are diagnosable by morphological data. However, different delineation methods are known to produce alternative interpretations for the same data (Luo et al., 2018). This is also the case here, leaving the question as to which of the putative GMYC and ABGD species should be retained. Future work will have to explore to what extent the putative species suggested by the present phylogenetic and species delineation analyses represent phenotypically diagnosable and reproductively isolated entities. In the same sense, it will be important to corroborate the current phylogenetic structuring by other molecular markers, for there is no *a priori* reason to expect that the DNA region surveyed here provides a complete picture of the entire phylogenetic and taxonomic structuring in *G. hermaphroditus*.

It has been suggested that species with non-visual mating signals and/or that are under morphological stasis are more likely to show cryptic diversity (Bickford et al., 2007). Both possibilities may be applicable to *G. hermaphroditus*: (a) chemical signals are probably involved in mate attraction as the copulation of *G. hermaphroditus* is preceded by some sort of 'sniffing' behaviour (Graff, 1908; Tessens, 2012) and in captivity a couple engaged in mating behaviour quickly attracts other specimens, which will start to circle the mating couple (personal observations by the first and last author); (b) the male copulatory organ is the main taxonomic character of rhabdocoel flatworms. Species level identification is often based exclusively on this character. However, *G. hermaphroditus* does not only use its male copulatory organ for mating (Graff, 1908; Heitkamp, 1978; Reisinger, 1923; Reuter, 1961), but also for prey capture (Tessens, 2012). Perhaps this dual function for mating and feeding imposes constraints on the shape of the male copulatory organ, thus allowing only the sort of subtle variations reported here.

Asexual reproduction is also a driver of cryptic diversification, with sympatric divergence occurring more easily (Fontaneto et al., 2009). However, asexual reproduction involves the production of clones, of which the taxonomic interpretation is contentious. As most other flatworms, *G.*

hermaphroditus is presumed to reproduce sexually because mating is frequently observed (Graff, 1908; Heitkamp, 1978; Reisinger, 1923; Reuter, 1961). This does not exclude the possibility of parthenogenesis or self-fertilisation, both of which are known to occur in other hermaphroditic flatworms (Casu et al., 2012; D'Souza & Michiels, 2009; Jarne & Auld, 2006; Noreña et al., 2015; Pandian, 2020; Ramm, 2017). Hence, more detailed information on the reproduction strategies of *G. hermaphroditus* throughout its range is needed to interpret their role in the evolution of this species complex.

Other isolation mechanisms as drivers of speciation have also been proposed. Extensive studies on numerous freshwater and marine populations of *G. hermaphroditus* from Europe, the Caribbean and Australia have demonstrated clusters of specimens characterised by karyotype and the size and morphology of the sclerotised structures in the male copulatory organ (Curini-Galletti & Puccinelli, 1989, 1990, 1994, 1998; Heitkamp, 1978; l'Hardy, 1986; Puccinelli & Curini-Galletti, 1987; Puccinelli et al., 1990). Morphological evolution of the male copulatory structure and different examples of karyological evolution, including changes in genome size, chromosome number and chromosome structure, have been suggested as pre- and postzygotic isolation mechanisms (Curini-Galletti & Puccinelli, 1998). Freshwater populations and some marine populations have chromosome numbers $2n = 4$, while most marine populations have $2n = 6$. In addition, close relatives of *G. hermaphroditus sensu lato*, *G. proavis* and *Gyratricella attemsi* (Graff, 1913) Karling, 1955 also have $2n = 6$. It was therefore hypothesised that three pairs of chromosomes in an ancestral marine form is the plesiomorphic condition (Puccinelli & Curini-Galletti, 1987). Karyological data for our specimens are currently lacking. Consequently, it remains to be tested whether the putative marine species forming a clade with the putative freshwater species (clades I and J) have two pairs of chromosomes, while all other putative marine clades have three pairs of chromosomes. If so, then this would suggest that chromosome fusion could have led to the early separation of most marine and freshwater clades (node 1). In an alternative scenario, putative marine species with two pairs of chromosomes are more scattered among the marine clades, indicating that chromosome fusion has occurred multiple times within the species complex.

Clusters of up to 12 putative species belonging to the same morphotype based on the copulation organ (clades C and L) were retrieved in our analyses. This suggests that morphological evolution might have been a next step in the speciation process of the *G. hermaphroditus* complex. Several putative species with different morphotypes occur sympatrically in some of our sampling localities (e.g. Cerbère, Doñana; Table S1). Differences in the sclerotised copulation structures may prevent hybridisation between these sympatric species. Other isolation mechanisms are likely responsible

for co-occurring putative species with a similar morphotype based on the copulation structures. This would be consistent with previous findings that some marine areas contain high levels of sympatric putative species in the *Gyratrix hermaphroditus* complex based on karyotype differentiation. Pericentric chromosome inversions and ecological and physical barriers have been proposed to explain patterns of high local diversity and regional differences in putative species occurrences, preventing local hybridisation and dispersal across areas with different types of sediments or water temperature, respectively (Curini-Galletti & Puccinelli, 1998). Integrating karyological and ecological data into future studies on the *G. hermaphroditus* complex may provide clues about which isolation mechanisms have acted during speciation in this complex.

4.2 | Geographic distribution

The cosmopolitan distribution of many micrometazoans has long been attributed to the lumping of cryptic taxa with a more limited geographic distribution (Darling & Carlton, 2018 and references therein). Often these conclusions are based on surveys in only part of the entire range of a cosmopolitan morphospecies (e.g. Jiu et al., 2017; Kordbacheh et al., 2017; Tomioka et al., 2016). However, by screening *G. hermaphroditus* on a global scale, we did not only find indications of high local cryptic diversity consistent with previous studies, but we also observed several putative species with a very wide (>500 km apart; 11/13% GMYC/ABGD) or even multicontinental distribution (4/10% GMYC/ABGD). This indicates that, while part of the global distribution of *G. hermaphroditus* might be due to lumping unrecognised cryptic species, some cryptic species themselves can have widespread distributions. For example, one of the putative freshwater species (GMYC: D4; ABGD: D2) was collected in South Africa, India and Australia, while some of the putative marine species have an Indo-Pacific distribution (GMYC: M1; ABGD: M1, N1). Indeed, our results suggest that widespread micrometazoans do exist, even in marine meiobenthos, and do not comply with the meiofauna paradox. However, the reported wide distributions could be caused by limitations in signal of the ribosomal genes analysed here. In addition, none of these widespread putative species share haplotypes between continents. This is why further screening of phylogenetic structuring using additional DNA markers is imperative.

The current data indicate that *G. hermaphroditus* comprises a complex mix of sympatric and allopatric species, with instances of sympatry in both marine and freshwater environments. The co-occurrence of regional species and more widespread species was also reported in the marine rhabdocoel *Astrotorhynchus bifidus* (McIntosh, 1874) Graff, 1905 (Van Steenkiste et al., 2018). Mechanisms to explain co-occurrence and/or co-existence in this complex were speculated to include habitat partitioning and life history traits. In *G. hermaphroditus*, sexual selection pressure might (partially) account for the distribution patterns observed. Indeed, the majority of cases of sympatry involved species with distinct copulatory organs. As the copulatory organ is also used for prey capture (Tessens, 2012), morphological differences in this structure could also be linked to trophic niche variation. However, as for all meiofauna, it remains highly difficult to pinpoint what exactly constitutes an ecological niche for these animals. Moreover, the fact that species carrying (seemingly) identical copulatory organs also co-occur implies that alternative and/or additional mechanisms are at work. Re-examination of sclerotised structures with high-resolution imaging methods such as scanning electron microscopy might also reveal additional morphological variations.

Because *G. hermaphroditus* lives both epiphytically in freshwater and interstitially in marine environments, biogeographic patterns should be compared to those of both zooplankton and marine meiobenthos. In freshwater environments, the wide distribution of microscopic species is thought to be the result of passive long-distance transport of resting stages through vectors such as wind, water and waterfowl, maintaining extensive gene flow (Fontaneto, 2019). Resting eggs or cocoons are also present in many limnic and limnoterrestrial microturbellarians (Artois et al., 2004; Domenici & Gremigni, 1977; Graff, 1913; Hand, 1991; Heitkamp, 1972a, 1972b, 1988; Hyman, 1951; Ingole, 1987; Luther, 1955; Pandian, 2020; Rietzler et al., 2018; Schockaert et al., 2008; Young, 1974). In *G. hermaphroditus*, resting eggs with a hard eggshell are present in limnic populations. Passive long-distance transport modes such as displacement by wind, animals or humans (Steinböck, 1931; Vanschoenwinkel et al., 2008, 2008, 2009; Vanschoenwinkel, Waterkeyn et al., 2008; Young & Young, 1976) could also ensure dispersal and connectivity of freshwater microturbellarians, but this remains to be tested (see Artois et al., 2011; Balsamo et al., 2020). One of the best-studied examples among freshwater meiofauna is the species complex of the monogonont rotifer *Brachionus plicatilis* Müller, 1786, in which passive dispersal led to the apparent global distribution of many cryptic species (Gómez et al., 2002; Mills et al., 2017; Suatoni et al., 2006). Similarly, there is evidence of globally distributed putative cryptic species of bdelloid rotifers (Fontaneto et al., 2008), even though most of them were distributed at continental or smaller scales (<2,000 km), presumably through passive transport of resting stages. However, cosmopolitan cryptic species of *B. plicatilis* are extensively substructured into geographically restricted lineages and with a strong global signal of isolation by distance (Gómez et al., 2000, 2002, 2007; Gómez, Serra et al., 2002; Mills et al., 2007, 2017). The same is true for bdelloid rotifers (Fontaneto et al., 2008; Robeson et al., 2011).

It is not clear whether there is ongoing gene flow between distant populations of the widespread cryptic species in *G. hermaphroditus*. Dispersal capacities of marine flatworms have hardly been studied, but rafting on floating materials such as driftweed (Gerlach, 1977; Goldstein et al., 2014; Van Steenkiste et al., 2018) and anthropogenic spread (Faubel & Gollasch, 1996) has been suggested and observed anecdotally. Some populations of *G. hermaphroditus* overwinter in eggs attached to the substrate (Brunet, 1965; Heitkamp, 1978; Hoxhold, 1974), which might promote passive dispersal through rafting. Yet, our data do not provide evidence of shared haplotypes between populations on different continents, suggesting complete isolation (Figure 2). However, a more densely sampled dataset including more specimens per putative target species is imperative before drawing definite conclusions on this matter.

4.3 | Conclusions and future perspectives

Our analyses of a large nuclear ribosomal dataset of *Gyratrix hermaphroditus* show an unprecedented amount of cryptic diversity. Depending on the method used, 62 or 78 putative species are delineated. Part of this diversity can be correlated to morphological and morphometric variations in the sclerotised copulatory organ. Disentangling this species complex has revealed an intricate mixture of sympatric and allopatric species distributed over all aquatic habitats. The uncovered cryptic species vary widely in their distribution ranges, with some species occurring in single localities and others over vast distances or even across different continents.

The species delineated in this study need to be further explored and corroborated by new lines of evidence and based on a far more comprehensive genomic survey and denser geographic sampling. Population genomic work can elucidate to what extent the entities delineated by our methods reflect gene flow. Moreover, almost nothing is known on the mating system of *G. hermaphroditus*, and *in vitro* cross-breeding experiments hold much potential to reveal prezygotic or postzygotic isolation mechanisms. Irrespective of the outcomes of such in-depth analyses of the species complex, our current data already demonstrate that even organisms with high dispersal potential can show substantial geographic subdivision and it should be clear that the biogeography of meiofauna is not as simple as 'everything is everywhere'. Rather, distribution patterns most likely span the whole range of alternatives, from full cosmopolitanism to local endemism.

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REFERENCES
Abramoff, M. D., Magalhaes, P. J., & Ram, S. J. (2004). Image Processing with ImageJ. *Biophotonics International*, *11*, 36–42.
Artois, T., Fontaneto, D., Hummon, W. D., McInnes, S. J., Todaro, M. A., Sørensen, M. V., & Zullini, A. (2011). Ubiquity of microscopic animals? Evidence from the morphological approach in species identification. In D. Fontaneto (Ed.), *Biogeography of microscopic organisms: Is everything small everywhere?* (pp. 244–283). Cambridge University Press.
Artois, T. J., & Tessens, B. S. (2008). Polycystididae (Rhabditophora: Rhabdocoela: Kalyptorhynchia) from the Indian Ocean, with the description of twelve species. *Zootaxa*, *1849*, 1–27. https://doi.org/10.11646/zootaxa.1849.1.1

Artois, T., Vermin, W., & Schockaert, E. (2000). Rhabdocoela (Platyhelminthes) from the Weddell Sea (Antarctica) with the description of eight new species. *Belgian Journal of Zoology*, *130*, 103–110.

Artois, T., Willems, W., De Roeck, E., Jocqué, M., & Brendonck, L. (2004). Freshwater Rhabdocoela (Platyhelminthes) from ephemeral rock pools from Botswana, with the description of four new species and one new genus. *Zoological Science*, *21*, 1063–1072. https://doi.org/10.2108/zsj.21.1063

Baas Becking, L. G. M. (1934). *Geobiologie of inleiding tot de milieukunde*. W.P. Van Stockum & Zoon N.V.

Balsamo, M., Artois, T., Smith, J. P. S. III, Todaro, M. A., Guidi, L., Leander, B. S., & Van Steenkiste, N. W. L. (2020). The curious and neglected soft-bodied meiofauna: Rouphozoa (Gastrotricha and Platyhelminthes). *Hydrobiologia*, *847*, 2613–2644. https://doi.org/10.1007/s10750-020-04287-x

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., & Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, *22*, 148–155. https://doi.org/10.1016/j.tree.2006.11.004

Brown, S. D. J., Collins, R. A., Boyer, S., Lefort, M.-C., Malumbres-Olarte, J., Vink, C. J., & Cruickshank, H. (2012). Spider: An R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. *Molecular Ecology Resources*, *12*, 562–565. https://doi.org/10.1111/j.1755-0998.2011.03108.x

Brown, S. D. J., Collins, R. A., Boyer, S., Lefort, M.-C., Malumbres-Olarte, J., Vink, C. J., & Cruickshank, H. (2018). *Spider: SPecies IDentity and Evolution in R*. R package version 1.4-2. Available from: https://rdrr.io/rforge/spider/

Brunet, M. (1965). Turbellariés calyptorhynques de substrats meubles de la région de Marseille. *Recueil Des Travaux De La Station Marine D'endoume*, *39*, 127–220.

Casu, M., Cossu, P., Lai, T., Scarpa, F., Sanna, D., Dedola, G. L., & Curini-Galletti, M. (2012). First evidence of self-fertilization in a marine microturbellarian (*Platyhelminthes*). *Journal of Experimental Marine Biology and Ecology*, *428*, 32–38. https://doi.org/10.1016/j.jembe.2012.05.026

Cerca, J., Meyer, C., Purschke, G., & Struck, T. H. (2020). Delimitation of cryptic species drastically reduces the geographical ranges of marine interstitial ghost-worms (*Stygocapitella*; Annelida, Sedentaria). *Molecular Phylogenetics and Evolution*, *143*, 106663. https://doi.org/10.1016/j.ympev.2019.106663

Cerca, J., Purschke, G., & Struck, T. H. (2018). Marine connectivity dynamics: Clarifying cosmopolitan distributions of marine interstitial invertebrates and the meiofauna paradox. *Marine Biology*, *165*, 123. https://doi.org/10.1007/s00227-018-3383-2

Clement, M., Posada, D., & Crandall, K. A. (2001). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, *9*, 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Colwell, R. K. (2005). *EstimateS: Statistical estimation of species richness and shared species from samples*. http://purl.oclc.org/estimates

Cong, Q., Shen, J., Borek, D., Robbins, R. K., Opler, P. A., Otwinowski, Z., & Grishin, N. V. (2017). When COI barcodes deceive: Complete genomes reveal introgression in hairstreaks. *Proceedings of the Royal Society B*, *284*, 20161735. https://doi.org/10.1098/rspb.2016.1735

Cornils, A., & Held, C. (2014). Evidence of cryptic and pseudocryptic speciation in the *Paracalanus parvus* species complex (Crustacea, Copepoda, Calanoida). *Frontiers in Zoology*, *11*, 19. https://doi.org/10.1186/1742-9994-11-19

Curini-Galletti, M. C., & Puccinelli, I. (1989). Karyometric and morphological analysis of two sympatric marine species of the *Gyratrix hermaphroditus* complex (Platyhelminthes: Kalyptorhynchia) occurring at Roscoff (Brittany, France). *Hydrobiologia*, *173*, 63–68. https://doi.org/10.1007/BF00008599

Curini-Galletti, M., & Puccinelli, I. (1990). The *Gyratrix hermaphroditus* species complex (Platyhelminthes: Kalyptorhynchia) in the Darwin Area (Northern Territory, Australia). *Transactions of the American Microscopic Society*, *109*, 368–379. https://doi.org/10.2307/3226691

Curini-Galletti, M., & Puccinelli, I. (1994). The *Gyratrix hermaphroditus* species-complex (Platyhelminthes Kalyptorhynchia) in marine tropical areas: First data from the Caribbean. *Belgian Journal of Zoology*, *124*, 157–166.

Curini-Galletti, M., & Puccinelli, I. (1998). The *Gyratrix hermaphroditus* species complex (Kalyptorhynchia: Polycystididae) in marine habitats of eastern Australia. *Hydrobiologia*, *383*, 287–298. https://doi.org/10.1023/A:1003456102035

D'Souza, T. G., & Michiels, N. K. (2009). Sex in parthenogenetic planarians: Phylogenetic relic or evolutionary resurrection? In I. Schön, K. Martens, & P. van Dijk (Eds.), *Lost sex. The evolutionary biology of parthenogenesis* (pp. 377–397). Springer.

Darling, J. A., & Carlton, J. T. (2018). A framework for understanding marine cosmopolitanism in the Anthropocene. *Frontiers in Marine Science*, *5*, 293. https://doi.org/10.3389/fmars.2018.00293

Derycke, S., Backeljau, T., & Moens, T. (2013). Dispersal and gene flow in free-living marine nematodes. *Frontiers in Zoology*, *10*, 1. https://doi.org/10.1186/1742-9994-10-1

Derycke, S., Remerie, T., Backeljau, T., Vierstraete, A., Vanfleteren, J., Vincx, M., & Moens, T. (2008). Phylogeography of the *Rhabditis* (*Pellioditis*) *marina* species complex: Evidence for long-distance dispersal, and for range expansions and restricted gene flow in the northeast Atlantic. *Molecular Ecology*, *17*, 3306–3322. https://doi.org/10.1111/j.1365-294X.2008.03846.x

Domenici, L., & Gremigni, V. (1977). Fine structure and functional role of the coverings of the eggs in *Mesostoma ehrenbergii* (Focke) (Turbellaria, Neorhabdocoela). *Zoomorphologie*, *88*, 247–257. https://doi.org/10.1007/BF00995475

Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, *7*, 214. https://doi.org/10.1186/1471-2148-7-214

Ezard, T., Fujisawa, T., & Barraclough, T. G. (2009). *SPLITS: SPecies' LImits by Threshold Statistics*. R package version 1.0-19. Available from http://rdrr.io/rforge/splits/

Faubel, A., & Gollasch, S. (1996). *Cryptostylochus hullensis* sp. nov. (Polycladida, Acotylea, Platyhelminthes): A possible case of transoceanic dispersal on a ship's hull. *Helgoländer Meeresuntersuchungen*, *50*, 533–537. https://doi.org/10.1007/BF02367165

Fontaneto, D. (2013). Molecular phylogenies as a tool to understand diversity in rotifers. *International Review of Hydrobiology*, *99*, 178–187. https://doi.org/10.1002/iroh.201301719

Fontaneto, D. (2019). Long-distance passive dispersal in microscopic aquatic animals. *Movement Ecology*, *7*, 10. https://doi.org/10.1186/s40462-019-0155-7

Fontaneto, D., Barraclough, T. G., Chen, K., Ricci, C., & Herniou, E. A. (2008). Molecular evidence for broad-scale distributions in bdelloid rotifers: Everything is not everywhere but most things are very widespread. *Molecular Ecology*, *17*, 3136–3146. https://doi.org/10.1111/j.1365-294X.2008.03806.x

Fontaneto, D., Kaya, M., Herniou, E. A., & Barraclough, T. G. (2009). Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. *Molecular Phylogenetics and Evolution*, *53*, 182–189. https://doi.org/10.1016/j.ympev.2009.04.011

Fujisawa, T., & Barraclough, T. G. (2013). Delimiting species using single-locus data and the generalized mixed Yule coalescent approach: A revised method and evaluation on simulated data sets. *Systematic Biology*, *62*, 707–724. https://doi.org/10.1093/sysbio/syt033

George, K. H. (2013). Faunistic research on metazoan meiofauna from seamounts – a review. *Meiofauna Marina*, *20*, 1–32.

Gerlach, S. A. (1977). Means of Meiofauna Dispersal. In W. Sterrer, & P. Ax (Eds.), *The Meiofauna Species in Time and Space. Mikrofauna Meeresboden*, Vol. *61* (pp. 89–103). Wiesbaden, Germany: Akademie der Wissenschaften und der Literatur.

Giere, O. (2009). *Meiobenthology. The microscopic motile fauna of aquatic sediments*. Springer-Verlag.

Giere, O. (2019). *Perspectives in meiobenthology. Reviews, reflections and conclusions*. Springer.

Girguis, P. (2016). Here, there and everywhere. *Microbial Ecology*, *1*, 16123. https://doi.org/10.1038/nmicrobiol.2016.123

Goldstein, M. C., Carson, H. S., & Eriksen, M. (2014). Relationship of diversity and habitat area in North Pacific plastic-associated rafting communities. *Marine Biology*, *161*, 1441–1453. https://doi.org/10.1007/s00227-014-2432-8

Gómez, A., Adcock, G. J., Lunt, D. H., & Carvalho, G. R. (2002). The interplay between colonization history and gene flow in passively dispersing zooplankton: Microsatellite analysis of rotifer resting egg banks. *Journal of Evolutionary Biology*, *15*, 158–171. https://doi.org/10.1046/j.1420-9101.2002.00368.x

Gómez, A., Carvalho, G. R., & Lunt, D. H. (2000). Phylogeography and regional endemism of a passively dispersing zooplankter: Mitochondrial DNA variation in rotifer resting egg banks. *Proceedings of the Royal Society of London B: Biological Sciences*, *267*, 2189–2197. https://doi.org/10.1098/rspb.2000.1268

Gómez, A., Montero-Pau, J., Lunt, D. H., Serra, M., & Campillo, S. (2007). Persistent genetic signatures of colonization in *Brachionus manjavacas* rotifers in the Iberian Peninsula. *Molecular Ecology*, *16*, 3228–3240. https://doi.org/10.1111/j.1365-294X.2007.03372.x

Gómez, A., Serra, M., Carvalho, G. R., & Lunt, D. H. (2002). Speciation in ancient cryptic species complexes: Evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution*, *56*, 1431–1444. https://doi.org/10.1111/j.0014-3820.2002.tb01455.x

Graff, L. v. (1908). *Turbellaria*. Leipzig, Germany: Winter.

Graff, L. v. (1913). *Das Tierreich: Turbellaria II. Rhabdocoelida*. Berlin, Germany: Königlich-Preußische Akademie der Wissenschaften.

Hallez, P. (1873). Observations sur le *Prostomum lineare*. *Archives De Zoologie Expérimentale & Générale*, *2*, 559–586.

Hand, S. C. (1991). Metabolic dormancy in aquatic invertebrates. In R. Gilles (Ed.), *Advances in comparative & environmental physiology*. (pp. 1–40). Springer-Verlag. https://doi.org/10.1007/978-3-642-75900-0_1

Heitkamp, U. (1972a). Die Mechanismen der Subitan- und Dauereibildung bei *Mesostoma lingua* (Abildgaard, 1789) (Turbellaria, Neorhabdocoela). *Zeitschrift Für Morphologie Der Tiere*, *71*, 203–289. https://doi.org/10.1007/BF00280167

Heitkamp, U. (1972b). Entwicklungsdauer und Lebenszyklen von *Mesostoma productum* (O. Schmidt, 1848) (Turbellaria, Neorhabdocoela). *Oecologia*, *10*, 59–68. https://doi.org/10.1007/BF00822761

Heitkamp, U. (1978). Speciation processes of *Gyratrix hermaphroditus* Ehrenberg, 1931 (Turbellaria, Kalyptorhynchia). *Zoomorphologie*, *90*, 227–251.

Heitkamp, U. (1988). Life-cycles of microturbellarians of pools and their strategies of adaptation to their habitats. *Progress in Zoology*, *36*, 449–456.

Hillis, D. M., & Dixon, M. T. (1991). Ribosomal DNA: Molecular evolution and phylogenetic inference. *The Quarterly Review of Biology*, *66*, 411–453. https://doi.org/10.1086/417338

Hoxhold, S. (1974). Zur Populationsstruktur und Abundanzdynamik interstitieller Kalyptorhynchia (Turbellaria, Neorhabdocoela). *Mikrofauna Des Meeresbodens*, *41*, 3–133.

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

Hyman, L. H. (1951). *The invertebrates: Platyhelminthes and Rhynchocoela. The acoelomate Bilateria*. New York, United States: McGraw-Hill.

IBM Corp. Released (2011). *IBM SPSS Statistics for Windows, Version 20.0*. IBM Corp.

Ingole, B. S. (1987). Occurrence of resting eggs in *Macrostomum orthostylum* (M.Braun, 1885) (Turbellaria: Macrostomida). *Zoologischer Anzeiger*, *219*, 19–24.

Jarne, P., & Auld, J. R. (2006). Animals mix it up too: The distribution of self-fertilization among hermaphroditic animals. *Evolution*, *60*, 1816–1824. https://doi.org/10.1111/j.0014-3820.2006.tb00525.x

Janssen, T., Karssen, G., Orlando, V., Subbotin, S. A., & Bert, W. (2017). Molecular characterization and species delimiting of plant-parasitic nematodes of the genus Pratylenchus from the penetrans group (Nematoda: Pratylenchidae). *Molecular Phylogenetics and Evolution*, *117*, 30–48. http://dx.doi.org/10.1016/j.ympev.2017.07.027

Jiu, M., Hu, J., Wang, L.-J., Dong, J.-F., Song, Y.-Q., & Sun, H.-Z. (2017). Cryptic species identification and composition of *Bemisia tabaci* (Hemiptera: Aleyrodidae) complex in Henan province, China. *Journal of Insect Science*, *17*, 78. https://doi.org/10.1093/jisesa/iex048

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780. https://doi.org/10.1093/molbev/mst010

Kordbacheh, A., Garbalena, G., & Walsh, E. J. (2017). Population structure and cryptic species in the cosmopolitan rotifer *Euchlanis dilatata*. *Zoological Journal of the Linnean Society*, *181*, 757–777. https://doi.org/10.1093/zoolinnean/zlx027

Kück, P. (2009). *ALICUT: a Perlscript which cuts ALISCORE identified RSS*. Department of Bioinformatics, Zoologisches Forschungsmuseum A. Koenig (ZFMK), Version 2.0 edition. https://www.zfmk.de/en/research/research-centres-and-groups/utilities

Kück, P., Meusemann, K., Dambach, J., Thormann, B., von Reumont, B. M., Wägele, J. W., & Misof, B. (2010). Parametric and nonparametric masking of randomness in sequence alignments can be improved and leads to better resolved trees. *Frontiers in Zoology*, *7*, 10. https://doi.org/10.1186/1742-9994-7-10

Leasi, F., & Norenburg, J. L. (2014). The necessity of DNA taxonomy to reveal cryptic diversity and spatial distribution of meiofauna, with a focus on Nemertea. *PLoS One*, *9*, e104385. https://doi.org/10.1371/journal.pone.0104385

l'Hardy, J.-P. (1986). Karyology of a marine population of *Gyratrix hermaphroditus* (Turbellaria, Rhabdocoela) and chromosomal

evolution in this species complex. *Hydrobiologia*, *132*, 233–238. https://doi.org/10.1007/BF00046254

Littlewood, D. T. J., Curini-Galletti, M., & Heniou, E. A. (2000). The interrelationships of Proseriata (Platyhelminthes: Seriata) tested with molecules and morphology. *Molecular Phylogenetics and Evolution*, *16*, 449–466. https://doi.org/10.1006/mpev.2000.0802

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

Luo, A., Ling, C., Ho, S. Y. W., & Zhu, C. D. (2018). Comparison of methods for molecular species delimitation across a range of speciation scenarios. *Systematic Biology*, *67*, 830–846. https://doi.org/10.1093/sysbio/syy011

Luther, A. (1955). Die Dalyelliiden (Turbellaria Neorhabdocoela). Eine Monographie. *Acta Zoologica Fennica*, *87*, 1–337.

Mallo, D., & Posada, D. (2016). Multilocus inference of species trees and DNA barcoding. *Philosophical Transactions of the Royal Society B*, *371*, 20150335. https://doi.org/10.1098/rstb.2015.0335

Mills, S., Alcántara-Rodríguez, J. A., Ciros-Pérez, J., Gómez, A., Hagiwara, A., Galindo, K. H., Jersabek, C. D., Malekzadeh-Viayeh, R., Leasi, F., Lee, J.-S., Mark Welch, D. B., Papakostas, S., Riss, S., Segers, H., Serra, M., Shiel, R., Smolak, R., Snell, T. W., Stelzer, C.-P., … Walsh, E. J. (2017). Fifteen species in one: Deciphering the *Brachionus plicatilis* species complex (Rotifera, Monogononta) through DNA taxonomy. *Hydrobiologia*, *796*, 39–58. https://doi.org/10.1007/s10750-016-2725-7

Mills, S., Lunt, D. H., & Gómez, A. (2007). Global isolation by distance despite strong regional phylogeography in a small metazoan. *BMC Evolutionary Biology*, *7*, 225. https://doi.org/10.1186/1471-2148-7-225

Misof, B., & Misof, K. (2009). A Monte Carlo approach succesfully identifies randomness in multiple sequence alignments: A more objective means of data exclusion. *Systematic Biology*, *58*, 21–34. https://doi.org/10.1093/sysbio/syp006

Monaghan, M. T., Wild, R., Elliot, M., Fujisawa, T., Balke, M., Inward, D. J. G., Lees, D. C., Ranaivosolo, R., Eggleton, P., Barraclough, T. G., & Vogler, A. P. (2009). Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Systematic Biology*, *58*, 298–311. https://doi.org/10.1093/sysbio/syp027

Norén, M., & Jondelius, U. (1999). Phylogeny of the Prolecithophora (Platyhelminthes) Inferred from 18S rDNA Sequences. *Cladistics*, *15*, 103–112. http://dx.doi.org/10.1111/j.1096-0031.1999.tb00252.x

Noreña, C., Damborenea, C., & Brusa, F. (2015). Phylum Platyhelminthes. In J. H. Thorp, & D. C. Rogers (Eds.), *Ecology and general biology: Thorp and Covich's freshwater invertebrates* (pp. 181–203). Academic Press. https://doi.org/10.1016/B978-0-12-385026-3.00010-3

Packmor, J., & Riedl, T. (2016). Records of Normanellidae Lang, 1944 (Copepoda, Harpacticoida) from Madeira island support the hypothetical role of seamounts and oceanic islands as "stepping stones" in the dispersal of marine meiofauna. *Marine Biodiversity*, *46*, 861–877. https://doi.org/10.1007/s12526-016-0448-7

Pandian, T. J. (2020). Turbellaria. In T. J. Pandian (Ed.), *Reproduction and development in Platyhelminthes* (pp. 47–92). Boca Raton, Florida, United States: CRC Press.

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

Posada, D. (2008). jModelTest: Phylogenetic Model Averaging. *Molecular Biology and Evolution*, *25*, 1253–1256. https://doi.org/10.1093/molbev/msn083

Puccinelli, I., & Curini-Galletti, M. C. (1987). Chromosomal evolution and speciation in marine populations of *Gyratrix hermaphroditus sensu lato* (Platyhelminthes: Kalyptorhynchia) and in other species of the Gyratricinae. *Transactions of the American Microscopical Society*, *106*, 311–320. https://doi.org/10.2307/3226222

Puccinelli, I., Curini-Galletti, M. C., Mariotti, G., & Moretti, I. (1990). Chromosomal evolution and speciation in the *Gyratrix hermaphroditus* species complex (Platyhelminthes, Kalyptorhynchia): Karyometric and morphological analyses of fifteen fresh-water populations from Western Europe. *Hydrobiologia*, *190*, 83–92. https://doi.org/10.1007/BF00020690

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, *21*, 1864–1877. https://doi.org/10.1111/j.1365-294x.2011.05239.x

R Core Team (2017). *R: A language and environment for statistical computing*. http://www.R-project.org/

Rambaut, A., & Drummond, A. J. (2007). *Tracer v1.4*. http://beast.community/tracer

Ramm, S. A. (2017). Exploring the sexual diversity of flatworms: Ecology, evolution, and the molecular biology of reproduction. *Molecular Reproduction & Development*, *84*, 120–131. https://doi.org/10.1002/mrd.22669

Reisinger, E. (1923). *Turbellaria*. Berlin, Germany: Barntraeger.

Reuter, M. (1961). Untersuchungen über Rassenbildung bei *Gyratrix hermaphroditus* (Turbellaria Neorhabdocoela). *Acta Zoologica Fennica*, *100*, 1–32.

Ribeiro, K. F., Duarte, L., & Crossetti, L. O. (2018). Everything is not everywhere: A tale on the biogeography of Cyanobacteria. *Hydrobiologia*, *820*, 23–48. https://doi.org/10.1007/s10750-018-3669-x

Rietzler, A. C., Dumont, H. J., Rocha, O., & Ribeiro, M. M. (2018). Predation and reproductive performance in two pelagic typhloplanid turbellarians. *PLoS One*, *13*, e0193472. https://doi.org/10.1371/journal.pone.0193472

Robeson, M. S., King, A. J., Freeman, K. R., Birky, C. W., Martin, A. P., & Schmidt, S. K. (2011). Soil rotifer communities are extremely diverse globally but spatially autocorrelated locally. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 4406–4410. https://doi.org/10.1073/pnas.1012678108

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*, 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Schockaert, E. R. (1996). Turbellarians: The importance of turbellarians in ecosystems. In G. Hall (Ed.), *Methods for the examination of organismal diversity in soils and sediments* (pp. 211–225). Wallingford, United Kingdom: CAB International in association with UNESCO and the IUBS.

Schockaert, E. R., Hooge, M., Sluys, R., Schilling, S., Tyler, S., & Artois, T. (2008). Global diversity of free living flatworms (Platyhelminthes, "Turbellaria") in freshwater. *Hydrobiologia*, *595*, 41–48. https://doi.org/10.1007/978-1-4020-8259-7_5

Spatharis, S., Lamprinou, V., Meziti, A., Kormas, K. A., Danielidis, D. D., Smeti, E., Roelke, D. L., Mancy, R., & Tsirtsis, G. (2019).

Everything is not everywhere: Can marine compartments shape phytoplankton assemblages? *Proceedings of the Royal Society B*, *286*, 20191890. https://doi.org/10.1098/rspb.2019.1890

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*, 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Steinböck, O. (1931). Marine Turbellaria. *Zoology of the Faroes VII*, *I*, 46023.

Suatoni, E., Vicario, S., Rice, S., Snell, T., & Caccone, A. (2006). An analysis of species boundaries and biogeographic patterns in a cryptic species complex: The rotifer—*Brachionus plicatilis*. *Molecular Phylogenetics and Evolution*, *41*, 86–98. https://doi.org/10.1016/j.ympev.2006.04.025

Talavera, G., Dincă, V., & Vila, R. (2013). Factors affecting species delimitations with the GMYC model: Insights from a butterfly survey. *Methods in Ecology and Evolution*, *4*, 1101–1110. https://doi.org/10.1111/2041-210X.12107

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*, 2731–2739. https://doi.org/10.1093/molbev/msr121

Tessens, B. (2012). *Dealing with a taxonomic Gordian knot: Phylogeny of and cryptic diversity in* Gyratrix hermaphroditus *Ehrenberg, 1831 (Platyhelminthes: Rhabdocoela, Kalyptorhynchia)* (pp. 145). PhD thesis. Centre for Environmental Sciences, Hasselt University.

Timoshkin, O. A., Kawakatsu, M., Korgina, E. M., & Vvedenskaya, T. L. (2004). 5. Preliminary analysis of the stylets of the *Gyratrix hermaphroditus* Ehrenberg, 1831 species complex (Platyhelminthes, Neorhabdocoela, Kalyptorhynchia) from lakes of central Russia, Pribaikalye and Kamchatka, Lakes Baikal and Biwa. In O. A. Timoshkin (Ed.), *Index of animal species inhabiting Lake Baikal and its catchment area* (pp. 1321–1343). Russian Academy of Sciences, Siberian Division.

Tomioka, S., Kondoh, T., Sato-Okoshi, W., Ito, K., Kakui, K., & Kajihara, H. (2016). Cosmopolitan or cryptic species? A case study of *Capitella teleta* (Annelida: Capitellidae). *Zoological Science*, *33*, 545–554. https://doi.org/10.2108/zs160059

Van Steenkiste, N. W. L., Herbert, E. R., & Leander, B. S. (2018). Species diversity in the marine microturbellarian *Astrotorhynchus bifidus* sensu lato (Platyhelminthes: Rhabdocoela) from the Northeast Pacific Ocean. *Molecular Phylogenetics and Evolution*, *120*, 259–273. https://doi.org/10.1016/j.ympev.2017.12.012

Vanschoenwinkel, B., Gielen, S., Seaman, M., & Brendonck, L. (2008). Any way the wind blows - frequent wind dispersal drives species sorting in ephemeral aquatic communities. *Oikos*, *117*, 125–134. https://doi.org/10.1111/j.2007.0030-1299.16349.x

Vanschoenwinkel, B., Gielen, S., Seaman, M., & Brendonck, L. (2009). Wind mediated dispersal of freshwater invertebrates in a rock pool metacommunity: Differences in dispersal capacities and modes. *Hydrobiologia*, *635*, 363–372. https://doi.org/10.1007/s10750-009-9929-z

Vanschoenwinkel, B., Gielen, S., Vandewaerde, H., Seaman, M., & Brendonck, L. (2008). Relative importance of different dispersal vectors for small aquatic invertebrates in a rock pool metacommunity. *Ecography*, *31*, 567–577. https://doi.org/10.1111/j.0906-7590.2008.05442.x

Vanschoenwinkel, B., Waterkeyn, A., Vandecaetsbeek, T., Pineau, O., Grillas, P., & Brendonck, L. (2008). Dispersal of freshwater invertebrates by large terrestrial mammals: A case study with wild boar (*Sus scrofa*) in Mediterranean wetlands. *Freshwater Biology*, *53*, 2264–2273. https://doi.org/10.1111/j.1365-2427.2008.02071.x

Willems, W. R., Wallberg, A., Jondelius, U., Littlewood, D. T. J., Backeljau, T., Schockaert, E. R., & Artois, T. (2006). Filling a gap in the phylogeny of flatworms: Relationships within the Rhabdocoela (Platyhelminthes), inferred from 18S ribosomal DNA sequences. *Zoologica Scripta*, *35*, 1–17. https://doi.org/10.1111/j.1463-6409.2005.00216.x

Worsaae, K., Kerbl, A., Vang, Á., & Gonzalez, B. C. (2019). Broad North Atlantic distribution of a meiobenthic annelid – against all odds. *Scientific Reports*, *9*, 15497. https://doi.org/10.1038/s41598-019-51765-x

Xia, X., & Xie, Z. (2001). DAMBE: Software package for data analysis in molecular biology and evolution. *Journal of Heredity*, *92*, 371–373. https://doi.org/10.1093/jhered/92.4.371

Yeates, D. K., Seago, A., Nelson, L., Cameron, S. L., Joseph, L., & Trueman, J. W. H. (2011). Integrative taxonomy, or iterative taxonomy? *Systematic Entomology*, *36*, 209–217. https://doi.org/10.1111/j.1365-3113.2010.00558.x

Young, J. O. (1974). The occurrence of diapause in the egg stage of the life-cycle of *Phaenocora typhlops* (Vejdovsk*y*) (Turbellaria: Neorhabdocoela). *Journal of Animal Ecology*, *43*, 719–731. https://doi.org/10.2307/3533

Young, J. O., & Young, B. M. (1976). First records of eight species and new records of four species of freshwater microturbellaria from East Africa, with comments on modes of dispersal of the group. *Zoologischer Anzeiger*, *196*, 93–108.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.