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Oligochaeta (Annelida) of the profundal of Lake Hazar (Turkey), with description of *Potamothrix alatus hazaricus* n. ssp.

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Abstract

Lake Hazar is an alkaline oligotrophic lake of tectonic origin, located in the Eastern Anatolia region in Turkey, 1248 m a.s.l. Its surface area is 80 km², the average depth 93 m and maximum depth 205 m. The lake and its surroundings are under protection as a region of historical value. During the present study (2007–2012), samples were taken from 15 stations located at a depth of 2–200 m. Oligochaeta comprised 69% of the total invertebrate abundance. The profundal oligochaete fauna was found to consist of only three tubificid taxa, all of the subfamily Tubificinae. *Potamothrix alatus hazaricus* Timm & Arslan, **n. ssp.** was dominating anywhere down to maximum depths while *Psammoryctides barbatus* (Grube) and *Ilyodrilus*(?) sp. occurred seldom. All three are new records for Lake Hazar. *Potamothrix alatus hazaricus* shares the "winged" body shape in its genital region with the nominal, brackish-water subspecies *P. a. alatus* Finogenova, 1972, and the lateral position of the spermathecal pores and the shape of the ventral chaetae with the freshwater subspecies *P. a. paravanicus* Poddubnaja & Pataridze, 1989 known from Transcaucasian lakes. The mitochondrial COI barcoding gene suggests long separation between the two taxa, but the nuclear ITS region shows no variation. The generic position of *Ilyodrilus* (?) sp. remains obscure since its internal genitalia could not be studied.

Key words: Oligochaeta, Tubificidae, Potamothrix, new taxon, oligotrophic lakes, Ponto-Caspian Basin, ITS region

Introduction

Owing to its geomorphological structure, Turkey has been recognized as one of the most important countries in the Palearctic in terms of its aquatic ecosystems, water sources, important bird areas and wetlands. There are about 900 natural lakes and ponds in Turkey covering about 5150 km² area with a high level of endemism and species diversity due to habitat diversity and lack of major disturbances (e.g. glaciation) in the Anatolian basin (Magnin & Yarar 1997). Many of the lakes have internationally important wetland status due to high diversity of waterfowl and fish. Lake Hazar (Figure 1), formed tectonically by the Eastern Anatolia Fault, is one of the most important natural lakes, one of the largest and deepest in eastern Turkey, located at about 39.2 °E and 38.2 °N, 1248 m above sea level. Its surface area is 80 km², the average depth 93 m and maximum depth 205 m; the catchment area excluding the lake surface covers 73 km². The lake's geologic formation is tectonic, with the East Anatolian Fault Zone passing through its bottom. The bedrocks are generally of igneous, shale, limestone, and metamorphic origin (Yaman *et al.* 2011). The lake was earlier drained by the Maden Stream into the Tigris River, of the Persian Gulf drainage system, but after construction of the Hazar Hydroelectric Central in 1957, this outflow dried up (DSI 1971). Ten running water bodies are discharging into Lake Hazar: the Behremaz and Kürk Streams; the Matar, Melem, Salık, Değirmen, Mogal, Baharın, Sevsak and Zıkkım Brooks. A few of these have a permanent flow while most of them are intermittent (Şen *et al.* 2002, Aksoy *et al.* 2007, Fig. 1).



FIGURE 1. Geographical location, inflows and sampling stations of Lake Hazar.

The lake is monomictic, with an incomplete mixing in spring and full turnover between autumn and early winter. It is stratified from June to September, when a thermocline between 10 and 20 m depths is formed. No winter ice cover has been observed. According to the trophic status classification by Vollenweider & Kerekes (1982), the surface water is oligotrophic in terms of total nitrogen and mean and maximum chlorophyll a concentrations, but mesotrophic in terms of total phosphorus; the water transparency measured with Secchi disk ranged from 190 cm in July to 670 cm in May (Tellioğlu *et al.* 2008). During the present study, in the summer months (August 2011 and June 2011), the temperature of surface water varied between 22–23 °C, pH 9.3–9.4, conductivity 2.20–2.23 mS cm⁻¹, oxidation reduction potential 33.30–94.60, and dissolved oxygen content 8.0–8.3 mg 1^{-1} (unpublished data). In general, Lake Hazar is an oligotrophic, hard-water, alkaline soda lake with regard to the surface water of the pelagic zone (Koçer & Şen 2012). The oxygen content of near-bottom water has not been measured but even the deepest zone is inhabited by zoobenthos.

Aquatic Oligochaeta are widely distributed benthic animals often used for biomonitoring, as well as in hazard and risk assessments (Brinkhurst & Jamieson 1971; Rosenberg & Resh 1993; Lang 1998; Rodriguez & Reynoldson 2011). The early, occasional biological (especially fish, algae and rotifers) and water quality papers on Lake Hazar (Şen & Duman 1991; Şen *et al.* 1999, 2001, 2007; Canpolat & Çalta 2001; Tellioğlu & Şen 2003; Aksoy *et al.* 2006) did not consider oligochaetes, with two studies as exceptions: Şahin & Baysal (1972) recorded a tubificid, *Monopylephorus irroratus* (Boldt), while Türkgülü (2010) found *Rhynchelmis* sp. and *Haplotaxis* sp. from the lake littoral. The objective of the present study was to examine both the taxonomic composition and distribution of profundal Oligochaeta in Lake Hazar as a component of the diversity of Turkish lacustrine fauna. A rich population of a new taxon, dominating in the profundal, was discovered and is described herein.

In this paper we use the traditional classification of family Tubificidae (see Timm 2012), not the phylogenybased one (with Tubificidae included in an enlarged family Naididae) proposed and preferred by the last two authors (see Erséus *et al.* 2008).

Material and methods

Sampling and morphological investigation. The first, pilot samples of the Oligochaeta were taken in Lake Hazar by the second author in July 2007, with about 148 specimens collected. During the more recent campaign, 4,522 specimens of aquatic Oligochaeta were collected by the second and third author in August 2011, October 2011 and June 2012 from 15 stations at depths of 2–200 m (Fig. 1). In addition, physico-chemical parameters of surface water were measured in situ, see introduction. Samples of zoobenthos were collected with an Ekman grab sampler, one haul per station. The samples were washed in the field on a series of sieves with decreasing mesh sizes and preserved in plastic bottles with 70% or 96% ethanol, sometimes also with formalin. Sorting of animals proceeded in the laboratory. Specimens of Oligochaeta were studied as temporary whole mounts in glycerin or polyvinyl lactophenol, some of them were later mounted into Canada balsam, and 9 specimens were serially sectioned in paraffine (cross and sagittal sections, stained after Mallory). Type and voucher specimens are deposited in the Museum of the Department of Hydrobiology, Eskişehir Osmangazi University, Turkey (EOGU), in the Centre for Limnology of the Estonian University of Life Sciences, Rannu, Estonia (VLS), and in the Swedish Museum of Natural History, Stockholm (SMNH).

For comparison, 162 specimens of another freshwater oligochaete, *Potamothrix alatus paravanicus* Poddubnaja & Pataridze, 1989, collected by Dr. S. Hakobyan from the profundal of Lake Sevan, Armenia, in March and October 2010, were studied; four of them were serially sectioned.

Hereinafter, segment range numbers are marked with Roman numerals (e.g., X, XI) and the respective intersegmental furrows or dissepiments with Arabic numerals (e.g., 10/11). All measurements and observations were made on preserved specimens.

Genetic analysis. Individuals and sequences used for molecular study are specified in Table 1. DNA was extracted from the posterior ends of ethanol-preserved worms: nine specimens of the form here described as *Potamothrix alatus hazaricus* **n. ssp.**, three of *P. alatus paravanicus*, six specimens representing other *Potamothrix* species, and as functional outgroups one specimen each of *Tubifex tubifex* (Müller, 1774) (lineage III *sensu* Beauchamp *et al.* 2001) and *Ilyodrilus templetoni* (Southern, 1909).

DNA extraction, PCR amplification and primers follow Matamoros *et al.* (2012). Two markers, the mitochondrial cytochrome c oxidase subunit I (COI) gene and the nuclear ribosomal Internal Transcribed Spacer (ITS) region, were amplified. For *P. a. hazaricus*, however, ITS was amplified for only five of the nine specimens, and for *I. templetoni*, the COI sequence (Kvist *et al.* 2010) was downloaded from GenBank. Sequencing was carried out by Macrogen Inc. (Seoul, Korea) and Eurofins MWG Operon (Ebersberg, Germany). Sequences were assembled in Geneious Pro v. 6.0.3 (Biomatters Ltd.; http://www.geneious.com). All sequences produced in this study are deposited in GenBank (Acc. nos. in Table 1). Vouchers of sequenced specimens (i.e., anterior body parts mounted on microscope slides) are deposited in the SMNH, Stockholm (see Table 1).

COI is suggested as the DNA barcoding gene for distinguishing animal species (Hebert *et al.* 2003), on the assumption that variation is considerably greater between than within species (the "barcoding gap"). To investigate the COI variation within and between *P. a. paravanicus* and *P. a. hazaricus*, as well as among all *Potamothrix* species included in this study, the *Potamothrix* COI sequences were aligned using MAFFT v6.814b (Katoh *et al.* 2002) as implemented in Geneious using the auto algorithm. Pairwise genetic distances were calculated for the COI-data set in MEGA 5.1 (Tamura *et al.* 2011) using both uncorrected p-distances and the TN93 model: the latter being the best fitting substitution model according to a model test conducted in MEGA 5.1. Pairwise deletion was used for missing data; a few sequences were incomplete at one or both ends. Histograms of pairwise distances were drawn in Microsoft Excel.

COI and ITS sequences from all specimens for which both were available were aligned using *MAFFT v6.814b* as implemented in Geneious using the auto algorithm, and then the two alignments were concatenated into one data matrix. To find the best fitting model, testing was conducted in MEGA 5.1 (Tamura *et al.* 2011) giving GTR+ Γ as the best model. A phylogenetic tree was estimated using Maximum Likelihood; the analysis was performed with PHYML 3.0 (Guindon & Gascuel 2003; Guindon *et al.* 2010) as implemented at the ATGC Montpellier bioinformatics platform (http://www.atgc-montpellier.fr/), using the GTR substitution model with Γ parameter estimated from the data, SPR+NNI was used for tree improvement. Branch support was calculated with the Chi²-based approximative Likelihood Ratio Test (aLRT) (Anisimova and Gascuel 2006) in PHYML. The tree was drawn in FigTree v.1.3.1 (Rambaut 2009).

| | | | GenBank Accession # | | |
|-------------------------------|---------|--|---------------------|----------|----------------|
| Taxa | Id. #: | Locality data | COI | ITS | Voucher # |
| Potamothrix a. hazaricus | CE14301 | Lake Hazar, Turkey, stn 8, N. Arslan & M. Rüzgar, 4 Oct 2011 | KF366623 | KF366642 | SMNH Type 8528 |
| Potamothrix a. hazaricus | CE14303 | Lake Hazar, Turkey, stn 8, N. Arslan & M. Rüzgar, 4 Oct 2011 | KF366624 | KF366643 | SMNH 132864 |
| Potamothrix a. hazaricus | CE14306 | Lake Hazar, Turkey, stn 3, N. Arslan & M. Rüzgar, 6 Oct 2011 | KF366625 | KF366645 | SMNH 132865 |
| Potamothrix a. hazaricus | CE14310 | Lake Hazar, Turkey, stn 10, N. Arslan & M. Rüzgar, 14 Aug 2011 | KF366626 | KF366646 | No voucher |
| Potamothrix a. hazaricus | CE14312 | Lake Hazar, Turkey, stn 6, N. Arslan & M. Rüzgar, 14 Aug 2011 | KF366627 | KF366644 | SMNH 132866 |
| Potamothrix a. hazaricus | CE14318 | Lake Hazar, Turkey, stn 3, N. Arslan & M. Rüzgar, 14 Aug 2011 | KF366628 | | SMNH 132867 |
| Potamothrix a. hazaricus | CE14319 | Lake Hazar, Turkey, stn 3, N. Arslan & M. Rüzgar, 14 Aug 2011 | KF366629 | | SMNH 132868 |
| Potamothrix a. hazaricus | CE14323 | Lake Hazar, Turkey, stn 1, N. Arslan & M. Rüzgar, 14 Aug 2011 | KF366630 | | SMNH 132869 |
| Potamothrix a. hazaricus | CE14324 | Lake Hazar, Turkey, stn 1, N. Arslan & M. Rüzgar, 14 Aug 2011 | KF366631 | | SMNH 132870 |
| Potamothrix a. paravanicus | CE10712 | Lake Sevan, Major Sevan, Armenia, S. Hakobyan, 27 Mar 2010 | KF366620 | KF366639 | SMNH 132871 |
| Potamothrix a. paravanicus | CE10713 | Lake Sevan, Major Sevan, Armenia, S. Hakobyan, 27 Mar 2010 | KF366621 | KF366640 | SMNH 132872 |
| Potamothrix a. paravanicus | CE10714 | Lake Sevan, Major Sevan, Armenia, S. Hakobyan, 27 Mar 2010 | KF366622 | KF366641 | SMNH 132873 |
| Potamothrix bavaricus | CE7212 | Sentino River, Genga/Ancona, Marche, Italy, C. Erséus, 9 Sep 2009 | KF366635 | KF366650 | SMNH 132874 |
| Potamothrix bedoti | CE275 | Lab culture, from Lake Võrtsjärv, Estonia, T. Timm, Dec 2000 | KF366636 | KF366653 | No voucher |
| Potamothrix hammoniensis | CE8587 | Aufsess/Bayreuth, Bavaria, Germany, D. Kallert, 18 May 2010 | KF366634 | KF366647 | SMNH 132875 |
| Potamothrix heuscheri | CE3945 | Lake Aspen, Lerum, Västergötland, Sweden, C. Erséus, 28 Apr 2008 | KF366637 | KF366648 | SMNH 132876 |
| Potamothrix moldaviensis | CE6597 | Åker Canal, Åkersberga, Uppland, Sweden, C. Erséus, 6 Jun 2009 | KF366633 | KF366651 | SMNH 132877 |
| Potamothrix vejdovskyi | CE274 | Lab culture, from Rybinsk Reservoir, Russia, T. Timm, Dec 2000 | KF366632 | KF366649 | No voucher |
| Ilyodrilus templetoni | CE282 | Lab culture, from Lake Võrtsjärv, Estonia, T. Timm, Dec 2000 | HM460269 | KF366654 | No voucher |
| Tubifex tubifex | CE2130 | Lab culture, Osnabrück, Germany, A. Bergter, 16 Nov 2006 | KF366638 | KF366652 | SMNH 1328788 |

TABLE 1. Specimens of *Potamothrix alatus* sspp. and outgroups included in the molecular study, collection localities, and GenBank accession numbers. Sequence numbers in **bold face** are new.

Results

The zoobenthos samples from Lake Hazar contained the following invertebrate groups: Oligochaeta, Chironomidae, Gastropoda, Isopoda, Coleoptera (larvae), Gammaridae, and, in few numbers, Bivalvia, Ceratopogonidae, Ephemeroptera and Odonata. Total abundance and species diversity were not calculated but

apparently low. The greatest part of the material was represented by oligochaetes (about 69%) and chironomid larvae (19%). Apart from these dominant groups, Coleoptera comprised 5%, Gastropoda 3%, Gammaridae 2% and Isopoda 1%, all others together about 1%. The oligochaetes and chironomids were more or less uniformly distributed down to the maximum depths sampled (200 m); the oligochaetes were less abundant only at the shallowest, littoral station (2 m) where chironomids dominated. The abundance of oligochaetes was 178–9067 ind. m⁻² at different stations in Lake Hazar (the abundance of other groups was not calculated). Only three oligochaete taxa were found in Lake Hazar during this study, and only two of them were identified to species level. Almost all individuals belonged to *Potamothrix alatus hazaricus* **n. ssp**. The second taxon, *Psammoryctides barbatus* (Grube) was found only once (1 specimen), at a depth of 60 m, and the third one, *Ilyodrilus*(?) sp., three times (3 specimens), at depths of 70–135 m.

Potamothrix alatus hazaricus Timm & Arslan, n. ssp.

(Fig. 2)

Holotype. VLS, Types-32.1: sexually mature specimen as a whole mount, Lake Hazar, station 7, depth 20 m, 4.06.2012. Collected by N. Arslan.

Type locality. Lake Hazar in eastern Anatolia (Turkey), 39.2 °E, 38.2 °N, 1248 m a.s.l.

Paratypes. All three from Lake Hazar, collected by N. Arslan. VLS, Types-32.2: cross sections on 3 slides, station not established, depth 50 m, 20.07.2007. ESOGU, (H) No 1: one sexually mature specimen in a whole mount, station 9, depth 100 m, 04.06.2012. SMNH Type 8528: one whole-mounted, sexually mature specimen (CE14301 in Table 1), station 8, depth 65 m, 04.10.2011; with sequenced COI barcode, GenBank Acc. No. KF366623.

Other material. About 280 specimens, all from Lake Hazar, studied as whole mounts; seven of them with sequenced barcodes, deposited in SMNH, see Table 1. Further 77 whole-mounted specimens on 17 slides, and 8 serially sectioned specimens are deposited in VLS; the rest of material in ESOGU.

Etymology. Named after Lake Hazar, the type locality.

Description. Length of sexually mature individuals after fixation in 96% ethanol 10–21 mm, most often 12–13 mm; after fixation in formalin or 70% ethanol, ranging between 11–26 mm, in single cases 33 or 34 mm. Segment number from 45 to about 130, the maximum number not always in the longest individuals since the tail portion sometimes with short, just developing segments. Body wall smooth and transparent. Prostomium short, conical, separated from peristomium by a groove. Preclitellar portion of body widening gradually, in segment VIII 0.5–1.3 mm wide, the genital segments usually wider. Postclitellar portion gradually narrowing to 0.25–0.35 mm while intersegmental furrows become deeper. The genital segments X–XI almost always bear wide, lateral wing-like outgrowths produced by contraction of dorsoventral muscles. These "wings" begin anteriorly in the dorsolateral part of X as narrow strips (Figs 2.1, 2.8), proceed diagonally downwards, and continue in XI as thick lateral folds directed downwards and containing many internal organs; usually they do not reach XII. As a result, the ventral side of segment XI appears deeply concave in fixed specimens (Figs 2.1, 2.9, 2.10). Body dorso-ventrally flattened and thin in X–XI, the thinnest between X and XI where a distinct transversal groove crosses the ventral side (here body often only about 0.28–0.45 mm high). Spermathecal pores in X lateral, inconspicuous. Male pores in line of ventral chaetae, large, sometimes up to 100 µm wide when penes are protruded. Both spermathecal and male pores open under the edges of the "wings". Female pores not observed. Clitellum in XI–XII, thin.

Hair chaetae (Fig. 2.2) present along the whole body, smooth, in preclitellar segments (0,1)2-3(4) per bundle, $(100)250-450 \mu m$ long and about 2 μm thick, longest in IV–VI, shortest in II. In postclitellar segments 0–2 hair chaetae, up to 160–190 μm long. Pectinate chaetae (Fig. 2.3) in preclitellar segments with equally long teeth and several short intermediate denticles, (2,3)4-6 per bundle, $(80)100-170 \mu m$ long and 3.5 μm thick, longest in IV–VI, shortest in II. In postclitellar dorsal bundles pectinates replaced by (1,2)3(4) small bifid chaetae, $80-120 \mu m$ long. Preclitellar ventral chaetae (Fig. 2.4) bifid, with slightly longer and thinner upper tooth, (2,3)4-6(7,8) per bundle. Postclitellar ventral chaetae (Fig. 2.5) 3–4(5) per bundle, $100-140 \mu m$ long, with equal teeth, similar to the dorsal ones.

Spermathecal chaetae (Fig. 2.6) in X, one on each side, sitting in thick (50–73 μ m) muscular-glandular pouches; sometimes absent, even in mature individuals. These chaetae straight or slightly sigmoid with bent

proximal end, 90–170 μ m long and 4–6.5 μ m thick. The distal portion (20–40% of the chaetal length) is nibshaped, with a broad median groove and sharp, bent tip. At each male pore in XI, either 1–2 very small but not modified bifid chaetae, or none.



FIGURE 2. *Potamothrix alatus hazaricus* n. ssp. 1. External view of forebody. 2. Hair chaeta. 3. Pectinate chaeta 4. Anterior ventral chaeta. 5. Posterior ventral chaeta. 6. Spermathecal chaeta. 7. Scheme of male duct, freehand drawing. 8. Cross section of the segment X. 9. Cross section of the anterior part of XI. 10. Cross section of the posterior part of XI. a1, a2, a3— consecutive parts of atrium, bv—blood vessel, i—intestine, mf—male funnel, mp—male pore, pr—prostate gland, ps—penial sheath with penis, sp—sperm bundles in body cavity, spt—spermathecal ampulla with spermatozeugmata, vd—vas deferens, w—lateral "wings" formed by the body wall.

Chloragogen tissue beginning in VI, the wide midgut in VIII. Dissepiments thin. Lateral vessels forming loops in ventral side of IV–VII; single dilated lateral vessels observed only in two sectioned individuals, in VII or VIII, but no regular "hearts". Testes in X, ovaries in XI. Sperm sacs anteriorly often reaching IX (or even VIII) and posteriorly XI–XII, egg sac with eggs sometimes as far as XIV or XV. Spermatozoa in X and in sperm sacs organized in large, spindle-shaped bundles (Figs 2.8, 2.9). Male funnels at the anterior side of dissepiment 10/11, large and of variable shape, their posterior end sometimes piercing the dissepiment and reaching XI. Vasa deferentia (Figs 2.7, 2.9) straight, ascending from male funnels, and about 200–300 μ m long and 32–60 μ m wide, wall about 10 μ m thick, and lumen densely ciliated.

Paired atria tripartite (Fig. 2.7). The first part in XI (Fig. 2.7: a1) short but curved or winding, about 20–85 μ m wide, with lumen only 5–6 μ m wide, and consisting of dark-staining glandular cells similar to those of the prostate gland. Prostate gland attached to the first part of atrium and sometimes surrounded by a loop of the latter, slightly lobate, up to 125–250 μ m in diameter. Second part of atrium (Figs 2.7, 2.10: a2) several times longer than body diameter, forming loops in the coelomic cavity of XI–XII; diameter variable (37–110 μ m), wall consisting of vacuolized epithelium, lumen irregularly wide. Third part of atrium (Figs 2.7, 2.9, 2.10: a3) localized vertically in the lateral part ("wing") of XI, 200–400 μ m long and 45–170 μ m wide, with dense wall consisting of narrow epithelial cells; its lumen 10–100 μ m wide, sometimes winding; the external muscle layer about 3 μ m thick. This terminal portion of atrium continuous with a 100–200 μ m long and proximally 65–145 μ m wide conical penis consisting of similar tissue; penis lumen sometimes dilated up to a width of 45 μ m. Penis usually retracted into spacious penial sac but often partially or completely protruded from the male pore. Penial sac 100–175 μ m wide in its proximal portion, with an inner circular fold near the base of the penis (Figs 2.7, 2.10).

Paired spermathecae in X. Spermathecal ampulla roundish or bilobate (when extending above or below intestine into opposite side), usually about 300–400 μ m, maximally 600–700 μ m wide, with thin (5 μ m) and smooth wall when full of spermatozoa but with thicker (up to 20 μ m) and internally folded wall in slack portions (Fig. 2.8). Spermatozeugmata numerous, spindle-shaped, 55–105 μ m wide, longer than the diameter of ampulla. The filled ampullae can be pressed into XI. Spermathecal duct short (about 300 μ m), up to 100 μ m wide in proximal part and narrowing to 25–40 μ m distally, distinctly three-layered, with lumen of variable width. The funnel-shaped spermathecal pores lie in the posterior part of X, lateral to the spermathecal chaetae and below the body "wings".

Ecology. A taxon with mass occurrence in the strongly alkaline Lake Hazar, abundant in the profundal at all depths. Not known in any other water bodies.

Psammoryctides barbatus (Grube, 1861)

A single immature individual was found at station 2 (depth 60 m) and recognized by its typically broad, comb-like dorsal chaetae and thick, curved posterior crotchets. It has been recorded in Turkey previously from Lake Sazlıgöl (Balık *et al.* 2001) Lake Eğrigöl (Yıldız *et al.* 2005), Lakes Karin and Dipsiz (Yıldız *et al.* 2007a), Lake Sapanca (Şahin & Yıldız 2011) and from the Gediz Delta (Balık *et al.* 2004).

Ilyodrilus (?) sp.

(Fig. 3)

Three individuals of this undetermined taxon were collected in 2011 at depths of 70 m (station 3, sexually mature individual), 100 m (station 9, maturing individual) and 135 m (station 11, maturing individual) and studied as unstained whole mounts. Length 8–16 mm, segment number about 60–80. Body wall smooth, intersegmental furrows weak, prostomium obtusely conical. Most chaetae were broken during the sampling process. In anterior dorsal bundles 1–2 thin hair chaetae (one of them 220 μ m long) and 4–5 pectinates (?), about 120 μ m long, with equal thin teeth, intermediate denticles not clearly seen. In anterior ventral bundles (3)4–6 bifid chaetae, 120–125 μ m long, with slightly longer and thinner upper tooth. No ventral chaetae in XI. In the midbody and tail regions bundles of 2–3 bifid chaetae, with upper tooth slightly longer or equal to lower. Chloragogen tissue on esophagus beginning in VIII; small lateral "hearts" present at the dissepiment 8/9. Clitellum in the

mature individual thin, traceable in X–XII. Penis sheaths in XI as thin-walled, prolonged symmetrical truncate cones, in the mature individual measuring 160 x 40 μ m, in the maturing ones 80 x 20 or 90 x 30 μ m; the distal pore terminal. Yolk-rich eggs in XI–XII. Male ducts and spermathecae not studied.



FIGURE 3. *Ilyodrilus*(?) **sp.** from Lake Hazar. **1.** External view of forebody. **2.** Pectinate chaeta. **3.** Anterior ventral chaeta. **4.** Penis sheath. e—eggs, h—"heart", ps—penis sheath.

The general shape of the penis sheaths fits the genus *Ilyodrilus* but the sheaths are more slender than those in the widely distributed species *I. templetoni* (Southern, 1909). *Tubifex montanus parvus* Giani, Martínez-Ansemil & Brinkhurst, 1984 has penis sheaths very similar to those of our form; however, its anterior dorsal chaetae are almost palmate, with rough intermediate denticles (Giani *et al.* 1984). In the nominotypical subspecies of *Tubifex montanus* Kowalewski, 1919, chaetae are more similar to those of our form while the penis sheath is depicted by Kowalewski (1919) as more funnel-shaped, with dilated proximal and tubular distal portion. Assessing the correct systematic position of our form would need a study of internal reproductive organs, as well as of DNA data. A COI barcode (not more closely accounted for here) of the sexually mature specimen from site 3 suggests that *Ilyodrilus* (?) sp. is a member of the subfamily Tubificinae, but it shows no close affinity to any of the barcodes of other tubificine taxa available to us (including GenBank data).

Both *I. templetoni* and *T. montanus* (the latter without subspecies name) have been repeatedly recorded from fresh waters in Turkey, although without any description: the former by Balık *et al.* (2004), Yıldız & Balık (2005) and Yıldız *et al.* (2005, 2007b, 2008, 2012); and the latter by Yıldız & Balık (2005, 2006) and Yıldız *et al.* (2005, 2007b, 2012). Records of another species of *Ilyodrilus*, *I. frantzi* Brinkhurst, 1965, from the Turkish inland waters (Yıldız & Balık 2005; Yıldız *et al.* 2005, 2007), are dubious since it is a North American species unknown from elsewhere in the eastern hemisphere. The penis sheath of *I. frantzi* is depicted as much shorter than broad by Brinkhurst & Jamieson (1971).





Genetic analyses of *Potamothrix*. For the COI gene, the maximum pairwise distance (p-distance as well as TN93 distance) within *Potamothix alatus paravanicus* is 0.9 %, within *P. a. hazaricus* 0.8 %. Between these taxa, the p-distances are 5.3–6.2 %, the TN93 distances 5.6–6.6%. The distribution of all these distances is shown in Fig. 4, exposing a distinct barcoding gap. The p-distances between all *Potamothrix* species (with *P. a. paravanicus* and *P. a. hazaricus* together considered as one of them) vary between 13.9 and 20.5 %, and the TN93 distances between 15.6 and 24.7 %. The p and TN93 distances between the two subspecies of *P. alatus* and their suggested sister taxon *P. bavaricus* (see below and Fig. 5) are 16.1–17.3 % and 18.6–20.2 %, respectively.

The ITS sequences are identical in all specimens of *P. a. paravanicus* and *P. a. hazaricus*; otherwise this marker shows considerable variation between the taxa included in the study.



FIGURE 5. Maximum Likelihood tree based on combined COI and ITS data from representatives of *Potamothrix* obtained with PhyML, numbers at branches are aLRT branch support. Scale shows expected numbers of mutations per site.

Discussion

As mentioned in the introduction, only three species of Oligochaeta have previously been recorded from the littoral of Lake Hazar: *Monopylephorus irroratus, Rhynchelmis* sp. and *Haplotaxis* sp. The first of them is probably misidentified as it is known mostly in brackish water. None of them was met in our study, which however did not consider the littoral. Instead, three tubificid taxa, all new for Lake Hazar and one of them new for science, were found in profundal. *Potamothrix alatus hazaricus* **n. ssp.** appeared to be absolutely dominating at all stations deeper than 2 m while the two other occurred very seldom.

P. a. hazaricus is rather similar to the nominotypical subspecies *P. alatus alatus* Finogenova, 1972 known from brackish waters of the Dnepr-Bug Estuary (Black Sea) and the Caspian Sea (Finogenova 1972, 1975). It differs from that form, apart from the freshwater habitat, in the longer upper tooth in the anterior ventral chaetae (the teeth of each chaeta being described as equally long in *P. a. alatus*), the more lateral position of the spermathecal pores (in line of ventral chaetae in *P. a. alatus*), and the thin, curved distal end of the spermathecal chaetae (tip of spermathecal chaetae not curved in *P. a. alatus*). Finogenova (1972) and Finogenova & Poddubnaja (1990) also mention the shorter (125–170 μm) vas deferens in *P. a. alatus*, in comparison with the third subspecies, *P. a. paravanicus* Poddubnaja & Pataridze, 1989 (150–310 μm; about the same, 200–300 μm, was found in the new form).

Potamothrix a. paravanicus was described from the profundal of the Transcaucasian lakes Paravani, Sagamo and Sevan, as a separate species, but which soon was attested to be a subspecies of *P. alatus* (Poddubnaja & Pataridze 1989; Finogenova & Poddubnaja 1990). It differs morphologically from the nominotypical subspecies and the Lake Hazar form in the complete lack of the lateral "wings" in the genital region of the body. For the individuals from Lakes Paravani and Sagamo in Georgia, Poddubnaja & Pataridze (1989) noted a large number of preclitellar ventral chaetae (6–10, seldom up to 12, per bundle) while we found much smaller numbers of them,

only 2–6 per bundle, in Lake Sevan. Finogenova & Podubnaja (1990) noted the smaller number of dorsal chaetae in the Lake Sevan worms, in comparison with those from the Georgian lakes, but did not discuss it further. The upper tooth of the anterior ventral chaetae is longer in all lacustrine forms, i.e. those from Lakes Sevan, Hazar, and the Georgian lakes. Finogenova & Podubnaja (1990) described a single, thickened ventral chaeta (not observed by us) at the male pores in XI, in the Sevan material.

Spermathecal chaetae have been described as distally straight in *P. a. alatus*, with slightly curved distal end in *P. a. paravanicus* both from Lake Paravani and Lake Sevan (our new material) but with thin and much curved tip in *P. a. hazaricus* **n. ssp.**; this difference, however, may depend on a different angle of observation. The lateral position of the spermathecal pores and the structure of the male ducts are similar in *P. a. paravanicus* from Lake Sevan and *P. a. hazaricus*. In the original description of *P. a. paravanicus*, the third, vertical portion the of atrium was depicted as shorter and interpreted as a muscular "penial bulb" (Poddubnaja & Pataridze 1989); the latter term, however, was not repeated by Finogenova & Poddubnaja (1990). Thus, *P. a. paravanicus* from Lake Sevan differs from *P. a. hazaricus* **n. ssp.** morphologically in the lack of "wings" (even in the case of similar fixation with strong alcohol) and in the slightly different shape of the spermathecal chaetae, while the structure of the reproductive system is virtually identical.

The two freshwater subspecies, *P. a. paravanicus* (with populations in the lakes of Georgia and Armenia also being slightly different from each other) and *P. a. hazaricus* appear to be ecologically similar. They are abundant in the profundal of oligotrophic mountain lakes with alkaline water: 1100–3500 ind. m⁻² in Lake Sevan (Jenderedjian 1994) and 178–9067 ind. m⁻² in Lake Hazar, without the co-occurence of any other form of *Potamothrix*. Almost no other tubificids occur in this biotope in Lake Hazar, while the oligochaete fauna was found to be slightly more diverse in the profundal of Lake Sevan, and yet *P. a. paravanicus* comprised as much as 95.7% of all individuals (Jenderedjan & Poddubnaja 1987). In greater depths of Lake Sevan, the individuals of *P. a. paravanicus* can grow very large, up to 55 mm long (Finogenova & Poddubnaja 1990), and reach an individual age of 20 years (Jenderedjian 1994). No enlarged individuals were observed in the deepest zone of Lake Hazar, while the lakes of Paravani and Sagamo are shallower.

Species concepts and species delimitation have been issues of endless disputes in the biological literature. De Queiroz (2007) tried to alleviate the discussion by introducing his "unified species concept", which defines species as "separately evolving metapopulation lineages". He also pointed out that speciation is usually a gradual process, and that species delimitation therefore is a matter of finding as much evidence as possible (e.g., geographical separation, mitochondrial and nuclear gene differences, and morphological differences) that any two putative sister species indeed are evolving separately. In a more traditional approach, Mayr (1969, pp. 41–42) advocated the use of subspecies to taxonomically handle what may be regarded as incipient speciation, by recognizing subspecies as aggregates of "phenotypically similar populations of a species, inhabiting a geographic subdivision of the range of the species, and differing taxonomically from other populations of the species"; taxonomically here meaning "sufficient diagnostic morphological characters". Mayr also noted that there may be both variation and overlap in these characters between subspecies. The subspecies category is still in common use for some vertebrate groups, but less so for most invertebrates, megadrile Oligochaeta (earthworms, Crassiclitellata) being a notable exception.

The *Potamothrix alatus* case has a history of subspecies designations (Poddubnaja & Pataridze 1989; Finogenova & Poddubnaja 1990), and we faced the question whether to regard the new form from Lake Hazar as yet another subspecies, and, in particular, whether we were able to assess the latter's taxonomic affinity to *P. a. paravanicus*. Our genetic data clearly shows that the worms from Lake Sevan (morphologically identified as *paravanicus*) and Lake Hazar (*hazaricus*) are closely related, and it is unfortunate that individuals of neither the nominotypical subspecies nor of *P. a. paravanicus* from its type locality were available to us. The COI barcoding gap (Fig. 4), associated with an approx. 6% genetic distance suggests a considerable time of separation of the populations of *P. a. paravanicus* and *P. a. hazaricus*, although these genetic differences are much smaller than those observed between other species of *Potamothrix*, with COI distances of 16–25% (TN93). For the nuclear ITS region, however, the time of isolation apparently has not been long enough to develop variation. Our ITS data show no conclusive evidence that *paravanicus* and *hazaricus* do not belong to the same panmictic metapopulation. Nevertheless, the geographical distribution, and the slight differences in mitochondrial and morphological characters suggest that the two forms are in a phase of at least incipient speciation, and we find it justified to regard them as separate taxonomic entities. With De Queiroz' (2007) reasoning, we find it likely that they represent two separately evolving lineages, i.e., we could propose them to be different species. However, as we have no genetic

information on *P. alatus alatus*, and no direct or indirect evidence of reproductive isolation between any of the forms, we have chosen the conservative alternative of assigning the Lake Hazar form to a third (new) subspecies of *P. alatus*. Future research will hopefully resolve the taxonomic uncertainties about this complex.

Without genetic information of *P. alatus* also from the brackish-water populations in the Ponto-Caspian Basin, it is difficult to judge whether the common ancestor of this complex was of euryhaline or freshwater origin. The isolation, dispersal and differentiation of the separate forms may have had a complex history, considering the geological past of the modern large Ponto-Caspian Seas. Many other *Potamothrix* spp. are diverse and abundant in this area, both in fresh and brackish water (Finogenova 1972, 1975), but their lack in these Transcaucasian and Turkish mountain lakes where *P. alatus* is present, remains an enigma. On the other hand, *P. alatus* has not been found in any of the many smaller Anatolian mountain and lowland lakes. Some of the latter are inhabited by a rich oligochaete fauna including several other species of *Potamothrix* (Yıldız & Balık 2005; Yıldız *et al.* 2005, 2007a, 2012; Şahin & Yıldız 2011).

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