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Maintaining taxonomic accuracy in genetic databases: A duty for taxonomists—Reanalysis of the DNA sequences from Mercan *et al.* (2024) on the genus *Potamothrix* (Annelida, Clitellata) in Turkish lakes

RÉGIS VIVIEN[1*] & PATRICK MARTIN[2]
[1]*Swiss Center for Applied Ecotoxicology (Ecotox Center), EPFL ENAC IIE-GE, 1015 Lausanne, Switzerland*
[2]*Royal Belgian Institute of Natural Sciences, Taxonomy and Phylogeny, 29 rue Vautier, B-1000 Brussels, Belgium*
pmartin@naturalsciences.be; https://orcid.org/0000-0002-6033-8412
**Corresponding author: regis.vivien@centreecotox.ch; https://orcid.org/0000-0003-3842-1970*

Abstract

Public DNA sequence databases such as GenBank are widely used for identification of organisms in ecological and taxonomic studies. It is important that these public databases contain as few mistakes as possible and that any errors detected in these databases are reported. Here, we reanalyzed the COI sequences of Mercan *et al.* (2024) and showed that they were mistakenly considered by these authors as belonging to different populations (haplotypes) within the species *Potamothrix hammoniensis* (Tubificinae). We found that they corresponded to four distinct Tubificinae lineages (species), *Pothamothrix alatus paravanicus*, *Potamothrix bavaricus*, *Tubifex* sp. and *Potamothrix* sp. Despite these identification errors, the data from Mercan *et al.* (2024) remain interesting as they provide new information on the diversity of the genus *Potamothrix* in Turkey. Prompt measures must be taken to correct these errors and prevent them from being detrimental to future studies.

Key words: Aquatic oligochaetes, Turkey, *Potamothrix*, diversity, public DNA databases

Introduction

Public DNA sequence databases such as GenBank (NCBI Resource Coordinators, 2016) or BOLD (www.boldsystems.org) are widely used by the scientific community to identify sequences through query tools like BLAST (Basic Local Alignment Search Tool) (e.g. Ismailaj *et al.* 2024). These resources are particularly valuable for taxonomic assignments of sequences obtained with high-throughput sequencing (e.g. Elbrecht *et al.* 2017; Carew *et al.* 2018; Vivien *et al.* 2019, 2020, 2023). It is important that these public databases contain as few mistakes as possible and that any errors detected in these databases are reported.

Recently, we observed in GenBank that many sequences of the COI mitochondrial marker, commonly used in DNA barcoding (Hebert *et al.* 2003), were assigned by Mercan *et al.* (2024) to the species *Potamothrix hammoniensis* (Michaelsen, 1901) (Michaelsen 1901) while they significantly differed from all other sequences previously attributed to the same species by various authors (Liu *et al.* 2017; Timm *et al.* 2013; Vivien *et al.* 2017). They were obtained in the framework of a study on the allopatric differentiation of populations of the species *Potamothrix hammoniensis* in eight lakes, spread over the entire national territory of Turkey. Four morphologically and genetically different populations of the species were distinguished. Two of them were found in one lake only: one population for Lake Nemrut in the mountainous far east of the country and another population for Lake Gala in the far west, close to the Greek border. The other two populations were found in more than one lake: one population for Lakes Sapanca and Egirdir, and another one for Lakes Mogan, Gölbasi, Cernek, and Büyük Akgöl. The specimens collected in these lakes differed morphologically from the original description of *P. hammoniensis* to varying degrees, which led the authors to the conclusion that evolutionary differentiations of *P. hammoniensis* had occurred in these lakes and that these populations were indicative of geographic isolation preceding allopatric speciation.

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In this paper, we reanalysed the 35 COI sequences published by Mercan *et al.* (2024) and all other *Potamothrix* sequences available in GenBank together, and found that the Mercan *et al.* (2024) sequences belong to five different Molecular Operational Taxonomic Units (MOTUs), representative of at least four different species, and that only one of them could possibly belong to *P. hammoniensis*.

Material and methods

Currently, a search on GenBank using the query "((Potamothrix[Organism]) AND COI[Gene Name]) OR ((Potamothrix[Organism]) AND COX1[Gene Name]) NOT Mercan" yields a total of 92 hits. Of these, we selected 89 sequences of *Potamothrix* spp. that resulted either from studies co-authored by recognized authorities in aquatic oligochaete taxonomy (Liu *et al.* 2017; Timm *et al.* 2013) or from our own works (Vivien *et al.* 2015, 2017). Eight sequences of *Tubifex tubifex* and *Tubifex* sp. (Tubificinae) were included and used as outgroups in further analyses, resulting in a whole dataset of 132 COI sequences. All these COI sequences had the same length, i.e. 658 base pairs.

Uncorrected pairwise genetic distances (p-distances) in COI were calculated using MEGA 11 (Tamura *et al.* 2011), after trivial alignment of COI sequences facilitated using the MUSCLE algorithm (default options) (Edgar 2004) implemented in Seaview v. 5.0.5 (Gouy *et al.* 2010). The mean p-distances within each MOTU, as identified by the single-locus approach ASAP (see below), and between MOTUs were calculated.

Species were delineated following a distance-based method, ASAP ("Assembling Species by Automatic Partitioning") (Puillandre *et al.* 2021). ASAP was run using p-distances as well as both the Jukes-Cantor (JC69) and the Kimura 2-parameter (K80) substitution models to compute the distances, in order to investigate the possible impact of different distance models on the partitioning. Analyses were performed on the dedicated public web server (https://bioinfo.mnhn.fr/abi/public/asap/).

A phylogenetic tree was inferred by maximum likelihood (ML) using W-IQ-TREE (Trifinopoulos *et al.* 2016), the web interface and server for IQ-TREE (Nguyen *et al.* 2015), with the best fit model, GTR+F+I+G4, automatically selected by the software, according to the Baysian Information Criterion, via ModelFinder (Kalyaanamoorthy *et al.* 2017), as well as optimization of its parameters. Branch support was obtained with the ultrafast bootstrap with 1000 replicates (Hoang *et al.* 2018).

The species partitioning obtained with ASAP was then mapped onto the phylogenetic tree reconstructed via IQTREE to better visualize the taxonomic assignment of the sequences from Mercan *et al.* (2024).

Results and discussion

The ASAP analyses consistently delineated the same 11 different MOTUs within *Potamothrix*, regardless how the distances were estimated (p-distances, JC69, K80). This partitioning corresponds to strongly supported singletons or clades in the ML tree (ultrafast bootstrap values (uBV): 95-100) (Fig. 1). Uncorrected pairwise distances between specimens ranged between 0.0 and 24.8%. Considering the 11 *Potamothrix* MOTUs delimitated according to ASAP, the mean distances within MOTUs varied between 0.18% (M10) and 4.8% (M05) while the mean distances between MOTUs varied between 5.7% (M01-M02) and 20.3% (M01-M07) (Table 1).

It is generally acknowledged that, as a rule of thumb in clitellates, clusters differing by more than 10% in uncorrected distances are likely to represent different species, whereas those differing by less than 5% are likely to belong to the same species (Schmelz *et al.* 2017). In this regard, the species delimitations obtained with ASAP are consistent with this empirical rule, as all MOTUs, except for M01 vs. M02 (5.7%) and M09 vs. M10 (7.8%), are not only separated by distances well over 10% (Table 1) but also correspond to distinct nominal species (Fig. 1).

The specimens from Mercan *et al.* (2024) correspond to 5 different MOTUs (Table 2), representing at least four different species, three of them belonging to *Potamothrix* and one, unexpectedly, to *Tubifex*: all specimens from Lake Gala (Fig. 1 bottom, "Gla") branched deeply within the *Tubifex* outgroup, indicating that they correspond to a species of this genus, but are in no way related to *Potamothrix*. The closest sequence to this MOTU (Lake Gala) found in GenBank (blast search) was *Tubifex tubifex* LT810423, separated by about 13–14% of genetic variation. This MOTU from Lake Gala can be considered as a new lineage of *Tubifex* in GenBank database.

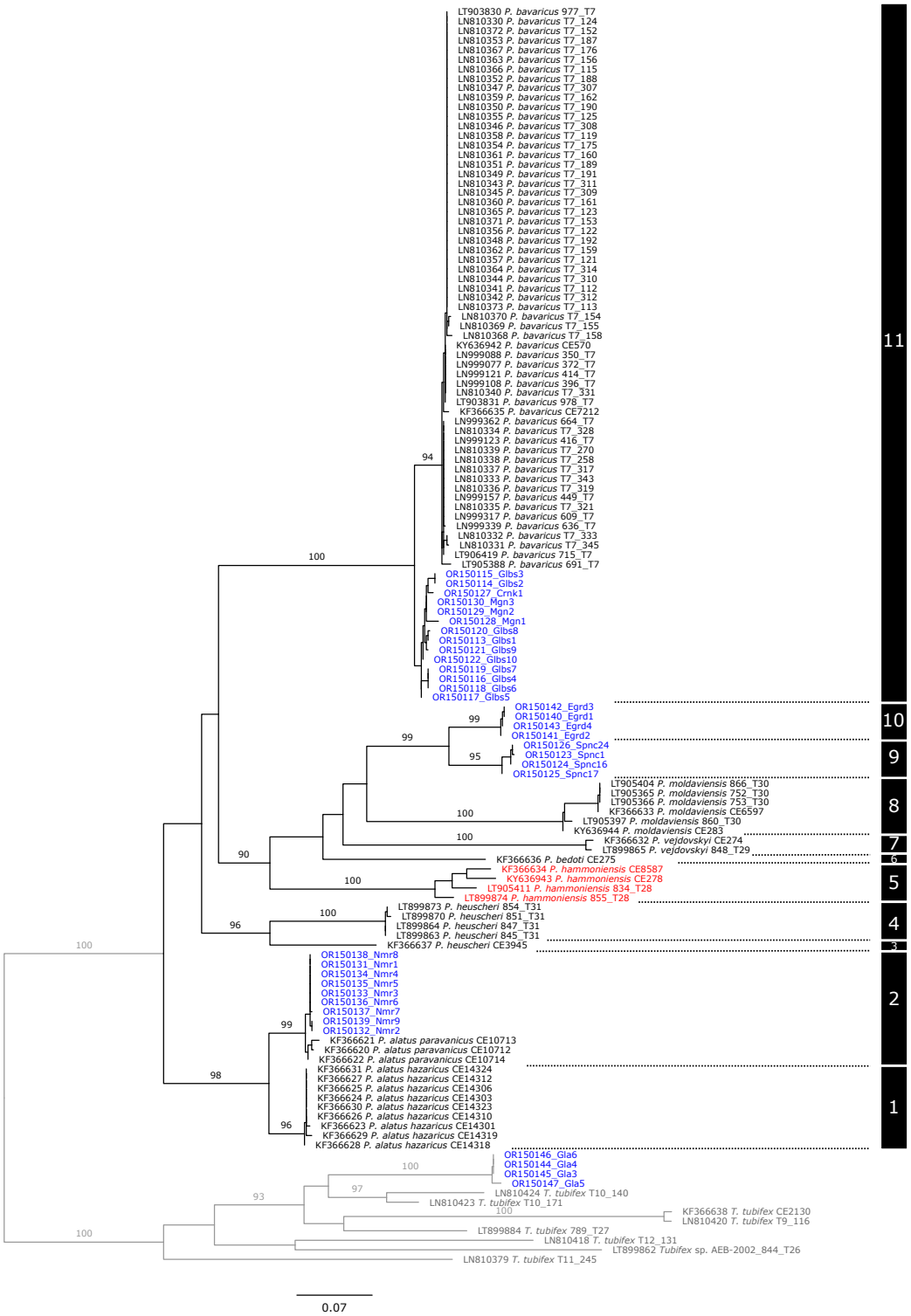

FIGURE 1. Molecular phylogeny constructed using the maximum likelihood method, COI gene sequence fragments of *Potamothrix hammoniensis sensu* Mercan *et al.* (2024), and external COI sequences (deposited in GenBank as part of anterior studies). External sequences include *Potamothix* sequences as well as *Tubifex* spp. sequences (outgroup). Sequences from Mercan *et al.* (2024) are indicated in blue with accession numbers beginning with "OR". Abbreviations of Lake Nemrut is "Nmr", Lake Gala "Gla", Lakes Egidir and Sapanca "Egrd" and "Spnc" and Lakes Mogan, Gölbasi, Cernek "Mgn", "Glbs", "Crnk". All the other sequences are external. Partitions at the right side of the figure represent the results of the species delimitation analysis for MOTUs within the *Potamothrix* clade; here the sequences from Lake Gala are excluded. Numbers at nodes are ultrafast bootstrap values (uBV). Nodes were considered as supported if uBVs were higher or equal to 90 (Hoang *et al.* 2018). For the sake of clarity, uBVs are not shown within delimited MOTUs. For further details, see text.

The *Potamothrix* population from Lake Nemrut (NMr) belongs to the same MOTU that corresponds to specimens identified in Timm *et al.* 2013 as *P. alatus paravanicus* Poddubnaja & Pataridze, 1989, while the populations from Lakes Gölbasi (Glbs), Cernek (Crnk), and Mogan (Mgn) group within the MOTU corresponding to the species *P. bavaricus* (Oschmann, 1913) (Liu *et al.* 2017; Timm *et al.* 2013; Vivien *et al.* 2015, 2017). In contrast, the populations from Lakes Egirdir (Egrd) and Sapanca (Spnc) are unassigned to any known species included in the analysis and regroup two MOTUs (M09 and M10) differing from each other by an average distance of 7.8% (Table 1).

TABLE 1. Mean intra-MOTU genetic variation (p-distances) for each of the 11 MOTUs of *Potamothrix* delimited using ASAP (numbers in bold type), mean inter-MOTU genetic variations (p-distances) between the 11 MOTUs (regular type) and standard deviations of these mean inter-MOTU genetic variations (in italics). n/c = not calculated.

		M01	M02	M03	M04	M05	M06	M07	M08	M09	M10	M11
M01	**0.0020**		*0.0091*	*0.0135*	*0.0143*	*0.0136*	*0.0149*	*0.0152*	*0.0145*	*0.0152*	*0.0145*	*0.0145*
M02	**0.0046**	0.0572		*0.0137*	*0.0138*	*0.0133*	*0.0148*	*0.0151*	*0.0145*	*0.0144*	*0.0144*	*0.0145*
M03	**n/c**	0.1438	0.1418		*0.0129*	*0.0145*	*0.0153*	*0.0156*	*0.0149*	*0.0147*	*0.0142*	*0.0148*
M04	**0.0025**	0.1617	0.1570	0.1199		*0.0140*	*0.0151*	*0.0142*	*0.0157*	*0.0146*	*0.0148*	*0.0141*
M05	**0.0484**	0.1665	0.1614	0.1601	0.1636		*0.0151*	*0.0139*	*0.0143*	*0.0141*	*0.0131*	*0.0143*
M06	**n/c**	0.1695	0.1762	0.1738	0.1734	0.1779		*0.0151*	*0.0156*	*0.0145*	*0.0137*	*0.0149*
M07	**0.0106**	0.2028	0.1952	0.1845	0.1809	0.1596	0.1770		*0.0146*	*0.0142*	*0.0140*	*0.0150*
M08	**0.0172**	0.1903	0.1985	0.1781	0.2006	0.1752	0.1872	0.1874		*0.0145*	*0.0136*	*0.0150*
M09	**0.0056**	0.1706	0.1629	0.1641	0.1695	0.1812	0.1529	0.1676	0.1574		*0.0099*	*0.0140*
M10	**0.0018**	0.1672	0.1615	0.1538	0.1650	0.1546	0.1496	0.1622	0.1579	0.0781		*0.0147*
M11	**0.0145**	0.1667	0.1662	0.1708	0.1753	0.1774	0.1738	0.1815	0.1845	0.1560	0.1698	

Whether considering M01 vs. M02 (5.7%; *P. alatus*) or M09 vs. M10 (7.8%), the average p-distances fall into this gray area where the decision to consider MOTUs as distinct species must be supported by additional data, particularly by complementing mitochondrial gene analysis with nuclear genes. This is crucial to avoid the common pitfalls associated with a single mitochondrial gene approach, such as introgression and incomplete lineage sorting (Puillandre *et al.* 2021). For a similar reason, Timm *et al.* (2013), despite a thorough morphological study, refrained from assigning full species status to the *P. alatus* populations from Lake Hazar (Turkey) and instead recognized them as a subspecies (*P. alatus hazaricus* Timm & Arslan, 2013), in line with previous studies that assigned a similar status for *P. alatus paravanicus* and *P. alatus alatus* Finogenova, 1972. Despite the greater distance between M09 and M10, and the fact that these populations are currently known only from two distinct lakes (Lake Sapanca and Lake Egirdir, respectively), it seems more prudent to consider them as a single species, in the absence of further evidence.

Most *Potamothrix* species are identifiable only when specimens are sexually mature and when the spermathecal chaetae are clearly visible (Timm & Veldhuijzen van Zanten 2002; Timm 2009; Timm & Martin 2019). In some cases, morphological differences between *Potamothrix* species may not be evident, which can lead to uncertain identifications. The DNA barcoding approach was developed to address such situations and has since proven its effectiveness (Lawley *et al.* 2021; Martinsson & Erséus 2021). Therefore, it is surprising that, despite the availability of tools like GenBank or BOLD, the identifications in Mercan *et al.* (2024) are incorrect.

However, as useful as databases like GenBank or BOLD may be, their effectiveness is closely tied to the richness of the data they contain. For instance, the first COI sequences for the genus *Potamothrix* only became available starting in late 2013 (Timm *et al.* 2013). Thus, we cannot exclude the possibility that the paper by Mercan *et al.* (2024) is based on older data, with some aspects not having been updated before publication.

Although Mercan *et al.* (2024) provide a detailed description of the dorsal chaetae of the anterior segments for their various *Potamothrix* populations, these characters are mostly ineffective for species discrimination. Within *Potamothrix*, qualitative and quantitative variation in somatic chaetae can overlap with interspecific differences, depending on the specimen's maturity, as discussed by Harman (1980) for the Naididae s. str. (= Naidinae).

TABLE 2. Identity of specimens identified as *P. hammoniensis* in Mercan *et al.* (2024), based on comparisons of the respective COI sequences with COI sequences deposited in GenBank before 2023, together with the lake of origin and the respective MOTU number (see Fig. 1). The last column indicates whether the spermathecal chaetae as described in Mercan *et al.* (2024) correspond to the DNA-based identification or not. s/n = sine numero.

GenBank accession number isolate (Mercan *et al.* 2024)	Lake name	Identification (this study)	MOTU numbers (see Fig. 1)	Spermathecal chaetae as described in Mercan *et al.* (2024)
OR150127_Crnk1	Cernek	*P. bavaricus*	11	as in *P. bavaricus*
OR150130_Mgn3	Mogan	*P. bavaricus*	11	as in *P. bavaricus*
OR150129_Mgn2	Mogan	*P. bavaricus*	11	as in *P. bavaricus*
OR150128_Mgn1	Mogan	*P. bavaricus*	11	as in *P. bavaricus*
OR150115_Glbs3	Gölbasi	*P. bavaricus*	11	as in *P. bavaricus*
OR150114_Glbs2	Gölbasi	*P. bavaricus*	11	as in *P. bavaricus*
OR150120_Glbs8	Gölbasi	*P. bavaricus*	11	as in *P. bavaricus*
OR150113_Glbs1	Gölbasi	*P. bavaricus*	11	as in *P. bavaricus*
OR150121_Glbs9	Gölbasi	*P. bavaricus*	11	as in *P. bavaricus*
OR150122_Glbs10	Gölbasi	*P. bavaricus*	11	as in *P. bavaricus*
OR150119_Glbs7	Gölbasi	*P. bavaricus*	11	as in *P. bavaricus*
OR150116_Glbs4	Gölbasi	*P. bavaricus*	11	as in *P. bavaricus*
OR150118_Glbs6	Gölbasi	*P. bavaricus*	11	as in *P. bavaricus*
OR150117_Glbs5	Gölbasi	*P. bavaricus*	11	as in *P. bavaricus*
OR150142_Egrd3	Egirdir	*Potamothrix* sp.	10	possibly as in *P. hammoniensis* [1]
OR150140_Egrd1	Egirdir	*Potamothrix* sp.	10	possibly as in *P. hammoniensis* [1]
OR150143_Egrd4	Egirdir	*Potamothrix* sp.	10	possibly as in *P. hammoniensis* [1]
OR150141_Egrd2	Egirdir	*Potamothrix* sp.	10	possibly as in *P. hammoniensis* [1]
OR150126_Spnc24	Sapanca	*Potamothrix* sp.	9	possibly as in *P. hammoniensis* [1]
OR150123_Spnc1	Sapanca	*Potamothrix* sp.	9	possibly as in *P. hammoniensis* [1]
OR150124_Spnc16	Sapanca	*Potamothrix* sp.	9	possibly as in *P. hammoniensis* [1]
OR150125_Spnc17	Sapanca	*Potamothrix* sp.	9	possibly as in *P. hammoniensis* [1]
OR150138_Nmr8	Nemrut	*P. alatus paravanicus*	2	different from *P. hammoniensis* [2]
OR150131_Nmr1	Nemrut	*P. alatus paravanicus*	2	different from *P. hammoniensis* [2]
OR150134_Nmr4	Nemrut	*P. alatus paravanicus*	2	different from *P. hammoniensis* [2]
OR150135_Nmr5	Nemrut	*P. alatus paravanicus*	2	different from *P. hammoniensis* [2]
OR150133_Nmr3	Nemrut	*P. alatus paravanicus*	2	different from *P. hammoniensis* [2]
OR150136_Nmr6	Nemrut	*P. alatus paravanicus*	2	different from *P. hammoniensis* [2]
OR150137_Nmr7	Nemrut	*P. alatus paravanicus*	2	different from *P. hammoniensis* [2]
OR150139_Nmr9	Nemrut	*P. alatus paravanicus*	2	different from *P. hammoniensis* [2]
OR150132_Nmr2	Nemrut	*P. alatus paravanicus*	2	different from *P. hammoniensis* [2]
OR150146_Gla6	Gala	*Tubifex* sp.	s/n	possibly as in *P. hammoniensis* [3]
OR150144_Gla4	Gala	*Tubifex* sp.	s/n	possibly as in *P. hammoniensis* [3]
OR150145_Gla3	Gala	*Tubifex* sp.	s/n	possibly as in *P. hammoniensis* [3]
OR150147_Gla5	Gala	*Tubifex* sp.	s/n	possibly as in *P. hammoniensis* [3]

[1] Spermathecal chaetae seem to correspond to *P. hammoniensis*, but photos or other illustrations would be needed to confirm identity.

[2] Spermathecal chaetae do not correspond to *P. hammoniensis*. They could correspond to *P. alatus paravanicus*, but photos or other illustrations would be needed.

[3] Inconsistency between COI sequences and morphological descriptions: *Tubifex* is without spermathecal chaetae.

Mercan *et al.* (2024) described the spermathecal chaetae of specimens from Lakes Mogan, Gölbasi, Cernek, Büyük and Akgöl (genetically identified here as *P. bavaricus*) as having a distinctly enlarged distal part, wider than those from other lakes. In contrast, specimens from Lake Nemrut (genetically *P. alatus paravanicus*) had straight, thin spermathecal chaetae with only minor distal enlargement. These descriptions confirm that the specimens from these lakes are not *P. hammoniensis* and that the specimens from Lakes Mogan, Gölbasi, Cernek, Büyük and Akgöl belong to *P. bavaricus* (Table 2). Indeed, *P. bavaricus* is characterized by spermathecal chaetae with a clearly enlarged distal region (spearhead-shaped), while *P. hammoniensis* has straight, not thin spermathecal chaetae (Martin 1991; Timm & Veldhuijzen van Zanten 2002; Timm 2009; Timm & Martin 2019).

Interestingly, the description of the spermathecal chaetae of specimens from Lakes Eğirdir and Sapanca closely resemble those of *Potamothrix hammoniensis*. These populations (Fig. 1: M09, M10) belong to a well-supported clade (uBV=90) that includes several MOTUs (M05–10), including *P. hammoniensis.* Within this clade, they form a sister relationship with *P. moldaviensis* and are separated from *P. hammoniensis* by several intervening branches. However, the relationships within this clade are not well supported, leaving open the possibility that the lineage consisting of MOTUs M09 and M10 is more closely related to *P. hammoniensis* than Fig. 1 suggests. Results suggest that MOTUs M09 and M10 represent a cryptic species of *P. hammoniensis* and that this nominal species is polyphyletic. If subsequent studies were to confirm this observation, it would serve as an additional example already documented in the Tubificinae species *Limnodrilus hoffmeisteri* Claparède, 1862 (Liu *et al.* 2017; Vivien *et al.* 2017). Mercan *et al.* (2024) described spermathecal chaetae in specimens from Lake Gala, similar to those of specimens of MOTUs M09-M10. However, *Tubifex* species (e.g. *Tubifex tubifex*) lack spermathecal chaetae (Timm 2009), highlighting an inconsistency between COI sequences and morphological descriptions for this lake.

The data from Mercan *et al.* (2024) remain very valuable as they provide new information on the diversity of *Potamothrix* in Turkey. Of the 25 validly described *Potamothrix* species known to date (Martin *et al.* 2024), the presence of 6 were reported in Turkey (Timm & Abarenkov 2024): *P. moldaviensis* Vejdovský & Mrázek, 1903; *P. heuscheri* (Bretscher, 1900); *P. hammoniensis*; *P. bedoti* (Piguet, 1913); *P. bavaricus*; *P. alatus* (with subspecies *hazaricus* and *alatus*). MOTUs M09 and M10 could constitute either a new *Potamothrix* species (different from *P. hammoniensis*) or a newly reported cryptic species of *P. hammoniensis*. The presence of *P. alatus paravanicus* in Lake Nemrut also represents a new occurrence for the Turkish fauna, as it had previously been reported only in the Transcaucasian lakes Paravani, Sagamo, and Sevan (Timm *et al.* 2013). It is noteworthy that this geographic occurrence is inconsistent with a subspecific status for this taxon, as it indicates a dispersal capacity that makes possible contact with at least the subspecies *P. alatus hazaricus,* also present in Turkish lakes. The fact that these two taxa form homogeneous and distinct clades in our analysis, separated by a clear genetic distance, suggests that their populations actually constitute separately evolving lineages, i.e., two distinct species according to the unified species concept of de Queiroz (2007). However, mitochondrial divergence is not always correlated with reproductive isolation or speciation, and a study of nuclear markers would be desirable to assess whether genetic flow still exists in the nuclear genome.

Conclusion

We began this article by emphasizing the importance of ensuring that public genetic databases contain as few errors as possible, as such errors can weaken the conclusions of studies based on them or even lead to entirely incorrect conclusions. As taxonomists, it is our duty to report any misidentification of sequences as soon as they are detected. Given that the GenBank database is widely used for identification of organisms in ecological and taxonomic studies, we respectfully encourage the authors of Mercan *et al.* (2024) to take appropriate measures to address this issue. Beyond correcting the taxonomic assignments of GenBank sequences, it is also essential to reexamine voucher specimens for morphological traits. With a more rigorous approach, the authors may identify diagnostic characters to formally describe some of these MOTUs and/or validate our results.

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