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AKIFUMI OHTAKA, KIMIO HIRABAYASHI, MARK J. WETZEL,
JANA SCHENKOVÁ & ADRIAN PINDER (Eds)

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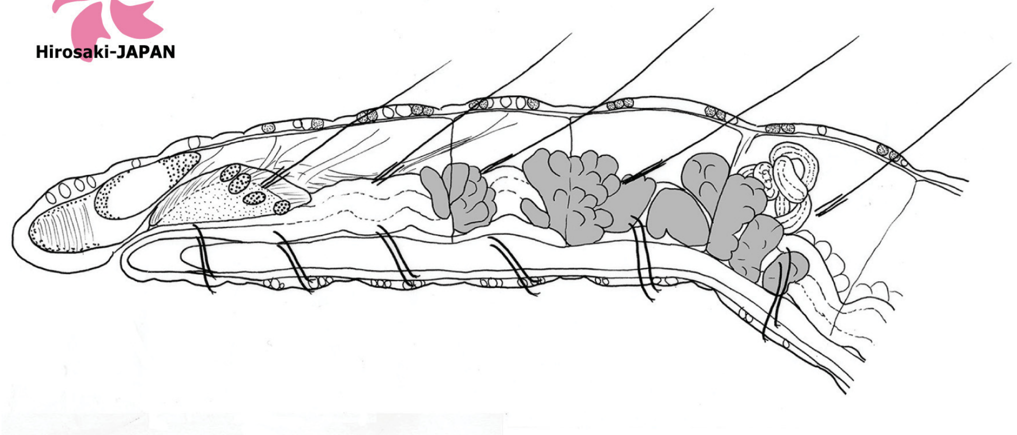

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PREFACE: 14th International Symposium on Aquatic Oligochaeta

AKIFUMI OHTAKA[1] & MARK J. WETZEL[2]

[1] *Faculty of Education, Hirosaki University, Hirosaki 036-8560, Japan. Email: ohtaka@hirosaki-u.ac.jp*
[2] *General Secretary, ISAO. Illinois Natural History Survey, Champaign, IL 61801 USA. E-mail: mjwetzel@illinois.edu*

This volume is the proceedings for the 14th International Symposium on Aquatic Oligochaeta (ISAO), held in Hirosaki, Aomori Prefecture, Honshu, Japan, from 9 through 14 September 2018, organized by the Department of Natural Science, Faculty of Education, Hirosaki University, Hirosaki. The symposium was attended by 43 participants from 16 countries who presented 30 oral and 6 poster contributions. The scientific sessions were convened at the Iwaki Conference Hall in the 50th Anniversary Auditorium, Hirosaki University. In addition to the scientific sessions, there was a one-day excursion to visit a traditional playhouse Kouraku-Kan, lunch along the edge of Lake Towada – an oligotrophic caldera lake, and hiking along the lake's outlet stream, Oirase-Keiryu, flowing through a cool temperate forest typical of northern Honshu. The symposium organizing committee consisted of Akifumi Ohtaka, Kimio Hirabayashi, Katsutoshi Ito, Mana Ito, Takaaki Torii, Naoya Kimura, Stuart R. Gelder, and Mark J. Wetzel, with invaluable support from students from Hirosaki University.

This symposium was dedicated to the memory of Dr. Rut Collado de la Peña (1967–2015), and to participants of our previous ISAO symposia who have passed away. A memoriam honoring Rut is presented elsewhere in this volume.

The symposium was opened with a welcoming presentation by Dr. Manabu Totsuka, the Dean of the Faculty of Education, Hirosaki University, and a performance by the Hirosaki University Tsugaru Shamisen Circle playing classic regional music on Tsugaru Shamisen, traditional Japanese 3-stringed instruments. The program consisted of the following eight sessions, which covered a wide range of branches of knowledge dealing with the biology of aquatic oligochaetes and branchiobdellidans: taxonomy, phylogeny, community ecology, environmental studies, biogeography, morphology, branchiobdellidan biology and other topics on aquatic oligochaetes and other groups within the Annelida. Seven experts from different fields of oligochaetology presented keynote lectures: Dr. Steven Fend: New Japanese taxa associated with the microdrile families Lumbriculidae and Haplotaxidae; Dr. Christer Erséus: Reconstruction of the evolutionary history of Clitellata using transcriptome genetic data; Dr. Göran Milbrink: Composition and abundance of aquatic oligochaetes in Scandinavian lakes well correlated with the "morpho-edaphic index"– Do these relationships still hold 40 years later?; Dr. Pilar Rodriguez: Metal tissue residues in aquatic oligochaetes and environmental risk assessment in a Zn-Pb mining affected river catchment; Dr. Patrick Martin: DNA barcoding and diversity of groundwater oligochaetes in the Ouémé basin, Benin, West Africa; Dr. Stuart Gelder: Endemic branchiobdellidans (Annelida: Clitellata) on commercial North American crayfish: Potential and actual translocated associations, including the ectosymbiont's adoption of exotic host species; and Dr. Tarmo Timm: Observations on the life cycles of Oligochaeta in aquaria.

A half-day special microscopy session was attended by many symposium participants, with discussions about morphological characters and variable kinds of taxonomic and systematic issues. Social events during this scientific meeting began with an afternoon city walk through Hirosaki – with visits to the Hirosaki Castle, experiencing a simplified Japanese tea ceremony in the Fujita Memorial Garden, visiting the Hiroski Municipal Tourist Center, and dinner at a Japanese style pub in the evening. One of the many highlights of this symposium was the participation by the founder of our ISAO group, Dr. Ralph O. Brinkhurst – who hosted the first of our 14 triennial meetings in Sidney, British Columbia, Canada in 1979. Since that first gathering of oligochaetologists, Dr. Brinkhurst has maintained a critical role in oligochaete research at the global level, serving unselfishly and enthusiastically as mentor for and as a collaborator with so many of our past and contemporary oligochaetologists.

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The symposium sessions concluded with a business meeting to discuss issues affecting our global community of aquatic oligochaetologists:

1) Position of General Secretary, ISAO: After a short introduction of duties associated with this position, Mark Wetzel, who has held this position since 2007, nominated Adrian Pinder to serve as the next General Secretary; Adrian accepted the nomination, and was elected by unanimous voice vote. Along with Mark and past ISAO hosts and planning committee members, Adrian has participated in and played a key role in the organization and conduct of past triennial symposia and served as host for our 12th ISAO meeting in Fremantle, Western Australia in 2012. Adrian will be assisting Mark and other ISAO members between now and the opening ceremony of our next, ISAO15 meeting in 2021, when he will be inducted as the new General Secretary.

2) Publication of the ISAO14 proceedings: During the year preceding the ISAO14 meeting in Japan, the ISAO International Scientific Advisory Committee discussed possible journals in which to publish the ISAO14 proceedings via several email communications. Zoosymposia was tentatively chosen, and after some discussion, affirmed by members during this business meeting.

After the editorial policies of Zoosymposia were summarized, the ISAO14 editorial committee issued the official call for submission of manuscripts to be considered for publication in the official proceedings of this symposium. Of the 36 scientific presentations during this symposium, 10 contributions were prepared as manuscripts for publication in Zoosymposia. All papers in this ISAO14 proceedings volume were reviewed by two or more referees, followed by revisions by members of the ISAO14 editorial committee.

3) Venue for the next, ISAO15 meeting: Invitations to host the next triennial symposium were presented by Dr. Irina Kaygorodova (Limnological Institute, Irkutsk, Russia), and Dr. Patrick Martin (Royal Belgian Institute of Natural Sciences, Brussels, Belgium). After discussion of these two exceptional invitations, a written ballot vote by ISAO members present during this business meeting resulted in the decision to convene the next, ISAO15 symposium in Brussels, Belgium in September 2021.

The Closing Banquet of the ISAO14 symposium was held in the Amethyst Hall of the Art Hotel Hirosaki. During the banquet, ISAO Founder Dr. Ralph O. Brinkhurst shared some retrospect on his career, entitled “Some Personal Reminiscences” – touching each of us both personally and professionally by bringing forth positive memories of our individual as well as collaborative experiences and discoveries to date, and encouraging our continued focus towards the future of our group and our science.

During the banquet an entertainment was provided by the student Ohayashi (Japanese musical band) members to play traditional local music accompanied by Fue (Japanese flute) and Taiko (Japanese drums). Many oligochaetologists enjoyed playing the Fue and Taiko.

As in each of the 13 previous symposia, this 14th triennial meeting in Hirosaki provided numerous unique opportunities for aquatic oligochaetologists to renew and expand our knowledge, exchange ideas, discuss continuing projects, establish new as well as continuing friendships and collaborations, and embrace the unique culture of Aomori Prefecture in northern Honshu, Japan.

We would like to thank to all who attended the ISAO14 symposium in Hirosaki, thus contributing to the success of whole symposium. Our sincere thanks go to all authors of presentations during the symposium, and for their diligence in preparing their contributions for publication. We also extend our appreciation to the referees of the papers published in this volume, and for their constructive comments and recommendations to improve the scientific and linguistic components of those submissions. Finally, we wish continued success, safe travels, and good health for all ISAO members. We look forward to our next symposium in Belgium.

FIGURE 1. Group photo of ISAO14 participants, taken in front of Kouraku-Kan, Japan's oldest traditional wooden playhouse, during our one-day excursion through Akita and Aomori Prefectures on Wednesday 12 September 2018.
1–Keiko Ohtaka; 2–Irina Kaygorodova; 3–Haifa Jawad Jabir Jaweir; 4–Lynette Pitman; 5–Adrian Pinder; 6–Tarmo Timm; 7–Naime Arslan; 8–Steve Fend; 9–Pilar Rodriguez; 10–Ralph Brinkhurst; 11–Seray Yildiz; 12–Deniz Mercan; 13–Patrick Martin; 14–Takaaki Torii; 15–Naoya Kimura; 16–Chiharu Goto; 17–Katsutoshi Ito; 18–Maria Baturina; 19–Ege Arslan; 20–Rüdiger Schmelz; 21–Marta Schmelz Collado; 22–Anna Schmelz Collado; 23–Mark Wetzel; 24–Mana Ito; 25–Christer Erséus; 26–Jana Schenková; 27–Michal Schenk; 28–Hanna Świątek; 29–Piotr Świątek; 30–Anna Urbisz; 31–Lukasz Urbisz; 32–Saelon Renkes; 33–Jean-François Parpet; 34–Stuart Gelder; 35–Hongzhu Wang; 36–Göran Milbrink; 37–Yongde Cui; 38–Akifumi Ohtaka; 39–Kimio Hirabayashi; 40–Mohammed Ibrahim Naveed Rasheed.

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In Memoriam: Dr. Rut Collado de la Peña (1967–2015)

Our dear friend and colleague, Dr. Rut Collado de la Peña, passed away on 14 November 2015, in A Coruña, Galicia, Spain, at the age of 48.

She was an associate professor ("Profesora Contratada Doctora") and member of the Science Faculty within the Department of Animal Biology, Plant Biology and Ecology at the Universidade da Coruña, a Spanish public university in the city of A Coruña, in Galicia, northwestern Spain.

Rut is survived by her husband, Rüdiger M. Schmelz, and daughters Anna Schmelz Collado and Marta Schmelz Collado.

Rut was born and grew up in A Coruña, where she completed primary and secondary education. She studied biology at the Universidade de Santiago de Compostela. Rut received her PhD from the Universidade da Coruña in 1994, thesis title: "Oligoquetos acuáticos del cuadrante noroccidental de la Península Ibérica: Taxonomía, biología y ecología", [*Aquatic oligochaetes of the north-west of the Iberian Peninsula: taxonomy, biology and ecology*] directed by our colleague Enrique Martínez-Ansemil. In 1995 she obtained a Master's degree in "Gestión Medioambiental" (Environmental Management) from the Instituto de Investigaciones Ecológicas, Málaga, with her thesis entitled: Los lagos: contaminación y eutrofización. Impacto sobre las comunidades acuáticas y posibilidades de recuperación [*Lakes: contamination and eutrophication. Impact on aquatic communities and possibilities of recovery*].

Prior to her position as Profesora Contratada Doctora at the Universidade da Coruña (1998–2015), Rut pursued research in a postdoctoral position (1994–1996) at the Institute of Freshwater and Fish Ecology (Berlin, Germany), where she focused on oligochaetes occurring in littoral and profundal habitats of eutrophic and oligotrophic lakes. She then attained a research position (1996–1998) at the Universität Osnabrück (Osnabrück, Germany), where her research focused on developing the Enchytraeid Reproduction Test (ERT), in collaboration with the German company ECT Oekotoxikologie GmbH, Flörsheim, and on use of protein patterns (isozymes) as taxonomic markers.

Throughout her scientific career, Rut was involved in numerous cooperative projects focusing on the

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ecology and taxonomy of aquatic and terrestrial oligochaetes (especially Enchytraeidae and Naididae). Her diverse focus on the taxonomy and systematics of enchytraeids and other oligochaete families, often in collaboration with others, resulted in the descriptions of 31 species new to science. These include four from watercourses in NW Spain, described in her PhD thesis: *Stylodrilus curvithecus* Collado *et al.*, 1993 (Lumbriculidae), *Mesenchytraeus lusitanicus* Collado *et al.*, 1993, *Krenedrilus realis* Martínez-Ansemil & Collado, 1996 (Tubificidae), and *Cernosvitoviella bulboducta* Martínez-Ansemil & Collado, 1996.

Six species of aquatic oligochaetes, collected from terrestrial sites, were described during her involvement with Project SHIFT (in the Amazonian rain forest, also in collaboration with ECT): *Pedonais crassifaucis* Collado & Schmelz, 2000 (Naididae), *Bothrioneurum righii* Collado & Schmelz, 2000 (Tubificidae), and *Pristina silvicola* Collado & Schmelz, 2000, *Pristina terrena* Collado & Schmelz, 2000, *Pristina marcusi* Collado & Schmelz, 2001, and *Pristina trifida* Collado & Schmelz, 2002 (all Naididae); 11 described during Project SOLOBIOMA in Brazil: *Achaeta becki* Schmelz & Collado, 2005, *Achaeta hanagarthi* Schmelz *et al.*, 2008, *Achaeta singularis* Schmelz *et al.*, 2008, *Achaeta paranensis* Schmelz *et al.*, 2008, *Guaranidrilus andreolii* Schmelz *et al.*, 2011, *Guaranidrilus cingulatus* Schmelz *et al.*, 2011, *Guaranidrilus hoeferi* Schmelz *et al.*, 2011, *Guaranidrilus marquesi* Schmelz *et al.*, 2011, *Xetadrilus fabryi* Schmelz *et al.*, 2011, *Xetadrilus maacki* Schmelz *et al.*, 2011, and *Xetadrilus aphanus* Schmelz *et al.*, 2011; and 10 described during other independent projects: *Enchytraeus luxuriosus* Schmelz & Collado, 1999, *Fridericia larix* Schmelz & Collado, 2005, *Bryodrilus hondurensis* Schmelz, 2015, *Hemienchytraeus phytotelmatus* Schmelz, 2015, *Achaeta coimbrensis* Schmelz & Collado, 2013, *Fridericia sousai* Schmelz & Collado, 2013, *Fridericia ciliotheca* Schmelz & Collado, 2013, *Fridericia sousai* Schmelz & Collado, 2013, *Fridericia roembkei* Schmelz & Collado, 2013, and *Fridericia marginata* Schmelz & Collado, 2013. A milestone was the book *A guide to European terrestrial and freshwater species of Enchytraeidae (Oligochaeta)*, published in 2010, together with her husband Rüdiger.

In addition to her independent and collaborative research projects, Rut was an instructor of classes in animal physiology, and assisted in the organization and presentation of lectures and preparation of classroom and lab practica during her professional career. Teaching was not a 'load' for her; she enjoyed teaching others as an opportunity rather than as an expectation, and took it very seriously. She resourcefully integrated newly published research results with her lectures and classroom materials.

In lab practica aligned with her classroom presentations, she substituted the black worm *Lumbriculus variegatus* for lethal experiments previously conducted using laboratory rats—following the initial designs and recommendations by our late colleague, Charlie Drewes (Drewes 2005). Students were very thankful for her commitment at all levels of teaching. Rut was a perennial member of our International Symposia on Aquatic Oligochaetes (ISAO) group, participating in several of our triennial meetings (either in person or as co-author of presentations).

left: Brenda Healy and Rut in front of the Flower Puppy (Topiary sculpture by Jeff Koons) in the outdoor terrace of the Guggenheim Museum during a city tour; ***right:*** Rut (far left) with Patty Gillis, René Hessling, Mark Wetzel, Mónica Joao de Barros Amorim, Regine Bönsch, Adrian Pinder, and Rüdiger Schmelz, during a mid-week social gathering on the patio of the Universitary Colegio Mayor Miguel de Unamuno (symposium hotel); both pictures taken during the 8th ISAO meeting hosted by Pilar Rodriguez at the Universidad del País Vasco in July 2000.

Rut also was a member of the Société d'Histoire Naturelle de Toulouse and of the Sociedade Galega de Historia Natural. She liked horse-riding, was at ease with dogs from her early childhood and enjoyed reading good literature of all sorts, from D.F. Wallace, J.K. Toole, to G. García Márquez, Jane Austen, J.R.R. Tolkien, and others. She had a weakness for fantasy—including that presented in novels, movies and TV series; apart from a diverse library, her daughters as children had an almost complete collection of the then popular preteen and teenage movies and TV series at home. She was fluent in Spanish, Galician, German, English, and French. She loved Germany, but as a true Galician, was really most happy with her family in her hometown, A Coruña.

During each of the preceding international symposia on aquatic oligochaete biology, and by inclusion of memoria in our published proceedings, we have honored our deceased colleagues, mentors, and friends—those who have participated in our ISAO group meetings and those who have provided significant influence, insight, and direction for our own scientific endeavors. On behalf of our fellow oligochaetologists worldwide who have known Rut both personally and professionally and benefited from her sincere kindness, professional guidance, and scientific contributions, the ISAO14 organizing and scientific committees dedicate this 14th International Symposium on Aquatic Oligochaetes to the memory of our dear friend and colleague, Rut Collado de la Peña.

Acknowledgements

The author extends his appreciation to Rut's husband, Rüdiger, to their daughters, Anna Schmelz Collado and Marta Schmelz Collado, to Enrique Martínez-Ansemil, and to the perennial members of the ISAO family for sharing some of the more personal and professional information presented in this memoriam.

Publications of Rut Collado de la Peña [*listed chronologically*]

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MARK J. WETZEL
General Secretary, ISAO
Illinois Natural History Survey,
Champaign, Illinois 61820, USA;
E-mail: mjwetzel@illinois.edu

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New species and new records of Japanese Lumbriculidae (Annelida, Clitellata)

STEVEN V. FEND[1], AKIFUMI OHTAKA[2] & TAKAAKI TORII[3]
[1] *PO Box 5674, Eugene, Oregon, USA, e-mail stevenfend@gmail.com*
[2] *Hirosaki University, Hirosaki, Japan*
[3] *IDEA Consultants Inc., Riemon, Yaizu-shi, Shizuoka, Japan*

Abstract

Most Japanese records and descriptions of the family Lumbriculidae are from the work of H. Yamaguchi, who described 6 endemic species and one genus, in addition to reporting the cosmopolitan *Lumbriculus variegatus* (Müller, 1774). Yamaguchi's work focused largely on the northern island of Hokkaido; since then, only one new Japanese lumbriculid genus and species has been described, also from Hokkaido. Recent collections from Honshu Island include several species not recorded in earlier literature, and here we report 4 new species of Lumbriculidae, plus a range extension of the Korean *Lamprortus orientalis* Rodriguez, 1994. Of the new species, only *Styloscolex* (*Styloscolex*) *tazawaensis* can be confidently assigned to an accepted genus using current morphological diagnoses. *Styloscolex* (*Neoscolex*) *tatsukoae* n. sp. is provisionally assigned to that Asian genus and subgenus based on the prosoporous male duct, spermathecae in the preatrial segment, and penial sheath—despite differing from congeners in having a single, median atrium in X, and single spermatheca in IX. Two additional species are here attributed to a new genus, *Honshudrilus*, which is distinguished by a combination of characters: spermathecae 2 or more segments anterior to the male pores, non-functional anterior male ducts (with loss of anterior testes), elongate-petiolate atria, penes formed by extruding the atrial duct lining, and spermathecal ducts with vestibules.

Key words: Clitellata, Oligochaeta, Lumbriculidae, taxonomy, Japan

Introduction

The largely Holarctic family Lumbriculidae includes approximately 240 described species and subspecies, most of which are regionally endemic. Excluding the nearly 60 Lake Baikal endemics, only about 25 species have been described from eastern Asia. The Japanese records and descriptions are largely the work of Hideji Yamaguchi (1936a, 1936b, 1937a, 1937b, 1953), who described 6 endemic species, including the monotypic *Hrabea ogumai* Yamaguchi, 1936, and also reported the apparently cosmopolitan *Lumbriculus variegatus* (Müller, 1774). Since then, additional lumbriculid distribution records have been presented by Ohtaka & Nishino (1995), Torii & Ohtaka (2007), Fend & Brinkhurst (2010) and Ohtaka (2014); and a single new species, *Yamaguchia toyensis* Fend & Ohtaka, 2004, has been described.

Most of the lumbriculid work by Yamaguchi was based on collections from the northern island of Hokkaido, with two exceptions: *Lumbriculus multiatriatus* Yamaguchi, 1937 from Sakhalin, Russia (Yamaguchi 1937a), and *Lumbriculus mukoensis* Yamaguchi, 1953 from Hyogo Prefecture, in southern Honshu. *Yamaguchia toyensis* is also known only from Hokkaido. Examination of recent collections from sites on Honshu Island has revealed a number of unusual species belonging to the family Lumbriculidae, and some of these new species are described in this contribution. Additionally, *Lamprortus orientalis* Rodriguez, 1994, described from Korea (Brinkhurst et al. 1994), is represented by new Japanese records.

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Methods

Sampling methods. Specimens examined in the present study were collected from streams, brooks or springs in several localities in Honshu Island, Japan. Bottom substrates in lotic habitats were disturbed to a depth of 10–20 cm, and worms displaced from the sediment were collected in 250–500 µm mesh dip nets; alternatively, bottom sediments from streams and wet soils were repeatedly stirred in water in a tray or bucket, and the suspended worms were collected with a 250 µm screen. Most specimens collected were relaxed in dilute ethanol and fixed in 8–10 % formalin solution for morphological study, or fixed in 90% ethanol for molecular study.

Laboratory methods. Specimens used for morphological study were usually stained in either hematoxylin or borax carmine, partially destained in acidified alcohol, dehydrated in a graded series of ethanol solutions, cleared in methyl salicylate and slide-mounted in Canada balsam. Some larger specimens were sagittally dissected, and where sufficient material was available, sagittal sections were cut at 7 µm and stained with Harris' hematoxylin and eosin Y. Unless otherwise indicated, descriptions of reproductive structures are based on mature specimens, with sperm in the spermathecae, and usually with well-developed eggs. Measurements of chaetae are based on slide-mounted worms, but body length and width measurements were supplemented with unmounted worms in alcohol; unless otherwise noted, measurements of somatic characters were based on specimens with at least partially-developed reproductive pores. Segment number is indicated by Roman numerals, and intersegments by Arabic numerals (as 9/10 to indicate septum between IX and X).

Type specimens are deposited in the National Museum of Nature and Science, Tokyo, Japan (NSMT) and the U.S. National Museum of Natural History, Smithsonian Institution (USNM).

Abbreviations used in the figures:

aa—atrial ampulla
ad—atrial duct
ff—female funnel
fp—female pore
mf—male funnel (mf1—anterior, mf2—posterior)
mp—male pore
ov—ovary
pe—penis
ph—pharynx
pg—pharyngeal gland
pr—prostates
ps—penis sheath
sa—spermathecal ampulla
sd—spermathecal duct
sp—spermathecal pore
sv—spermathecal vestibule
te—testis
vd—vas deferens

Results

Taxonomy: The current list of lumbriculid species known from Japan is given below. In this paper, we discuss new species and a new record for Japan, indicated with an asterisk (*), and the section where described in this paper. Many lumbriculid specimens from the Yamaguchi collection have only recently been rediscovered, and a later contribution will evaluate and supplement descriptions by Yamaguchi (1936a, 1937a, b, 1953), based on original collections and new material.

**Honshudrilus gudariensis* n. gen., n. sp. 3.3.1
**Honshudrilus yuzawaensis* n. gen., n. sp. 3.3.2
Hrabea ogumai Yamaguchi, 1936
  (Yamaguchi 1936a, 1953; Fend & Ohtaka 2004)
**Lamprortus orientalis* Rodriguez, 1994 3.1
  New record
Lumbriculus multiatriatus Yamaguchi, 1937
  (Yamaguchi 1937a, 1953, Cook 1971, Sokolskaya 1972a, Timm & Rodriguez 1994, Timm 1999)
Lumbriculus japonicus Yamaguchi, 1936
  (Yamaguchi 1953, Timm 1997, 1999)
  =*Lumbriculus variegatus japonicus* Yamaguchi, 1936 (Cook 1971)
Lumbriculus mukoensis Yamaguchi,1953
  (Timm 1997, 1999, Torii & Ohtaka 2007)
  =*Lumbriculus variegatus mukoensis* Yamaguchi, 1953 (Cook 1971)
Lumbriculus variegatus (Müller, 1774)
  (Yamaguchi 1953)
Rhynchelmis orientalis Yamaguchi, 1936
  (Cook 1971, Kaygorodova & Liventseva 2007, Fend & Brinkhurst 2010, Fend & Lenat 2010)
Styloscolex (*Styloscolex*) *japonicus* Yamaguchi, 1937
  = *Styloscolex japonicus* Yamaguchi, 1937 (Yamaguchi 1937b, 1953, Cook 1971, Ohtaka 2014, Sokolskaya 1976, Timm 1997, 1999, Timm & Všivkova 2007, Rodriguez et al. 2014)
**Styloscolex* (*Styloscolex*) *tazawaensis* n. sp. 3.2.1
**Styloscolex* (*Neoscolex*) *tatsukoae* n. sp. 3.2.2
Yamaguchia toyensis Fend & Ohtaka, 2004

3.1 *Lamprortus orientalis* Rodriguez, 1994

(Figure 1)

Material examined: Japan, Hiroshima Prefecture, outlet stream from Haizuka Reservoir, 28 Jan 2010, collected by T. Torii, 5 sagittally dissected and slide mounted. Aomori Prefecture, a spring in Hirosaki, 40.596586N, 140.471261E, elev. 38.8 m, 5 Apr. 2014, 4 mature slide mounts. 9 Apr. 2018, 9 mature slide mounts. All collected by A. Ohtaka.

Supplemental Description: Diameter in segment X 0.85–1.0 mm; length of 1 complete specimen 19 mm; clitellum IX–XV. Chaetae in anterior to middle segments 142–181 µm, nodulus median to slightly distal, 0.33–0.49 from the distal end. Some specimens with a single pair of short, blind lateral blood vessels in posterior segments. Spermathecal ducts tubular, length 140–280 µm in Haizuka streams (Fig. 1C), 250–380 µm in the Hirosaki spring; spermathecal ampullae large, sacciform, extending into adjacent segments (Fig. 1B). Atrium length 590–990 µm in the Haizuka worms and 500–650 µm in Hirosaki specimens; conical penes within spherical, glandular bulbs to about 200 µm diameter.

Remarks: Brinkhurst et al. (1994) justified this monotypic genus "on the basis of the unique plan of the reproductive organs, particularly the possession of a second pair of ovaries, making the taxon hologynous". *Lamprortus* closely resembles the large, Palearctic genus *Lamprodrilus* Michaelsen, 1901 (Michaelsen 1901a) in having elongate atria paired in two segments (X and XI), prosoporous male ducts (without anterior vasa deferentia or testes), posterior vasa deferentia not penetrating the posterior septum, and spermathecae posterior to the male pores. It differs in having two ovarian segments with associated female funnels (in XII and XIII), and in having the single pair of spermathecae opening three segments posterior to the atria, in XIV. In *Lamprodrilus* the spermathecae usually begin in XIII (Cook 1971).

The present collection represents a new record for Japan. The new specimens appear quite similar to the material described by Rodriguez (in Brinkhurst et al. 1994): positions of genital pores and gonads are as described (Fig. 1A), and basic morphology of reproductive structures appears identical (compare Fig. 1B with Fig. 2 in the original description). Spermathecal ducts (Fig. 1C) were longer than in the original description (reported to 160 µm); however, orientation made length difficult to measure in most specimens (Fig. 1B). Atrium length was not given in the original description, but appears to be about 900 µm in one specimen (Fig.

2 in Brinkhurst et al., 1994), similar to some of the new material. The short, lateral blood vessels seen in posterior segments of some new specimens were difficult to observe. We consider these differences to be minor or subject to preservation and individual variation, and therefore the genus remains monotypic.

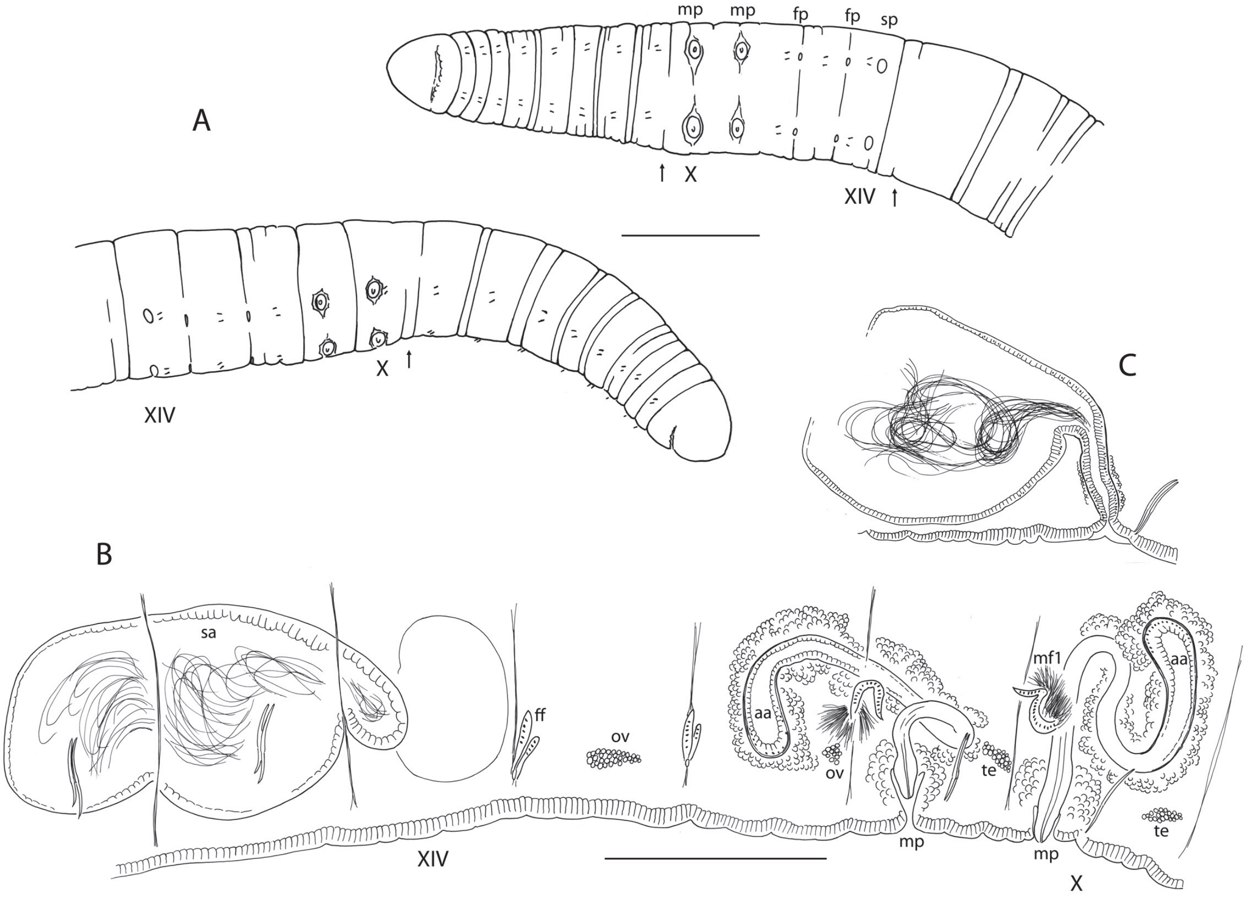

FIGURE 1. *Lamprortus orientalis* Rodriguez 1994. Specimens from Hiroshima Prefecture, outlet stream from Haizuka Reservoir. A. Whole worms, ventral and ventrolateral views, showing locations of genital pores; arrows indicate beginning and end of clitellum. Scale bar = 1 mm. B. Reproductive organs, segments X–XVI, from a dissected worm (vasa deferentia not illustrated); scale bar = 500 µm. C. Spermatheca, lateral view; scale same as (B).

Habitat: The outlet streams from Haizuka-dam in Hiroshima have a gravel bottom with water depth 20–30 cm; filamentous cyanobacteria, mainly *Homoeothrix janthina*, were dominant on cobbles. This cyanobacterium is common in oligotrophic streams in Japan; however, it was not well represented in *L. orientalis* gut contents, which were dominated by filamentous algae, diatoms and fine organic matter. The small stream in Hirosaki lies within an urban landscape; it is spring-fed, with gravel bottom and water depth 10–20 cm; water temperature was 9.2º C and pH 6.2 at the time of collection.

Lamprortus orientalis was described from the Suyong River, Pusan, Korea. The type locality, and other sites within the Suyong drainage, are in areas with agricultural and urban land use, and associated with pollution-tolerant species (Brinkhurst et al. 1994). Occurrence of this species in Hiroshima Prefecture in Honshu, Japan is not surprising considering its proximity to the type locality. The Korean Strait, separating the Korean Peninsula and Honshu Island is relatively recent, dating to about 100,000 ybp in late Diluvial epoch of the Quaternary Era (Ohshima 1990). We suggest that *L. orientalis* may be distributed widely in Honshu Island, based on the additional collections in Aomori Prefecture, located in the northernmost part of Honshu. Additionally, as this species occurs in anthropogenically-modified habitats, its range may have been further increased by human activities (e.g., transfer of plants among drainages).

3.2 *Styloscolex* Michaelsen, 1901

Styloscolex Michaelsen, 1901 (Michaelsen 1901b, Cook 1971, Holmquist 1974, Sokolskaya 1972b, 1978, Timm 1994, Timm & Všivkova 2007)
Styloscolex s. str. Sokolskaya, 1976
Styloscolex (*Styloscolex*) Sokolskaya, 1983
Styloscolex (*Neoscolex*) Sokolskaya, 1976

Remarks: A diagnosis for the genus is given by Cook (1971), and has since then been modified to include two subgenera, *Styloscolex* (*Styloscolex*) Sokolskaya, 1983 (= *Styloscolex* s. str. in Sokolskaya 1976) and *Styloscolex* (*Neoscolex*) Sokolskaya, 1976. *Styloscolex* species are prosoporous, with only one testicular segment. The previously described species all have the reproductive segments shifted anteriad (i.e., testes and male pores in VII or VIII), and are distributed in Lake Baikal and eastern Asia, with one record from northern Alaska (Holmquist 1974). *Styloscolex* (*Styloscolex*) species usually have elongate atria and cuticular penes, and all have an intervening, sterile segment between the testicular and ovarian segments (a character otherwise unknown in the Lumbriculidae). Another remarkable characteristic of the subgenus is that different species have spermathecae in either pre- or post-atrial segments, or both (Sokolskaya 1969, Timm 1994). Two Japanese species conform to *S.* (*Styloscolex*): *Styloscolex japonicus* Yamaguchi, 1937 will be discussed in a future contribution, based on the rediscovered Yamaguchi collection plus several new records from Hokkaido, and a new species is described herein.

The three or four species attributed to *Styloscolex* (*Neoscolex*) by Sokolskaya (1983) resemble *S.* (*Styloscolex*) in having the series of reproductive segments anterior to the usual position, i.e. with male pores VII or VIII instead of X; spermathecae are in the preatrial segment; male ducts are prosoporous, and the short vasa deferentia do not enter the post-atrial segment. They are primarily distinguished from *S.* (*Styloscolex*) by the position of the ovaries, in the post-atrial segment—and thus without an intervening sterile segment. Atrial morphology is variable in this subgenus: atria are sacciform in *S.* (*Neoscolex*) *macer* Sokolskaya, 1976 and in an undescribed species (Sokolskaya 1983), and club-shaped in *S.* (*Neoscolex*) *sokolskajae* Morev, 1978. Only *S.* (*Neoscolex*) *levanidovi* (Sokolskaya, 1977) has the elongate atria and cuticular penes that characterize the nominate subgenus. Members of this subgenus are known only from northeastern Asia; a single Japanese species, described below, is provisionally assigned to this group.

3.2.1 *Styloscolex* (*Styloscolex*) *tazawaensis* n. sp.

(Figure 2)

Holotype: NSMT-An 522. A dissected worm (anterior segments only), stained with carmine and slide-mounted in Canada balsam.

Type locality: Japan, Akita Prefecture. A spring at Kata, near Lake Tazawa, 39.696440N, 140.662897E, elev. 264 m, 11 May 2008, collected by A. Ohtaka.

Paratype: NSMT-An 523. From the type locality, same collection. One unstained whole mount (anterior segments only) in Canada balsam.

Etymology: Derived from the type locality, Lake Tazawa.

Description: Both specimens incomplete; the longer (paratype) with 47 segments. Diameter 0.46 mm in X, maximum diameter to 0.63 mm. Prostomium rounded, length and width both about 300 µm (Fig. 2A). Epidermis in anterior segments 11–14 µm thick, in clitellum 26–50 µm, and 6–7 µm posteriorly. Clitellum well-developed and glandular VII–XII, cells unordered (not in rows). Secondary annulation may be present as a narrow anterior ring from about IV–VII.

Chaetae simple-pointed, moderately sigmoid, dorsal and ventral pairs similar; length 120–151 µm in anterior region, 122–144 µm posteriorly; thickness 4–5 µm; nodulus approximately 0.37–0.43 from the distal end (Fig. 2E, F). Pharynx moderately thickened in II–IV, without distinct dorsal pad. Pharyngeal glands small in IV (V). Chloragogen tissue covers gut beginning in VI. First nephridia paired on 6/7, the second pair on 11/12 or 12/13; nephridia variable in posterior segments. Blood vessels were not clearly observed.

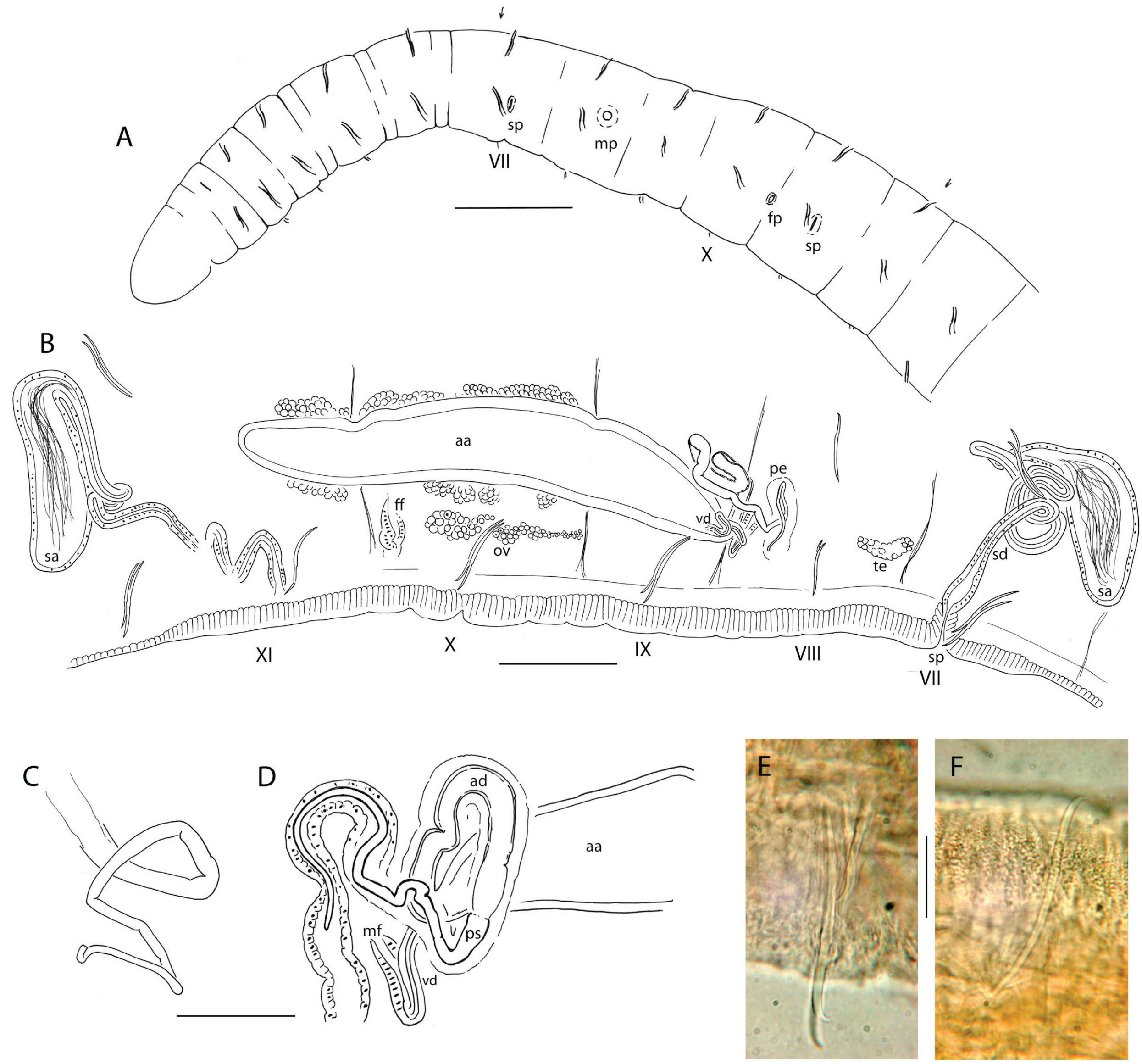

FIGURE 2. *Styloscolex* (*Styloscolex*) *tazawaensis* n. sp. from Akita Prefecture, spring at Kata, near Lake Tazawa. A. Whole worm, ventrolateral view showing location of genital pores, scale bar = 500 µm. B. Reproductive organs in segments VII–XI, from a dissected worm; scale bar = 200 µm. C. Penis sheath, from a whole mount; scale bar = 100 µm. D. Detail of atrial duct, male funnel, penis in sac, from a dissected worm. E. Ventral chaeta in IV; scale bar = 50 µm. F. Dorsal chaeta in XII.

Spermathecal pores are transverse slits, immediately behind ventral chaetae in VII and XI; male pores round, may have small conical papilla, on ventral chaetal line in VIII midway between chaetae and 8/9 (Fig. 2A); female pores on chaetal line at intersegment 10/11. Testes small, in VIII; ovaries in X, may be small or elongate, extending through posterior septum into XI. Egg sacs extending back as far as XV; female funnel 80 µm high.

Spermathecae similar in VII and XI; the ones in XI may extend back into XII. Spermathecal duct ending in a short expansion (to 50 µm wide), the remaining duct tubular, 17–25 µm in diameter, with columnar epithelium; duct may be highly folded, total length 550–700 µm (Fig. 2B). Spermathecal ampulla elongate-sacciform, length 290–390 µm, width 90–100 µm. Ampulla with thin, non-glandular epithelium (8–15 µm); sperm loosely arranged.

Male funnels small and conical, on 8/9 only. Vasa deferentia difficult to see in the available material; apparently do not penetrate the posterior septum; junction with atrium near base (ectal end) of ampulla (Fig. 2B,D). Atrial duct long and convoluted; ectal 420–550 µm forming a penis with a thin, folded cuticular covering ("penial sheath"), sheath diameter about 5 µm at the male pore, expanding to 20–28 µm entally, within a narrow sac (Fig. 2B–D). Remainder (170–190 µm) of duct gradually widens and muscle layer becomes thicker, eventually expanding to form the atrial ampulla. Ampulla elongate-fusiform (almost tubular), extending back to XI or XII; length 850–1000 µm, maximum diameter 75–155 µm; distinct muscle layer 12–22 µm; outer layer of small prostate glands 25–35 µm high; ampulla filled with sperm (Fig. 2B).

Remarks: Despite the limited material available, the new species is clearly attributable to *S.* (*Styloscolex*) by a combination of: prosoporous male ducts with male pores in VIII, a preatrial pair of spermathecae in VII, elongate atria, and ovaries and female funnels in the second postatrial segment (XI). Within the subgenus *Styloscolex*, diagnostic characters of *S. tazawaensis* n. sp. include: male pores paired in VIII, spermathecal pores paired in both VII and XI; atrial duct very long and convoluted; elongate, thin, folded penis sheath; atrial ampulla elongate-fusiform, length about 8x diameter; spermathecal duct tubular, long and folded.

The presence of a second pair of spermathecae in XI is shared only with *Styloscolex tetrathecus* Burow, 1931, described from Lake Baikal by Burov (1931), from northeastern China by Yamaguchi (1940), and from Lake Potatgytkhyn, Koryak upland by Sokolskaya (1978, 1983). The original description of that species is not very detailed; the penes are "long and narrow" and ducts are "distinctly differentiated" from the ampullae (no measurements of these structures were given). However, the very long atrial and spermathecal ducts of *S. tazawaensis* n. sp. seem to differ from both the original description and redescriptions of *S. tetrathecus*; the atrial ampulla is also much longer than the 250 µm given by Sokolskaya (1983) (see Fig. 20 in Sokolskaya 1983 = Fig. 1 in Sokolskaya 1978). Burov's (1931) *S. tetrathecus* description, as well as Sokolskaya's redescription, indicate a much shorter atrial duct, barely passing through the septum into the adjacent segment before widening into the ampulla. Other redescriptions (Yamaguchi 1940, Isossimov 1962) do not provide much detail, focusing on segmental position of reproductive organs. Another widespread species (from Kamchatka and Alaska), *Styloscolex opisthothecus* Sokolskaya, 1969, differs from most congeners in having only post-atrial spermathecae, in XI. The atrial and spermathecal ducts (e.g., Figs. 3–4 in Holmquist 1974) are short, resembling those of *S. tetrathecus*.

Of the other *Styloscolex* species, only *Styloscolex hankensis* Timm & Všivkova, 2007, described from Lake Hanka (Russia/China) has been described as having a penis sheath "very long, highly elastic, and doubly folded". The latter has only one spermathecal segment (VII), and as it was described from a single, unmated specimen, it is difficult to compare the spermathecal morphology. As in the new species, *Styloscolex tubulatus* Timm, 1994, from the Magadan Region (eastern Russia) has elongate spermathecal ducts, but has spermathecae only in two post-atrial segments (X and XI). It is further distinguished from the new species by the shorter atrial duct and penes, and by a very large glandular mass surrounding the penial sac.

Habitat: The type locality is a small, spring-fed, muddy *Alnus* swamp in Lake Tazawa basin, Akita Prefecture, Honshu. Water depth was less than 10 cm, and temperature exceeds 20° C in summer. Forest floor vegetation is dominated by the emergent Asian skunk cabbage (*Lysichiton camtschatcensis*). Chironomine and tanypodine chironomids dominated the bottom fauna, and the tubificine oligochaete, *Embolocephalus yamaguchii* (Brinkhurst, 1971) has also been recorded (Ohtaka & Martin 2011). *Styloscolex* (*Styloscolex*) *tazawaensis* n. sp., *Styloscolex* (*Neoscolex*) *tatsukoae* n. sp., and some other lumbriculids were collected by scooping the bottom sediment.

3.2.2 *Styloscolex* (*Neoscolex*) *tatsukoae* n. sp.

(Figure 3)

Holotype: NSMT-An 524. Anteriormost 24 segments, stained with carmine and slide-mounted in Canada balsam.

Type locality: Japan, Akita Prefecture. A spring at Kata, near Lake Tazawa, 39.696440N, 140.662897E, elev. 264 m, 11 May 2008, collected by A. Ohtaka.

Paratype: NSMT-An 525. From the type locality, same collection. One whole mount of anterior segments, in Canada balsam.

Etymology: From Tatsuko, a local girl who became the spirit of Lake Tazawa.

Description: Both specimens incomplete; the longer (paratype) about 12 mm long, with 57 segments; diameter (slide-mounted) 0.4–0.47 mm in X. Prostomium rounded-conical, length about equal to width (Fig. 3A). Clitellum 1/2 IX–XIII, similar thickness to anterior epidermis (10–12 µm), but distinctly glandular.

Chaetae simple-pointed, sigmoid; directed forward in segments anterior to X; length 67–122 µm in anterior segments, 80–98 µm posteriorly; nodulus at 0.36–0.44 from the distal end.

Pharynx in II–III, mostly thickened dorsally; pharyngeal glands in IV–VI. First nephridia on 6/7, with duct terminating in a small, rounded vesicle at the pore, just in front of the chaetae in VII. Next nephridia on 12/13, then in a few segments posteriorly. Ventral blood vessel forms in III; convoluted commissural blood vessels in anterior segments at least to X. No obvious lateral blood vessels or caecae in posterior segments.

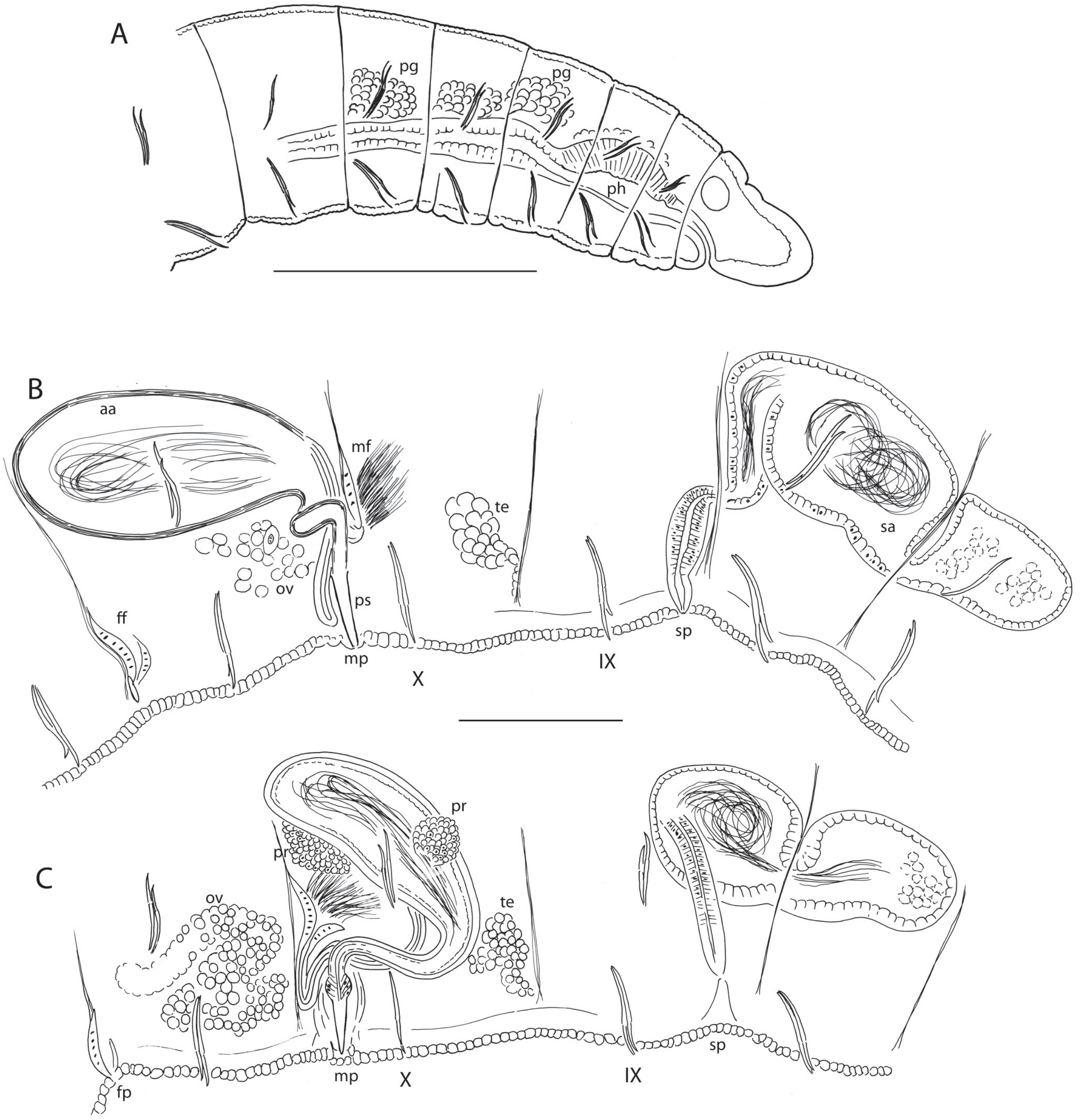

FIGURE 3. *Styloscolex* (*Neoscolex*) *tatsukoae* n. sp. from Akita Prefecture, spring at Kata, near Lake Tazawa. A. Anterior segments, showing pharynx and anteriorly-directed chaetae; scale bar = 500 µm. B. Reproductive organs; scale bar = 200 µm. C. Reproductive organs from another specimen; showing apparent prostate glands.

Spermathecal pore single, midventral in IX, just behind septum 8/9. Male pore single, midventral in X, near 10/11. Female funnels and pores paired near the ventral chaetal line at 11/12. Testes paired in X, sperm sacs IX–XVII; ovaries long, extending to posterior XI, egg sac to XIX. All genital pores simple, slightly recessed, inconspicuous.

Spermathecal duct 130–140 µm long, 30–43 µm wide, formed of thick (to 20 µm), columnar epithelium with indistinct muscle layer and narrow lumen. Duct penetrates 8/9, and ampulla enters VIII. Spermathecal ampulla ovate-sacciform; length 370–480 µm, width 150–170 µm (Fig. 3B,C); irregular epithelium 7–24 µm thick. Sperm loosely distributed, unordered within ampulla.

Male funnels apparently paired on 10/11, may be directed back into XI, within sperm sac. Vas deferens diameter 12–19 µm; not penetrating the posterior septum; joining atrium near base of ampulla. Atrium single in X, petiolate, the atrial duct 120–170 µm long by 25–29 µm diameter, in X or extending into XI; ampulla broadly club-shaped (length 290–360 µm, width 95–170 µm) (Fig. 3B,C). Atrial muscle layer 6–12 µm thick; epithelium thinner than muscle layer, not clearly seen in either specimen. Prostate glands were not clearly seen, but 3–4 faint masses of glandular tissue were apparently associated with the atrial ampullae (Fig. 3C). Atrial duct ends in a narrow-conical penis, 80–95 µm long by 14–18 µm wide; apparently covered by a cuticular sheath, cuticular layer thin and inconspicuous.

Habitat: See above, same locality as *Styloscolex tazwaensis* n. sp.

Remarks: The combination of characters is unique within the family: male pore single, midventral behind ventral chaetae in X, just in front of 10/11; spermathecal pore single, midventral, anterior to ventral chaetae in IX, just behind 8/9; atrium single, petiolate, with ovate ampulla; duct ending in conical penis with a thin, cuticular sheath; male ducts prosoporous, vasa deferentia joining the atrium without penetrating the posterior septum. Although some of these characters are unusual, all occur independently in other lumbriculid taxa. The unpaired, median atrium and spermatheca are shared with the Palearctic *Tatriella* Hrabě, 1936 and some Nearctic *Eclipidrilus* Eisen, 1881 [*Eclipidrilus lacustris* (Verrill, 1871) and *Eclipidrilus macphersonae* Fend & Lenat, 2012]. Cuticular penis sheaths are rare in the family; although well-developed and apparently rigid only in some *S.* (*Styloscolex*) species and perhaps in *S.* (*Neoscolex*) *levanidovi*, thin sheaths have also been described in *Sylphella puccoon* Rodriguez et al., 2014, *Eclipidrilus* (*Premnodrilus*) species (Wassell 1984), and in some *Lumbriculus* Grube, 1844 (e.g., Holmquist 1976). The position of the spermathecal pores in the new species, at the anterior margin of IX, is unusual within the family; however, *Eclipidrilus pacificus* Fend, 2005 has (paired) spermathecal pores in the anterior part of IX, and spermathecal pores are somewhat anterior to the chaetae in other taxa, such as *Rhynchelmis* (*Sutroa*) species.

The petiolate atrium and the posterior vas deferens of the new species resemble those of the two *Tatriella* species, both of which have limited distributions in northern and central Europe. However, the new species differs from *Tatriella* in several characters considered to have generic significance: prosoporous, rather than semiprosoporous male ducts; spermathecal pores located in IX (although anteriorly, near the anterior septum), rather than in the posterior part of VIII; and a comparatively long penis, with a weak cuticular sheath.

In addition to a few species having unpaired atria and/or spermathecae, the Nearctic genus *Eclipidrilus* has spermathecae in the pre-atrial segment (IX) and elongate-petiolate atria. *Eclipidrilus* is a diverse genus that has become difficult to define (see discussion in Fend & Lenat 2012), and at least some species have been assigned to different subgenera (Wassell 1984). As in the new species, the posterior vasa deferentia do not form a loop in the post-atrial segment; however, most *Eclipidrilus* species differ in being semiprosoporous (each atrium with anterior and posterior vasa deferentia), with varying degrees of reduction of the anterior duct. *Eclipidrilus* species have been considered to have characteristic atrial morphology (petiolate, with a very elongate ampulla) and histology (muscle layer spiral, cross-hatched or two-layered), and this has been considered diagnostic for the genus (Cook 1971, Sokolskaya 1983, Fend 2005). Although these characters are inconsistently expressed in some species assigned to *Eclipidrilus* (Fend & Lenat 2012), it does not seem appropriate to assign the new species to that genus.

Styloscolex (*Neoscolex*) resembles the new species in having spermathecae in the pre-atrial segment, prosoporous male ducts, and a single species with a cuticular penis sheath. As it also has an eastern Asian distribution, we provisionally assign the new species to that genus and subgenus. The genus *Styloscolex* has been defined in part by a forward shift in reproductive segments (i.e., the testes and atrium in a segment anterior to X); consequently, this tentative attribution is likely to be modified as future collections support more detailed descriptions.

Some characters were not clear in the available material (two whole-mounted, anterior ends), and should be confirmed with additional, sectioned or dissected specimens. In particular, details of the male reproductive organs were obscured by the densely filled sperm sacs. There appeared to be two sperm funnels, although only the uppermost was clearly visible, but only one vas deferens could be clearly seen on either specimen. Paired sperm ducts occur in other lumbriculids having median, unpaired atria, e.g. *Tatriella slovenica* Hrabě, 1936 (cf. Fig. 13 in Hrabě 1939) and *E. pacificus* (cf. Fig. 9B in Fend 2005). The apparent prostate glands were not clearly visible on either specimen, and the structures shown in Fig. 3C may have been unassociated glandular tissue. Absence of prostate glands on the atrial ampulla has only occasionally been reported in lumbriculid descriptions, e.g., in *Eclipidrilus breviatriatus* Fend & Lenat, 2012, and in *Styloscolex baicalensis* Michaelsen, 1901. Prostate glands are present on the atrial duct of in *T. slovenica*, but they are absent from most of the ampulla.

3.3 *Honshudrilus* n. gen.

Type species. *Honshudrilus gudariensis* n. sp.

Diagnosis: (Possible apomorphies underlined.) Small worms without a proboscis. All chaetae simple-pointed. No lateral blood vessels in posterior segments**.** First pair of nephridia in VII (on 6/7). Testes paired in X, ovaries paired in XI. Spermathecal pores on the ventral chaetal line, more than one segment anterior to male pores (in VI or VIII); spermathecal ducts with ectal vestibules. Female pores on ventral chaetal line at 11/12. Male pores behind ventral chaetae in X; penes formed by extruded lining cells of atrial duct. Atria petiolate, with long duct, and elongate ampulla covered with multicellular, petiolate prostate glands. Male ducts semiprosoporous, with nonfunctional anterior vasa deferentia not associated with testes; posterior vasa deferentia penetrate posterior septum, forming a loop in XI.

Included species: *Honshudrilus gudariensis* n. sp. Ohtaka, Fend & Torii, *Honshudrilus yuzawaensis* n. sp. Fend, Ohtaka & Torii.

Etymology: These worms appear to be endemic to the Island of Honshu.

Remarks: Attribution of the two included species to a single genus is somewhat problematic, yet it is reasonably consistent with generic diagnoses outlined by Cook (1968, 1971) and modified by more recent literature. The first major division in Cook's (1971) key separates lumbriculid genera into those with spermathecae opening in segments anterior to the atrial segments vs. spermathecae in the atrial or post-atrial segments. Most species with pre-atrial spermathecae have them in the first pre-atrial segment (Brinkhurst 1989), but (like *Honshudrilus yuzawaensis* n. sp.), they are in the second pre-atrial segment in *Rhynchelmis* Hoffmeister, 1843, *Pseudorhynchelmis* Hrabě, 1982, *Tatriella* Hrabě, 1936, *Pararhynchelmis* Fend & Lenat, 2010, and *Secubelmis* Fend & Gustafson, 2001. A single genus, *Martinidrilus* Fend & Lenat, 2007 has spermathecae opening in either the third or fourth pre-atrial segment. Relative position and number of spermathecae is invariant in many lumbriculid genera, differing most notably within *Styloscolex* (see above), *Rhynchelmis*, *Martinidrilus*, *Lamprodrilus*, and *Lumbriculus*.

Characters distinguishing *Honshudrilus* from similar genera are summarized in Table 1. Possible synapomorphies for the genus, including the penes, the spermathecal vestibules, and the non-functional anterior male ducts, are admittedly weak, as they can vary within other accepted genera. Male pores of both species have "type 2" penes, as defined by Rodriguez & Giani (1994); these temporary structures are formed by extruding the elongate lining cells in the atrial duct (Cook 1967, Fig. 2a; Rodriguez & Giani 1994, Fig. 17F). While spermathecal ducts of both *Honshudrilus* are distinctly widened into "vestibules" at the pores, these structures are morphologically dissimilar in the two species (see below). The nonfunctional anterior male funnels and vasa deferentia seem to be a good apomorphy, as they imply a reduction of the supposedly ancestral semiprosoporous condition for the family (Brinkhurst 1989). Nevertheless, a range of similar, apparently intermediate forms occurs within both *Rhynchelmis* and *Eclipidrilus*.

The two described *Martinidrilus* species, both from southeastern North America, are distinguished from *Honshudrilus* by additional synapomorphies, e.g., spermathecae opening laterally, vasa deferentia joining before entering short atria, and numerous short lateral blood vessels in posterior segments. As none of these unusual characters occurs in either of these two Japanese species, it seems preferable to erect a new genus, rather than compromising the diagnosis of *Martinidrilus*. *Honshudrilus* differs from both described *Martinidrilus* species in having 1) spermathecal pores on the chaetal line, with distinct vestibules, 2) the anterior male duct and funnel are small and nonfunctional, 3) atria have elongate ducts and ampullae, 4) vasa deferentia do not join before joining the atria, 5) posterior lateral blood vessels appear to be entirely lacking, and 6) lining cells in the ectal part of the atrial duct can apparently be extruded, forming a type 2 penis.

TABLE 1. Comparison of *Honshudrilus* n. gen. species with other lumbriculids having similar characters. Underlined characters are considered likely apomorphic within the family; characters with asterisk (*) were considered possible apomorphies for *Pseudorhynchelmis* by Martin & Kaygorodova (2008).

	Honshudrilus gudariensis	*Honshudrilus yuzawaensis*	*Pseudorhynchelmis* (Baikal spp.)	*Martinidrilus*	*Eclipidrilus frigidus*
spermathecae relative to atrial segment	4 segments anterior (VI)	2 segments anterior (VIII, usually)	2 segments anterior (VIII)	2–4 segments anterior (VII–VIII or VI)	1 segment anterior (IX)
spermathecal duct	small vestibule	large vestibule	1 species with vestibule or bulb	not modified	large vestibule
spermathecal position in segment	paired, on chaetal line	paired, on chaetal line	paired, on (or slightly inside) chaetal line	paired, lateral or dorsolateral	paired, on chaetal line
testes	1 pair, in X	1 pair, in X	2 pairs, IX, X	2 pairs, IX, X	2 pairs, IX–X
anterior male duct	non-functional, reduced	non-functional, reduced	functional	functional	usually functional, reduced
male duct enters post-atrial segment	yes	yes	yes	yes	no
vas deferens structure	thin, tubular	thin, tubular	*widened entally (usually)	thin, tubular; joining before atrium	thin, tubular
atrium shape	elongate-petiolate	elongate-petiolate	ovate to elongate-tubular	short-ovate	elongate-petiolate
atrial musculature	thin	thin	thin	thin	thick, spiral
atrial epithelium	somewhat thickened	somewhat thickened	*thick and glandular	somewhat thickened	thin
penes	type 2	type 2	*type 1 (usually)	small papilla	large type 2 (in sac)
modified genital chaetae	none	none	spermathecal, penial, or none	none	none
posterior lateral blood vessels	none	none	none	many short lateral vessels	2 pairs, branched
distribution	Japan, Honshu	Japan, Honshu	Lake Baikal	Southeastern North America	Western North America

An alternative classification would place *Honshudrilus* in *Pseudorhynchelmis*, a group of taxa largely restricted to Lake Baikal (with one exception in European groundwater); *Pseudorhynchelmis* species also have atria in X and spermathecae are always two segments anteriad, in VIII. Atria vary from ovate to elongate in the different species; where the atrium is elongate, it is tubular, rather than petiolate, and both vasa deferentia are functional. Martin & Kaygorodova (2008) discussed the difficulty of defining consistently-expressed apomorphies for *Pseudorhynchelmis*, but suggested (1) ental widening of the vasa deferentia, (2) thick-glandular atrial epithelium, and (3) pendant penes in sacs (“type 1” penes, as defined by Rodriguez & Giani 1994) as possibly diagnostic. *Pseudorhynchelmis alyonae* (Martin et al., 1998) also has an elaborate spermathecal duct, but the large expansion at the spermathecal pore appears to be a solid "bulb", rather than a deep vestibule. Additionally, genital chaetae occur in several species. As *Honshudrilus* species lack all of these diagnostic characters, and also have non-functional anterior male ducts (not associated with testes), it does not seem appropriate to combine them with *Pseudorhynchelmis* at this point.

Several *Rhynchelmis* species have vestigial or nonfunctional anterior male funnels and ducts, but these differ from *Honshudrilus* species in most other respects. For example, *Rhynchelmis* species are large, robust worms, usually with a proboscis, and well-developed lateral blood vessels; atria are usually tubular; spermathecae usually join the gut; and even the expanded spermathecal ducts of *Rhynchelmis* (*Sutroa*) species are glandular "bulbs", rather than vestibules.

3.3.1 *Honshudrilus gudariensis* Ohtaka, Fend & Torii
(Figure 4)

Holotype: NSMT-An 526. A whole worm, stained with hematoxylin, slide-mounted in Canada balsam. Collected 1 March 2017 by A. Ohtaka.

Type locality: Japan: Aomori Prefecture, spring-fed Gudari-numa Stream in Hakkōda Mountains, Komagome 40.669844N, 140.952302E, elev. 589 m.

Paratypes: All from the type locality. NSMT-An 527–529, 17 March 2017, 1 dissected and slide mounted. 11 February 2016, 1 whole mount. 4 June 2014, 1 sagittally sectioned on slide. USNM 1610892-1610894, 4 June 2014, 1 whole mount. 17 March 2017, 2 whole mounts in Canada balsam.

Other material: Type locality, collected by A. Ohtaka, 19 April 2014, 2 whole mounts. 4 June 2014, 4 whole mounts. 10 August 2014, 5 whole mounts. 31 August 2014, 1 whole mount. 2 November 2014, 3 whole mounts. 17 March 2017, 2 whole mounts and 1 dissected on slide. 2 November 2014, 4 sagittal sections. Akita Prefecture, a spring-fed brook at O-mori, near Lake Tazawa, 39.719979N, 140.697021E, with gravel bottom, depth 5−10 cm, 20 September, 2015, 1 whole mount. Takamatsu, Yuzawa-shi, Yamagata, 1 May 2013, collected by T. Torii, 1 whole mount.

Etymology: From the type locality, Gudari-numa.

Description: Body whitish in color without pigment. Length of preserved worms 22 to 32 mm; maximum diameter around X, 0.28 to 0.36 mm; 64 to 75 segments. Prostomium rounded or conical, as long as or slightly shorter than wide (Fig. 4A). Secondary segmentation typically a narrow anterior ring in IV–VIII, otherwise inconspicuous throughout the body. Epidermis 8–16 µm thick in anterior segments. Clitellum inconspicuous, with slightly thickened (13–20 µm) and glandular epidermis, usually from beginning of X to end of XII. Circular muscle of body wall 2–4 µm thick, and longitudinal muscles 10–16 µm thick.

Chaetae paired, in 4 bundles in each segment from II. Chaetae sigmoid and simple-pointed, with nodulus at ⅓ from the distal end, shape and size not different between dorsal and ventral bundles (Fig. 4B). Chaetae 80 to 98 µm long in anterior segments and 72 to 88 µm in posterior segments.

Pharynx thickened dorsally in II–III (sometimes IV), pharyngeal glands developed in IV–VI. Chloragogen begins in VII. First pair of nephridia on 6/7; nephridia absent in X–XIII, occurring irregularly in middle and posterior segments. Main dorsal and ventral vessels prominent, without secondary longitudinal vessels. Commissural blood vessels prominent in II, III, IV; blind lateral vessels not observed in posterior segments.

One pair of male pores on the chaetal line in X, posterior to ventral chaetae, within a 60–70 µm ring of slightly thickened epidermis (Fig. 4C,D). One pair of spermathecal pores on the chaetal line in VI, posterior to ventral chaetae. Female pores inconspicuous, on chaetal lines on intersegment 11/12; female funnels small, about 50 µm high. Paired testes on anterior septa in X, usually extending to mid-segment. Ovaries in XI, usually extending to posterior septum. Sperm sacs may extend anteriorly into IX and posteriorly as far as XVI; egg sacs extend as far back as XVIII. Two pairs male funnels, on 9/10 and 10/11; the posterior pair functional (with sperm), large (to 90–100 µm high) and extending forward into X; the anterior pair smaller (to 50–60 µm high) and non-functional (Fig. 4C). Anterior vasa deferentia nonfunctional, lumen not obviously ciliated, 8–12 µm in diameter; posterior vasa deferentia about 350 µm long, 14–20 µm thick throughout their length, with ciliated lumen 8–11µm wide, forming a short loop in the postatrial segment before entering X; both anterior and posterior vasa deferentia follow atrial duct and join basal part of atrial ampulla (Fig. 4C). Atrial ampulla pyriform or elongate-ovate in shape, 110–150 µm long and 55–75 µm wide; irregularly transverse outer muscle layer 3–7 µm thick; lumen to 30 µm wide. Epithelium of ampulla appears to vary with stage of development: in some specimens it is cuboidal, 5–10 µm thick (Fig. 4F); in other specimens it is thicker (12–20 µm), granular, with cell boundaries obscure (Fig. 4G). Atrial ampulla loosely covered with non-glandular cells and 6 to 12 petiolate prostate glands 32–50 µm high (Fig. 4H). Atrial duct long and thick, well marked off from ampulla, 300–350 µm long and 40–50 µm wide; duct epithelium less glandular than in ampulla, 10–15 µm thick and lumen 3–8 µm wide. Terminal ⅓ of atrial duct slightly thicker than ental part, projecting vertically within body; epithelial cells in this section usually columnar; near the pore they are directed outward, and may project out of the body, forming a penis (Fig. 4E).

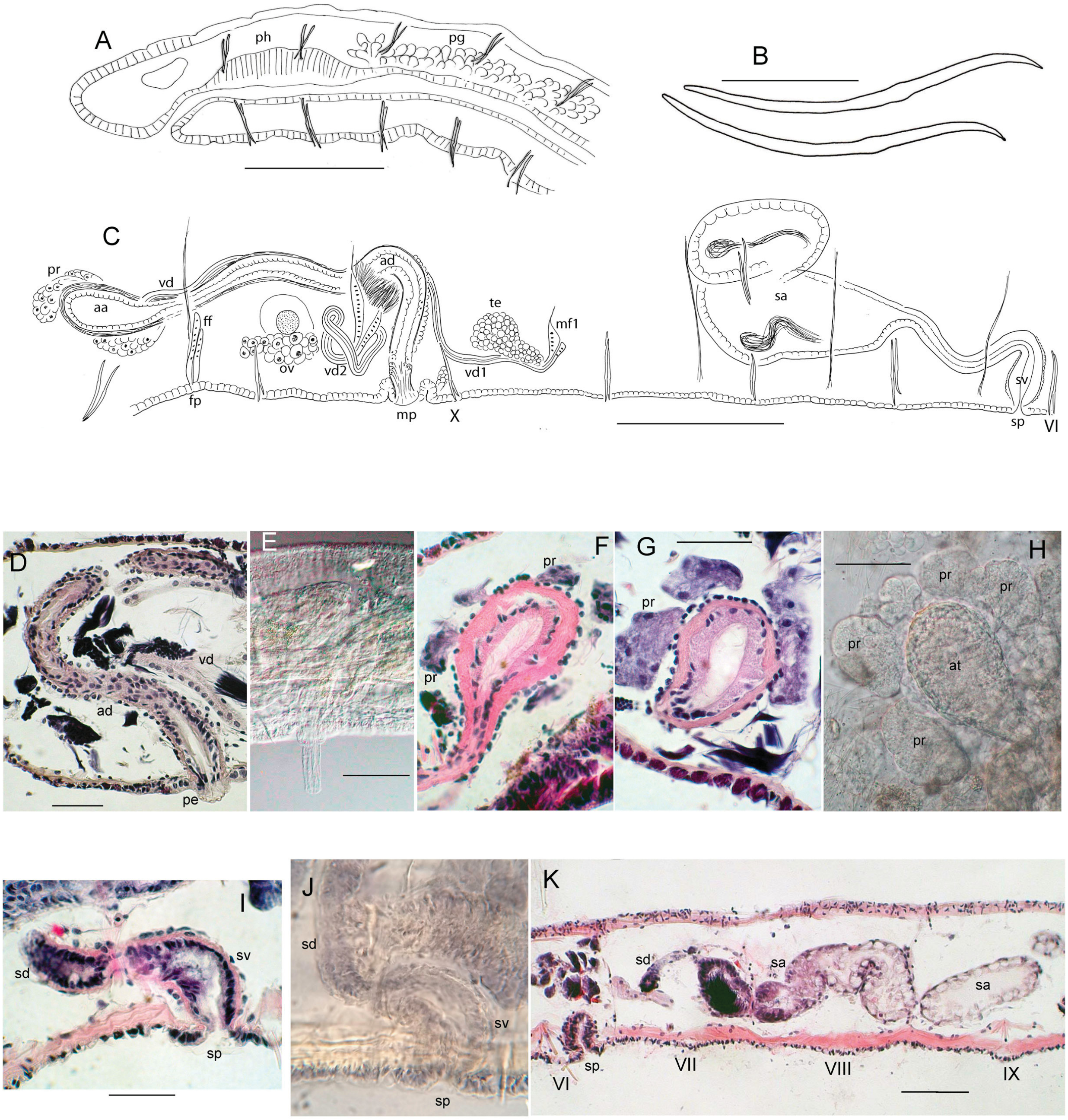

FIGURE 4. *Honshudrilus gudariensis* n. sp. from Aomori Prefecture, spring-fed Gudari-numa Stream in Hakkōda Mountains, Komagome. A. Anterior segments, showing pharynx; scale bar = 200 µm. B. Paired chaetae; scale bar = 20 µm. C. Reproductive organs in segments VI–XII, from a dissected worm; scale bar = 200 µm. D. Atrial duct with partially-extruded penis, from a sagittal section; scale = 50 µm. E. An extruded penis; scale = 100 µm. F, G. Atrial ampullae with thin and thick epithelium, from sectioned worms; scale = 50 µm. H. Atrial ampulla with prostates; scale = 50 µm. I, J. Ectal end of spermathecal duct with vestibule; (I) is from a section; scale = 50 µm. K. Spermatheca, with ampulla extending into IX, from a section; sperm is concentrated in ectal part of ampulla, ental part with vacuoles in epithelium; scale = 100 µm.

Spermathecal ducts 260–400 µm long, opening ventrally between chaetal bundle and posterior septa in VI; ectal part of duct widened into a vestibule, 60–90 x 35–50 µm, with thin but distinct muscle layer (Fig.

4I,J); ental part of duct tubular, 130–210 x 20–26 µm, nearly filled by densely-packed, columnar epithelium. Spermathecal ampulla elongate-sacciform, 200–350 µm long, 50–105 µm wide, extending backward to VII–IX; about the ectal ⅓ thin-walled, densely filled with unordered sperm; the remainder with thicker, vacuolated epithelium and very sparse sperm (Fig. 4K); vacuoles may be sorptive, apparently containing some sperm.

Remarks: The anterior position of spermathecae in VI, four segments anterior to the atria in X is shared only with *Martinidrilus carolinensis* Fend & Lenat, 2007. As stated above, the two genera differ in morphology of atria and vasa deferentia, and in the lateral position of spermathecal pores.

The spermathecal ampulla was relatively small, and not differentiated in one specimen; instead, the entire sac was thin-walled, and sperm was distributed throughout. The specimen also lacked mature eggs in the egg sacs, suggesting that the worm had recently mated, and was at an earlier stage of development. The large, vacuolar cells in the ental part of the ampulla of mature specimens suggest sperm sorption, which occurs commonly in the family (see Černosvitov 1930, Cook 1975, Fend & Lenat 2012).

Histology of the atrial ampulla is quite variable, and appearance of the epithelial layer probably varies with stage of development. A thick, granular layer likely represents secretions of the prostate glands. Similar variation has been noted in other lumbriculid species (Timm 1998).

Gut contents were dominated by pennate and centric diatoms along with detritus. The material resembled the bottom deposit in the habitat, Gudari-numa Stream, suggesting the detritivorous nature of this species.

Habitat: Spring-fed Gudari-numa Stream is located in the Hakkōda Mountains, Aomori Prefecture, Honshu. Water temperature is around 7° C throughout the year (Baba and Ohtaka 2017). The substrate consists of gravels and sand, with clusters of submerged *Ranunculus nipponicus*. The bottom fauna is numerically dominated by diamesine chironomids; other cool-water or stygobiont invertebrates include flatworms, nerillid polychaetes, bathynellaceans, and harpacticoid copepods. An endemic, subterranean amphipod, *Pseudocrangonyx gudariensis* Tomikawa & Sato, 2016 was also described from the site (Tomikawa et al. 2016). Dominant oligochaetaes were the lumbriculids *H. gudariensis*, *Lumbriculus* sp. and another species in an uncertain genus. A single specimen was collected at the type locality for *H. yuzawaensis* n. sp. (see below) and another from a spring-fed brook near Lake Tazawa in Akita Prefecture.

3.3.2 *Honshudrilus yuzawaensis* n. sp.

(Figure 5)

Holotype: NSMT-An 530. A whole worm, not stained, slide-mounted in Canada balsam.

Type locality: Japan: Akita Prefecture: a mountain stream in Takamatsu, Yuzawa, 38.976783N, 140.538141E, elev. 540 m, 1 May 2013. Collected by T. Torii.

Paratypes: NSMT-An 531–533. From the type locality, same collection. 1 whole mount, on same slide as holotype. 2 whole mounts, both on a different slide. USNM 1610895. 1 whole mount.

Other material: From the type locality, same collection. 3 whole mounts on 1 slide.

Etymology: From the type locality.

Description: Length of 3 slide-mounted worms 14.1–16.3 mm; 59–64 segments; maximum diameter 0.30–0.42 mm; prostomium rounded-conical, length about equal to width; secondary annulation IV–VII. Chaetae simple-pointed, sigmoid; length 75–109 µm in anterior segments, nodulus 0.33–0.43 from the distal end (Fig. 5D). Epidermis 7–12 µm thick in anterior segments, 12–18 µm in clitellum; clitellum X–XII(XIII). Longitudinal muscle 10–12 µm thick anteriorly.

Pharynx thickened dorsally in II–III, pharyngeal glands in IV–VI. Gut filled with fine particulate organic matter. First nephridia on 6/7. Brain deeply bilobed. Commissural blood vessels in preclitellar segments, lateral blood vessels absent posteriorly.

Both male and spermathecal pores on ventral chaetal line. Spermathecal pores inconspicuous, usually paired behind ventral chaetae in VIII, rarely also in VI or IX (Fig. 5B). Male pores paired in X (Fig. 5A,B), midway between chaetae and posterior septum within a 90 µm, slightly concave ring of thickened epidermis, most with a small papilla, but cylindrical penis is extruded to 135 µm in 1 specimen (Fig. 5C,F). Testes medium-size, in X; ovaries in XI, extending to mid-segment. Female funnel 70–100 µm high, pore intersegmental at 11/12. Sperm sacs to XVI, mature eggs to XVIII.

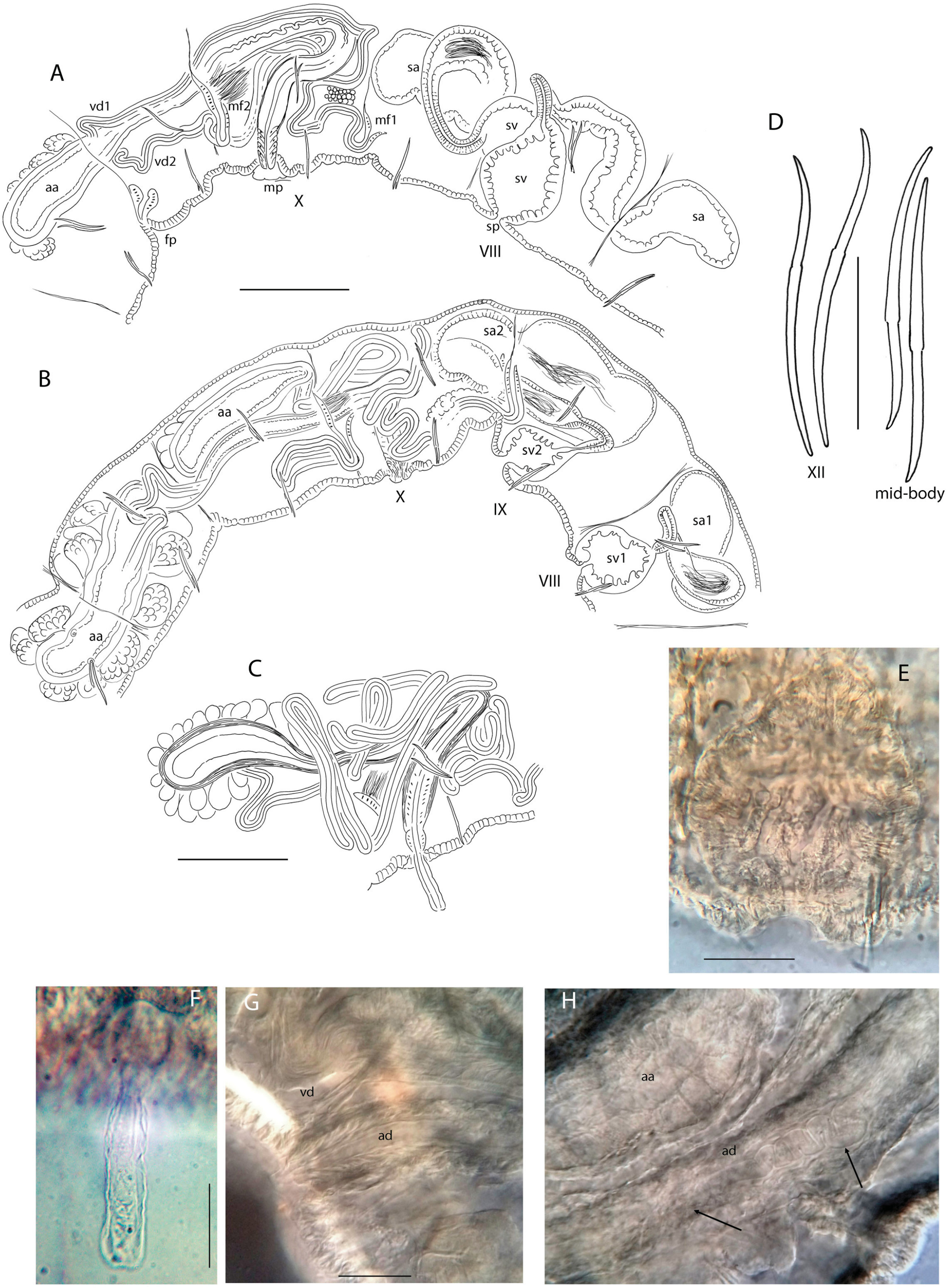

FIGURE 5. *Honshudrilus yuzawaensis* n. sp. from Akita Prefecture, mountain stream in Takamatsu, Yuzawa. A, B. Reproductive organs, segments VIII–XII or XIII, from whole-mounted worms; (A) has the normal spermathecal configuration; (B) with an additional spermatheca in IX; scale = 200 µm. C. Detail of atrium (with extruded penis) and vasa deferentia; scale = 200 µm. D. Dorsal (left) and ventral chaetal pairs; scale = 50 µm. E. Spermathecal vestibule; scale = 50 µm. F. Extruded penis; scale = 50 µm. G. Male pore and duct, penis not extruded; scale = 50 µm. H. Detail of atrial duct, with detached lining indicated by arrows, same scale as G.

Ectal end of spermatheca with a large (to 200 x 140 µm), ovoid or turnip-shaped vestibule (Fig. 5A,B,E), lined with thick, folded epithelium, apparently with a thin, cuticular lining in some specimens. Vestibule joins a tubular duct, 125–170 µm long, diameter 20–26 µm; duct with columnar epithelium and narrow lumen. Spermathecal ampulla sacciform, commonly extending into adjacent segments; thin-walled (4–10 µm) in mated specimens; sperm loosely dispersed throughout, but denser at ectal end; ampulla length 300–400 µm, diameter to 170 µm (Fig. 5A,B).

Male funnels on 9/10 and 10/11 (semiprosoporous), the anterior pair small and nonfunctional (60 µm high), the posterior pair larger (100 µm), with sperm (Fig. 5A,B). Vasa deferentia ciliated (or only posterior ciliated), both anterior and posterior pairs long and winding in X, following the atria posteriorly within sperm sacs; diameter of anterior pair 18–24 µm, posterior (functional) pair 22–26 µm. Posterior vasa deferentia penetrate the posterior septum, forming a large loop in XI; they approach and apparently join the atrial ampulla near the midpoint.

Atrial duct very long and narrow, total length 610–830 µm, extending back into XI or XII. About the ectal 200 µm of the duct is oriented vertically, relatively straight and tubular, diameter 40–50 µm (Fig. 5A,G); entally, diameter varies from about 50 µm down to about 20 µm, narrowest near junction with ampulla. Near the male pore, the atrial duct has elongated lining cells, which may be extruded as a type 2 penis (Fig. 5F,G); the remainder of duct with thick epithelium and a diffuse muscle layer; internally, the duct may be lined with a (cuticular?) tubule, which may be straight or somewhat folded (Fig. 5H). Ental end of the atrial duct expands to form the well-defined atrial ampulla.

Atrial ampulla usually extending back into XI or XII; elongate (length 230–330 µm, diameter 90–130 µm). Ampullar epithelium thick (to 25 µm), surrounded by a dense muscle layer 10–12 µm thick. Prostate glands 10 or more multicellular bundles 50–80 µm high.

Habitat: The type locality is a small stream in Akita Prefecture, Honshu. Water depth was less than 20 cm and temperature around 20° C in summer, or 2° C in winter. The substrate consists of gravels and sand, covered with diatoms and the cyanobacteria *Homoeothrix janthina*. Nemouridae and Chironominae dominated the bottom fauna, and the naidine oligochaete, *Piguetiella denticulata* Liang & Xie, 1997 has also been recorded.

Remarks: Location of spermathecae may be variable. Of the 8 specimens, one had spermathecae paired in VIII and single in VI; another had paired spermathecae in both VIII and IX; the remaining 6 had one pair in VIII only (cf. Fig. 5A). The very large spermathecal vestibule is unusual within the Lumbriculidae, and the apparently folded lining suggests that it can be expanded or even everted.

A cuticular lining of the atrial duct has also been described (Fend 2005) in *Eclipidrilus frigidus* Eisen, 1881 and *Eclipidrilus palustris* (Smith, 1900); in both of these species the cuticular layer appears coiled within the duct, but straightens when the penis is extruded. A similar cuticular lining within the atrial duct of *Uktena riparia* Fend et al., 2015 appears to have a different function: spermatophores are formed by the detached lining, and apparently trasferred to the spermatheca of the partner.

Development of the (non-functional) anterior vasa deferentia appears to vary; in most specimens they are distinctly narrower than the posterior vasa, and in some specimens they do not appear to be ciliated. Nevertheless, the anterior male funnels are always smaller than the posterior ones, and lack sperm. The junction of the long and winding vasa deferentia with the atrial ampulla was difficult to see in the available material.

Discussion

Most Japanese lumbriculid descriptions have been the legacy of Yamaguchi (1936a, b; 1937a, b, 1953), and were based largely on collections from Hokkaido. Thus it is not surprising that the fauna of Honshu (and perhaps other Japanese islands) includes undiscovered taxa. The traditional generic diagnoses, outlined by Cook (1971) and based largely on number, relative positions, and morphology of reproductive organs, have been generally upheld by both the morphology-based cladistic analysis of Brinkhurst (1989) and by the more limited molecular analyses to date (e.g., Kaygorodova et al. 2007, Zhou et al. 2010). Cook's morphology-based taxonomy was based largely on well-studied regions, particularly Europe, eastern Russia, and parts of North America. Most subsequently described genera have been from less-studied areas, or from subterranean habitats (Cook 1975, Rodriguez & Giani 1987).

With the exception of *Styloscolex tazawaensis* n. sp., taxonomic affinities of the new species discussed here are not clear, and they cannot confidently be assigned to existing genera defined on the basis of species from other regions. As material is limited, we have attempted a conservative solution, erecting a single genus (*Honshudrilus*) for two rather different species, provisionally assigning *S. tatsukoae* n. sp. to the closest matching taxon *Styloscolex* (*Neoscolex*), and postponing the formal descriptions of two additional species until sufficient material is collected. In addition to eventually clarifying these issues, it seems likely that further sampling will show that the lumbriculid fauna of Japan is not only diverse, but represents undescribed lineages. This may be comparable to the situation in southeastern North America, where several genera have only recently been described, based on recent collections (Fend & Lenat 2007, 2010; Rodriguez et al. 2014; Fend et al. 2015).

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Astacopsidrilus hibernicus sp. nov. (Phreodrilidae, Oligochaeta, Annelida) from Irish peatlands

RÜDIGER M. SCHMELZ[1,2*], MÅRTEN J. KLINTH[3], RACHEL WISDOM[4,5] & THOMAS BOLGER[4]

[1] *IfAB Institute of Applied Soil Biology, Tomberg 24a, 22337 Hamburg, Germany*

[2] *University of A Coruña, Science Faculty, GIBE, Evolutionary Biology Group, CICA, Centro de Investigaciones Científicas Avanzadas, As Carballeiras s/n, Campus de Elviña, 15071 A Coruña, Spain*

[3] *Department of Biological & Environmental Sciences, University of Gothenburg, P.O. Box 463, SE-405 30 Gothenburg, Sweden. E-mail: marten.klinth@bioenv.gu.se*

[4] *School of Biology and Environmental Science, University College Dublin, Belfield, Dublin*

[5] *Department of Agriculture, Food and the Marine, Backweston Campus, Celbridge, Kildare, Ireland*

[*] *Corresponding author, Email: rmschmelz@gmail.com*

Abstract

The discovery of a large and flourishing population of Phreodrilidae in terrestrial peatlands in northwest Ireland was surprising on two counts: these oligochaete worms are usually aquatic and most of the species occur in the Southern Hemisphere. The phreodrilids were discovered in a project that targeted Enchytraeidae, therefore methods adapted to the investigation of enchytraeids could be applied, including the study of living animals and properly fixed whole mounts. DNA sequencing was also performed. All worms identified here belong to one species, new to science, and placed in the genus *Astacopsidrilus*, because of the ventral position of the spermathecal pores and the opening of the female funnels inside the spermathecal vestibule. *Astacopsidrilus hibernicus* **sp. nov.** is mainly distinguished by thick segmental cushions of epidermal gland cells on the dorsal side of the posterior body half. Male sexual organs and spermathecae are comparatively small and without the often-observed bizarre modifications common in species of this family. DNA sequencing yielded a fragment of the 16S rRNA gene. This is the first description of a phreodrilid species from Europe; the few previous recordings of this family in Ireland and the United Kingdom had been left unidentified.

Keywords: Clitellata, Irish fauna, taxonomy, Enchytraeidae

Introduction

Phreodrilidae are a small family of aquatic oligochaetes mainly distributed in the Southern Hemisphere; southern Australia and New Zealand harbour most of the species. The global distribution, which includes South America, Africa, the Arabian Peninsula, Sri Lanka, and subantarctic islands, indicates a Gondwanan origin of the family (Martin *et al.* 2008). Phreodrilids are mainly cool-adapted freshwater species; when recorded from warmer environments, their habitat is groundwater (Martínez-Ansemil *et al.* 2002; Pinder 2008). There are also records from acid (dystrophic) peatland waters (Pinder & Brinkhurst 1997), from brackish water (Pinder & Erséus 2000), and from beech forest litter (Stout 1958)—the latter being the only record thus far documenting the presence of phreodrilids in a terrestrial habitat. Records of this family outside the former Gondwana landmass are very scarce, and they are based on a very limited number of specimens: *Insulodrilus ryuteki* Martin & Ohtaka, 2008 from the profundal of Lake Biwa, Japan (one specimen); *Insulodrilus* sp. from the Thames estuary in London, U.K. (Pinder *et al.* 2013) (eight specimens), and one unidentified phreodrilid specimen from a small stream in Northern Ireland (Gunn *et al*. 2003); all specimens were amputees, i.e. the posterior end of each specimen was missing.

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Schmelz *et al.* (2015) reported an unexpectedly large and flourishing population of Phreodrilidae in terrestrial peatlands in northwest Ireland. More than 100 specimens of phreodrilids were sampled in the framework of the project *BOGLAND: Sustainable Management of Peatlands in Ireland* (Renou-Wilson *et al.* 2011), which included the study of invertebrate taxa (Wisdom 2010) at intact, damaged and restored peatland sites. Among the targeted groups, which included mites, beetles and spiders, were Enchytraeidae, which are abundant and dominant in peatlands not only in Ireland (Healy 1979; Healy & Bolger 1984). Enchytraeids are obtained for study by extracting them from soil samples in the laboratory, and they are then identified *in vivo* because the taxonomically important structures, mostly soft-bodied internal organs, are best seen in living animals. Suitable specimens may later be fixed in Bouin's fluid, stained, and whole-mounted for more detailed investigation (see Schmelz & Collado 2010 for details). This procedure was also applied to the phreodrilids found in the samples, which allowed an investigation in greater detail than would have been possible with ethanol-preserved specimens. All specimens belonged to one species, which is described as new to science in this paper. It was first considered to belong to *Insulodrilus* Brinkhurst, 1965 (Schmelz *et al.* 2015); its placement here in *Astacopsidrilus* Goddard, 1909 is explained further below.

Material and methods

Sampling began in October 2008 with a preliminary screening of the oligochaete fauna at three different peatland sites and was extended in May 2009 to a systematic sampling of 11 different peatland sites (Fig. 1A). The sites were located in fens, raised bogs, and blanket bogs. Sampling at each site was limited to the upper 5 cm of soil. Extraction, investigation and identification of worms in each sample was completed at the University of A Coruña, Science Faculty. Worms were extracted from soil samples using a modified version of O'Connor's (1967) wet/heat funnel device, then stored in water for a maximum of 3 days. Specimens were then studied *in vivo* and as stained whole mounts using dissecting microscope and light microscope with differential interference contrast (Nomarski) optics, following protocols as outlined in Schmelz and Collado (2010).

The dominant species in all samples was *Cognettia sphagnetorum* (Vejdovský, 1878). However, this species was recently split into several species based on DNA-sequences, of which one has retained the name *sphagnetorum* (Martinsson & Erséus 2015, there as *Chamaedrilus sphagnetorum*). It is most probably this species that was present in the peatland samples; it is congruent with the morphological "variant B" in Chalupský (1992) and Schmelz and Collado (2010: 81f.)—a form that prefers moist to wet habitats. Phreodrilids were found in large numbers in samples collected from two sites, Knockmoyle and Owenanirragh. Knockmoyle, the type locality for *Astacopsidrilus hibernicus* **sp. nov.**, is a state-owned site at Bellacorick, Co. Mayo, 1,198 hectares in area (Fig. 1B). It has extensive pool systems, flushes, shrub and grassland communities, dominated by the fen sedges *Schoenus nigricans*, *Eriophorum angustifolium* and *Rhynchospora fusca* (Cyperaceae). Owenanirragh (Fig. 1C) is an isolated area of Atlantic blanket bog, located 6 km NE of Glenamoy, Co. Mayo—approx.14 km NNW of the type locality. The dominant plant species is *Schoenus nigricans*. Restored sites, dominated by *Molinia caerulea*, *Calluna* sp. and *Rubus* sp., are present as well, although these sites were not sampled for oligochaetes.

DNA sequencing was carried out at the Department of Biological and Environmental Sciences, University of Gothenburg, Sweden. DNA was extracted from amputated posterior ends of four specimens, using QuickExtract DNA Extraction Solution 1.0 (Epicentre, Madison, WI, USA). The extracts were then used to run PCR for Cytochrome C Oxidase C subunit I (COI) (following Folmer *et al.* 1994), and a shorter part of 16S using the primers Ann16SF and Ann16SR following Sjölin *et al.* (2005). Sequencing was conducted by MWG Eurofins Operon (Edersberg, Germany), sequences were assembled in Genious 6.1.8 (https://www.geneious.com, Kearse *et al.* 2012), and deposited in GenBank.

In the description, *ectal* and *ental* parts of a structure are oriented towards the periphery and the center of the body, respectively. Type material is deposited at the British Museum of Natural History (NHMUK ANEA) and at the National Museum of Ireland (NMINH). Further reference material is in the first author's personal collection.

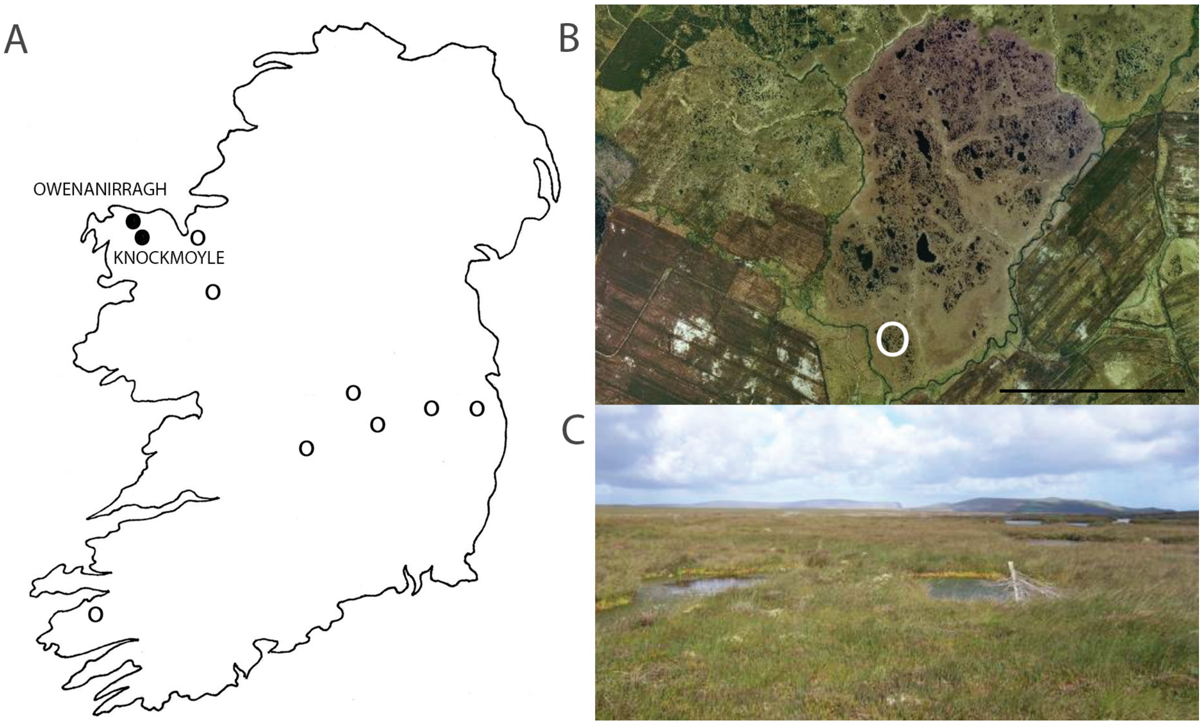

FIGURE 1. *Astacopsidrilus hibernicus* **sp. nov.**, sampling localities. **A.** Map of Ireland with localities of bogs and fens sampled for invertebrates in the BOGLAND project. Filled circles: Sites with *Astacopsidrilus hibernicus* **sp. nov.** The two sites are 14 km apart. The third site where this species was found (Bellacorick, 1 specimen) is too close to Knockmoyle, the type locality, to be indicated separately. Open circles: Sites without that species. **B.** Aerial photograph of the type locality, the Atlantic blanket bog at Knockmoyle, Co. Mayo. White circle: Sampling site. Scale bar: 1 km. **C.** View of the site at Owenanirragh. **B**,**C** from Wisdom (2010).

Abbreviations used in Figures 2, 3

aa atrium apically (blind-ending), **ab** atrium basally (connecting with vas deferens, closer to male pore), **br** brain, **ch** chloragocytes, **cy** spermatogonial cysts in different stages of sperm development, **eg** epidermal gland cells, **ff** female funnel, **ga** ganglia on pharyngeal pad, **gc** clitellum, granulocytes, **hc** clitellum, hyalocytes, **im** intracoelomic dorso-ventral muscle strands, **ne** nephridium, **ov** ovary, **pe** penis, **pm** dorsoventral intra-prostomial musculature, **pp** pharnyngeal pad, **rm** retractor muscles of pharyngeal pad (Fig. 2A,B) or penis and spermathecal vestibule (Fig. 3A), **sa** spermathecal ampulla, **sf** sperm funnel, **st** spermathecal sperm trap, **sv** spermathecal vestibule, **te** testis, **vn** ventral nerve cord.

Results

Astacopsidrilus hibernicus Schmelz, sp. nov.

(Figures 2, 3)

Holotype. NHMUK ANEA 2019.7340, adult specimen, fixed in hot Bouin's fluid, stained with Paracarmin, whole-mounted in Canada-Balsam between two coverslips. Type locality: Ireland, Knockmoyle, Co. Mayo, 54°09'N, 9°34'W. Habitat: Atlantic blanket bog. Soil sample taken by R. Wisdom, Oct. 2008.

Paratypes. NHMUK ANEA 2019.7341–7346, 6 specimens (4 adult, 2 juvenile), same data as holotype. NMINH 2019.3.1–3.6, 6 specimens (4 adult, 2 juvenile), one per slide and accession number, same data as holotype.

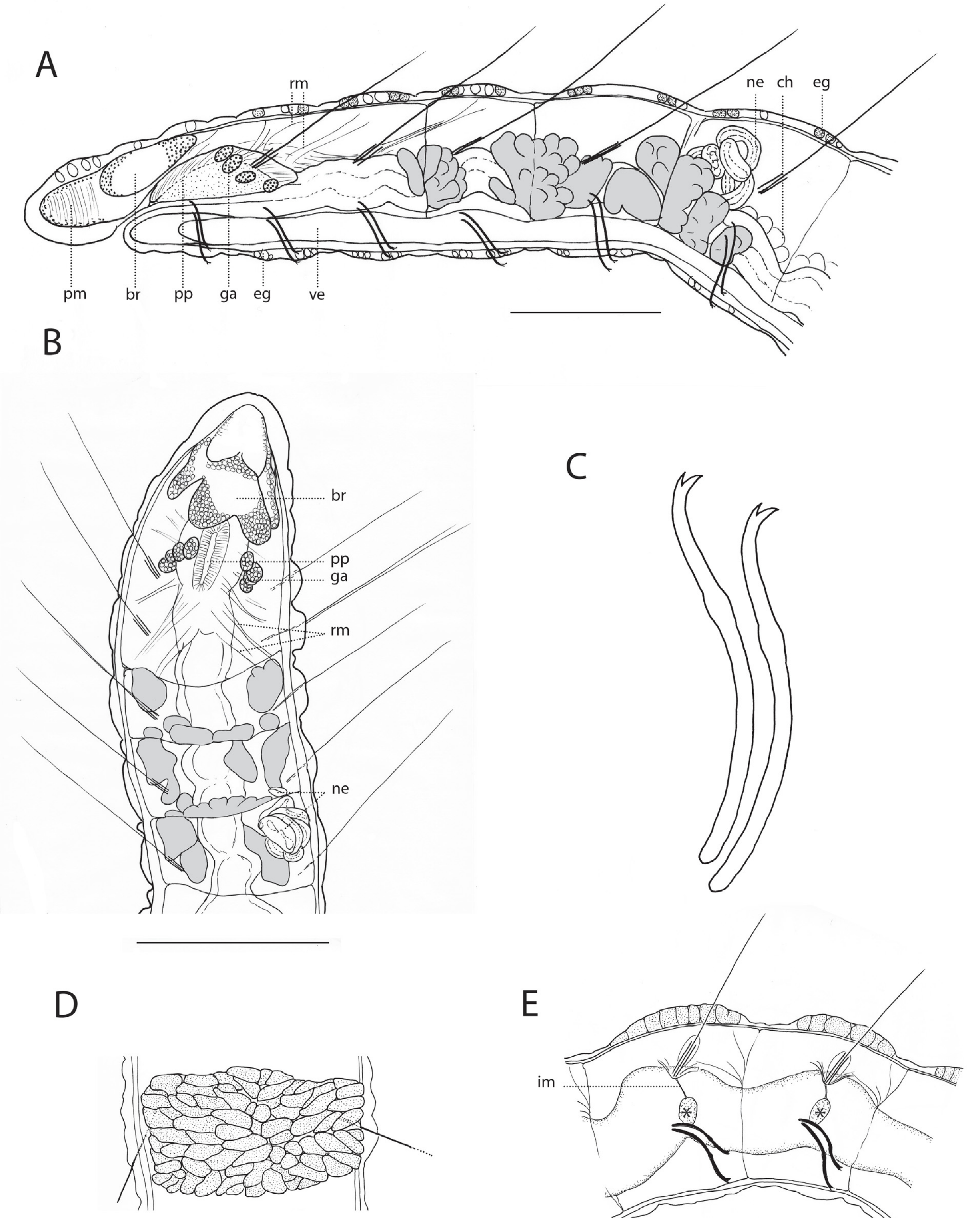

FIGURE 2. *Astacopsidrilus hibernicus* **sp. nov.**, drawings from stained whole mounts. **A.** Anterior body, lateral view. The nephridium in VII is on the right side, the nephrostome therefore hidden behind the pharyngeal glands (shaded grey). **B.** Anterior body, dorsal view. Circles in brain and pharyngeal ganglia represent perikarya, their size being approximately accurate. **C.** Ventral chaetae of VII. **D.** Dorsal cushion of epidermal gland cells in a posterior segment, dorsal view. **E.** Posterior body region, 2.5 segments, lateral view, with dorsal cushions of epidermal gland cells. Asterisks: parachaetal bodies. All scale bars: 100 µm.

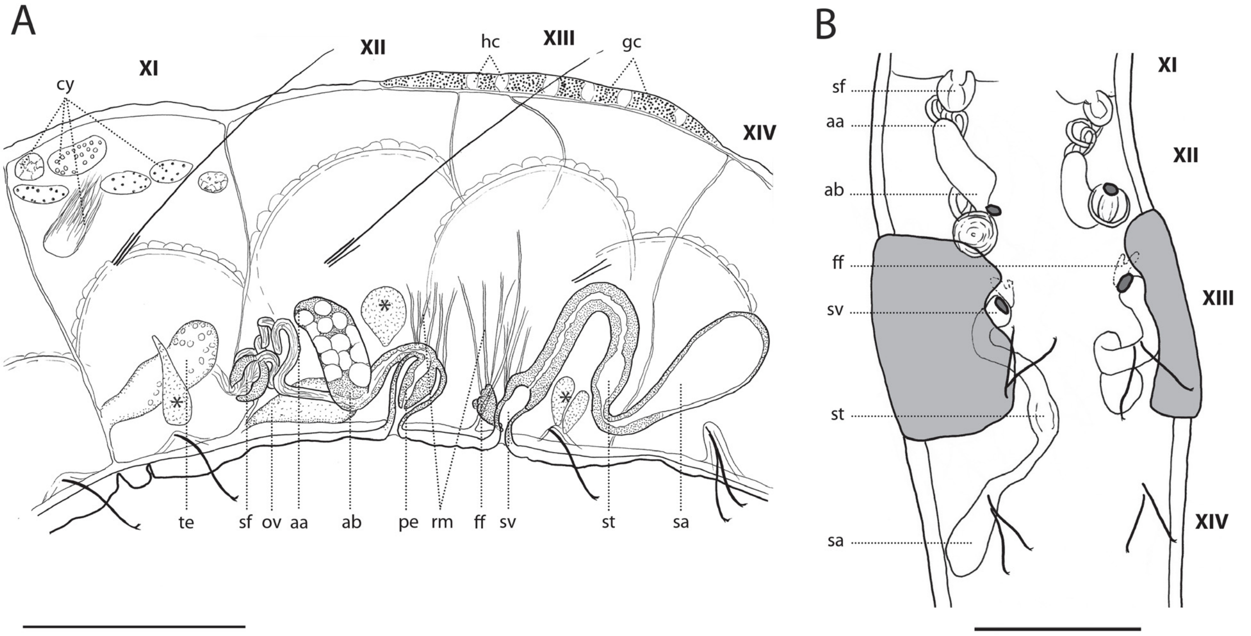

FIGURE 3. *Astacopsidrilus hibernicus* **sp. nov.**, gonadal and clitellar region (segments XI–XIV), drawings from stained whole mounts. **A.** Lateral view. The canal of the spermathecal ectal duct is less conspicuous than shown here. Asterisks: parachaetal bodies. **B.** Ventral view, semi-schematic. Shaded grey: clitellum. Dark-grey: male pores in XII and pores of spermathecal vestibules in XIII. Dotted lines: female funnels. All scale bars: 100 µm.

Additional material. 36 specimens (21 adult or subadult, 15 juvenile), stained whole mounts, same data as holotype.—15 specimens, adult, fixed and preserved in 70% ethanol, Owenanirragh, Co. Mayo, 54°16'N, 9°37'W, Atlantic blanket bog; soil samples taken by R. Wisdom, V 2009. Of these, four specimens processed for DNA sequencing, GenBank accession numbers of 16S rRNA gene sequence fragments MK837018, MK837019, MK837020, MK837021.—Three adult specimens, fixed in Bouin's fluid, preserved in 70% ethanol, Owenanirragh, same sampling data as above.—110 specimens, identified *in vivo*, not preserved, Owenanirragh, same sampling data as above.—One subadult specimen, investigated and identified *in vivo*, not preserved; Bellacorick flush, Co. Mayo, 53°11'N, 6°50'W, fen; soil sample taken by R. Wisdom, V 2009.

Etymology. "hibernicus" (Latin) = Irish.

Diagnosis. Small phreodrilid worms, live body length of adult specimens c. 3–4 mm; length in whole mounts 1.5–3 mm, diameter at V 0.12–0.15 mm, at XIII 0.17–0.23 mm, at XVIII 0.14–0.2 mm; 31–42 segments. Dorsal chaetae from III, one hair per bundle with a pair of support chaetae, hairs 120–180 µm long in IV–VIII, shorter in posterior segments. Ventral chaetae from II, two bifids per bundle with upper tooth half as long and half as thick as lower tooth. In adults, hairs absent in XIII and ventral chaetae in absent XII, present in XIII, not modified. Epidermal gland cells circumferal in anterior segments, forming thick dorsal cushions in posterior segments. Pharyngeal glands in (IV)V–VII. Nephridia unpaired, at VII and X in preclitellar segments, positions variable in postclitellar segments. Clitellum in 1/2 XII–XIII, saddle-shaped. Testes in XI, ovaries in XII. Developing sperm not abundant. Male efferent apparatus comparatively small, with spherical funnel at 11/12, vas deferens entering atrium basally, and short conical penis in XII. Male pores in line with ventral chaetae. Spermathecae comparatively small, confined to XIII or extending into adjacent segment, with ampulla, sperm trap and short vestibule, opening ventrally behind 12/13. Female funnel and duct plesiopore, opening into vestibule, close to body surface. One mature egg present at a time.

Description. Living worms transparent, habitus *Cernosvitoviella*-like. Live body length 3–4 mm, diameter 0.15–0.18 mm; clitellar region conspicuously widened in both living and preserved adult animals. Preserved adult worms 120–150 µm at V, 170–250 µm at XII, 140–180 µm at XVIII (N=19). Segments 30–42 in adults, fewer in juveniles (18–24 counted).

Dorsal chaetae from III, inserting latero-dorsally, above lateral lines; one hair (rarely 2) per bundle, each hair with a pair of minute and thin support chaetae entally, confined in the chaetal sacs. Hairs largest in anterior segments, maximum length 150–190 µm in IV–VIII, width at base c. 2 µm; hairs in posterior segments shorter and thinner, length c. 80–120 µm. Hairs continuously tapering ectad. Support chaetae always parallel to hairs, on opposite sides, confined to chaetal sacs, i.e. not protruding, c. 30–36 µm long. Ventral chaetae 2 per bundle, all alike, sigmoid with nodulus below ectal 1/3, bifid with upper tooth half as long and thick as lower tooth (Fig. 2C); measured lengths 45–52 µm in III–XI and in posterior segments, 30–40 µm in II and in mid-body segments posterior to clitellum. Rarely a supernumerary bundle with 1 or 2 extra-chaetae. Chaetae shifted backwards in a segment, i.e. nearer to posterior than to anterior septum. At sexual maturity ventral chaetae absent in XII, dorsal hair chaetae absent in XIII, but support chaetae still observed in XIII (Fig. 3A). No modified (spermathecal or penial) chaetae.

Prostomium rounded, slightly longer than wide *in vivo*, as long as or shorter than wide in fixed (contracted) specimens, here length c. 50–60 µm. Epithelium with smooth outer and inner surface, prostomial lumen with transverse dorso-ventral musculature at peripheries (Fig. 2A: pm). Proboscis absent. Body wall thin (3–8 µm), consisting of cuticle (visible at x400 magnification, thickness < 1 µm), epidermis, ring muscles (single-layered, continuous) and longitudinal muscles. Lateral lines slightly shifted dorsad. Epidermal gland cells of two types, staining and non-staining. Staining cells oval, hyaline, absent at intersegmental furrows; in anterior segments present dorsally, laterally and ventrally, most dense in anterior 7 segments, here in two segmental rows, one anterior, the other one posterior to the chaetae. Between the rows, at chaetal level, non-staining cells present (Fig. 2A: eg). From VIII on increasingly fewer cells, scattered over body surface, not in rows. In posterior segments, beginning from XV or further back, epidermal gland cells forming thick segmental cushions, conspicuous in living and preserved material; cells densely packed with non-staining vesicles or secretory bodies (Fig. 2D,E). Cushions increasing in size and thickness caudad. Body surface below cuticle and cells staining.

One or two segmental drop-shaped or ellipsoid bodies latero-ventral beneath body wall, extending from ventral chaetae dorsad, with smooth outline and non-staining vesicular content similar to the dorsal epidermal cushions; named here 'parachaetal bodies' (Figs. 2E, 3A). Thin intra-coelomic dorso-ventral muscle strands present, close to inner lateral sides of body wall, extending from ventral chaetae to dorsal chaetae; insertion dorsally below lateral line (Fig. 2E). Septa not closed, developed as dorso-ventral strands open dorsally and ventro-laterally. Coelom almost empty, coelomocytes probably absent, but some cells visible in coelom (detached chloragocytes?), free or attached to coelomic lining, transparent, with vesicles of irregular size and shape.

Brain (Fig. 2A,B: br) with four posterior lobes and three incisions; the outer lobes attached to dorsal side of circumoesophageal connectives. Ventral nerve cord medullar in I–VI, from VII on perikarya increasingly concentrated in mid-segmental region as ganglia.

Pharyngeal pad in 1/2I–1/2III to II–III, longer than wide, as a dome-shaped upfolding of the dorsal epithelium, with retractor muscles attached that extend as far back as into V (Fig. 2A,B: pp). Pharyngeal glands paired, in (IV),V–VII, as more or less compact, racemose aggregations of cells, shape, position and number of aggregations ("lobes") differing among specimens, attached to anterior and/or posterior sides of septa, or to body wall, or free (Fig. 2A,B). Four pairs of pea-shaped ganglia laterally on pharyngeal pad (Fig. 2A,B: ga), arranged in a line from antero-dorsal to postero-ventral, lined by retractor muscles. Pharynx and esophagus densely ciliated; ciliation abruptly less dense (cilia shorter?) at 1/2 VIII, here gut often widened abruptly and with thin walls. Intestinal walls thicker and with inclusions of unknown nature from XIV on.

Nephridia (Fig. 2A,B: ne) unpaired, with small ventro-lateral nephrostome attached to septum, a postseptal thickening and numerous subsequent densely arranged loops with almost no interstitial tissue; nephropore ventro-lateral anterior to ventral chaetae, terminal vesicle absent. Nephridial loops usually confined to the right or the left half of a segment, where the nephroporus is located, i.e. rarely extending into the opposite half, or into an adjacent segment. Location of anterior nephridia always in VII and X, juveniles included. Location of posterior nephridia variable: third nephridium in XVII in 11 out of 20 investigated specimens, further back in the rest (XX–XXVII); nephridia never in adjacent segments, never two nephridia in one segment. Altogether 4 to 8 nephridia in an individual.

Dorsal blood vessel running through all segments, closely attached to gut surface except in foremost segments (point of detachment not seen). One lateral commissure seen in III. Ventral blood vessel not attached to gut. Chloragocytes from VII.

Clitellum (Fig. 3A,B) conspicuous, elevated, saddle-shaped (= absent mid-ventrally), short, beginning immediately behind chaetae of XII, ending at level of septum 13/14. Total extension slightly less than 1.5 segment lengths. Cells in reticulate pattern, more granulocytes than hyalocytes (Fig. 3A: gr, hc). Gonadal region in the usual position, not shifted anteriad; all reproductive organs except clitellum paired.

Testes (Fig. 3A: te) paired in XI, club-shaped, widely separate, attached to posterior face of septum 10/11. Developing sperm not abundant, few cysts floating freely in coelom of XI (Fig. 3A: cy), or further anteriorly. No seminal vesicle. Sperm funnels (Fig. 3A,B: sf) attached to 11/12 ventro-laterally; each funnel an oval body with smooth outline (length c. 20 µm, diameter c. 15 µm), lumen with medial constriction. Vas deferens (Fig. 3A,B) ciliated throughout, densely and irregularly coiled immediately behind funnel, diameter 4 µm. Atrium (Fig. 3A,B: aa, ab) rounded, compact and with smooth outline, length c. 50–60 µm, 2–3x as long as wide, apically blind-ending, basally attached to distal stretch of vas deferens. Atrial walls muscular, atrial lumen densely filled with c. 20, roughly spherical, non-staining gland cells; no lumen distinguished in atrium. No gland cells at junction of atrium with vas deferens. Ectal stretch of vas deferens widened (c. 8 µm), shorter than length of atrium, attached to penis sac apically. Penis (Fig. 3A,B: pe) lemon-shaped, longer than wide, pointed distally, length c. 20 µm, spherical in top view (Fig. 3B), embedded in penial sac with walls 2–3 µm thick. Dorso-ventral retractor muscles inserting at ental roof of penis sacs (Fig. 3A: rm). Male pores in line with ventral chaetae, anterior to ventral clitellum (Fig. 3B).

Ovaries in XII (Fig. 3A: ov). Female funnels (Fig. 3A,B: ff) ventro-laterally at 12/13, adjacently anterior to spermathecal vestibules; female pores close to body surface but still inside vestibules, plesiopore. Spermathecae (Fig. 3A,B: sa, st, sv) simple, slightly longer than body diameter, confined to XIII or extending into XIV (rarely into XII), tube-like, with three thickened areas: vestibule ectally, sperm trap medially and ampulla entally. Vestibule not elongate or enlarged, c. 1.5x as long as wide (e.g. 30 µm by 18 µm), c. 1.5x as wide as duct, ciliated. Dorso-ventral retractor muscles inserting on and near anterior face of vestibule. Duct c. 80 µm long, with smooth outer and undulating, inconspicuous, inner surface, i.e. duct lumen indistinct. Sperm trap slightly wider than duct, with distinct lumen and flat walls, followed by club-shaped ampulla, diameter 20–60 µm. Few spermatozoa seen in sperm trap, ampulla filled with a hyaline yellowish mass, here spermatozoa not distinguished. One mature egg at a time, extending over 2–3 segments, posteriorly into XIV or XV.

DNA-barcoding. All four specimens were successfully sequenced for the shorter 16S fragment (GenBank) accession numbers MK837018, MK837019, MK837020, MK837021), but did not produce any successful COI sequences, possibly due to DNA degradation or primer mismatch. The 16S sequences could not be matched with any published material on GenBank. However, when compared with 16S sequences from Australian phreodrilids and some European Naididae (C. Erséus, unpublished data), the Irish species was clearly a member of Phreodrilidae, but with a genetic distance of about 20 % from the Australian specimens.

Discussion

Generic placement. Among the two subfamilies currently recognized in Phreodrilidae (Pinder & Brinkhurst 1997), the new species belongs to Phreodriloidinae, characterized by a ventral position of spermathecal pores (Brinkhurst 1991). (In the second subfamily, Phreodrilinae, spermathecal pores are located laterally or dorso-laterally.) Within this subfamily we place this species in *Astacopsidrilus* Goddard, 1909 because it is the only genus that includes species where the female pores are located inside the spermathecal vestibules and not separately on the body surface, as is usual in Oligochaeta. In this we follow Pinder & Brinkhurst (1997) who use this character to decide whether a species is to be placed in *Astacopsidrilus* or in the otherwise very similar *Insulodrilus* Brinkhurst, 1965. In a morpho-cladistic analysis (Brinkhurst 1991), the intra-vestibular position of the female pores qualified as an autapomorphy of a monophyletic *Astacopsidrilus*, whereas *Insulodrilus* remained paraphyletic. The two genera in their current circumscription are problematic and in need of revision (Pinder & Brinkhurst 1997; Pinder 2008). A main difficulty here is the fact that the key character to distinguish the genera—the location of the female pore—is unknown in many species; it is not even known in the type species of both genera. (The type species of *Insulodrilus* is *Phreodrilus lacustris* Benham, 1903, that of *Astacopsidrilus* is *Astacopsidrilus notablis* Goddard, 1909). Furthermore the distinction 'inside vs. outside the spermathecal vestibule' is a "character of degree" (Pinder 2008), and often quite subtle, especially when the female pores are located near the opening of the vestibule (compare Pinder & Brinkhurst 1997, Figs. 86, 90 for *Insulodrilus* with Pinder & Brinkhurst 1997, Figs. 149, 164 and Pinder 2003,

Fig. 2 for *Astacopsidrilus*). In the new species, however, its intravestibular position is beyond doubt, as there are only four pores on the body surface of the gonadal region, one pair of male pores and one pair of vestibular pores (Fig. 3B). It should also be mentioned that—comparing all available species descriptions—modified genital chaetae are absent more often in *Astacopsidrilus* than in *Insulodrilus*: we counted 6 out of 11 species, 55%, for *Astacopsidrilus*, and 3 out of 14 species, 21%, for *Insulodrilus.*

On the other hand, the moderate size of the spermathecal vestibule of the new species is in marked contrast to most species of *Astacopsidrilus*, where the vestibules are usually large, deep, and with strong muscular investment that connects them with the dorsal or dorso-lateral side of the body wall. Here, species of *Insulodrilus* are more similar. But this difference is not absolute, as species with large vestibules and strong musculature have been allocated in *Insulodrilus*, e.g., *I. tanganyikae* (Brinkhurst, 1970), while *Astacopsidrilus* includes also species with modest vestibules and poor musculature, e.g., *A. naceri* Giani *et al.*, 1995. Note that vestibular musculature is also present in the new species (Fig. 3A: rm).

[Linguistic side note: We prefer 'vestibules' over the—in our opinion—erroneous latinized plural 'vestibulae', which has become customary in the taxonomic literature on phreodrilids since Brinkhurst (1970). 'Vestibulae' is feminine, while the Latin noun is neuter: 'vestibulum', with the nominative plural 'vestibula' (correctly in Brinkhurst 1965)].

Species separation, character discussion. *Astacopsidrilus hibernicus* **sp. nov.** is among the smallest species of Phreodrilidae described to date. Only *Antarctodrilus micros* Pinder & Brinkhurst, 1997 is similarly small, with up to 34 segments and a recorded body size of 1.4–3.7 mm length and 0.14 mm diameter. This species differs from *A. hibernicus* in dorso-lateral spermathecal pores, sperm sacs extending over several segments, and a forward shift of the gonadal region, among other characters. The smallest species within *Astacopsidrilus* are those with an ectocommensal lifestyle on crayfish—*A. notabilis* Goddard, 1909 (53 segments, length 5.5. mm, width up to 0.65 mm), *A. fusiformis* Goddard, 1909 (46 segments, length 2.8 mm, width 0.6 mm) and *A. jamiesoni* Brinkhurst, 1991 (<50 segments, length up to 4.5 mm, width 0.6 mm). Conspicuous differences to *A. hibernicus* are stoutness of the body (or contractility, if the low length:width ratio is a fixation artefact), much reduced teeth of the ventral chaetae, and much longer atria. *Astacopsidrilus ryuteki* Martin & Ohtaka, 2008 is also small (length 3.1 mm, width up to 0.35 mm, 24 segments, one posteriorly incomplete specimen). Conspicuous differences of this species, found in the profundal of Lake Biwa, Japan, to *A. hibernicus* are presence of spermathecal chaetae, a very long atrium, and dorsal chaetae that resemble the ventral ones. The smallest species in *Insulodrilus* is *I. angela* Pinder, 2008 (c. 6 mm by up to 0.375 mm, segment number unknown). Conspicuous differences to *A. hibernicus* are a long and folded atrium, presence of spermathecal chaetae, and a vestibule with narrow, duct-like lumen.

The fragments of two hitherto unnamed phreodrilid taxa previously recorded from Europe are also very small, but clearly different from *A. hibernicus*. The single amputee (with the posterior body end missing), described in Gunn *et al.* (2003) from a small river in Northern Ireland, is 2.8 mm long, it has spermathecal chaetae and dorsal support chaetae with a flared distal tip. Eight amputees collected in an intertidal area of the upper Thames estuary in London (Pinder *et al.* 2013) are c. 3 mm long and up to 0.25 mm wide; subadult specimens have spermathecal chaetae and an elongate though small spermathecal vestibule. They resemble *Insulodrilus lacustris* (Benham, 1903) or related species (Pinder *et al.* 2013).

Astacopsidrilus hibernicus **sp. nov.** has sperm-related sexual organs (atria, penes, vestibules, spermathecae) that are smaller or shorter than in the other species of the family, and much less convoluted. For example, there is only one other species, *Antarctodrilus micros* Pinder & Brinkhurst, 1997, in which the atrium is no more than 3 times longer than the width, and even in this species it is so big that it occupies most of the segment. In fact, the gonadal region of the new species looks like the 'bauplan' from which all the other variations and sometimes bizarre modifications—at least in *Antarctodrilus*—can be derived. Likewise, the chaetal equipment, which is without peculiarities, can also be considered as the unmodified basic condition in Phreodrilidae. With respect to this 'classical' set of characters (i.e. those commonly considered in species descriptions: chaetae and reproductive organs), and viewed from a phylogenetic perspective, *A. hibernicus* seems to be characterized mainly by plesiomorphies. However, the opposite is possible as well, namely apomorphic reduction of structures.

The truly distinguishing character of this new species is the thick dorsal cushion of epidermal gland cells in posterior body segments (Fig. 2D,E). The two types of epidermal gland cells in the anterior body region are also noteworthy (Fig. 2A) as are the parachaetal bodies between the dorsal and ventral chaetae, attached to the inner face of the body wall (Figs. 2E, 3A). Unfortunately, we have not found any further consideration of these or comparable structures in species descriptions of other Phreodrilidae, so the uniqueness of these features cannot be demonstrated here—they may simply have been overlooked. In fact, parachaetal bodies were detectable *only* in the whole mounts stained with Bouin's fluid. The thick postero-dorsal glandular cushions, however, are as thick and as conspicuous as the clitellum, which is usually described or illustrated. It may therefore be a structure that is truly absent or at least very rare in the rest of Phreodrilidae that have been described to date. Their function is unknown, but their dorsal position—together with the terrestrial habitat of the species (see below)—suggests that these glandular cushions may secrete fluid as a lubrication to suppress desiccation.

Habitat and distribution. A further peculiarity of this new species is the terrestrial habitat. The description of the sites in Wisdom (2010) as well as the occurrence of oribatid and gamasine mites, carabid beetles, and spiders leave no doubt that the sites with *A. hibernicus* are terrestrial and not aquatic. The accompanying oligochaete fauna consisted only of enchytraeids, dominated by *Cognettia sphagnetorum* (Vejdovský, 1878), together with a few specimens of *Cernosvitoviella* sp., *Marionina clavata* Nielsen & Christensen, 1961, *Achaeta affinis* Nielsen & Christensen, 1959, and *Mesenchytraeus glandulosus* Nielsen & Christensen, 1959. Considering the indicator values for enchytraeid species as presented in Graefe & Schmelz (1999), the presence of these enchytraeid species confirms the character of our collecting sites as moist to wet, but terrestrial. Terrestrial phreodrilid species have previously been described from beech litter in New Zealand (Stout 1958). In all of these cases, the microhabitat is probably aquatic: for example, including minute pools or water films around mineral soil and humus particles. Pinder & Brinkhurst (1997) record phreodrilids from Australian peatlands but the sites were definitely aquatic (Pinder, pers. comm.). Sampling at the more terrestrial peat sites will probably yield more terrestrial species of this family in regions of the Southern Hemisphere that are rich in phreodrilid species.

Astacopsidrilus hibernicus **sp. nov.** was found only in the peatlands located in the outer northwest regions of Ireland, even though the other peatland sites sampled during this study (Fig. 1) supported a similar oligochaete fauna—restricted to the enchytraeids *Cognettia sphagnetorum* (the dominant species) and accompanied by *Cernosvitoviella* spp. and a few specimens representing some of the more terrestrial species. Furthermore, *A. hibernicus* was abundant at two localities that are about 14 km apart from each other. This suggests an established population in an extended though clearly confined area.

The implications and alternative explanations for the presence of a taxon (Family Phreodrilidae) in Ireland, whose domain is the Southern Hemisphere ("invasion from down-under or ancient relict?"), have been discussed in Schmelz *et al.* (2015) and need not be repeated here. General aspects of dispersal of phreodrilids are discussed in Pinder and Brinkhurst (1997) and in Martin and Ohtaka (2008). More intensive sampling plus DNA-sequencing may help to find the correct explanation. The species name chosen expresses our opinion that—whatever its origin, travel route or date of arrival—this species should be considered as an established component of the Irish fauna.

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Preliminary report on freshwater oligochaetes from some districts of Tamil Nadu (India), along with DNA barcode sequence of a commercially important oligochaete in Chennai

SANJIB CHAKMA[1], SVANTE MARTINSSON[2], SIVABALAN SRINIVASAN[1], AHMED ARFATH GHANI[1], ROHIT ADAM[1] & MOHAMMED IBRAHIM NAVEED[1]

[1] *Department of Zoology, The New College, Chennai-14, India.*
[2] *Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden.*
Email: naveed_newcollege@rediffmail.com

Abstract

A survey of aquatic Oligochaeta was conducted at Thiruvallur, Chennai, Kanchipuram, Villupuram and Nilgiris districts of Tamil Nadu from October 2015 to June 2016. Eleven taxa were recorded from a total of 922 specimens examined, eight of them were identified to species level. Five naidids identified during the present study: *Branchiodrilus semperi*, *Dero dorsalis*, *Dero digitata*, *Dero indica* and *Dero zeylanica*, and six tubificid taxa *Branchiura sowerbyi*, Tubificidae sp. 1, Tubificidae sp. 2, Tubificidae sp. 3, *Limnodrilus hoffmeisteri* and *L. udekemianus. Dero dorsalis* constitute the first report for Kanchipuram district. A DNA barcode sequence (GenBank accession no. MF125273) of the commercially important *Limnodrilus hoffmeisteri*, commonly known as 'Tubifex worms' in Chennai, was obtained and compared with other published COI sequences from that morphospecies from around the world.

Key words: Freshwater oligochaetes, DNA barcode, Clitellata, COI, Tamil Nadu

Introduction

Studies on aquatic Oligochaeta from Tamil Nadu are scanty (Srinivasan *et al.* 2016). DNA barcode sequences of this group are absolutely lacking from this region, hence the objectives of this work are to 1) obtain a DNA barcode sequence of the commercially important freshwater oligochaete *Limnodrilus hoffmeisteri* and 2) to report on the diversity of aquatic Oligochaeta from some water bodies of Thiruvallur, Chennai, Kanchipuram, Villuppuram and Nilgiris districts of Tamil Nadu.

Study area and sampling sites (Figure 1): To understand the diversity of freshwater oligochaetes in Tamil Nadu, samples were collected at random from (1) Korattur Lake (13º07'2.89"N 80º10'40.47''E), (2) Ambatur Lake (13º06'52.42''N 80º08'25.68'' E), (3) Porur Lake (13º02'3.82" N 80º08'53.71" E) in Thiruvallur district, (4) Adyar River near Kotturpuram (13º01'33.89"N 80º14'32.06"E), (5) Velachery Lake (12º59'13.2" N 80º13'8.65" E), (6) Kallikattu Lake (12º58'40.05" N 80º14'19.16" E), (7) Taramani Lake (12º58'33.65" N 80º14'25.27" E) in Chennai district, (8) Kilkattalai Lake (12º57'28.15" N 80º11'26.15" E), (9) Lakshmipuram Lake (12º55'14.65" N 80º08'6.32" E), (10) Sholinganalur Lake (12º53'24.16" N 80º13'36.87" E), (11) Chengalpattu Lake (12º41'31.62" N 79º58'57.89" E) in Kanchipuram district, (12) Tindivanam Lake (12º14'6.07" N 79º38'52.13" E) in Villuppuram district and (13) Ooty canal (11º24'43.19" N 76º42'33.19" E) in Nilgiri district. Specimens for DNA studies were obtained from aquarium shops in Kolathur, Chennai.

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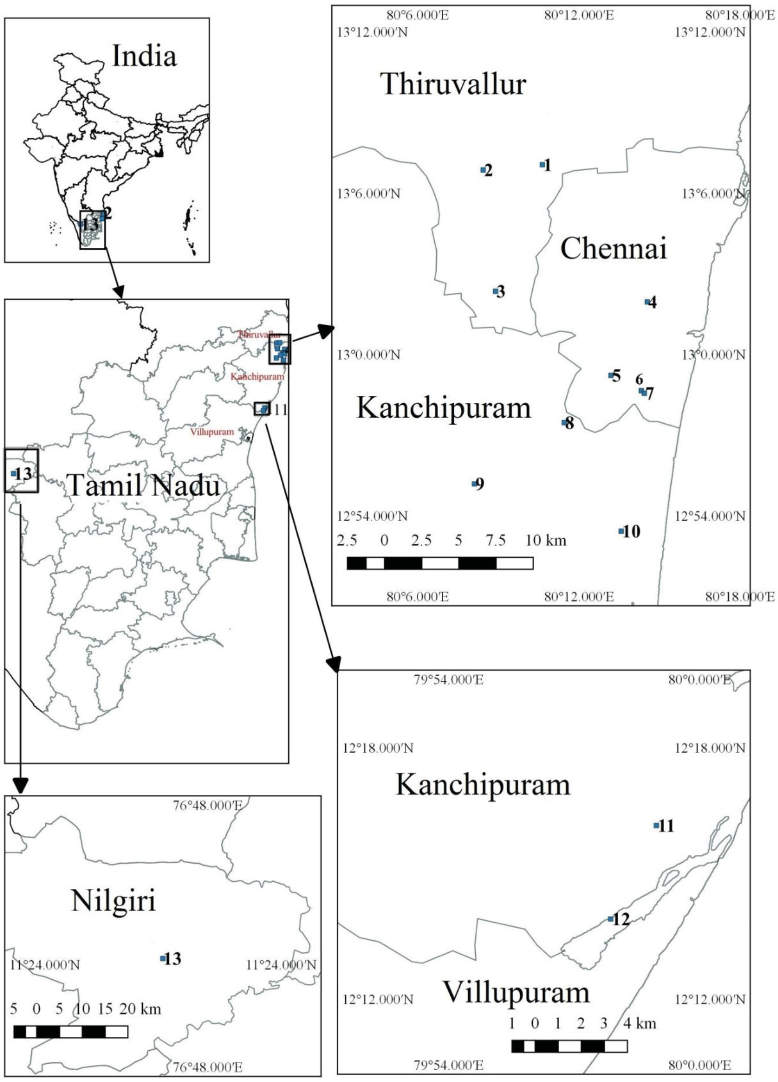

FIGURE 1. Map of sampling sites.

Materials and methods

Random sampling was done from October 2015 to June 2016, following the procedure of Srinivasan *et al.* (2016). Oligochaetes collected and separated from various aquatic vegetation and sediment samples were transferred into vials containing 10% formalin for preservation and storage. A few specimens collected from aquarium shops were preserved in 90% ethanol for sequencing studies as specimens preserved in formaldehyde cannot be used for sequencing (Haaren and Soors 2012). Live worms were examined under a cover slip in a drop of water. Preserved worms were mounted in a few drops of glycerin as temporary mounts for routine examination. A few permanent mounts were prepared using DMHF, also known as Hydantoin 739 or 5,5-Dimethylhydantoin Formaldehyde Resin. A phase contrast light microscope was used for examining the taxonomic characters. Identification of oligochaetes was done according to Brinkhurst and Jamieson (1971), Naidu (2005), Timm (2009) and Haaren and Soors (2012). Drawings were made using a compound microscope with camera lucida. For molecular studies the posterior tail region of a mature specimen identified as *Limnodrilus hoffmeisteri* was cut and preserved in 90% ethanol. Molecular analyses of the ethanol preserved specimens were done at the Biozone Lab, Chennai, following a phenol chloroform protocol Pachamuthu *et al.* 2000. The standard barcoding gene COI was amplified with the primers COI—5 FORWARD GGTCAACAAATCATAAAGATATTGG (25) and COI—5 REVERSE TAAACTTCAGGGTG

ACCAAAAAATCA (26), using the following PCR-program: initial denaturation at 94°C for 3 min followed with 32 cycles with 94°C for 1 min, 48°C for 1 min, and 72°C for 1 min 20 sec, and finishing with the final extension at 72°C for 7 min. DNA sequence obtained for identifying the specimen was compared to the database of known sequences in GenBank, using the Standard Nucleotide BLAST with default settings (search performed 2018-08-24). Analysis of the phylogenetic tree of our sequences and the 60 closest sequences from GenBank was estimated with Maximum Likelihood using MEGA 6 (Tamura *et al.* 2016). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The Tamura-Nei substitution model with a Gamma distribution were used. Branch support was estimated using 100 Bootstrap replicates.

The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Results

In total 11 taxa were recorded from a total of 922 specimens examined. Out of the 11 taxa, 8 were identified to species level and three to family level. *Dero dorsalis* constitutes the first report for Kanchipuram district. The systematic account of the taxa identified is given below:

Systematic account with taxonomic observations of the specimens examined

Family Naididae

Branchiodrilus semperi (Bourne, 1890)

Specimen examined: Chengalpattu Lake (Kanchipuram), 24.4.2016 (1 specimen).

Taxonomic observations: Length: about 4 mm. Worms brownish. Gills finger-like, dorso-lateral, two per segment starting from VI. Dorsal chaetae from VI. Hair chaetae smooth. Simple-pointed needle chaetae with a peculiar bayonet- shaped distal curve without nodulus (Figure 2A). Ventral chaetae 1–3 per bundle with distal tooth longer and thinner than proximal (Figure 2B). Posterior segments lacking gills from XX.

Remarks: Finger-like gills on the anterior part of the body and dark brown patch in the anterior segments were the most prominent character pointing towards *B. semperi*, which was originally described from Chennai (Madras) by Bourne (1890). However, it should be noted that Martin *et al.* (2018) concluded that the morphological characters used to separate the species in *Branchiodrilus* are not reliable, and that the number of species are much higher than previously believed. As our record is collected relatively close to the type locality it is possible that it represents the true *B. semperi*.

Dero dorsalis Ferronière, 1899

Specimens examined: Lakshmipuram Lake (Kanchipuram), 5.01.2016 (3 specimens) and 07.05.16 (10 specimens); Chengalpattu Lake (Kanchipuram), 24.4.2016 (3 specimens).

Taxonomic observations: Length 8–10 mm. Dorsal chaetae begin from IV with 1 hair and 1 needle. Hair chaetae about 250 to 350 µm long (Figure 2C). Needle chaetae bifid and bayonet-shaped, with distal tooth longer and thinner than proximal. Distal tooth longer and thinner than proximal also in the ventral chaetae (Figure 2D). Five pairs of gills observed in the branchial fossa.

Remarks: First report for Kanchipuram district.

Dero digitata (Müller, 1773)

Specimens examined: Porur Lake (Thiruvallur), 5.2.2016 (24 specimens).

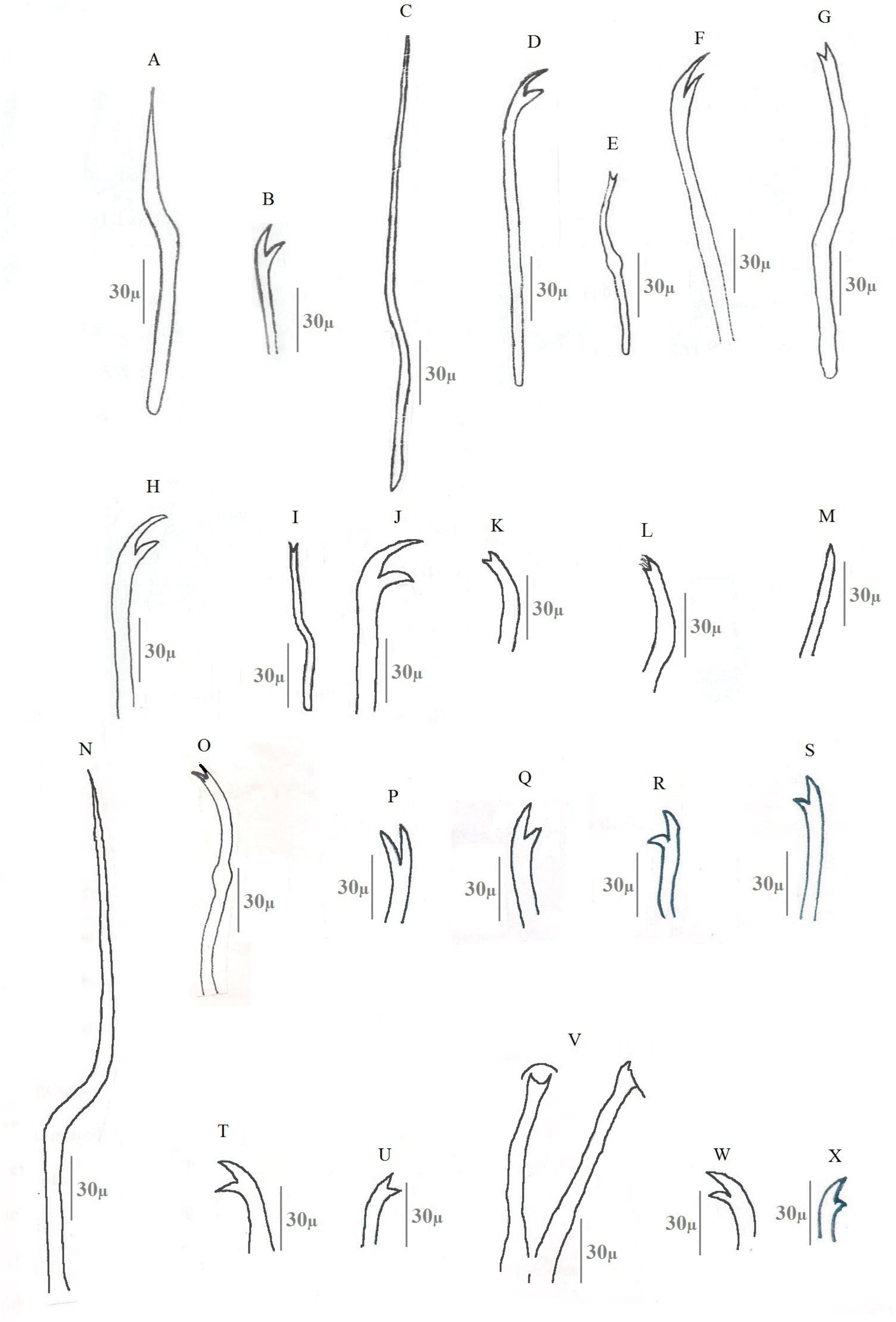

FIGURE 2. *Branchiodrilus semperi*: A) needle chaeta, B) ventral chaetae. *Dero dorsalis*: C) hair chaetae, D) ventral chaetae. *Dero digitata*: E) needle chaetae, F) ventral chaetae. *Dero indica*: G) needle chaetae, H) ventral chaetae. *Dero zeylanica*: I) needle chaetae, J) ventral chaetae *Branchiura sowerbyi*: K) Bifid chaetae, L) pectinate chaetae, M) Simple-pointed ventral chaetae. Tubificidae sp. 1: N) hair chaetae, O) dorsal needle chaetae. Tubificidae sp. 2 (*Illydrilus templetoni*?): P) dorsal chaetae, Q) ventral chaetae. Tubificidae sp. 3: R) dorsal chaetae, S) ventral chaetae. *Limnodrilus hoffmeisteri*: T) dorsal chaetae, U) ventral chaetae, V) penis sheaths. *Limnodrilus udekemianus*: W) dorsal chaetae, X) anterior ventral chaetae.

Taxonomic observations: Length 5 to 8 mm. Worms red in colour. Dorsal chaetae from VI, ventral chaetae from II. The dorsal bundles consist of 1 hair and 1 needle chaeta. Needle chaeta are bifid, sickle-shaped with distal nodulus (Figure 2E). Distal tooth of the needle chaeta is slightly thinner and longer than the proximal (Figure 2E). Ventral bundles in II–V consist of 4 long, thin and slightly curved chaeta per bundle with upper tooth longer than the lower (Figure 2F). In other segments 3–4 ventral chaetae per bundle, with upper tooth almost equal to lower. Four pairs of gills in the branchial fossa.

Dero indica Naidu, 1962

Specimens examined: Porur Lake (Thiruvallur),5.2.2016 (42 specimens), Lakshmipuram Lake (Kanchipuram), 7.5.2016 (12 specimens).

Taxonomic observations: Length 5 to 8 mm. Worms red in colour. Dorsal chaetae from VI. Dorsal bundles with 2 hair and 2 needle chaetae. Needle chaeta bifid, sickle-shaped with distal nodulus. Distal tooth of the needle chaeta slightly thinner and longer than the proximal (Figure 2G). Ventral bundles from II–V consist of 4 long, thin and slightly curved chaeta per bundle with upper tooth longer than the lower (Figure 2H). In other segments 3–4 ventral chaetae per bundle, with upper tooth almost equal to lower. Four pairs of gills observed in the branchial fossa.

Dero zeylanica Stephenson, 1913

Specimens examined: Porur Lake (Thiruvallur) on 5.2.2016 (10 specimens), Ambatur Lake (Thiruvallur) on 15.4.2016 (6 specimens), Chengalpattu Lake (Kanchipuram) on 20.4.2016 (9 specimens).

Taxonomic observations: Length 5–6 mm. Dorsal chaetae begin in VI, each bundle with 3 hair and 3 needle chaetae. Needle chaeta bifid, sickle-shaped with distal tooth longer than proximal (Figure 2I). Ventral chaetae in II–V 4–6 per bundle, longer and thinner, distal teeth twice as long as proximal (Figure 2J). In other segments ventral chaetae with almost equal teeth. Four pairs of gills observed in the branchial fossa.

Remarks: *Dero digitata, D. indica* and *D. zeylanica* differ in the number of dorsal chaetae. Further molecular studies are needed to distinguish them.

Family Tubificidae

Branchiura sowerbyi Beddard, 1892

Specimens examined: Lakshmipuram Lake, (Kanchipuram) on 16.12.2015 (7 specimens), Chengalpattu lake (Kanchipuram) on 24.04.2016 (2 specimens), Lakshmipuram Lake (Kanchipuram) on 07.05.2016 (15 specimens).

Taxonomic observations: Worms very large, about 90–120 mm long. Dorsal chaetae begin from II with 1–3 hairs and 2–8 needles. Needles bifid (Figure 2K). Bifid dorsal chaetae present (Figure 2L). Single-pointed ventral chaeta present (Figure 2M). One pair of gills per segment were observed in the posterior third of the body.

Tubificidae sp. 1

Specimens examined: Chengalpattu Lake (Kanchipuram) on 17.4.2016 (9 specimens).

Length about 12 to 16 mm. Prostomium conical**.** Dorsal chaetal bundles from II with 1–4 hair and 5–6 bifid needle chaetae. Hair chaetae bayonet shaped (Figure 2N). Dorsal and ventral crotchets with distal teeth almost equal to the proximal (Figure 2O). Tail region unsegmented.

Tubificidae sp. 2

Specimens examined: Ooty canal (Nilgiris) on 1.06.2016 (3 specimens).

Taxonomic observations: Length 11 to 13 mm. Segments 50 to 60. Dorsal crotchets bifid with about equal teeth (Figure 2P). Hair chaetae present. Anterior ventral chaetae 4–6 per bundle, with distal tooth much longer than proximal (Figure 2Q). In segments X–XII structures like egg sacs visible. Normal ventral chaetal bundles present in X. Well-developed, short clitellum in XI. No ventral chaetae visible in XI. No penis sheaths were observed but expanded (dorsal?) blood vessel in XII.

Remarks: These characters are insufficient to identify these specimens further, as no penis sheaths were observed, the identification remains with a question mark.

Tubificidae sp. 3

Specimen examined: Ooty canal, (Nilgiris) 01.06.2016 (1 specimen).

Taxanomic observations: Length about 12 mm, segments about 43. Prostomium slightly protruded. Dorsal chaetae with 2 to 3 hair and 2 to 3 bifid crotchets with almost equal teeth (Figure 2R). Ventral chaetae 2–4 per bundle, with distal tooth longer than proximal (Figure 2S).

Remarks: The specimen seems to be a tubificid: very probably belonging to the subfamily Tubificinae (which includes genera and species with hair and pectinate chaetae such as *Tubifex, Ilyodrilus, Potamothrix*).

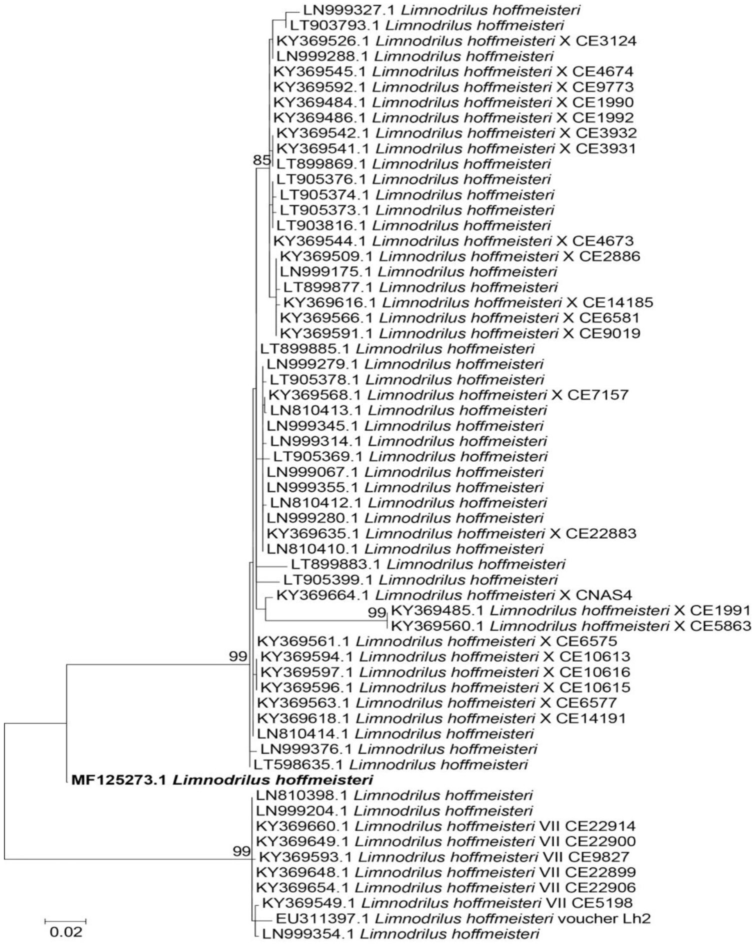

FIGURE 3. Phylogenetic Tree estimated using Maximum Likelihood. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

Limnodrilus hoffmeisteri Claparède, 1862

Specimens examined: Lakshmipuram Lake (Kanchipuram) on 5.1.2016. (18 specimens), 10.1.2016 (12 specimens), Kallikattu Lake (Chennai) on 7.4.2017 (40 specimens), Kallikattu lake (Chennai) on 11.4.2016 (75 specimens), 13.4.2016 (63 specimens), Korattur Lake (Thiruvallur) on 26.4.2016 (2 specimens), Kilkattalai Lake (Kanchipuram) on 1.5.2016 (1 specimen). Ooty canal (Nilgiris) on 1.6.2016 (17 specimens), aquaria (Chennai) on 6.11. 2015 (500 specimens), Ootycanal (Nilgiris) on 1.06.2016(1specimen).

Taxonomic observations: Length about 12–35 mm. Chloragogen beginning from V. Dorsal (Figure 2T) and ventral (Figure 2U) crotchets similar, with about equal teeth. Ventral chaetae absent in XI. Penial sheaths 11–12 times longer than proximal width, curved distally. The penial sheaths (Figure 2V) of the specimen collected from Ooty canal showed slight variation from that of the specimen collected from Chennai.

Results of barcoding / molecular studies: A DNA barcode sequence, 670 bp long (GenBank accession no. MF125273) was obtained from one *Limnodrilus hoffmeisteri*. The sequence was compared to the 60 best matching sequences in GenBank. The tree with the highest log likelihood (–1959.4745) is shown (Figure 3). The closest matches with our sequences, on GenBank, correspond with lineages VII and X of the *L. hoffmeisteri* complex *sensu* Liu *et al.* 2017, but our sequence does not fit directly in any of these two lineages.

Limnodrilus udekemianus Claparède, 1862

Specimens examined: Ooty canal (Nilgiris) on 1.6.2016 (4 specimens).

Taxonomic observations: Worms long, about 35–47 mm. Both dorsal (Figure 2W) and ventral (Figure 2X) crotchets similar. Anterior crotchets 3–8 per bundle with distal tooth thicker, much longer than proximal and curved. Crotchets in the posterior segments with teeth almost equal.

Remarks: This species differ from *L. hoffmeisteri* in having a long curved distal tooth in the anterior chaetae and in general the worms were much larger than *L. hoffmeisteri* and the chloragogen began on the intestine.

Discussion

Thirteen species of aquatic Oligochaeta are known from Chennai, 4 from Thiruvallur, 4 from Kanchipuram, 7 from Villuppuram and 15 from Nilgiris districts of Tamil Nadu. About 34 species of aquatic Oligochaeta have been reported from Tamil Nadu by Srinivasan *et al.* 2016. Out of the 5 naidids reported in the present study *Dero dorsalis* constitute the first report for Kanchipuram district. Hence, the present paper adds one species to Kanchipuram district.

Out of the 6 Tubificids reported in the present study, *Branchiura sowerbyi* and *Limnodrilus hoffmeisteri* are cosmopolitan in distribution. In Tubificidae sp. 1 the hair chaetae are like most *Aulodrilus* sp. Three species belonging to the genus *Aulodrilus* have been described from the Indian sub-continent by Naidu (2005). It is not excluded that this can be an undescribed species of *Aulodrilus.* In the specimen identified as Tubificidae sp. 2, the morphological characters do not contradict (but are insufficient for identification) as the widely distributed *Tubifex tubifex*, or some closely related species since no penis sheaths were observed. The species identified as Tubificidae sp. 2 needs further examination by collecting more such mature specimen with well-developed reproductive system. *Limnodrilus udekemianus* collected from Ooty canal was first reported in India by Naveed (2012). This species has longer proximal teeth in the crotchets when compared to *L. hoffmeisteri*.

Ragi and Jaya (2014) reported that *Dero* sp. were the most abundant in their study from Kerala, (South India) and a similar situation was also observed during the present survey in most of the district of Tamil Nadu except the cold region of Nilgiri district where tubificid worms were more common.

A DNA barcode sequence (mitochondrial COI (Cytochrome c Oxydase 1)) obtained from a mature specimen of *Limnodrilus hoffmeisteri* is closest to lineage VII and X of the *L. hoffmeisteri* complex *sensu* Liu *et al.* (2017). However, it is not a direct match to any of these two lineages and it is possible that it is an additional lineage within the complex that deserves to be treated as a separate species. To further test this more data is needed.

There are also several cryptic species known in *Branchiodrilus* (Martin *et al.* 2018) and *Dero* (Erseus *et al.* 2017) and molecular studies are needed to confirm the identifications of the species in these genera. The three *Dero* species *Dero digitata, D. indica* and *D. zeylanica* differ mainly in the number of dorsal chaeta, and molecular studies are needed to test if these differences are stable between species, or if it varies within species, blurring the lines between these species.

Though the present work is a humble attempt to explore the biodiversity of aquatic Oligochaeta from Thiruvallur, Chennai, Kanchipuram, Villupuram and Nilgiris it gives some idea about the composition of this group in the areas studied and suggests that more undescribed aquatic oligochaetes exist in Ooty and this area needs to be surveyed to discover many new species. This work is a humble beginning in the field of molecular taxonomy of aquatic oligochaetes from India and a lot of questions are yet to be understood. Though the present work is a preliminary attempt, it provides a DNA barcode sequence of the commercially important *Limnodrilus hoffmeisteri* commonly called as 'Tubifex worms' and provides sufficient support to the morphological identification of this species.

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The aquatic oligochaete fauna of Lake Çıldır, Ardahan-Kars, Turkey, including an updated checklist of freshwater annelids known to occur in the country

NAIME ARSLAN[1*] & DENIZ MERCAN[1]
[1] *Eskişehir Osmangazi University, Science and Art Faculty, Biology Department, Meşelik, 26480, Eskişehir, Turkey.*
**Corresponding author e-mail: oligo2009@gmail.com*

Abstract

In this paper, we present the results of the first survey for aquatic oligochaetes in Lake Çıldır, northeastern Turkey, during which 22 oligochaete species were recorded from the lake. The results of this survey were integrated into an updated and annotated list of oligochaetes and other aquatic annelids occurring in the country, summarized from historical and recent publications. Currently, the freshwater annelid fauna of Turkey includes 150 species of oligochaetes (1 Crassiclitellata, 21 Enchytraeidae, 1 Propappidae, 1 Haplotaxidae, 4 Lumbriculidae, 56 Naidinae, 64 Tubificinae, 2 Lumbricidae), 1 species of Branchiobdellida, and 6 species of Aphanoneura (1 Potamodrilidae and 5 Aeolosomatidae). Although studies focusing on the aquatic oligochaete fauna of Turkey have increased over the past 15–20 years, species diversity still remains unclear.

Keywords: Checklist, Annelida, Oligochaeta, oligochaetes, Turkey, Lake Çıldır

Introduction

Three distinct biogeographical areas are present in Turkey: Anatolian, Mediterranean, the Black Sea regions, and their transition zones. The diverse climatic and geographical features often vary greatly—even within short distances—due to their location in the country (https://www.iucn.org/content/biodiversity-turkey). Surrounded by three seas, Turkey (783,356 km2 in area) is uniquely positioned geographically with its mountain ranges such that terrestrial, fresh water, and marine biodiversity is considerably high. The biological diversity of the country can be compared to that of a small continent. Its territory includes forests, mountains, steppes, wetlands, coastal and marine ecosystems as well as different forms and combinations of these systems, supporting considerable species diversity. The faunal biodiversity of Turkey is quite high compared to that of other countries in the temperate zone. Despite the lack of extensive data, invertebrates constitute the largest group among the identified living species. The total number of invertebrate species in Turkey is estimated to be ~19,000; of these, ~4,000 species / subspecies are considered to be endemic (International Union for Conservation of Nature 2018). In addition, Turkey is blessed with a diversity of lentic and lotic resources, including 107 major rivers and 25 river basins, more than 120 natural lakes, and 135 wetlands of international significance. The map of Earth's freshwater ecosystems, as published by The Nature Conservancy (2015), is based on the data regarding the distribution and composition of freshwater fish species. According to data presented therein, Turkey has eight different freshwater ecoregions, but as previously stated, these regions are categorized based on their fish, amphibian and reptile species. Therefore in this study, the inland water categorization published by Timm (1980) was used for freshwater oligochaete species. Inland waters have been categorized into six different zoogeographical regions; Turkey is located entirely within the Holoarctic region. Timm (1980) divided the Holoarctic region into six subregions on the basis of the distribution of oligochaetes: Euro-Siberian, West Balkan, Ponto-Caspian (brackish water), east Siberian, Pacific, and Atlantic. Turkey is located within the Ponto-Caspian region.

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The Oligochaeta is a large group of annelid worms that inhabit terrestrial, freshwater and marine environments. While earthworms (Crassiclitellata and Moniligastridae) and enchytraeids (Enchytraeidae) are mainly terrestrial, taxa in the other 14 families are predominantly aquatic (Timm 2017). Timm (1980) reported that 700 limicolous and 100 true marine oligochaete species are known worldwide. However, this number has greatly increased because of other recent studies (e.g., Erséus 1979; Timm 1980; Erséus 1981; Dumnicka 1983; Erséus 1984; Brinkhurst *et al.* 1994; Erséus 1997; Arslan *et al.* 2006; Matamoros *et al.* 2007; Timm 2013; Arslan *et al.* 2018). Timm (2017) published an annoted catalogue, which included 1081 new nominal taxa and 372 new combinations or names with new ranks of available scientific names given to the oligochaetes in freshwater and marine families that were described since the publication of the monograph by Brinkhurst & Jamieson (1971) and its supplement (Brinkhurst & Wetzel 1984).

Although studies on the aquatic oligochaete fauna of Turkey have increased over the last 20+ years, the diversity of oligochaete species still remains unclear. To date, one branchiobdellidan, 150 oligochaete and six aphanoneuran species have been reported. Although several scientists have described new taxa from Turkey, this number is undoubtedly lower than estimated. The reason is that Turkey is the only country covering almost entirely 3 out of 34 global biodiversity hotspots (Caucasus, Irano-Anatolian, and Mediterranean). Their geographical locations, with mountains acting as an isolation barrier for aquatic organisms as well the characteristics of its peninsulas, support a high biodiversity. In Turkey, there are numerous disconnected rivers, and lakes are commonly separated from each other by mountains. One of those lakes is Lake Çıldır, located in the northern part of East Anatolia. In recent years, several studies have focused on the freshwater oligochaetes of the Turkish lakes and rivers, but to date no study has focused specifically on the oligochaete fauna of Lake Çıldır.

In this paper, we discuss the oligochaete fauna of Lake Çıldır in northeastern Turkey based on our recent survey, then we present an updated list of freshwater annelid species that have been recorded from the country, including distributional information and citations for papers in which those species were presented.

Material and Methods

Study area

Lake Çıldır (41° 04' N, 43° 12' E) is located in northeastern part of Turkey. It's a large freshwater lake between Kars and Ardahan provinces (Figure 1). The surface area of the Lake is 124 km^2 and has a maximum depth of around 40 m (Alkan *et al.* 2016). Its altitude is 1959 m. The surface of the lake is ice-covered for approximately 6–7 months. Lake Çıldır has IBA (important bird area) and IPA (important plant area) status due to the fact that it is home to two rare birds—the Ruddy shelduck (*Tadorna ferruginea* (Pallas)) and the Armenian gull (*Larus armenicus* Buturlin), and three rare plants—*Carex limosa* (Linnaeus)*, Potamogeton alpinus* (Balbis)*, Scholochloa festucacea* (Willd.), and *Sparganium minimum* Wallr.

Sampling

In this present study, 116 oligochaete specimens were collected from the lake between July–August 2017 using hand net or Ekman grab sampler (with coverage of 225 cm^2), one haul per station. In addition, some physico-chemical parameters of surface water were measured *in situ* by using Hach Lange HQ40D. After the samples were processed in the field using a series of sieves with decreasing mesh sizes, specimens were extracted from raw samples in lab, under a dissecting microscope, and transferred to 70% ethyl alcohol. Specimens were prepared for the identification either in glycerin or polyvinyl lactophenol. Taxonomic identifications of oligochaetes followed the keys and species diagnoses presented in Brinkhurst and Jamieson (1971), Brinkhurst and Wetzel (1984), Sperber (1948, 1950), Kathman and Brinkhurst (1998) and Timm (1999).

Literature review: aquatic Oligochaetes and other annelids occuring in Turkey

The first study on the freshwater oligochaete fauna of Turkey was published by Sperber (Sperber 1958). Martínez-Ansemil & Giani (1987) indicated that the occurrence of *Limnodrilus hoffmeisteri* Claparède in Turkey was mentioned in Naidu (Naidu 1965). Since 1949, several Turkish scientists have contributed to the knowledge of the aquatic oligochaetes of Turkey.

We completed an extensive review of historical and recent literature focusing on studies of aquatic fauna in Turkey, specifically those including freshwater oligochaetes and other annelids. Distributional and habitat information for these species as presented in those publications has been summarized and is presented in the Appendix. The list of oligochaetes in the Appendix does not include records or other information discussed in unpublished MSc and PhD theses, nor those pertinent to soil forms (e.g., Lumbricidae and Enchytraeidae). However, species considered to be primarily terrestrial, but are occasionally collected from semi-aquatic habitats have been included in the Appendix. The primary focus of this study was to evaluate the oligochaete fauna of Lake Çıldır, which had no previous studies on Annelida fauna, and to then integrate the results of that study to compile an updated list of oligochaete species that had been presented in historical as well as recent publications.

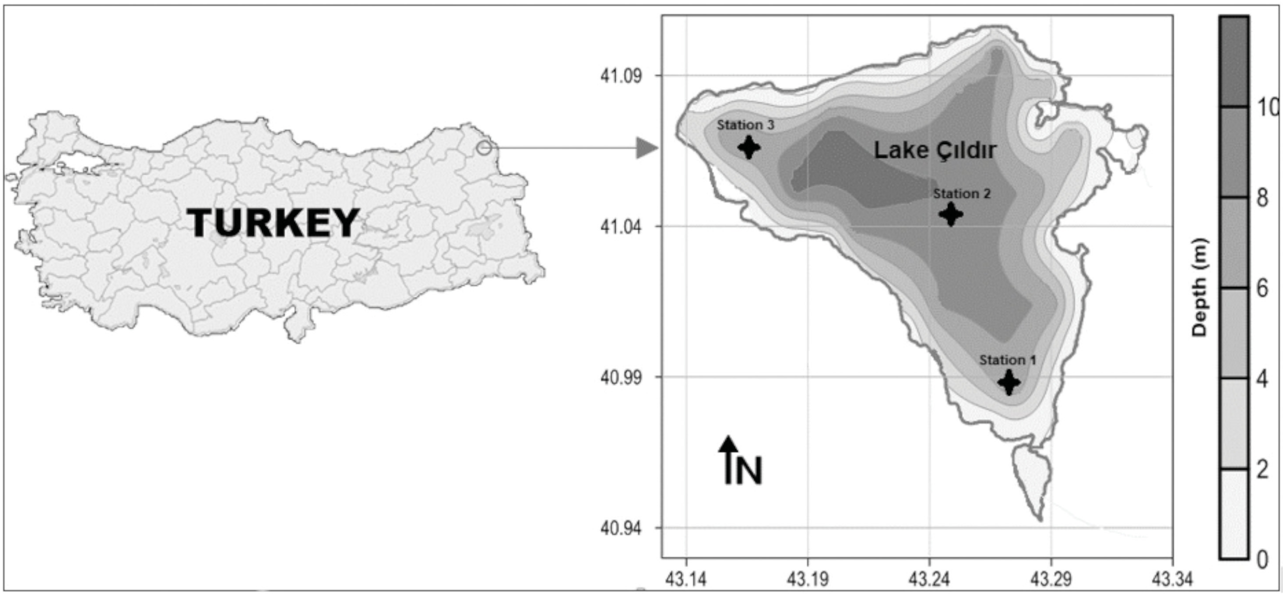

FIGURE 1: Geographical position of Lake Çıldır in Turkey, with location of sampling points (this map was modified from Alkan *et al.* 2016).

Results and Conclusions

The Oligochaete fauna of Lake Çıldır

In the present study, a total of 22 oligochaete species were identified from Lake Çıldır. All identified species are new records for Lake Çıldır (Table 1). Although oligochaete species identified in this study were reported several times from different parts of Turkey, four species—*Chaetogaster diastrophus* (Gruithuisen), *Uncinais uncinata* (Ørsted), *Spirosperma velutinus* (Grube) and *Stylodrilus parvus* (Hrabě & Cernosvitov)—were reported from only a few locations (see Appendix). Two other species, *Peipsidrilus libanus* (Giani *et al.*) and *Tubifex acuticularis* Martínez-Ansemil & Giani, first described as new to science from Lebanon by Giani *et al.* (1982) and Martínez-Ansemil & Giani (1983), are reported herein from Turkey for only the second time. Arslan *et al.* (2007) had previously collected each of these two species from Göksu River (Balıkdamı wetland). These two species were recorded during this present study from Lake Çıldır (Turkey), suggesting that *Tubifex acuticularis* and *Peipsidrilus libanus* may have a wider distribution in the Near East.

Remarks on two oligochaete taxa occurring in Lake Çıldır

***Tubifex* sp.:** Specimen length 5.3–9.8 mm, genital segments in X–XI. Segment number 42–63. Prostomium not obtuse and clitellum inconspicuous, no coelomocytes. In dorsal bundles 2–4 hair seta and 3–4 pectinate seta, upper and lower toot equal. Hair chaetae smooth anteriorly but slightly serrate posteriorly. Ventral bundles contain bifid chaetae, 4–5 per bundle (anteriorly), 2–3 (posteriorly). Male genitalia paired in X–XI, vas deferens longer than atrium. Atrium comma shaped. In segment X, testes paired, ovaries in XI, spermatozeugmata present.

This species is similar to *Tubifex blanchardi* Vejdovský form (Holmquist 1983; Marotta *et al.* 2014), but it has a few different structures. In this study this specimen was listed as *Tubifex* sp. since the exact species identification will be done following the DNA analysis.

Potamothrix alatus **Finogenova:** Among the identified oligochaete species in the Lake Çıldır, another interesting species is *Potamothrix alatus* whose taxonomic status based on specimens collected during this study is still uncertain. Several morphological features of the *P. alatus* specimens collected from Lake Çıldır are similar to two previously described subspecies (*P. alatus paravanicus* Poddubnaja & Pataridze and *P. alatus hazaricus* Timm & Arslan) yet have different morphological characteristics from these two subspecies. This taxon is listed as *P. alatus* for this study; we hope that DNA sequencing will determine whether specimens of this taxon collected during this study are identical to *P. alatus paravanicus* or *P. alatus hazaricus* or represent a separate subspecies. *Potamothrix a. paravanicus* was identified as a different species from Lakes Paravani, Sagamo and Sevan. However, it was then revised as a subspecies of *P. alatus* (Poddubnaja & Pataridze 1989; Finogenova & Poddubnaja 1990). *Potamothrix alatus hazaricus* was described in Lake Hazar (Arslan *et al.* 2013), which is close to Lake Çıldır. *Potamothrix alatus hazaricus* differs morphologically from *P. a. paravanicus*, the former has a different structure with lateral wings in the genital region of the body. The genital segments of *P. a. hazaricus* X–XI almost always have large, lateral wing-like protrusions. These "wings" start anteriorly in the dorsolateral part of X and continue in XI (Arslan *et al.* 2013), while lateral wing-like protrusions of *P. alatus*, a morphology observed on specimens collected from sites Çıldır Lake sites, are only present in XI and not as large or as wide as those observed on *P. a. hazaricus* specimens. The differences of *P. alatus* in Lake Çıldır were not only in the genital region, but also in the hair, pectinate and ventral chaetae that were located in the ventral and dorsal bundles. Nuclear TSI and mitochondrial COI analyses appear to be a prerequisite for them to be defined as a different subspecies.

TABLE 1. Composition of oligochaete species and some environmental parameters for 3 sampling points in Lake Çıldır during the research period (July–August 2017). Numerals across the species show the individual number of oligochaete species collected.

	Parameters	**Sampling sites**		
		1	**2**	**3**
	Latitude and longitude coordinates	40° 59'28.83''N 43° 56'16.72''E	41° 02'38.92''N 43° 15'46.57''E	41° 03'45.62''N 43° 10'12.96''E
	Hydrogen Ion Concentration (as pH)	7.8	8.2	8.1
	Dissolved oxygen (mg l^{-1})	7.6	8.7	9.3
	Depth (m)	3	12	5
	Surface water temperature (°C)	18	20	19
	Oligochaeta species			
	Naidinae			
1	*Chaetogaster diastrophus* (Gruithuisen, 1828)	4	-	1
2	*Paranais frici* Hrabě, 1941	2	1	-
3	*Uncinais uncinata* (Ørsted, 1842)	25	32	54
4	*Stylaria lacustris* (Linnaeus, 1767)	4	15	7
5	*Nais elinguis* Müller, 1773	2	5	3
6	*Nais communis* Piguet, 1906	-	1	4
7	*Nais variabilis* Piguet, 1906	2	-	-
8	*Nais pardalis* Piguet, 1906	3	-	4
9	*Nais simplex* Piguet, 1906	2	-	-

...*Continued on the next page*

TABLE 1. (Continued)

	Parameters	Sampling sites		
		1	2	3
	Tubificinae			
10	*Tubifex tubifex* (Müller, 1774)	3	5	2
11	*Tubifex* sp.	2	4	-
12	*Tubifex acuticularis* Martínez-Ansemil & Giani, 1983	3	2	-
13	*Peipsidrilus libanus* (Giani *et al.*, 1982)	-	-	4
14	*Limnodrilus hoffmeisteri* Claparède, 1862	6	2	-
15	*Spirosperma velutinus* (Grube, 1879)	2	-	-
16	*Haber speciosus* (Hrabě, 1931)	-	-	4
17	*Potamothrix hammoniensis* (Michaelsen, 1901)	3	-	3
18	*Potamothrix bavaricus* (Oschmann, 1913)	-	2	-
19	*Potamothrix alatus* Finogenova, 1972	2	1	2
20	*Psammoryctides albicola* (Michaelsen, 1901)	2	-	-
	Lumbriculidae			
21	*Stylodrilus parvus* (Hrabě & Černosvitov, 1927)	4	-	-
	Enchytraeidae			
22	*Enchytraeus albidus* (Henle, 1837)	2	-	-

Checklist of Turkish fauna

In summarizing the data presented in the publications annotated in Appendix and complimented by the survey of oligochaete species recently collected from Lake Çıldır (Table 1), 150 oligochaete, 1 branchiobdellidan and 6 aphanoneuran species are now known to occur in Turkey. Several scientists have described new taxa from Turkey, yet we believe additional taxa, including as yet undescribed species, have yet to be discovered in the country, which certainly could include one or more cosmopolitan species. According to the distribution charts prepared by Timm (Timm 1980), it can be emphasized that among these 157 freshwater annelid species recorded from Turkey, 23 species—*Aeolosoma variegatum* Vejdovský, *Slavina appendiculata* (D'Udekem), *Dero digitata* (Müller), *Dero furcatus* (Müller), *Dero obtusa* (d'Udekem), *Nais elinguis, Nais communis, Nais variabilis, Chaetogaster limnaei* von Baer, *Chaetogaster langi* Bretscher, *Pristina longiseta* Ehrenberg, *Pristina aequiseta* Bourne, *Pristina jenkinae* (Stephenson), *Pristina proboscidea* Beddard, *Tubifex tubifex, Limnodrilus hoffmeisteri, Limnodrilus udekemianus* Claparède, *Limnodrilus claparedeanus* Ratzel, *Rhyacodrilus coccineus* (Vejdovský), *Aulodrilus pigueti* Kowalewski, *Aulodrilus limnobius* Bretscher, *Lumbriculus variegatus* (Müller), and *Eiseniella tetraedra* (Savigny)—have cosmopolitan or wide distribution. Apart from these, *Chaetogaster diastrophus, Nais elinguis, Allonais pectinata* (Stephenson), and *Aulodrilus pluriseta* (Piguet) were also considered to be cosmopolitan species by Spencer (Spencer 1980). All of these species had previously been recorded from different areas in Turkey. In addition, many European species (e.g., *Paranais frici, Potamothrix hammoniensis, Ophidonais serpentina* (Müller), and *Vejdovskyella comata* (Vejdovský)) and transholarctic species (e.g., *Stylaria lacustris, Uncinais uncinata, Chaetogaster diaphanus, Limnodrilus profundicola* (Verrill)) were recorded from Turkey several times by different authors (see Appendix). These transholarctic species have also been reported from the Sino-Indian region (Timm 1980). Eight species (*Paranais litoralis* (Müller), *Paranais frici, Nais simplex, Nais pseudobtusa* Piguet, *Vejdovskyella comata, Tubifex ignotus* (Stolc), *Ilyodrilus templetoni* (Southern), and *Bothrioneurum vejdovskyanum* (Štolc) have been recorded as being Holarctic, yet their known distribution includes the Ethiopian region (Spencer 1980). These eight species were also recorded in Turkey. Several oligochaete species occurring in Turkey show similarities to the communities in neighboring regions.

The uniqueness of Turkey with respect to its mountainous regions act as an isolation barrier for aquatic organisms, and the characteristics of its peninsulas have contributed to the country's in high biodiversity. There are numerous disconnected rivers in the country, and lakes are separated from one another by mountains, including Lake Çıldır, which is located in the northern part of East Anatolia. Surveys of new as well as understudied aquatic and semi-aquatic habitats in Turkey will certainly result in the discovery of new distributional records and, as well, new species to science.

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Appendix. List of Aphanoneura, Branchiobdellida and Oligochaeta species reported from different aquatic systems of Turkey, including the results of this present study in Lake Çıldır (abbreviations used in this Appendix–S.: stream, L.: lake, R.: river, DL: Dam lake, TML: Taurus Mountains's lake, WBSL: West Black Sea Lakes, GL: Glasier Lake, CW: Coastal Wetland, EBSL: Eastern Black Sea Lakes; EHEBSL: Eastern High Elevation Black Sea Lakes, TTRR: Thrace Region Rivers).

	Taxa	**Literature data**
Phylum	**Annelida**	
Subclass	**Branchiobdellida**	
Order	**Branchiobdellida**	
1	*Branchiobdella kozarovi* Subchev, 1978	Yenikarpuzlu Pond- Edirne (Odabaşı *et al.* 2016);
Subclass	**Oligochaeta**	
Order	**Crassiclitellata**	
Family	**Criodrilidae**	
2	*Criodrilus lacuum* Hoffmeister, 1845	Emir L. (Geldiay, 1949);
Order	**Enchytraeida**	
Family	**Enchytraeidae**	
3	*Achaeta* sp.	Balıkdamı Wetland (Arslan *et al.* 2006);
4	*Cognettia sphagnetorum* (Vejdovský, 1878)	EBSL (Yıldız *et al.* 2010b); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Tigris R. (Zeybek *et al.* 2016); Tunca R. (Çamur-Elipek *et al.* 2006); Tunca R. (Kırgız *et al.* 2005);
5	*Cognettia glandulosa* (Michaelsen, 1889)	EBSL (Yıldız *et al.* 2010b); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Tigris R. (Zeybek *et al.* 2016); Tunca R (Çamur-Elipek *et al.* 2006); Tunca R. (Kırgız *et al.* 2005);
6	*Cognettia* sp.	TTRR (Taş *et al.* 2012); Tunca R. (Çamur-Elipek *et al.* 2006); Tunca R. (Kırgız *et al.* 2005);
7	*Enchytraeus polatdemiri* Arslan & Timm, 2018	Van L. (Arslan *et al.* 2018);
8	*Enchytraeus albidus* (Henle, 1837)	Sazlıgöl L. (Balık *et al.* 2001); Gediz Delta (Balık *et al.* 2004); Çıldır L. (Arslan & Mercan present study);
9	*Enchytraeus buchholzi* Vejdovský, 1879	Localities? (Pop 1974); Işıklı L. (Balık *et al.* 2000); TTRR (Taş *et al.* 2012); Tunca R. (Çamur-Elipek *et al.* 2006); Tunca R. (Kırgız *et al.* 2005);
10	*Enchytraeus coronatus* Nielsen and Christensen, 1959	Yuvarlak R. (Yıldız *et al.* 2007b);

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	Taxa	Literature data
Phylum	**Annelida**	
11	*Enchytraeus* sp.	Locality? (Pop 1974); Gölcük L. (Geldiay & Tareen 1972); Buldan L. (Ustaoğlu *et al.* 2004); Eğrigöl L. (Yıldız *et al.* 2005);
12	*Frederica* sp.	Ankara S. (Moubayed *et al.* 1987); TTRR (Taş *et al.* 2012); Tunca R. (Çamur-Elipek *et al.* 2006); Tunca R. (Kırgız *et al.* 2005); Denizli mountain lakes (Yıldız & Ustaoğlu 2016);
13	*Henlea ventriculosa* (d'Udekem, 1854)	Lake District Region (Yıldız & Balık 2005); Yuvarlak R. (Yıldız *et al.* 2007b); EBSL (Yıldız *et al.* 2010b); Tigris R. (Zeybek *et al.* 2016);
14	*Henlea nasuta* (Eisen, 1878)	Lake District Region (Yıldız & Balık 2005); Tigris R. (Zeybek *et al.* 2016);
15	*Henlea perpusilla* Friend, 1911	EBSL (Yıldız *et al.* 2010b); TTRR (Taş *et al.* 2012); Karagöl L. (Topkara *et al.* 2018); Tunca R. (Çamur-Elipek *et al.* 2006); Tunca R. (Kırgız *et al.* 2005);
16	*Henlea* sp.	EBSL (Yıldız *et al.* 2010b); EHEBSL (Yıldız *et al.* 2012);
17	*Lumbricillus lineatus* (Müller, 1774)	Gediz Delta (Balık *et al.* 2004);
18	*Lumbricillus tuba* Stephenson, 1911	Gediz Delta (Balık *et al.* 2004);
19	*Lumbricillus* sp.	TTRR (Taş *et al.* 2012); Tunca R. (Çamur-Elipek *et al.* 2006);
20	*Marionina argentea* (Michaelsen, 1889)	Specific locality information not included in publication (Pop 1974);
21	*Marionina triplex* Matamoros, Yıldız & Erséus, 2007	Sinop (Matamoros *et al.* 2007);
22	*Mesenchytraeus armatus* Levinsen, 1884	Lake District Region (Yıldız & Balık 2005); EBSL (Yıldız *et al.* 2010); EHEBSL (Yıldız *et al.* 2012);
23	*Mesenchytraeus* sp.	Yuvarlak R. (Yıldız *et al.* 2007b); EBSL (Yıldız *et al.* 2010b); EHEBSL (Yıldız *et al.* 2012); Tigris R. (Zeybek *et al.* 2016);
Family	**Propappidae**	
24	*Propappus volki* Michaelsen, 1916	Tunca R. (Kırgız *et al.* 2005); Tunca R. (Çamur-Elipek *et al.* 2006); TTRR (Taş *et al.* 2012);
Family	**Haplotaxidae**	
25	*Haplotaxis gordioides (Hartmann, 1821)*	Antakya (Omedeo 1987); Ankara S. (Kazancı & Girgin 1998); Lake District Region (Yıldız & Balık 2005); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Porsuk R. (Arslan & İlhan 2010);
Order	**Lumbriculida**	
Family	**Lumbriculidae**	
26	*Lumbriculus variegatus* (Müller, 1774)	Işıklı L. (Balık *et al.* 2000); Kelkit S. (Duran *et al.* 2003); Lake District Region (Yıldız & Balık 2005); TML (Yıldız *et al.* 2007a); Yuvarlak R. (Yıldız *et al.* 2007b); EBSL (Yıldız *et al.* 2010b); EHEBSL (Yıldız *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); İkizgöl (Yıldız *et al.* 2009); Eğirdir L. (Arslan 2006); Musaözü DL (Arslan *et al.* 2007); Porsuk R. (Arslan & İlhan 2010);
27	*Lumbriculus* sp.	Gölcük L. (Geldiay & Tareen 1972); Karamuk L. (Anonymous 1993); Eğirdir; Bafa and Eber L.; Büyük Menderes Delta (Kazancı *et al.* 2000);
28	*Trichodrilus* sp.	Balıkdamı Wetland (Arslan *et al.* 2006); Uluabat L. (Kökmen *et al.* 2007);

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	Taxa	Literature data
Phylum	**Annelida**	
29	*Rhynchelmis* sp.	Porsuk R. (Arslan & İlhan 2010);
Family	**Naidinae**	
30	*Allonais pectinata* (Stephenson, 1910)	Sakarya R. (Arslan & Şahin 2004);
31	*Allonais gwaliorensis* (Stephenson, 1910)	Sakarya R. (Arslan & Şahin 2004); Yuvarlak R. (Yıldız *et al.* 2007b);
32	*Amphichaeta leydigii* Tauber, 1879	Gediz Delta (Balık *et al.* 2004); Buldan L. (Ustaoğlu *et al.* 2004);
33	*Amphichaeta sannio* Kallstenius, 1892	Gediz Delta (Balık *et al.* 2004);
34	*Chaetogaster diaphanus* (Gruithuisen, 1828)	Işıklı L. (Balık *et al.* 2000); Sakarya R. (Arslan & Şahin 2004); TML (Yıldız *et al.* 2007a); Yuvarlak R. (Yıldız *et al.* 2007); EHEBSL (Yıldız *et al.* 2012); Aksu R. (Çapraz & Arslan 2005); Porsuk R. (Arslan & İlhan 2010);
35	*Chaetogaster diastrophus* (Gruithuisen, 1828)	Yuvarlak R. (Yıldız *et al.* 2007b); Balıkdamı Wetland (Arslan *et al.* 2006); Sazlıdere S. (Taş *et al.* 2011); Çıldır L. (Arslan & Mercan present study);
36	*Chaetogaster langi* Bretscher, 1896	Sakarya R. (Arslan & Şahin 2004);
37	*Chaetogaster limnaei* von Baer, 1827	Gölcük L. (Geldiay & Tareen 1972);
38	*Chaetogaster limnaei limnaei* von Baer, 1827	Tuzla S. (Odabaşı *et al.* 2015);
39	*Chaetogaster* sp.	EHEBSL (Yıldız *et al.* 2012);
40	*Dero borelli* Michaelsen, 1900	Sakarya R. (Arslan & Şahin 2004);
41	*Dero dorsalis* Ferroinére, 1899	Sazlıgöl L. (Balık *et al.* 2001); İkizgöl (Yıldız *et al.* 2009); Ceyhan River Basin Lakes (Fındık & Aras 2016);
42	*Dero digitata* (Müller, 1773)	Işıklı L. (Balık *et al.* 2000); Sazlıgöl L. (Balık *et al.* 2001); Gediz Delta (Balık *et al.* 2004); Kuş L. (Balık *et al.* 2005); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); Topçam DL (Yıldız & Balık 2006); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Gölcük L. (Yıldız *et al.* 2015); Akgöl L. (Yıldız *et al.* 2009); İkizgöl (Yıldız *et al.* 2009); Aksu R. (Çapraz & Arslan 2005); Kovada L. (Arslan & Şahin 2006); Tunca R. (Çamur-Elipek *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Uluabat L. (Kökmen *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Çorlu R. (Taş *et al.* 2008); Menderes R. (Akbulut *et al.* 2009); Sazlıdere S. (Taş *et al.* 2011); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Büyük Akgöl L. (Arslan *et al.* 2018); Euphrates R. (Aras & Fındık 2016); Ceyhan River Basin Lakes (Fındık & Aras 2016);
43	*Dero furcatus* (Müller, 1774)	Sakarya R. (Arslan & Şahin 2004); Buldan L. (Ustaoğlu *et al.* 2004); TTRR (Taş *et al.* 2012); Aksu R. (Çapraz & Arslan 2005); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Sazlıdere S. (Taş *et al.* 2011); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Ceyhan River Basin Lakes (Fındık & Aras 2016);
44	*Dero obtusa* d'Udekem, 1855	Gediz Delta (Balık *et al.* 2004); Lake District Region (Yıldız & Balık 2005); Topçam DL (Yıldız & Balık 2006); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Çorlu R. (Taş *et al.* 2008); Euphrates R. (Aras & Fındık 2016);

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	Taxa	Literature data
Phylum	**Annelida**	
45	*Homochaeta naidina* Bretscher, 1896	Gediz S. (Balık *et al.* 1999); Işıklı L. (Balık *et al.* 2000); Gediz Delta (Balık *et al.* 2004);
46	*Homochaeta setosa* (Moszynski, 1933)	Gediz Delta (Balık *et al.* 2004);
47	*Nais alpina* Sperber, 1948	Yuvarlak R. (Yıldız *et al.* 2007b);
48	*Nais barbata* Müller, 1773	Sakarya R. (Arslan & Şahin 2004); Lake District Region (Yıldız & Balık 2005); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Tigris R. (Zeybek *et al.* 2016); Kovada L. (Arslan & Şahin 2006); Musaözü DL (Arslan *et al.* 2007); Uluabat L. (Kökmen *et al.* 2007); Porsuk R. (Arslan & İlhan 2010); Sazlıdere S. (Taş *et al.* 2011); Küçük Menderes R. (Arslan *et al.* 2016);
49	*Nais behningi* Michaelsen, 1923	Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Tigris R. (Zeybek *et al.* 2016);
50	*Nais bretscheri* Michaelsen, 1899	Sakarya R. (Arslan & Şahin 2004); Gümüş S. (Öntürk & Arslan 2003); Yuvarlak R. (Yıldız *et al.* 2007b); Tigris R. (Zeybek *et al.* 2016); Aksu R. (Çapraz & Arslan 2005); Eğirdir L. (Arslan 2006); Musaözü DL (Arslan *et al.* 2007); Menderes R. (Akbulut *et al.* 2009); Porsuk R. (Arslan & İlhan 2010); Sazlıdere S. (Taş *et al.* 2011);
51	*Nais communis* Piguet, 1906	Sakarya R. (Arslan & Şahin 2004); Lake District Region (Yıldız & Balık 2005); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Sapanca L. (Şahin & Yıldız 2011); EHEBSL (Yıldız *et al.* 2012); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Tigris R. (Zeybek *et al.* 2016); Aksu R. (Çapraz & Arslan 2005); Kovada L. (Arslan & Şahin 2006); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Uluabat L. (Kökmen *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Menderes R. (Akbulut *et al.* 2009); Porsuk R. (Arslan & İlhan 2010); Gala L. (Çamur-Elipek *et al.* 2010); Büyük Akgöl L. (Arslan *et al.* 2018); Euphrates R. (Aras & Fındık 2016); Ceyhan River Basin Lakes (Fındık & Aras 2016); Çıldır L. (Arslan & Mercan present study);
52	*Nais christinae* Kasprzak, 1973	Gümüldür R. (Yıldız & Balık 2010);
53	*Nais elinguis* Müller, 1773	Ankara S. (Moubayed *et al.* 1987); Sakarya R. (Arslan & Şahin 2004); Gediz Delta (Balık *et al.* 2004); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); EHEBSL (Yıldız *et al.* 2012); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Tigris R. (Zeybek *et al.* 2016); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Çorlu R. (Taş *et al.* 2008); Menderes R. (Akbulut *et al.* 2009); Porsuk R. (Arslan & İlhan 2010); Sazlıdere S. (Taş *et al.* 2011); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Küçük Menderes R. (Arslan *et al.* 2016); Çıldır L. (Arslan & Mercan present study);

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	Taxa	Literature data
Phylum	**Annelida**	
54	*Nais pardalis* Piguet, 1906	Sivas (Sperber 1958); Sakarya R. (Polatdemir Arslan & Şahin 2003); Gümüş S. (Öntürk & Arslan 2003); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Kemer DL (Yıldız *et al.* 2008); Uludağ GL (Ustaoğlu *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); EHEBSL (Yıldız *et al.* 2012); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Adıgüzel DL (Özbek *et al.* 2016); Tigris R. (Zeybek *et al.* 2016); Aksu R. (Çapraz & Arslan 2005); Kovada L. (Arslan & Şahin 2006); Tunca R. (Çamur-Elipek *et al.* 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Porsuk R. (Arslan & İlhan 2010); Küçük Menderes R. (Arslan *et al.* 2016); Çıldır L. (Arslan & Mercan present study);
55	*Nais pseudobtusa* Piguet, 1906	Sakarya R. (Arslan & Şahin 2004); Lake District Region (Yıldız & Balık 2005); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); EHEBSL (Yıldız *et al.* 2012); EHEBSL (Yıldız *et al.* 2012); Tigris R. (Zeybek *et al.* 2016); Balıkdamı Wetland (Arslan *et al.* 2006); Porsuk R. (Arslan & İlhan 2010);
56	*Nais simplex* Piguet, 1906	Sakarya R. (Arslan & Şahin 2004); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); EHEBSL (Yıldız *et al.* 2012); EHEBSL (Yıldız *et al.* 2012); Tigris R. (Zeybek *et al.* 2016); Balıkdamı Wetland (Arslan *et al.* 2006); Euphrates R. (Aras & Fındık 2016); Ceyhan River Basin Lakes (Fındık & Aras 2016); Çıldır L. (Arslan & Mercan present study);
57	*Nais stolci* Hrabě, 1981	Tigris R. (Yıldız & Ahıska 2010);
58	*Nais variabilis* Piguet, 1906	Ayva keuy? (Sperber 1958); Sakarya R. (Polatdemir Arslan & Şahin 2003); Gümüş S. (Öntürk & Arslan 2003); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); TTRR (Taş *et al.* 2012); Tigris R. (Zeybek *et al.* 2016); Aksu R. (Çapraz & Arslan 2005); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Uluabat L. (Kökmen *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Menderes R. (Akbulut *et al.* 2009); Porsuk R. (Arslan & İlhan 2010); Gala L. (Çamur-Elipek *et al.* 2010); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Çıldır L. (Arslan & Mercan present study);
59	*Nais* sp.	Gölcük L. (Geldiay & Tareen 1972); Ankara S. (Kazancı & Girgin 1998); EHEBSL (Yıldız *et al.* 2012); EHEBSL (Yıldız *et al.* 2012); Ceyhan River Basin Lakes (Fındık & Aras 2016);
60	*Ophidonais serpentina* (Müller, 1773)	Afchin? (Sperber 1958); Gölcük L. (Geldiay & Tareen 1972); Tigris basin and West of Ceyhan channel (Moubayed *et al.* 1987); Sakarya R. (Polatdemir Arslan & Şahin 2003); Işıklı L. (Balık *et al.* 2000); Sazlıgöl L. (Balık *et al.* 2001); Lake District Region (Yıldız & Balık 2005); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Uludağ GL (Ustaoğlu *et al.* 2008); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012) ; Tigris R. (Zeybek *et al.* 2016); Kovada L. (Arslan & Şahin 2006); Eğirdir L. (Arslan 2006); Musaözü DL (Arslan *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Çorlu R (Taş *et al.* 2008); Porsuk R. (Arslan & İlhan 2010); Sazlıdere S. (Taş *et al.* 2011); Büyük Akgöl L. (Arslan *et al.* 2018);

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	Taxa	**Literature data**
Phylum	**Annelida**	
61	*Paranais botniensis* Sperber, 1948	Gediz Delta (Balık *et al.* 2004); Yuvarlak R. (Yıldız *et al.* 2007b);
62	*Paranais frici* Hrabě, 1941	Sakarya R. (Arslan & Şahin 2004); Gümüş S. (Öntürk & Arslan 2003); Gediz Delta (Balık *et al.* 2004); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); TML (Yıldız *et al.* 2007a); Yuvarlak R. (Yıldız *et al.* 2007b); Küçük Menderes CW (Yıldız *et al.* 2010a); Sapanca L. (Şahin & Yıldız 2011); TTRR (Taş *et al.* 2012); Kovada L. (Arslan & Şahin 2006); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Uluabat L. (Kökmen *et al.* 2007); Menderes R. (Akbulut *et al.* 2009); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Çıldır L. (Arslan & Mercan present study);
63	*Paranais litoralis* (Müller, 1784)	Gediz Delta (Balık *et al.* 2004); Yuvarlak R. (Yıldız *et al.* 2007b);
64	*Paranais simplex* (Hrabě, 1936)	Gediz Delta (Balık *et al.* 2004);
65	*Piguetiella blanci* Piguet, 1906	Gediz Delta (Balık *et al.* 2004);
66	*Pristina aequiseta* Bourne, 1891	Sakarya R. (Polatdemir Arslan & Şahin 2003); Yuvarlak R. (Yıldız *et al.* 2007b); Tigris R. (Zeybek *et al.* 2016); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Uluabat L. (Kökmen *et al.* 2007); Porsuk R. (Arslan & İlhan 2010); Büyük Akgöl L. (Arslan *et al.* 2018); Ceyhan River Basin Lakes (Fındık & Aras 2016);
67	*Pristina arcaliae* Pop, 1974	Bozova and Antalya (Pop 1974);
68	*Pristina foreli* Bourne, 1891	Erekli; Insirti? (Sperber 1958); locality? (Pop 1974); Sakarya R. (Polatdemir Arslan & Şahin 2003); Küçük Menderes R. (Balık *et al.* 2006a); WBSL (Yıldız *et al.* 2008);
69	*Pristina longiseta* Ehrenberg, 1931	Locality? (Pop 1974); Sakarya R. (Polatdemir Arslan & Şahin 2003); Porsuk R. (Arslan & İlhan 2010); Sazlıdere S. (Taş *et al.* 2011);
70	*Pristina proboscidea* Beddard, 1896	Muğla (Pop 1974); Sakarya R. (Polatdemir Arslan & Şahin 2003);
71	*Pristinella acuminata* Liang, 1958	Işıklı L. (Balık *et al.* 2000); Yuvarlak R. (Yıldız *et al.* 2007b); Lake District Region (Yıldız & Balık 2005);
72	*Pristinella amphibiotica* (Lastočkin, 1927)	Sakarya R. (Arslan & Şahin 2004); Gediz Delta (Balık *et al.* 2004);
73	*Pristinella bilobata* (Bretscher, 1903)	Sakarya R. (Arslan & Şahin 2004); Gediz S. (Balık *et al.* 1999); Sazlıgöl L. (Balık *et al.* 2001); Gediz Delta (Balık *et al.* 2004); TML (Yıldız *et al.* 2007a); Yuvarlak R. (Yıldız *et al.* 2007b); TTRR (Taş *et al.* 2012); Tunca R. (Çamur-Elipek *et al.* 2006);
74	*Pristinella jenkinae* (Stephenson, 1931)	Erekli; Khodja Ali? (Sperber 1958); Sakarya R. (Polatdemir Arslan & Şahin 2003); Yuvarlak R. (Yıldız *et al.* 2007b); TTRR (Taş *et al.* 2012); Aksu R. (Çapraz & Arslan 2005); Kovada L. (Arslan & Şahin 2006); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Porsuk R. (Arslan & İlhan 2010); Çatören and Kunduzlar DL (Arslan *et al.* 2014);
75	*Pristinella menoni* (Aiyer, 1930)	Yarim Bourghaz? (Sperber 1958); Sakarya R. (Arslan & Şahin 2004); Gediz S. (Balık *et al.* 1999); Buldan L. (Ustaoğlu *et al.* 2004); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008);

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	Taxa	**Literature data**
Phylum	**Annelida**	
76	*Pristinella osborni* (Walton,1906)	Işıklı L. (Balık *et al.* 2000); Sakarya R. (Arslan & Şahin 2004); Lake District Region (Yıldız & Balık 2005); WBSL (Yıldız *et al.* 2008); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Tigris R. (Zeybek *et al.* 2016); Aksu R. (Çapraz & Arslan 2005);
77	*Pristinella rosea* (Piguet, 1906)	Sakarya River (Arslan & Şahin 2004); Yuvarlak R. (Yıldız *et al.* 2007b); Porsuk R. (Arslan & İlhan 2010); Ceyhan River Basin Lakes (Fındık & Aras 2016);
78	*Pristinella sima* (Marcus, 1944)	Sakarya R. (Arslan & Şahin 2004); Yuvarlak R. (Yıldız *et al.* 2007b);
79	*Slavina appendiculata* (d'Udekem, 1855)	Sakarya R. (Arslan & Şahin 2004); Işıklı L. (Balık *et al.* 2000); Gediz Delta (Balık *et al.* 2004); WBSL (Yıldız *et al.* 2008); Sazlıdere S. (Taş *et al.* 2011);
80	*Spericaria josinae* (Vejdovský, 1884)	Sakarya R. (Arslan & Şahin 2004);
81	*Stylaria fossularis* Leidy, 1852	Gölcük L. (Geldiay & Tareen 1972); Sakarya R. (Arslan & Şahin 2004); Işıklı L. (Balık *et al.* 2000); Lake District Region (Yıldız & Balık 2005); WBSL (Yıldız *et al.* 2008);
82	*Stylaria lacustris* (Linnaeus, 1767)	Tigris basin (Moubayed *et al.* 1987); Gediz and Güzelhisar S. (Balık *et al.* 1999); Işıklı L. (Balık *et al.* 2000); Sakarya R. (Polatdemir Arslan & Şahin 2003); Gediz Delta (Balık *et al.* 2004); Lake District Region (Yıldız & Balık 2005); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); TTRR (Taş *et al.* 2012); Tigris R. (Zeybek *et al.* 2016); Aksu R. (Çapraz & Arslan 2005); Kovada L. (Arslan & Şahin 2006); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Uluabat L. (Kökmen *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Çorlu R. (Taş *et al.* 2008); Porsuk R. (Arslan & İlhan 2010); Sazlıdere S. (Taş *et al.* 2011); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Küçük Menderes R. (Arslan *et al.* 2016); Büyük Akgöl L. (Arslan *et al.* 2018); Euphrates R. (Aras & Fındık 2016); Ceyhan River Basin Lakes (Fındık & Aras 2016); Çıldır L. (Arslan & Mercan present study);
83	*Uncinais uncinata* (Ørsted, 1842)	Sakarya R. (Arslan & Şahin 2004); Gediz S. (Balık *et al.* 1999); Yuvarlak R. (Yıldız *et al.* 2007b); EHEBSL (Yıldız *et al.* 2012); EHEBSL (Yıldız *et al.* 2012); Uluabat L. (Kökmen *et al.* 2007); Çatören and Kunduzlar D.L. (Arslan *et al.* 2014); Çıldır L. (Arslan & Mercan present study);
84	*Vejdovskyella comata* (Vejdovský, 1884)	Işıklı L. (Balık *et al.* 2000); Gediz Delta (Balık *et al.* 2004);
85	*Vejdovskyella intermedia* (Bretscher, 1896)	Ayva keuy? (Sperber 1958);
Family	**Tubificinae**	
86	*Aulodrilus pigueti* Kowalewski, 1914	Upper Sakarya R. (Arslan & Şahin 2003); Gediz Delta (Balık *et al.* 2004); Lake District Region (Yıldız & Balık 2005); Topçam DL (Yıldız & Balık 2006); Some Lagune L. (Yıldız *et al.* 2007c); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Karasu R. (Zeybek *et al.* 2018); Akgöl L. (Yıldız *et al.* 2009); Aksu R. (Çapraz & Arslan 2005);

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	Taxa	Literature data
Phylum	**Annelida**	
87	*Aulodrilus pluriseta* (Piguet, 1906)	Upper Sakarya R. (Arslan & Şahin 2003); Gediz Delta (Balık *et al.* 2004); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); TML (Yıldız *et al.* 2007a); WBSL (Yıldız *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); Tahtalı DL (Taşdemir *et al.* 2010); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Tigris R. (Zeybek *et al.* 2016); Aksu R. (Çapraz & Arslan 2005); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006);
88	*Aulodrilus limnobius* Bretscher, 1899	Gediz Delta (Balık *et al.* 2004); Yuvarlak R. (Yıldız *et al.* 2007b); Sapanca L. (Şahin & Yıldız 2011); TTRR (Taş *et al.* 2012); Tunca R. (Çamur-Elipek *et al.* 2006);
89	*Bothrioneurum vejdovskyanum* Štolc, 1886	Karamenderes S. (Odabaşı *et al.* 2017);
90	*Branchiura sowerbyi* Beddard, 1892	Buldan L. (Ustaoğlu *et al.* 2004);
91	*Coralliodrilus amissus* Arslan, Timm & Erséus, 2007	Balıkdamı Wetland (Arslan *et al.* 2006);
92	*Epirodrilus moubayedi* Giani & Martínez-Ansemil, 1983	Balıkdamı Wetland (Arslan *et al.* 2006);
93	*Gianius anatolicus* Arslan, Timm & Erséus, 2007	Balıkdamı Wetland (Arslan *et al.* 2006);
94	*Haber speciosus* (Hrabě,1931)	Işıklı L. (Balık *et al.* 2000); Sazlıgöl L. (Balık *et al.* 2001); Gediz Delta (Balık *et al.* 2004); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Topçam DL (Yıldız & Balık 2006); WBSL (Yıldız *et al.* 2008); Uludağ GL (Ustaoğlu *et al.* 2008); Balıkdamı Wetland (Arslan *et al.* 2006); Porsuk R. (Arslan & İlhan 2010);
95	*Haber swirencowi* (Jaroschenko, 1948)	Gediz Delta (Balık *et al.* 2004);
96	*Heterochaeta costata* Claparède, 1863	Some Lagune L. (Yıldız *et al.* 2007c); Karasu R. (Zeybek *et al.* 2018);
97	*Ilyodrilus templetoni* (Southern,1909)	Gediz Delta (Balık *et al.* 2004); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); TML (Yıldız *et al.* 2007a); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Kemer DL (Yıldız *et al.* 2008); Uludağ GL (Ustaoğlu *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); Sapanca L. (Şahin & Yıldız 2011); EHEBSL (Yıldız *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Gölcük L. (Yıldız *et al.* 2015);
98	*Ilyodrilus frantzi* Brinkhurst, 1965	Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Yuvarlak R. (Yıldız *et al.* 2007b);
99	*Ilyodrilus* sp.	Hazar L. (Timm *et al.* 2013);
100	*Isochaetides* sp.	TTRR (Taş *et al.* 2012);
101	*Limnodrilus claparedeianus* Ratzel, 1868	Işıklı L. (Balık *et al.* 2000); Gediz Delta (Balık *et al.* 2004); Eğrigöl L. (Yıldız *et al.* 2005); Küçük Menderes R. (Balık *et al.* 2006a); TML (Yıldız *et al.* 2007a); Küçük Menderes CW (Yıldız *et al.* 2010a); Tigris R. (Zeybek *et al.* 2016); Gölcük L. (Yıldız *et al.* 2015); Porsuk R. (Arslan & İlhan 2010); Gala L. (Çamur-Elipek *et al.* 2010); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Küçük Menderes R. (Arslan *et al.* 2016);

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	Taxa	**Literature data**
Phylum	**Annelida**	
102	*Limnodrilus hoffmeisteri* Claparède, 1862	Locality? (Naidu 1965); Karagöl (Ustaoğlu 1980); Asi S. (Moubayed *et al.* 1987); Ankara S. (Kazancı & Girgin 1998); Işıklı L. (Balık *et al.* 2000); Sazlıgöl L. (Balık *et al.* 2001); Gümüş S. (Öntürk & Arslan 2003); Gediz Delta (Balık *et al.* 2004); Kuş L. (Balık *et al.* 2005); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Topçam DL (Yıldız & Balık 2006); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Kemer DL (Yıldız *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); Tahtalı DL (Taşdemir *et al.* 2010); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Tigris R. (Zeybek *et al.* 2016); Karasu R. (Zeybek *et al.* 2018); Akgöl L. (Yıldız *et al.* 2009); İkizgöl (Yıldız *et al.* 2009); Aksu R. (Çapraz & Arslan 2005); Kovada L. (Arslan & Şahin 2006); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Uluabat L. (Kökmen *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Çorlu R. (Taş *et al.* 2008); Menderes R. (Akbulut *et al.* 2009); Porsuk R. (Arslan & İlhan 2010); Gala L. (Çamur-Elipek *et al.* 2010); Sazlıdere S. (Taş *et al.* 2011); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Küçük Menderes R. (Arslan *et al.* 2016); Büyük Akgöl L. (Arslan *et al.* 2018); Euphrates R. (Aras & Fındık 2016); Ceyhan River Basin Lakes (Fındık & Aras 2016); Çıldır L. (Arslan & Mercan present study);
103	*Limnodrilus hoffmeisteri* f. *parvus* Southern, 1909	Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); Bozalan L. (Balık *et al.* 2006b); Some Lagune L. (Yıldız *et al.* 2007c); Sapanca L. (Şahin & Yıldız 2011); Gölcük L. (Yıldız *et al.* 2015); Kovada L. (Arslan & Şahin 2006);
104	*Limnodriloides pierantonii* (Hrabě, 1971)	Gediz Delta (Balık *et al.* 2004);
105	*Limnodrilus profundicola* (Verrill, 1871)	Işıklı L. (Balık *et al.* 2000); Gediz Delta (Balık *et al.* 2004); Kuş L. (Balık *et al.* 2005); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R.(Balık *et al.* 2006a); Yuvarlak R. (Yıldız *et al.* 2007b); Kemer DL (Yıldız *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Tigris R. (Zeybek *et al.* 2016); Gölcük L. (Yıldız *et al.* 2015); Uluabat L. (Kökmen *et al.* 2007); Gala L. (Çamur-Elipek *et al.* 2010);

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	Taxa	Literature data
Phylum	**Annelida**	
106	*Limnodrilus udekemianus* Claparède, 1862	Ankara S. (Kazancı & Girgin 1998); 1998; Işıklı L. (Balık *et al.* 2000); Gümüş S. (Öntürk & Arslan 2003); Gediz Delta (Balık *et al.* 2004); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); Topçam DL (Yıldız & Balık 2006); TML (Yıldız *et al.* 2007a); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Kemer DL (Yıldız *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); Tahtalı DL (Taşdemir *et al.* 2010); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Gölcük L. (Yıldız *et al.* 2015); İkizgöl (Yıldız *et al.* 2009); Aksu R. (Çapraz & Arslan 2005); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Musaözü DL (Arslan *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Çorlu R. (Taş *et al.* 2008); Menderes R. (Akbulut *et al.* 2009); Porsuk R. (Arslan & İlhan 2010); Sazlıdere S. (Taş *et al.* 2011); Küçük Menderes R. (Arslan *et al.* 2016);
107	*Limnodrilus* sp.	Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Menderes R. (Akbulut *et al.* 2009); Gala L. (Çamur-Elipek *et al.* 2010);
108	*Monopylephorus irroratus* (Verrill, 1873)	Hazar L. (Şahin & Baysal 1972);
109	*Peipsidrilus libanus* (Giani *et al.*, 1982)	Balıkdamı Wetland (Arslan *et al.* 2006); Çıldır L. (Arslan & Mercan present study);
110	*Peipsidrilus* sp.	TTRR (Taş *et al.* 2012); Tunca R. (Çamur-Elipek *et al.* 2006);
111	*Peloscolex arganoi* Pop, 1974	Mersin (Pop 1974);
112	*Peloscolex boitanii* Pop, 1974	Mersin (Pop 1974);
113	*Peloscolex cottarelli* Pop, 1974	Mersin (Pop 1974);
114	*Peloscolex euxinicus* Hrabě, 1966	Gediz Delta (Balık *et al.* 2004);
115	*Potamothrix alatus* Finogenova, 1972	Çıldır L. (Arslan & Mercan present study);
116	*Potamothrix alatus hazaricus* Timm & Arslan, 2013	Hazar L. (Timm *et al.* 2013);
117	*Potamothrix bavaricus* (Oschmann, 1913)	Seyhan Dam Lake (Kırgız 1989); Gediz Delta (Balık *et al.* 2004); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Yuvarlak R. (Yıldız *et al.* 2007b); Some Lagune L. (Yıldız *et al.* 2007c); WBSL (Yıldız *et al.* 2008); Kemer DL (Yıldız *et al.* 2008); Uludağ GL (Ustaoğlu *et al.* 2008); Karasu R. (Zeybek *et al.* 2018); Gebekirse L. (Yıldız *et al.* 2009); Kovada L. (Arslan & Şahin 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Euphrates R. (Aras & Fındık 2016); Çıldır L. (Arslan & Mercan present study);
118	*Potamothrix bedoti* (Piguet, 1913)	Kuş L. (Balık *et al.* 2005); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); Topçam DL (Yıldız & Balık 2006); Yuvarlak R. (Yıldız *et al.* 2007b); Some Lagune L. (Yıldız *et al.* 2007c); WBSL (Yıldız *et al.* 2008); Kemer DL (Yıldız *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); Karasu R. (Zeybek *et al.* 2018); Karagöl L. (Topkara *et al.* 2018); Büyük Akgöl L. (Arslan *et al.* 2018);

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	Taxa	**Literature data**
Phylum	**Annelida**	
119	*Potamothrix heuscheri* (Bretscher, 1900)	Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); Yuvarlak R. (Yıldız *et al.* 2007b); Some Lagune L. (Yıldız *et al.* 2007c); WBSL (Yıldız *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); Karasu R. (Zeybek *et al.* 2018); Akgöl L. (Yıldız *et al.* 2009); Küçük Menderes R. (Arslan *et al.* 2016);
120	*Potamothrix hammoniensis* (Michaelsen, 1901)	Seyhan Dam Lake (Kırgız 1989); Işıklı L. (Balık *et al.* 2000); Sazlıgöl L. (Balık *et al.* 2001); Gediz Delta (Balık *et al.* 2004); Kuş L. (Balık *et al.* 2005); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); Topçam DL (Yıldız & Balık 2006); TML (Yıldız *et al.* 2007a); Yuvarlak R. (Yıldız *et al.* 2007b); Some Lagune L. (Yıldız *et al.* 2007c); WBSL (Yıldız *et al.* 2008); Kemer DL (Yıldız *et al.* 2008); Uludağ GL (Ustaoğlu *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); Sapanca L. (Şahin & Yıldız 2011); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Karasu R. (Zeybek *et al.* 2018); Karagöl L. (Topkara *et al.* 2018); Gölcük L. (Yıldız *et al.* 2015); Gebekirse and Akgöl L. (Yıldız *et al.* 2009); Aksu R. (Çapraz & Arslan 2005); Kovada L. (Arslan & Şahin 2006); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Uluabat L. (Kökmen *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Menderes R. (Akbulut *et al.* 2009); Porsuk R. (Arslan & İlhan 2010); Sazlıdere S. (Taş *et al.* 2011); Gölbaşı L. (Arslan *et al.* 2013); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Küçük Menderes R. (Arslan *et al.* 2016); Büyük Akgöl L. (Arslan *et al.* 2018); Euphrates R. (Aras & Fındık 2016); Ceyhan River Basin Lakes (Fındık & Aras 2016); Çıldır L. (Arslan & Mercan present study);
121	*Potamothrix moldaviensis* Vejdovský & Mrazek, 1902	Eğrigöl L. (Yıldız *et al.* 2005);
122	*Potamothrix vejdovskyi* (Hrabě, 1941)	Gediz Delta (Balık *et al.* 2004); Sapanca L. (Şahin & Yıldız 2011);
123	*Potamothrix* sp.	Eğirdir L. (Arslan 2006);
124	*Psammoryctides albicola* (Michaelsen, 1901)	Işıklı L. (Balık *et al.* 2000); Sazlıgöl L. (Balık *et al.* 2001); Gümüş S. (Öntürk & Arslan 2003); Gediz Delta (Balık *et al.* 2004); Kuş L. (Balık *et al.* 2005); Lake District Region (Yıldız & Balık 2005); TML (Yıldız *et al.* 2007a); Yuvarlak R. (Yıldız *et al.* 2007b); TTRR (Taş *et al.* 2012); Gölcük L. (Yıldız *et al.* 2015); Aksu R. (Çapraz & Arslan 2005); Kovada L. (Arslan & Şahin 2006); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Uluabat L. (Kökmen *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Menderes R. (Akbulut *et al.* 2009); Porsuk R. (Arslan & İlhan 2010); Gölbaşı L. (Arslan *et al.* 2013); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Küçük Menderes R. (Arslan *et al.* 2016); Euphrates R. (Aras & Fındık 2016); Çıldır L. (Arslan & Mercan present study);
125	*Psammoryctides barbatus* (Grube, 1861)	Sazlıgöl L. (Balık *et al.* 2001); Gediz Delta (Balık *et al.* 2004); Eğrigöl L. (Yıldız *et al.* 2005); TML (Yıldız *et al.* 2007a); Sapanca L. (Şahin & Yıldız 2011); Hazar L. (Timm *et al.* 2013); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Ceyhan River Basin Lakes (Fındık & Aras 2016);

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	Taxa	Literature data
Phylum	**Annelida**	
126	*Psammoryctides deserticola* (Grimm, 1877)	Işıklı L. (Balık *et al.* 2000); Sazlıgöl L. (Balık *et al.* 2001); Gediz Delta (Balık *et al.* 2004); Kuş L. (Balık *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); Topçam DL (Yıldız & Balık 2006); TML (Yıldız *et al.* 2007a); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Kemer DL (Yıldız *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); Sapanca L. (Şahin & Yıldız 2011); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Tigris R. (Zeybek *et al.* 2016); Gölcük L. (Yıldız *et al.* 2015);
127	*Psammoryctides longicapillatus* Martínez-Ansemil & Giani, 1983	Euphrates (Moubayed *et al.* 1987);
128	*Psammoryctides moravicus* (Hrabě, 1934)	Ankara S. (Kazancı & Girgin 1998); Gediz Delta (Balık *et al.* 2004); TTRR (Taş *et al.* 2012); Tunca R. (Çamur-Elipek *et al.* 2006); Balıkdamı Wetland (Arslan *et al.* 2006);
129	*Psammoryctides* sp.	Eğirdir L. (Arslan 2006);
130	*Rhyacodrilus coccineus* (Vejdovský, 1876)	Gümüş S. (Öntürk & Arslan 2003); TTRR (Taş *et al.* 2012); Tunca R. (Çamur-Elipek *et al.* 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Uluabat L. (Kökmen *et al.* 2007); Porsuk R. (Arslan & İlhan 2010);
131	*Spirosperma ferox* (Eisen, 1879)	Ankara S. (Kazancı & Girgin 1998); Işıklı L. (Balık *et al.* 2000); Sazlıgöl L. (Balık *et al.* 2001); Gediz Delta (Balık *et al.* 2004); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Sapanca L. (Şahin & Yıldız 2011); EHEBSL (Yıldız *et al.* 2012);
132	*Spirosperma nikolskyi* Lastočkin & Sokolskaya, 1953	Lake District Region (Yıldız & Balık 2005); Denizli mountain lakes (Yıldız & Ustaoğlu 2016);
133	*Spirosperma velutinus* (Grube, 1879)	Gediz Delta (Balık *et al.* 2004); Balıkdamı Wetland (Arslan *et al.* 2006); Çıldır L. (Arslan & Mercan present study);
134	*Stylodrilus parvus* (Hrabe & Černosvitov, 1927)	EBSL (Yıldız *et al.* 2010b); EHEBSL (Yıldız *et al.* 2012); Balıkdamı Wetland (Arslan *et al.* 2006); Çıldır L. (Arslan & Mercan present study);
135	*Stylodrilus heringianus* Claparède, 1862	EBSL (Yıldız *et al.* 2010b); EHEBSL (Yıldız *et al.* 2012);
136	*Tubifex acuticularis* Martínez-Ansemil et Giani, 1983	Balıkdamı Wetland (Arslan *et al.* 2006); Çıldır L. (Arslan & Mercan present study);
137	*Tubifex blanchardi* Vejdovský, 1891	Denizli mountain lakes (Yıldız & Ustaoğlu 2016);
138	*Tubifex costatus* (Claparède, 1863)	Gediz Delta (Balık *et al.* 2004);
139	*Tubifex ignotus* (Stolć, 1886)	Sazlıgöl L. (Balık *et al.* 2001); Gediz Delta (Balık *et al.* 2004); Kuş L. (Balık *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); TML (Yıldız *et al.* 2007a); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); Sapanca L. (Şahin & Yıldız 2011); Balıkdamı Wetland (Arslan *et al.* 2006);
140	*Tubifex nerthus* Michaelsen, 1908	Sazlıgöl L. (Balık *et al.* 2001); Kuş L. (Balık *et al.* 2005); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Yuvarlak R. (Yıldız *et al.* 2007b); WBSL (Yıldız *et al.* 2008); Sapanca L. (Şahin & Yıldız 2011); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); İkizgöl (Yıldız *et al.* 2009);

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	Taxa	**Literature data**
Phylum	**Annelida**	
141	*Tubifex newaensis* (Michaelsen, 1903)	Yuvarlak R. (Yıldız *et al.* 2007b); Some Lagune L. (Yıldız *et al.* 2007c); Karasu R. (Zeybek *et al.* 2018);
142	*Tubifex montanus* Kowalewski, 1919	Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Topçam DL (Yıldız & Balık 2006); TML (Yıldız *et al.* 2007a); Yuvarlak R. (Yıldız *et al.* 2007b); EHEBSL (Yıldız *et al.* 2012);
143	*Tubifex tubifex* (Müller,1774)	Gölcük L. (Geldiay & Tareen 1972); Karagöl (Ustaoğlu 1980); Karasu S. (Çetinkaya *et al.* 1994); Ankara S. (Kazancı & Girgin 1998); Işıklı L. (Balık *et al.* 2000); Sazlıgöl L. (Balık *et al.* 2001); Gümüş S. (Öntürk & Arslan 2003); Gediz Delta (Balık *et al.* 2004); Kuş L. (Balık *et al.* 2005); Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Küçük Menderes R. (Balık *et al.* 2006a); Bozalan L. (Balık *et al.* 2006b); Topçam DL (Yıldız & Balık 2006); Yuvarlak R. (Yıldız *et al.* 2007b); Kemer DL (Yıldız *et al.* 2008); Uludağ GL (Ustaoğlu *et al.* 2008); Küçük Menderes CW (Yıldız *et al.* 2010a); Sapanca L. (Şahin & Yıldız 2011); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Tigris R. (Zeybek *et al.* 2016); Karagöl L. (Topkara *et al.* 2018); Gölcük L. (Yıldız *et al.* 2015); Gebekirse and Akgöl L. (Yıldız *et al.* 2009); İkizgöl (Yıldız *et al.* 2009); Aksu R. (Çapraz & Arslan 2005); Kovada L. (Arslan & Şahin 2006); Tunca R. (Çamur-Elipek *et al.* 2006); Eğirdir L. (Arslan 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Musaözü DL (Arslan *et al.* 2007); Uluabat L. (Kökmen *et al.* 2007); Manyas L. (Arslan & Ahıska 2007); Çorlu R. (Taş *et al.* 2008); Menderes R. (Akbulut *et al.* 2009); Porsuk R. (Arslan & İlhan 2010); Gala L. (Çamur-Elipek *et al.* 2010); Sazlıdere S. (Taş *et al.* 2011); Çatören and Kunduzlar DL (Arslan *et al.* 2014); Büyük Akgöl L. (Arslan *et al.* 2018); Euphrates R. (Aras & Fındık 2016); Ceyhan River Basin Lakes (Fındık & Aras 2016); Çıldır L. (Arslan & Mercan present study);
144	*Tubifex tubifex* f. *tubifex*	Locality? (Pop 1974); Upper Euphrates and Ankara S. (Moubayed *et al.* 1987); TML (Yıldız *et al.* 2007a);
145	*Tubifex tubifex* f. *bergi* (Müller, 1774)	Eğrigöl L. (Yıldız *et al.* 2005); Lake District Region (Yıldız & Balık 2005); Some Lagune L. (Yıldız *et al.* 2007); WBSL (Yıldız *et al.* 2008); Denizli mountain lakes (Yıldız & Ustaoğlu 2016); Gebekirse L. (Yıldız *et al.* 2009);
146	*Tubifex* sp.	Çıldır L. (Arslan & Mercan present study);
147	*Tubifex* spp.	Seyhan Dam Lake (Kırgız 1989); Enne S. (Tanatmış 1989); Akşehir L. (Sözen & Yiğit 1999); Karacaören Dam Lake (Karaşahin & Yıldırım 2000); EHEBSL (Yıldız *et al.* 2012); TTRR (Taş *et al.* 2012); Kovada L. (Arslan & Şahin 2006); Tunca R. (Çamur-Elipek *et al.* 2006);
148	*Quistadrilus multisetosus* (Smith, 1900)	Işıklı L. (Balık *et al.* 2000); Sazlıgöl L. (Balık *et al.* 2001); Lake District Region (Yıldız & Balık 2005); Denizli mountain lakes (Yıldız & Ustaoğlu 2016);
149	*Varichaetadrilus psammophilus* (Loden, 1977)	Gediz Delta (Balık *et al.* 2004);
Family	**Lumricidae**	
150	*Eisenella tetraedra* (Savigny, 1826)	Adana (Omodeo 1956); Ankara S. (Kazancı & Girgin 1998); Yuvarlak S. (Barlas *et al.* 2000); TTRR (Taş *et al.* 2012); Tunca R. (Çamur-Elipek *et al.* 2006); Balıkdamı Wetland (Arslan *et al.* 2006); Porsuk R. (Arslan & İlhan 2010);

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	Taxa	Literature data
Phylum	**Annelida**	
151	*Tatriella slovenica* Hrabě, 1936	Yuvarlak R. (Yıldız *et al.* 2007b);
Subphylum	**Annelida sbp Incertae sedis**	
Order	**APHANONEURA**	
Family	**Potamodrilidae**	
152	*Potamodrilus fluviatilis* (Lastočkin, 1935)	Gediz S. (Balık *et al.* 1999);
Family	**Aeolosomatidae**	
153	*Aeolosoma tenebrarum* Vejdovský, 1884	Gediz S. (Balık *et al.* 1999); Gediz Delta (Balık *et al.* 2004);
154	*Aeolosoma headleyi* Beddard, 1888	Tarsus (Pop 1974); Gediz S. (Balık *et al.* 1999);
155	*Aeolosoma variegatum* Vejdovský, 1886	Gediz S. (Balık *et al.* 1999);
156	*Aeolosoma leidyi* Cragin, 1887	Gediz Delta (Balık *et al.* 2004);
157	*Aeolosoma* sp.	Gölcük L. (Geldiay & Tareen 1972); Buldan L. (Ustaoğlu *et al.* 2004).

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Composition and abundance of oligochaetes in Scandinavian lakes in the 1970s in full compatibility with the ”morpho-edaphic index”. Do these relationships still hold 40 years later?

GÖRAN MILBRINK
Department of Ecology and Genetics/Animal Ecology, Evolutionary Biology Centre, Uppsala University, Sweden

Abstract

In the 1960s it became fairly well-known that biological indices through their supposedly integrative power could be more robust measures of the water quality than separate chemical data. Oligochaetes and chironomid larvae in the bottom fauna hereby played an important role. Precision, however, increased dramatically if the relationship between oligochaetes and the so-called morpho-edaphic index, i.e. the average total phosphorus content in µg/l divided by the mean depth (in meters) of the lake (Ryder *et al.*, 1974), was used in stead (Milbrink, 1978). The percentage composition and total abundance of oligochaetes in a number of lakes or selected basins of large lakes in southern Sweden and Norway were thus tested against this index in the 1970s. In logarithmic scales these relationships turned out to be more or less linear. The immediate conclusions were then that this method of classifying lakes could be of considerable scientific as well as practical value. Today we know a lot more about the ecological preferences of oligochaete species and characteristic species associations. The oligochaete fauna of the large lakes of southern Sweden, Mälaren, Vättern, Vänern and Hjälmaren, have been studied in detail over many years—over 100 years in the first three of these lakes and in the last lake for over 50-years. Effects of generally applied advanced sewage treatment in Sweden since the late 1960s are easily recognizable in the material. We have a situation of oligotrophication. With our new knowledge it comes natural today to study changes in species composition after trophic change and to investigate if the above close relationships in abundance are still largely linear.

Introduction

In the early 1960s the species composition of profundal freshwater oligochaetes largely proved to be indicative of the actual water quality (Brinkhurst, 1966). However, this relationship between oligochaetes and, for example, the average concentrations of total phosphorus in the water, was found to be rather unprecise. Precision increased dramatically if the relationship between oligochaetes and the so-called morpho-edaphic index (Ryder *et al.*, 1974), was used in stead (Milbrink, 1978). The morpho-edaphic index is the average total phosphorus content in µg/l in surfacial water strata divided by the mean depth (in meters) of the lake—or a particular basin of the lake.

In 1977 a comprehensive figure (Figure 1) was presented showing the actual relationships between profundal oligochaetes in the large lakes in southern Scandinavia (Figure 2) and a few additional lakes and the morpho-edaphic index (Milbrink, 1978). At that time Figure 1 was in black and white. Each lake locality is thus marked in the figure in the form of a histogram positioned along the abscissa showing the morpho-edaphic index (logarithmic scale). Each histogram shows the average percentage composition of oligochaetes with reference to their known sensitivity to eutrophication (cf. Table I). The ordinate which also has a logarithmic scale shows the mean abundance of oligochaetes in each locality and moment. Thus in a double-logarithmic scale abundance values representing the different localities roughly fell along a straight line. The original Figure 1 also shows oligochaete indicator communities marked Group I, Group II and so forth. In the present Figure 1 patterns in black-and-white symbolizing the different indicator communities have been

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replaced with a colour scale in accordance with the original ”Saprobien System” designed by Liebmann (1962) and Zelinka & Marvan (1961). In that system red stands for tolerant species and bad conditions and blue for the opposite, i.e. sensitive species and favourable conditions. Striking red colours dominate the right part of the figure, whereas shades of blue dominate the left (see below for further explanations). With reference to the morpho-edaphic index oligochaetes thereby would have an indicator value both qualitatively and quantitatively. This is quite obviously an advantage both from a scientific, as well as a practical point of view. We have today much more information from lakes of all kinds in Scandinavia and elsewhere. With all the new information of the profundal oligochaete fauna we have, the question is now, do these relationships based upon fairly preliminary data from the 1960s and 1970s hold 40–50 years later? The main hypothesis is that this is true.

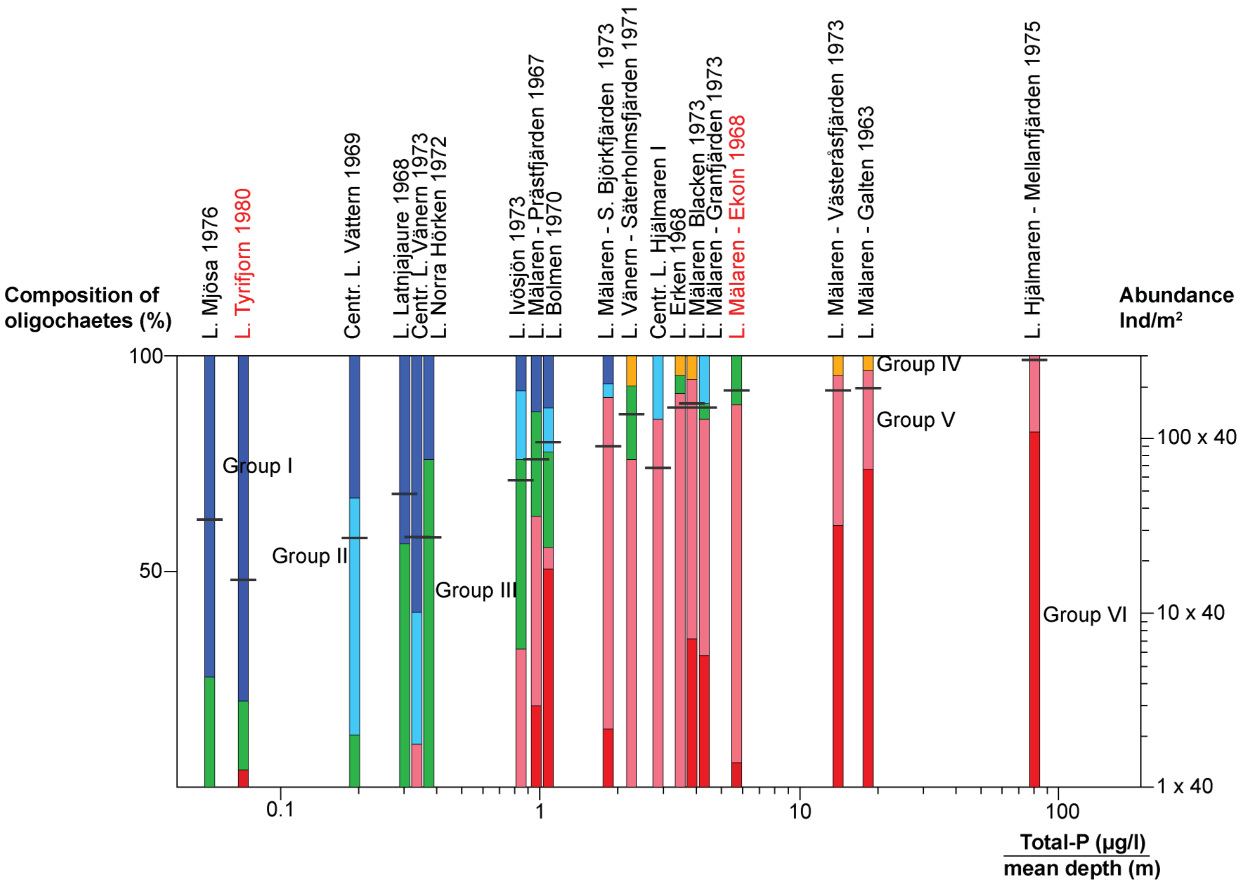

FIGURE 1. Percentage composition of profundal oligochaetes in lakes of different nutrient standard and size in Scandinavia (redrawn from Milbrink, 1978). Each histogram in the diagram shows the percentage composition of oligochaetes in a particular site at a particular time in accordance with the continental colour scale (Liebmann, 1962; Zelinka & Marvan, 1961) thus forming characteristic species groups with known specific sensitivity to oxygen deficiency (see explantion in text). Each diagram is positioned along the abscissa with reference to the morpho-edaphic index value (Ricker *et al.*, 1974). Oligochaete abundance is also given on the ordinate. Both axes are logarithmic. Abundance values form an approximate straight line.

Material and Methods

Information about profundal oligochaete communities in Scandinavian lakes were mainly based upon mean values obtained from 3–5 parallel Ekman grab samples taken in the 1960s and 1970s. From the large lakes of southern Sweden-Mälaren, Vänern and Vättern—oligochaete collections from representative station networks exist from the start of the 20th century. We thus have the Ekman collection from Lake Vättern (1911–1914), the Alm collection from Lake Mälaren (1915–1916), the Nybelin collection from the same lake (1933–1935), and the Nordqvist/Vallin collection from Lake Vänern (1922). All bottom samples were taken with the

Ekman grab sampler once in the spirit of getting as much information as possible about the bottom fauna as potential food-source for fish. The entire oligochaete material, which was mainly well-preserved in 70% alcohol, has been analyzed by the author.

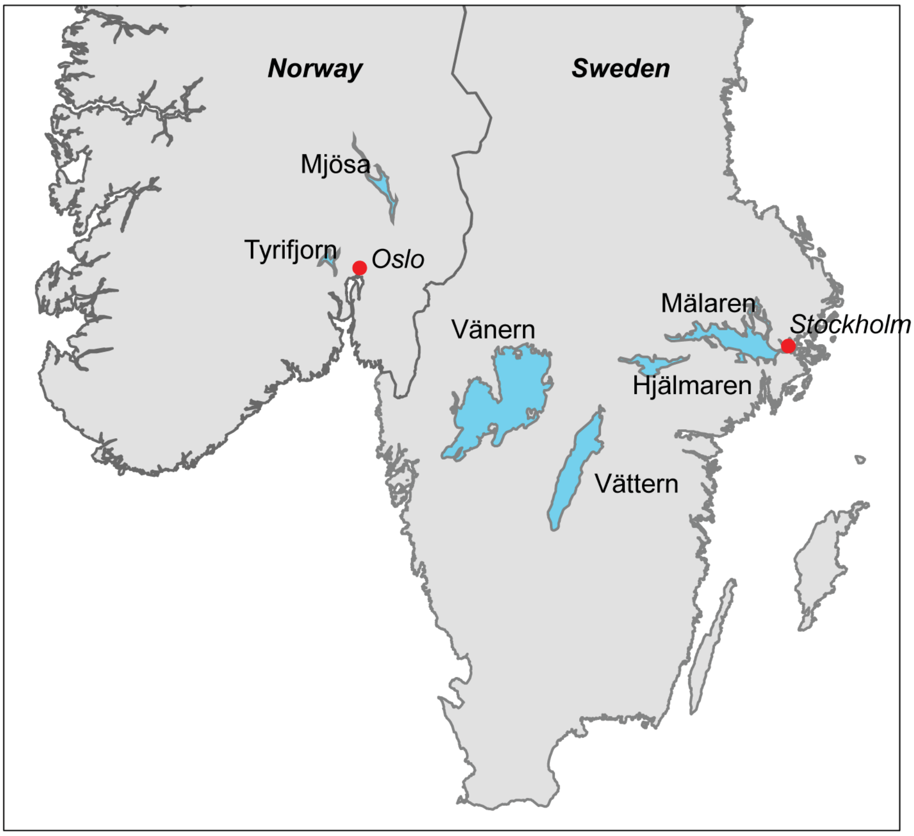

FIGURE 2. Map of southern Scandinavia showing the large lakes in south Sweden—Vänern, Vättern, Mälaren and Hjälmaren—and in south Norway—Mjösa and Tyrifjorn. The geographic positions of Oslo , the capital of Norway, is in WSG84, 59.914°N 10.752°E. Similar for Stockholm, the capital of Sweden, is 59.329°N 18.069°E.

Key localities within station nets in these large lakes plus Lake Hjälmaren in southern Sweden have since 1975 been sampled every three years as part of a routine—at least until 2010, often longer. Three parallel samples out of five on each locality each time have likewise been analyzed by the author. The bottom material has usually been sieved through 0.5 mm meshes and the animal residues have been sorted out and thereafter been kept in 70% alcohol.

It is, of course, fully understood that the below information is based upon the mean values of parallel representative samples. It also stands quite clear that if there had only been few parallel samples available for each analysis each time, for example from the early collections, the information value would be more limited. Statistical methods are on the whole hard to apply.

As said above, Group I in Figure 1 stands for the most sensitive oligochaete species we know, i.e. *Spirosperma ferox* (Eisen) and *Stylodrilus heringianus* Claparède*,* Group II stands for the likewise sensitive species *Psammoryctides barbatus* Grube. Group III stands for the ambivalent species *Tubifex tubifex* (Stolc) and *Eiseniella tetraedra* (Savigny)*,* Group IV for species with little known ecological requirements, Group V for the tolerant species *Potamothrix hammoniensis* (Michaelsen) and a number of species with similar ecological demands, for instance several Ponto-Caspian *Potamothrix*-species, and lastly Group VI for the very tolerant species *Limnodrilus hoffmeisteri* Claparède.

For references to the actual depth of each sampling station and to its geographical position, the reader is referred to the legends.

TABLE I. Mean occurrence of freshwater oligochaetes in Scandinavian lake-types (redrawn from Milbrink 1978). The mean percentual occurrence of each species in the oligotrophic, mesotrophic and eutrophic environments, respectively, as estimated by the author. Full names of the species in the table are, *Limnodrilus hoffmeisteri* Claparède, *Potamothrix hammoniensis* (Michaelsen), *Limnodrilus claparedeanus* (Ratzel), *Aulodrilus pluriseta* (Piguet), *Potamothrix heuscheri* (Bretscher), *Ilyodrilus templetoni* (Southern), *Potamothrix vejdovskyi* (Hrabè), *Potamothrix bedoti* (Piguet), *Limnodrilus udekemianus* Claparède, *Potamothrix moldaviensis* (Vejdovský & Mrázek), *Lophochaeta ignota* (Stolc), *Aulodrilus limnobius* Bretscher, *Bothrioneurum vejdovskyanum* Stolc, *Aulodrilus pigueti* Kowalewski, *Psammoryctides albicola* (Michaelsen), *Rhyacodrilus coccineus* Vejdovský, *Rhyacodrilus falciformis* Bretscher, *Psammoryctides barbatus* (Grube), *Limnodrilus profundicola* (Verrill), *Spirosperma ferox* (Eisen), *Rhynchelmis limosella* Hoffmeister, *Stylodrilus heringianus* Claparède, *Eiseniella tetraedra* (Savigny).

Species	Oligo	Meso	Eut	Species	Oligo	Meso	Eut
Limnodrilus hoffmeisteri	1	2	7	*Aulodrilus limnobius*	2	5	3
Potamothrix hammoniensis	1	3	6	*Bothrioneurum vejdovskyanum*	2	5	3
Limnodrilus claparedeanus	1	3	6	*Aulodrilus pigueti*	3	5	2
Aulodrilus pluriseta		4	6	*Psammoryctides albicola*	4	5	1
Potamothrix heuscheri		4	6	*Rhyacodrilus coccineus*	4	6	
Tubifex tubifex	5		5				
Eiseniella tetraedra	5		5	*Rhyacodrilus falciformis*	(5)	(5)	
Ilyodrilus templetoni		5	5				
Potamothrix vejdovskyi		5	5	*Psammoryctides barbatus*	5	5	
Potamothrix bedoti		5	5	*Limnodrilus profundicola*	7	3	
Limnodrilus udekemianus	1	4	5	*Spirosperma ferox*	8	2	
Potamothrix moldaviensis	2	4	4	*Rhynchelmis tetratheca*	8	2	
Lophochaeta ignota	2	4	4	*Stylodrilus heringianus*	9	1	

Presentation

Most information behind, for instance, Figure 1 (as well as Figure11 below) is the result of oligochaete analyses made on material obtained from station nets in the large lakes of southern Scandinavia (see Figure 2), Mälaren (1.072 km2), Vänern (5.650 km2), Vättern (1.893 km2), Hjälmaren (483 km2), Mjösa (368 km2), and Tyrifjorn (136 km2). All histograms and abundance values in the original Fig. 1 showed the situation in the 1960s and 1970s. Now some 50 years later we know a lot more. Figure 1 is largely based upon Table I from Milbrink (1978), now slightly modified. In this table each oligochaete species had been given 10 points in total—equivalent to 100% . The table shows how frequently each species, on the average, is found in oligotrophic, mesotrophic or eutrophic environments. The very sensitive species *S. ferox* and *S. heringianus* are thus given 8 and 9 points, respectively, in oligotrophic environments, 2 and 1, respectively, in mesotrophic environments and none in eutrophic environments. These species forming the Group I in the original figure have now been given a deep blue colour. Another fairly sensitive species, *P. barbatus*, was given 5 points in the oligotrophic enviroment and 5 in mesotrophy has been given a light blue colour in the histograms. On the other hand the very tolerant species *L. hoffmeisteri* was given 7 points in eutrophic environments, 2 in mesotrophy and 1 in oligotrophy. This species has been given a deep red colour in the histograms. The likewise very tolerant species *P. hammoniensis* was given 6 points in eutrophic environments, 3 in mesotrophic and 1 in oligotrophic environments, etc. The colour given to the latter species and to some other species with rather similar ecological requirements is light red. The somewhat ambivalent species *T. tubifex* and *E. tetraedra* forming Group III in the original figure were given a green colour and finally species with little shown preferences or those species with little known ecological demands have been given a yellow colour. Thus, as said above, the very first impression is that the left side of Figure 1 is mainly blue while the right side is mainly red.

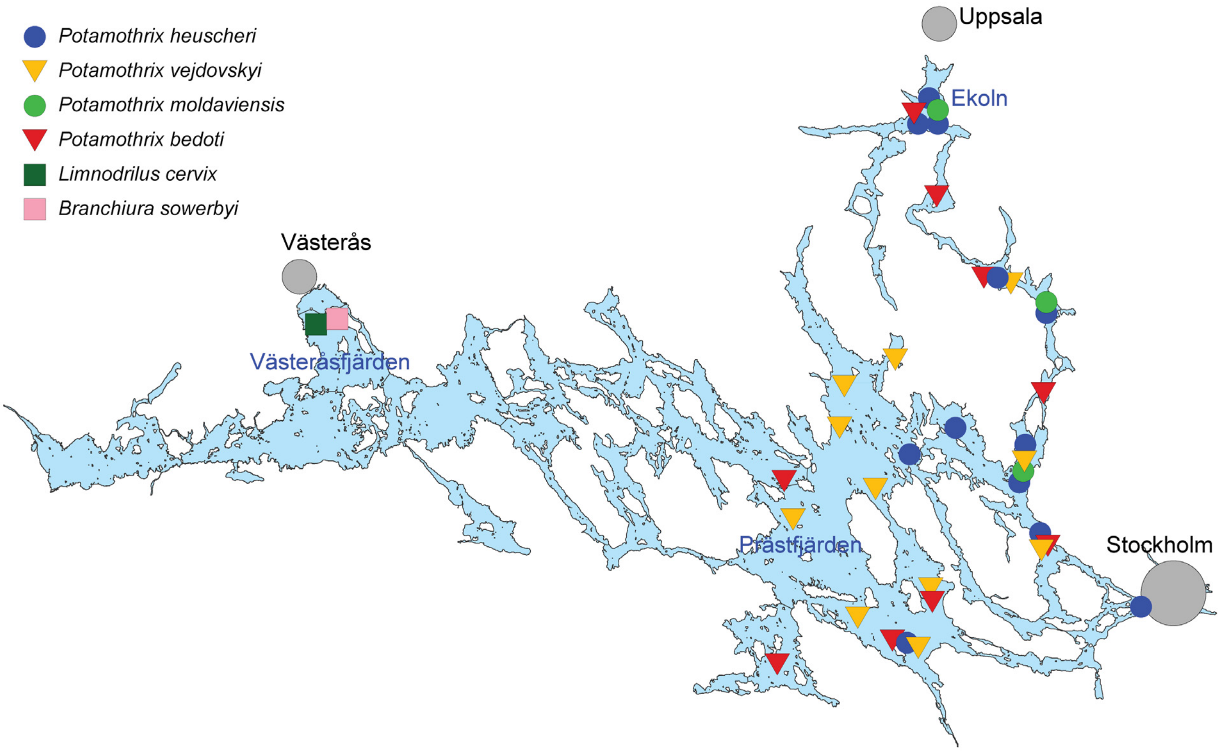

FIGURE 3. Map of Lake Mälaren showing its different basins and the sampling stations once defined by the Swedish EPA, such as the basins of Västeråsfjärden in the west (mean depth about15m, mean total-phoshorus concentrations about 40 µg/l), Prästfjärden in the centre (depths varying between 30 and 50 m, mean total-phosphorus concentrations about 15–20 µg/l), and Ekoln in the north (mean depth about 40 m, mean total-phosphrus concentration about 40 µg/l). Thus Lake Mälaren is eutrophic in its western and northern parts and mesotrophic in its central parts. The map also shows records of invading Ponto-Caspian *Potamothrix* species from the east and *Branchiura sowerbyi* Beddard from the sub-tropics (Milbrink, 1999).

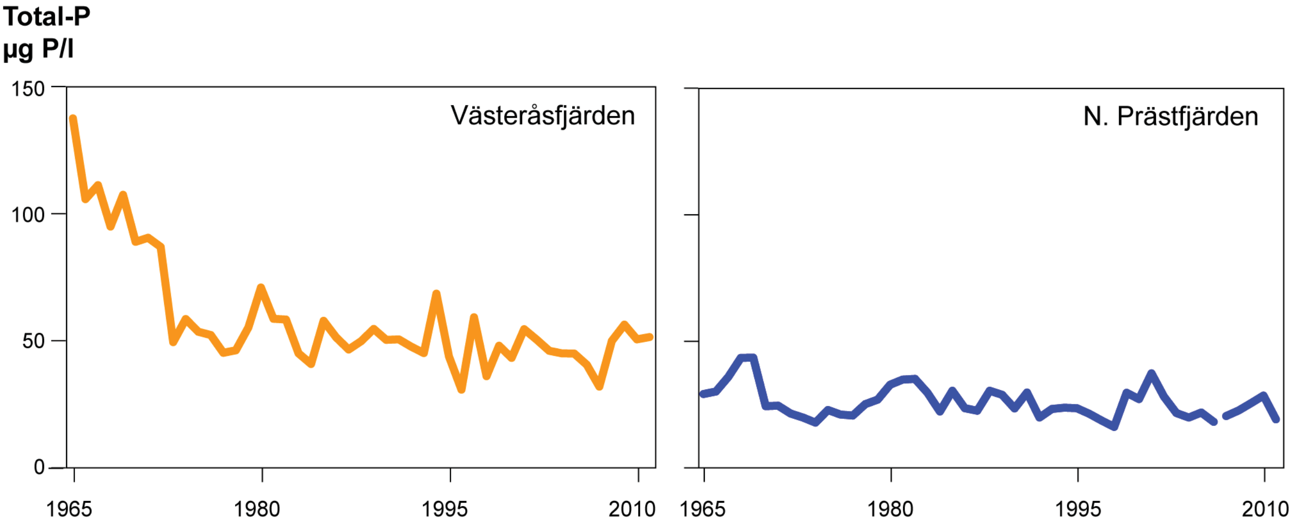

FIGURE 4. Total-phophorus concentrations (in µg/l) over time in surficial water layers in the basins of Västeråsfjärden and Prästfjärden in Lake Mälaren (1965–2011).

In the original Table I in Milbrink, 1978, however, a few very characteristic oligochaete species had been ommitted because of the above mentioned ambivalence. One of these species, *T. tubifex*, actually tends to occur on either side of the ecological spectrum (Milbrink, 1978) and would rather have obtained 5 points in both eutrophic and oligotrophic environments but none in mesotrophy (the present Table I). *E. tetraedra* is also a frequently occurring species but with little known preferences. This species is most often found in oligotrophy but can obviously also stand eutrophied environments. Also this species would be given similar points as *T. tubifex* (Table I).

Figure 3 is a map of Lake Mälaren primarily showing how far the Ponto-Caspian oligochaete fauna has reached into the lake (Milbrink, 1999). The names of some of the basins discussed here are also given. In Figure 1, for instance, the histograms of both Västeråsfjärden and Prästfjärden basins could be seen. In both basins the oligochaete fauna is dominated by *P. hammoniensis.* In Västeråsfjärden the very tolerant species *L. hoffmeisteri* complements the latter species, while in Prästfjärden there is a substantial share of the sensitive species *S. ferox* (about 20%) but also of *T. tubifex* and *L. hoffmeisteri*. Figure 4 shows the mean values of total-phosporus (in µg/l) in surficial water layers in the same two basins. In Västeråsfjärden in the 1960s and 1970s, i.e. before sewage treatment, values were very high reaching more than 100 µg/l which means that the water was not suitable for human consumption. Today values have come down to 30–40 µg/l implying ”nearly suitable water quality for human needs”. The very high total phosphorus values in Västeråsfjärden in the 1960s is the situation corresponding to the oligochaete histogram above from 1973 (cf. Figure 1). Oligochaete abundance values were at the same time very high also indicating a polluted situation. At the start of the new century the qualitative composition had remained nearly the same, whereas abundance had become considerably lower. Västeråsfjärden is thus still eutrophic. In Prästfjärden in central Lake Mälaren, on the other hand, eutrophication has been much milder and total phosphorus values have since the 1960s stayed between 15 and 20 µg/l indicating that the water is ”suitable for human consumption”. In Figure 1 the histogram for Prästfjärden from 1967 shows a typical mesotrophic situation, a substantial share of sensitive species, a similar share of tolerant species, and medium-high abundance values. Water chemistry and oligochaete composition and abundance would seem to go hand in hand.

FIGURE 5. Map of Lake Vättern with the two main sampling stations ”Jungfrun” (position 58.5008°N14.6784°E) and ”Omberg” (position 58.2450°N14.5784°E). Water depth for both stations is about 100 m.

Figure 5 shows a map of Lake Vättern, an ultra-oligotrophic lake, which became markedly eutrophied, mostly in its southern part, from the 1950s to the 1970s. due to outflows of untreated sewage water from the two cities of Jönköping and Husqvarna. Figure 6 is an aerial view of the city of Jönköping facing southern Lake Vättern. The lake is deep—more than 100 m, its contents of dissolved salts is particularly low, and the water transparency is very high (today around 4 µg of total-phosphorus/l and secchi disc values are again back to 15–16 m). The water retention time is also extreme—about 50 years. Lake Vättern is perhaps the best example of how effective sewage treatment starting at the end of the 1960s could be, and likewise the best example of how well this first phase of eutrophication and the succeding phase of oligotrophication is reflected in the oligochaete fauna (see Figure 11 below). From the histograms in Figure 1 it is easily recognizable that central Lake Vättern and the very deep Norwegian Lakes Mjösa (maximum depth about 475 m, total-phosphorus concentrations 4,5–6 µg/l) and Tyrifjorn (maximum depth about 295 m, total-phosphorus concentrations about 4 µg/l)) are all dominated by deep blue colour, which in its turn says that here is a dominance of *S. ferox* and *S. heringianus.* Values of total abundance of oligochaetes are also particularly low (see further text below).

FIGURE 6. Aerial view over Lake Vättern from the south with the city of Jönköping in the foreground and the Island of Visingsö in the back-ground (Photo: courtesy the County Administration in Jönköping).

In Figure 7 mean values of total-phosphorus, as well as water transparency in central Lake Vättern are shown over time (1978–2011). This would be the least affected part of the lake, far from sewage outfalls. Over this time-period total-phosphorus actually decreases from around 10 µg/l to 2–3 µg/l (sic.), while Secchi disc readings increase from about 10 m to 13,5 m. Figure 8 shows the total abundances of oligochaetes and chironomids in central Lake Vättern from about 1975 to 2003. Interestingly enough oligochaete abundance successively decreases from a maximum in the 1970s to very low values, whereas profundal chironomids, usually very reliable indicators of the environment, do not demonstrate the same trend as the oligochaetes at this time. Unfortunately abundance values from 2005 and on must be considered a bit uncertain due to changes in sampling methods (see legend to Figure 8).

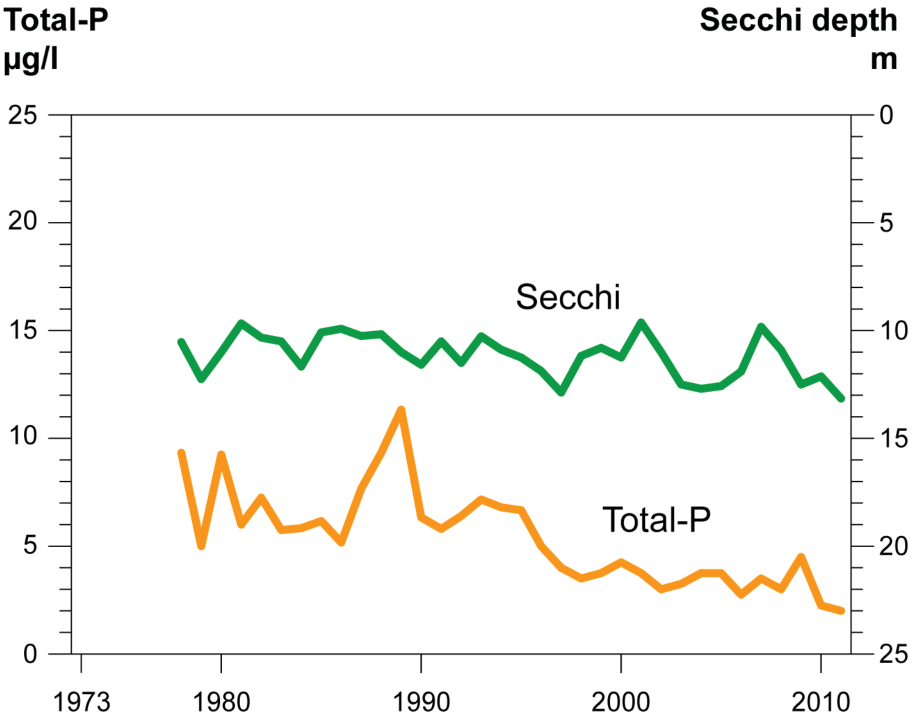

FIGURE 7. Total phosphorus concentrations (in µg/l) over time (1975–2011) in surficial water layers and Secchi disc readings (in m) in central Lake Vättern (station "Jungfrun" (position, see above).

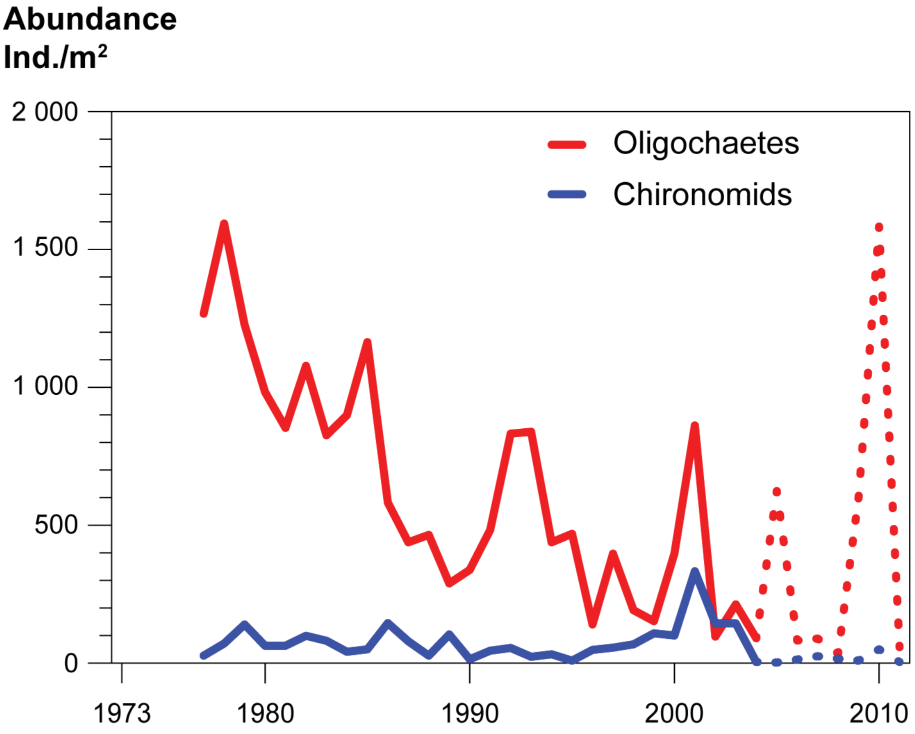

FIGURE 8. Mean abundance of oligochaetes and chironomid larvae over time (1973–2005) at a depth of about 100 m in central Lake Vättern (station "Jungfrun" (position, see obove). Due to changes in sampling methods applied, values from 2005 and on are considered less reliable and therefore connected by broken lines.

Figure 9 is a map over Lake Vänern, the largest lake of south Sweden. Maximum depth is about 60 m, and its mean total-phosphorus concentrations is about 6 µg/l. The water quality is oligotrophic and the water colour is slightly brownish due to humic substances from forest ground in the northern parts of its drainage area. In its northern part the lake is surrounded by bays and so-called ”fjords” heavily affected by domestic and industrial outfalls (Milbrink & Sonesten, unpublished). Like the other large lakes in south Sweden Lake Vänern became eutrophied in the 1950s and 1960s with values of total-phosphorus reaching 10–15 µg/l even in its central parts. Figure 10 shows the constant decrease in concentrations of total-phosphorus on the two open-water stations given in Figure 9—Tärnan and Megrundet. Today concentrations are down to 6–7 µg/l. The oligochaete fauna of most of Lake Vänern is totally dominated by *S. ferox, S. heringianus* and with *P. barbatus* in low abundance (see Figure1). On the other hand, those bordering bays and ”fjords” of the lake which have since long been affected by eutrophication and industrial outflows are characterized by *P. hammoniensis, L. hoffmeisteri* and other tolerant species in high abundance (Milbrink, 1983).

FIGURE 9. Map of Lake Vänern with two open-water reference stations marked ”Megrundet”(position 58.8462°N12.8140°E, depth about 40 m), and ”Tärnan” (position 59.0912°N13.4639°E, depth about 60 m).

Lake Hjälmaren, the smallest of the four large lakes, is eutrophic (cf. Figure 2). It is shallow (maximum depth about 20 m) and has no thermal stratification. In the 1950s the lake became heavily eutrophied in its western basins receiving sewage water from the city of Örebro. After efficient sewage treatment in the 1960s the lake has recovered quite substantially, but it is still eutrophic with total-phosphorus values steadily between 30 and 40 µg/l. The oligochaete fauna is characterized by *P. hammoniensis* but with a rising share of *P. barbatus* (from about 20% to nearly 40% today)—another good example of oligotrophication due to efficient sewage treatment (Milbrink, unpublished).

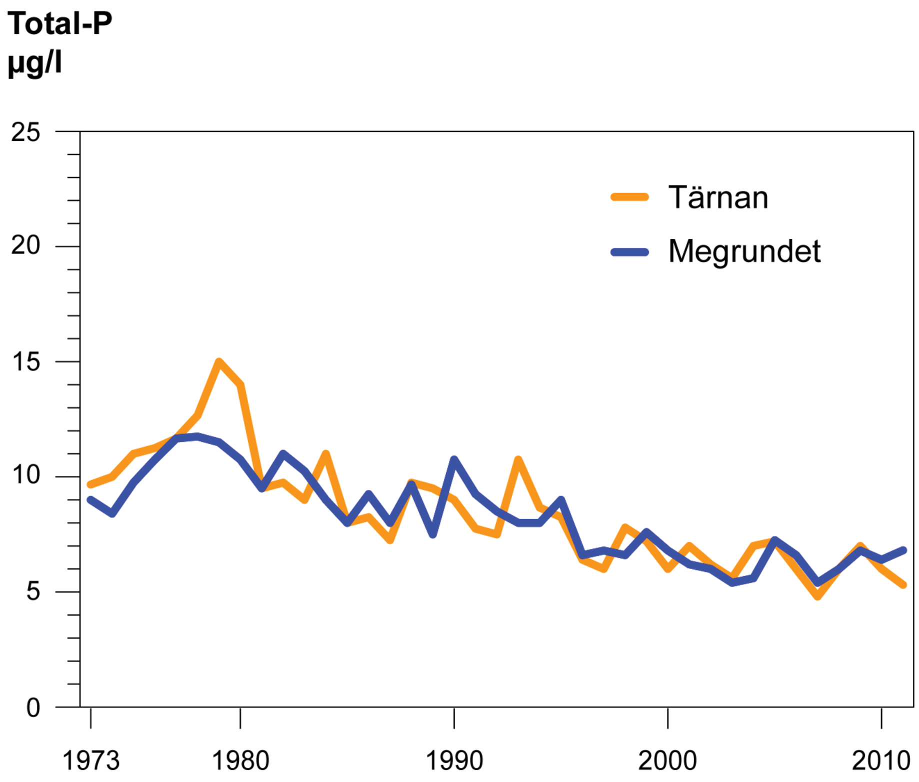

FIGURE 10. Total phosphorus concentrations (in µg/l) over time (1973–2011) in surficial water layers on two open-water reference stations in Lake Vänern (see Figure 9, above).

Thus due to advanced sewage treatment conditions in all the large lakes of south Sweden have improved quite significantly. It is, in deed, a success story.

Returning to Figure 1 the author found it important to scrutinize what had actually happened to the oligochaete fauna in the process, with first eutrophication and then oligotrophication. Changes over time over the whole trophic spectrum are quite obvious, especially in the oligotrophic part. Also in the eutrophic parts of the diagram differences are substantial. Remember that the abscissa in the figure showing the morpho-edaphic index is logarithmic, which means that the distance between the histograms representing different lakes and localities is in reality much wider. For the purpose let us take Lake Vättern as an example and compare the oligochaete composition and abundance in 1911–1914, 1969 and 2011(Figure 11). Due to the fact that total-phosphorus concentrations had changed very much during this period the morpho-edaphic index has changed, as well, and the histograms become widely spread. Because total-phosphorus concentrations in mesotrophic central Lake Mälaren or in eutrophic central Lake Hjälmaren had not changed that much over the same time period the histograms showing the situation before eutrophication took place and after sewage treatment do not diverge as much as for Lake Vättern.

In 1911–1914 Lake Vättern was clearly very oligotrophic with total-phosphorus concentrations likely to have been around 3 or 4 µg/l. We have, however, no chemical data from that time verifying this. As is shown in Figure 11, the oligochaete composition at that time was totally dominated by species of Group 1, i.e. *S. ferox* and *S. heringianus* (about 70 %), *P barbatus* (about 15%) and *Tubifex tubifex* (about 15%). In 1969, on the other hand, after a long period of eutrophication total-phosphorus values varied between 7 and 10 µg/l. Group 1-species had fallen to about 30% and finally after oligotrophication from the 1970s this group had again increased to about 50% in 2011. Total-phosphorus values had by then decreased to between 2 and 3 µg/l (Figure 7). Oligochaete abundance in central Lake Vättern had at the same time changed from about 500 specimens/m2 in 1911–1914, to about 1000 in the early 1970s, and again back to about 600 at the start of the 2000s. All abundance values plotted in Figure 11 tend to fall along a straight line irrespective of the trophic situation. It should be emphasized that even after the eutrophication maximum in the 1960s central Lake Vättern was still very oligotrophic in character.

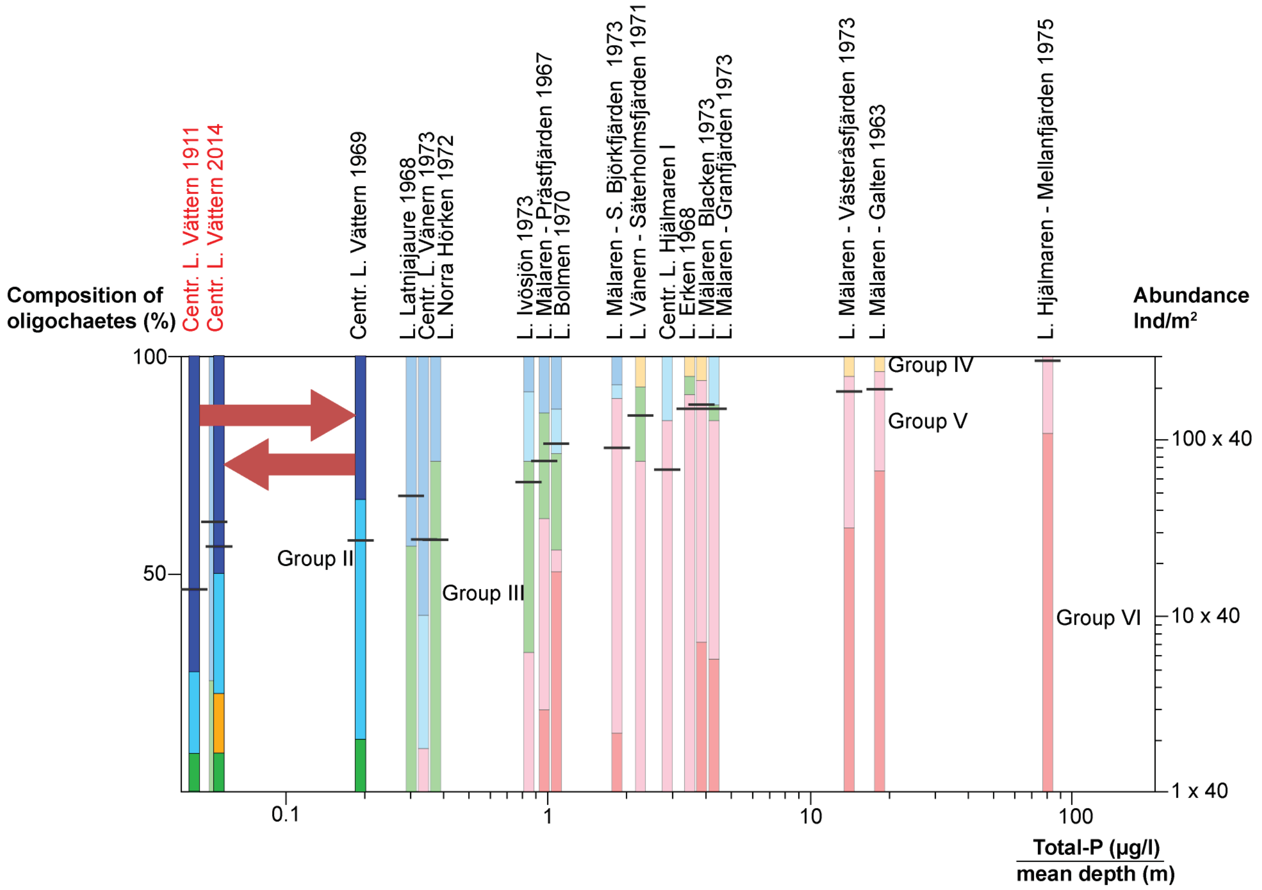

FIGURE 11. Modification of Figure 1 with particular reference to oligochaete bottom samples from Lake Vättern 1911–1914, 1969 and 2014. Oligochaete composition and abundance as in Figure 1. Arrows show the development from one time period to the next.

Discussion

The species composition of oligochaetes in relation to the morpho-edaphic index has greatly increased precision in the characterization of inland waters. Expressing this index along a logarithmic scale facilitates illustration such as in Figure 1. Furthermore putting abundance values on a logarithmic scale has likewise made it possible to combine oligochaete species identifications and abundance values by reducing the impact of extreme amplitudes. Figure 1 would seem to combine all that was known at that time about oligochaetes as indicators of the water quality. Many more lakes have been investigated with reference to oligochaetes since the 1960s and 1970s. Allthough the original Figure 1 was mainly based upon preliminary data from lakes all over Scandinavia, new data have since confirmed that the previous information was largely correct.

For a long time it has been well-known that profundal oligochaetes are reliable indicators of eutrophication (Milbrink, 1978;1980;1999). In the process the share of sensitive species decreases while the share of tolerant species increases. Few studies, however, have shown that under oligotrophication after sewage treatment, the reverse is possible, i.e. sensitive species increase while tolerant species yield. In this study it has clearly been demonstrated that the four large lakes of southern Sweden have behaved this way. The best example would definitely be Lake Vättern, in which eutrophication has had great consequences for the oligochaete fauna and also other fauna components. During the eutrophication phase in the 1960s, fisheries in Lake Vättern, for instance, was depenant upon big catches of whitefish (coregonids). Those fish populations have virtually dwindled during the succeding phase of oligotrophication and so had to a great extent local fisheries. Coarse fish like roach (*Rutilus rutilus*) have likewise retreated from the pelagic in the southern part of the lake, etc. Eutrophication in Lake Vättern was obviously a rather slow process whereas oligotrophication which came after sewage treatment and diversion in the 1970s was comparatively quick. There is a similar series of events described from Switzerland. In the early 1980s Lang (1984) could show that the eutrophication of the large Swiss Lakes Geneva and Neuchatel made tolerant oligochaete species like *L.hoffmeisteri* and several Ponto-Caspian *Potamothrix* species such as *P. vejdovskyi* and *P. moldaviensis* take over more and more. Lang & Reymond (1996) could later document that after large-scale sewage treatment both lakes had slowly recovered implying that sensitive oligochaete species such as *S. heringianus* had come back and *Potamothrix* species had decreased in proportion.

The question has been, to what extent had all the information gathered in Figure1 from 1978 survived till the present day, information that was mainly based on preliminary species identifications from the 1960s and 1970s. It is a time-span of no less than 40–50 years, and many new species analyses have been done during this period. In the opinion of the author there is no doubt that our information on oligochaete composition and abundance has survived and the hypothesis that new data would bring considerable changes to the figure and to our base of knowledge could be rejected.

Acknowledgements

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Long-term macrobenthic community structure changes in the Upper Sakarya River System (1995–2015)

NAIME ARSLAN[1] & DENIZ MERCAN[1]
[1]*Eskişehir Osmangazi University, Science and Art Faculty, Biology Department, Eskişehir, Turkey*
Email:oligo2009@gmail.com

Abstract

The Upper Sakarya River System (USRS) is one of the most important river systems in Turkey. Its primary drainage is provided by the Porsuk Seydi and Bardakçı Rivers and their tributaries. Long term benthic invertebrate community structure in the USRS was investigated from 1995–2015 (with sampling conducted every five years) in order to assess changes in their composition and in relation to water quality. Oligochaete specimens sorted from samples were identified to the species level when possible; all other invertebrate specimens sorted from samples were identified to order and family level. In addition, some environmental parameters (e.g., dissolved oxygen, temperature, and hydrogen ion concentration as *p*H) were measured *in situ*.

Although Ephemeroptera-Plecoptera-Trichoptera fauna were the most abundant group in fauna of USRS during the years 1995, 2000 and (in part) 2005 (18.80, 17.69, and 14.07%, respectively), this ratio decreased to 7.90% during the more recent years of monitoring. In 1995, 2000 and 2005, *Nais bretscheri, Chaetogaster diastrophus, Chaetogaster langi, Pristinella jenkinae, Aulodrilus pigueti, Aulodrilus pluriseta, Potamothrix hammoniensis*, and *Psammoryctides albicola* were the dominant oligochaete taxa. After 2005, tubificine species became more prevalent in samples. While 6 stations had high BMWP (Biological Monitoring Working Party) value in 1995, 2000 and 2005, only 1 station had high value after 2005. Values of Shannon Diversity Indices ranged from 2.00 to 3.05 for the years 1995–2000, 1.87 to 2.24 for the years 2000–2005, 1.06 to 1.85 for the years 2005–2010, and 0.97 to 1.80 for the years 2010–2015. In USRS, while values of dissolved oxygen were measured as 8.00 mg/l and 9.00 mg/l in 1995 and 2000, this high value was measured only at one station in 2015. It was found that numerical and proportional distributions of benthic invertebrates in the USRS have changed considerably between 1995 and 2015. It is obvious that these changes are the result of anthropogenic habitat degradation.

Key words: Upper Sakarya River, zoobenthos, Oligochaeta

Introduction

The Sakarya River Basin—which encompasses 3.4% of the riverine habitat of Turkey—constitutes approximately 7% (5800 ha) of the surface area of the country. The basin includes in total 13 provinces including mainly Eskişehir, Sakarya and Bilecik—either partially or completely. The basin is consisted of 3 sub-basins, namely Upper, Central and Lower Sakarya sub-basins. The headwaters of the upper Sakarya River drainage emanate from five different springs in a region called "Sakaryabaşı", all located in the southern areas of Çifteler District of Eskişehir Province. The waters originating from these springs flow into Bardakçı, Seydi and Sarısu Creeks, draining in a southeasterly direction, eventually becoming the political boundary between Ankara and Eskişehir provinces near the village of Çakmak. This drainage continues to flow in a northerly direction towards its confluence with Porsuk Creek. Porsuk, Seydi and Bardakçı Creeks are included in Upper Sakarya River System (USRS). The Sakarya River basin includes 16 reservoirs, the most important being the

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Porsuk, Çatören, Kunduzlar, Gökçekaya reservoirs. Additionally, Balıkdamı wetland, which is under protection, in located in Upper Sakarya sub-basin.

Water pollution due to the anthropogenic elements—and the decrease in and destruction of aquatic biological diversity as a result of that pollution—is not a problem specific to Turkey but a worldwide problem. Therefore activities were initiated to reveal the aquatic biodiversity, to determine the pollution level in surface waters, to execute biological monitoring, to identify reference sites in order to take measures, and to draft river basin management plans among European Union Member States in accordance with Water Framework Directive (WFD) (2000) with which Turkey aligns the national legislation. Numerous activities were carried out within this scope in Turkey, many of which continue today. One of the most important components of these activities is to determine five biological quality indicators (macroinvertebrates, fish, macrophytes, diatoms, and phytoplankton), identifying the organisms to the species level when possible. Among those components, macroinvertebrates have critical importance in terms of maintaining the aquatic life and sustainable use of the environment. Moreover, various species have bioindicator characteristics and they form an early warning mechanism for short term changes. Due to the fact that their population density is high and that they can occur in almost all types of waters in every period, it is critical to document changes through biological monitoring (Rosenberg and Resh 1993; Metcalfe-Smith 1994). In recent years, ecological quality assessments based on benthic macroinvertebrates were applied by numerous researchers in different river systems in Turkey (e.g., Duran *et al.* 2003; Kazancı *et al.* 2008; Camur-Elipek *et al.* 2006; Topkara *et al.* 2011; Arslan *et al.* 2016). Among the benthic macroinvertebrate groups, oligochaete species are considered one of the more important indicator taxa used for biological monitoring and to set the pollution levels of surface waters (Rosenberg and Resh 1993). Because tubificine taxa have ability to replace less tolerant groups of macroinvertebrates in organic polluted surface waters (Schenková and Helešic 2006), they are used as indicators of organic pollution in surface waters (Lin and Yo 2008).

Although extensive historical and recent research focusing on the water quality and fauna the in Upper Sakarya River System—one of the most essential river systems in Turkey—has been conducted, the assessment of water and habitat quality is performed for the first time in this present study by using physico-chemical variables along with biotic indices (Biological Monitoring Working Party (BMWP), Shannon Wiener and Margalef Index, Ephemeroptera-Plecoptera-Trichoptera% (EPT) and Oligochaeta%), and by monitoring macroinvertebrate fauna and their changes over time for a long time period of 20 years.

The objectives of this study were: i) to determine the fauna diversity of benthic invertebrates on the level of ordo-family in Upper Sakarya River System, ii) to identify the oligochaete fauna to the species level, iii) to determine the changes in fauna structure from 1995 to date (especially EPT% and Oligochaeta%), iv) to make an assessment of the fauna structure with biotic indices, and v) to reveal the changes in water quality with benthic invertebrates which are among the ecological quality elements. Thus, a river system was assessed in terms of ecological quality for 20 years and the structure of benthic invertebrates and their biodiversity were revealed for the first time in Turkey. The results of this study include data summarized past studies associated with the WFD (2000), providing substantive information that can be used during planning of future river basin activities.

Material and Methods

Study area

The Sakarya River (824 km) is the third largest river in Turkey, it is 810 km long and ranges from 60–150 m wide. It rises from five different springs, called Sakaryabaşı, all of which are located in the western Anatolian Plateau. Upper Sakarya River System includes the Porsuk River—one of the biggest tributaries in this system, and also the Sakarya, Seydi and Bardakçı Rivers and their tributaries. All the rivers are being used presently for irrigation and industrial water supply.

Sampling

Thirteen sampling stations (Figure 1) in the Upper Sakarya River System (USRS) and its tributaries were studied between 1995–2015; each station was sampled once every five years, and always during Autumn. Four stations initially surveyed in 1995 and again in 2000 were later eliminated from further study due dewatering and eventual desiccation due to road construction; data generated from samples collected from these four stations during the early years of this study were not included in the calculations. Benthic macroinvertebrates were collected using a hand net (500 µm mesh size) from different habitats present at each of the 13 stations in USRS: three stations on the Sakarya River, six stations on the Porsuk River and four stations on the Seydi River. After collection, samples were washed through a series of sieves (1.5 mm, 0.7 mm, and 0.3 mm mesh net sieves, respectively) then preserved in 80% ethyl alcohol.

In the laboratory, macroinvertebrate specimens were sorted from the raw, preserved samples under stereo dissecting microscopes, separated to order or family level, and then enumerated. The oligochaete specimens were mounted on microscope slides and identified to species level using the keys presented in Sperber (1948, 1950), Brinkhurst and Jamieson (1971), and Timm (1999). Several physico-chemical parameters were measured during each sampling period—water temperature, hydrogen ion concentration (as *p*H), dissolved oxygen (DO), biological oxygen demand (BOD), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonium nitrogen (NH_4-N) and sulfate ($S0_4$). The values resulting from field and laboratory analyses of water samples collected during this study were compared with values and limits established by inland water quality management in Turkey (Republic of Turkey Ministry of Environment and Forest 2015).

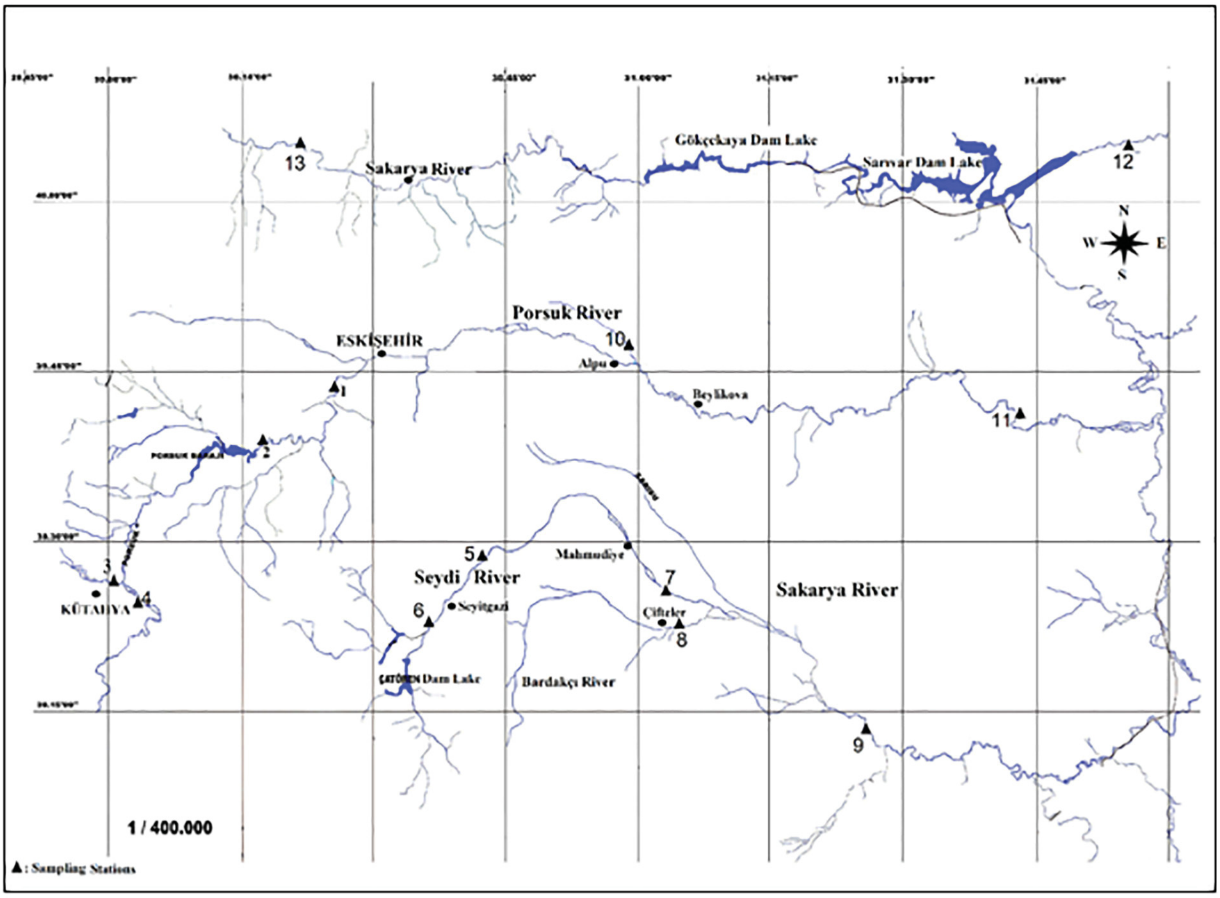

FIGURE 1. The Upper Sakarya River System in Turkey, noting 13 sampling stations surveyed during the research period (1995–2015) discussed in this paper.

Results

Sixty taxonomic groups of aquatic macroinvertebrates were present in samples collected from 13 stations during this study in the USRS. With the exception of the oligochaetes, all taxa were identified to class, order and family level (Table 1); in total, 53 oligochaete species were identified in samples collected during this study (Table 2). Shannon Wiener index values varied between 0.93–2.57 during the research period in the basin. The highest values were recorded from stations 1 and 7 on the sampling dates between 1995 and 2015. Shannon Wiener index values were recorded as 2.31, 2.58 and 2.53 at Station 1 in the years 2000, 2005 and

2015, respectively (Table 3). Values at Station 7 were recorded as 2.49 and 2.07 in the years 1995 and 2010, respectively (Table 3). The lowest value was reported at Station 3 (1.04 and 0.74, respectively) in the years 1995 and 2000, and at Station 10 (1.08 and 1.37) in the years 2005 and 2010, respectively (Table 3).

TABLE 1. Dominance values of taxa individual numbers by years which were identified in the sampling stations during the research period (1995-2015) in the Upper Sakarya River System (as %).

	Years				
Taxa	**1995**	**2000**	**2005**	**2010**	**2015**
Turbellaria	0.40	0.20	0.20	0.04	0.03
Total Gastropoda	11.80	8.90	7.50	12.20	8.40
Planorbidae	2.40	3.60	2.30	3.10	4.20
Physidae	3.60	2.10	3.90	7.40	3.50
Melanopsidae	3.40	2.30	1.10	0.90	0.20
Lymnaeidae	2.40	0.90	0.20	0.80	0.50
Total Bivalvia	4.05	3.02	4.10	6.09	5.10
Unionidae	2.40	2.10	1.30	2.30	0.70
Dreissenidae	0.20	0.40	1.60	2.70	3.50
Sphaeriidae	1.40	0.70	1.20	1.10	0.90
Oligochaeta	27.40	33.70	27.80	32.20	33.80
Total Hirudinae	1.60	0.70	1.10	1.40	1.90
Erpobdellidae	0.70	0.20	0.20	0.10	0.10
Glossiphoniidae	0.70	0.00	0.30	0.20	0.30
Hirudinidae	0.20	0.50	0.40	0.80	1.20
Erpobdellidae	0.00	0.00	0.20	0.30	0.30
Hydracarina	0.40	0.50	0.90	0.60	0.80
Nematoda	5.20	2.30	5.60	4.50	3.90
Total Ephemeroptera	11.90	13.10	8.90	6.70	5.70
Baetidae	1.10	2.10	3.20	2.80	4.20
Oligoneuriidae	2.30	2.70	0.70	0.50	0.20
Heptageniidae	2.20	3.20	1.10	0.60	0.10
Ephemerellidae	1.80	1.50	1.30	1.30	0.60
Caenidae	1.80	1.20	1.10	0.40	0.20
Ephemeridae	1.60	1.30	0.70	0.40	0.30
Potamantidae	1.10	1.10	0.80	0.70	0.10
Total Odonata	2.40	2.00	0.80	1.20	1.30
Calopterygidae	0.80	1.10	0.50	0.60	0.80
Coenagrionidae	0.40	0.00	0.20	0.00	0.10
Platycnemididae	0.30	0.00	0.10	0.20	0.20
Aeshnidae	0.50	0.30	0.00	0.30	0.10
Gomphidae	0.40	0.20	0.10	0.10	0.00
Libellulidae	0.00	0.40	0.00	0.00	0.10

...Continued on the next page

TABLE 1. (Continued)

	Years				
Taxa	**1995**	**2000**	**2005**	**2010**	**2015**
Total Hemiptera	0.70	0.40	0.30	0.40	0.10
Gerridae	0.20	0.20	0.10	0.20	0.10
Corixidae	0.10	0.10	0.20	0.10	0.00
Notonectidae	0.20	0.10	0.00	0.00	0.00
Pleidae	0.10	0.00	0.00	0.10	0.00
Isonychiidae	0.10	0.00	0.00	0.00	0.00
Total Coleoptera	4.10	1.10	5.06	0.80	1.07
Dytiscidae	0.30	0.20	1.30	0.30	0.40
Haliplidae	0.50	0.30	0.60	0.00	0.10
Hydraenidae	0.70	0.40	1.20	0.10	0.20
Hydrophilidae	0.40	0.10	1.40	0.00	0.30
Gyrinidae	2.20	0.10	0.50	0.40	0.00
Chironomidae	9.80	17.30	18.40	13.10	19.40
Tipulidae	0.20	0.20	0.34	0.59	0.30
Simuliidae	4.30	1.80	2.50	1.10	0.70
Culicidae	0.06	0.51	0.23	0.17	0.05
Tabanidae	1.00	1.40	0.80	0.50	0.60
Ceratopogonidae	0.87	1.73	1.09	2.06	1.09
Psychodidae	0.01	0.07	0.10	0.20	0.10
Ephydridae	0.01	0.02	0.04	0.00	0.02
Dixidae	0.02	0.02	0.01	0.01	0.01
Stratiomyidae	0.00	0.00	0.11	0.06	0.04
Total Trichoptera	5.60	4.10	4.60	4.50	2.10
Hydropsychidae	2.20	3.20	3.10	2.90	1.90
Hydroptilidae	0.70	0.40	0.90	0.90	0.10
Rhyacophilidae	0.80	0.40	0.30	0.10	0.10
Sericostomatidae	1.00	0.00	0.20	0.40	0.00
Limnephilidae	0.90	0.10	0.10	0.20	0.00
Total Plecoptera	1.30	0.40	0.40	0.20	0.07
Capniidae	0.20	0.00	0.10	0.00	0.07
Nemouridae	0.30	0.00	0.10	0.00	0.00
Perlidae	0.80	0.40	0.20	0.20	0.00
Total Megaloptera	0.10	0.07	0.20	0.01	0.00
Sialidae	0.10	0.07	0.20	0.01	0.00
Asellidae	2.10	0.80	1.50	2.60	3.60
Gammaridae	3.60	4.30	6.40	8.00	9.10
Decapoda	0.30	0.50	0.20	0.10	0.07

TABLE 2. Minimum, maximum and mean dominance values of Oligochaeta species' individual numbers in 13 stations during the research period (1995-2015) in the Upper Sakarya River System in Turkey (as %).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Cognettia sphagnetorum	-	-	-	-	-	-	-	-	(0.0-2.6) 0.5	-	-	-	-
Cognettia sp.	-	(0.0-2.7) 0.5	-	-	-	(0.0-3.0) 0.6	-	-	-	-	-	-	-
Enchytraeus albidus	-	-	-	-	-	(0.0-3.4) 0.7	-	-	-	-	-	-	-
Enchytraeus buchholzi	-	(0.0-4.1) 1.6	-	(0.0-2.5) 0.5	(0.0-11.2) 3.3	(0.0-2.2) 0.4	-	-	(0.0-3.2) 0.6	-	-	-	-
Henlea ventriculosa	-	-	-	-	(0.0-3.7) 1.0	-	-	-	-	-	-	-	-
Henlea perpusilla	-	-	-	-	(0.0-3.4) 0.7	-	-	-	(0.0-3.8) 1.2	-	-	-	-
Henlea sp.	-	-	-	-	-	-	-	-	-	-	-	-	-
Mesenchytraeus sp.	-	-	-	-	(0.0-5.6) 1.4	-	-	-	-	-	-	-	-
Haplotaxis gordioides	-	-	-	-	(0.0-4.1) 1.5	-	-	-	(0.0-1.3) 0.3	-	-	-	-
Lumbriculus variegatus	-	(0.0-0.7) 0.1	-	-	(0.0-11.2) 4.4	(0.0-5.1) 2.3	(0.0-13.8) 3.7	(0.0-8.6) 2.5	-	-	(0.0-2.4) 0.48	(0.0-2.7) 0.7	-
Lumbriculus sp.	-	-	-	-	-	-	(0.0-2.9) 1.1	-	-	-	-	-	-
Trichodrilus sp.	(0.0-4.6) 2.0	-	-	-	-	(0.0-9.0) 1.8	-	(0.0-8.2) 2.5	-	-	-	-	-
Rhynchelmis sp.	(0.0-3.3) 1.4	-	-	-	-	-	-	-	(0.0-5.2) 1.0	-	-	-	-
Chaetogaster diaphanus	(0.0-10.5) 3.8	-	-	-	(0.0-10.6) 4.4	-	(0.0-6.2) 4.0	-	-	-	-	(0.0-4.0) 1.5	-
Chaetogaster diastrophus	-	-	-	-	-	(0.0-10.2) 2.0	-	-	-	-	-	-	-
Chaetogaster langi	(0.0-5.9) 2.2	-	-	-	-	(0.0-5.7) 3.2	-	-	-	-	-	-	-
Dero digitata	-	(0.0-8.1) 1.8	-	(0.0-2.1) 0.7	-	-	-	-	(0.0-14) 4.5	(0.0-11.4) 2.3	-	-	-
Dero furcatus	-	(0.0-8.9) 4.8	(0.0-9.1) 4.8	-	-	-	-	-	(0.0-10.4) 2.8	(0.0-20.0) 5.1	-	-	-
Dero obtusa	-	(0.0-6.1) 1.2	-	(0.0-2.6) 0.5	-	-	-	-	-	-	(0.0-8.3) 3.1	-	-
Nais barbata	(0.0-10.5) 6.0	-	-	-	(2.5-9.4) 5.4	(0.0-6.1) 1.8	(1.2-9.9) 5.3	(0.0-5.9) 2.3	(0.0-5.1) 1.7	-	-	(0.0-9.2) 5.2	(0.0-12.0) 5.8
Nais bretscheri	(0.0-22.2) 9.6	-	-	-	(0.0-18.8) 9.0	(0.0-12.8) 5.3	(0.0-19.8) 4.7	(0.0-8.8) 4.8	-	-	-	(0.0-4.7) 2.3	(0.0-7.1) 3.5
Nais communis	-	(0.0-19.3) 7.6	(0.0-6.9) 2.7	(0.0-12.0) 5.0	(4.7-22.1) 11.6	(0.0-11.5) 3.9	(0.0-8.5) 3.0	(0.0-6.1) 2.3	(6.6-14.6) 8.9	-	(0.0-4.2) 0.83	(5.0-16.0) 8.0	(0.0-20.7) 7.8
Nais elinguis	-	(2.1-10.5) 6.9	(0.0-24.2) 11.3	(2.3-14.4) 8.6	-	-	(0.0-18.1) 6.5	-	(9.8-17.9) 14.5	(0.0-20.0) 5.1	(0.0-21.9) 9.71	(5.0-16.0) 8.0	-
Nais pardalis	(7.9-11.0) 12.2	(2.3-30.0) 12.9	-	(0.0-10.1) 3.7	(7.6-23.9) 12.5	(12.6-28.4) 21.6	(0.0-15.8) 9.7	(0.0-48.9) 9.8	(0.0-17.3) 8.3	-	(0.0-2.4) 0.48	(4.9-10.3) 7.7	(7.8-22.8) 14.4
Nais pseudobtusa	(0.7-4.4) 2.4	-	-	-	(0.0-7.1) 2.1	(0.0-9.1) 2.7	(0.0-4.7) 1.1	(9.2-34) 23.2	(0.0-15.4) 3.1	-	-	-	(0.0-3.7) 1.2
Nais variabilis	-	(0.0-1.1) 0.2	-	-	-	(0.0-4.9) 1.0	-	-	-	-	-	-	-
Ophidonais serpentina	-	(0.0-15.4) 4.0	(0.0-4.4) 0.9	(0.0-7.4) 2.6	-	(0.0-4.5) 1.5	-	-	(0.0-10.3) 4.7	-	(0.0-4.8) 0.95	(0.0-5.1) 1.7	(0.0-4.9) 1.4

...Continued on the next page

TABLE 2. (Continued)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Paranais frici	(0.0-8.5) 4.6	(0.0-4.1) 1.3	-	(0.0-5.1) 1.7	-	(0.0-4.8) 1.2	(0.0-4.7) 2.8	-	(0.0-13.9) 6.8	(0.0-2.9) 0.6	-	(0.0-9.0) 3.9	(0-2.2) 0.8
Pristina aequiseta	-	-	-	-	(0.0-12.4) 4.4	-	(0.0-6.8) 3.7	(0.0-6.1) 1.2	-	-	-	(0.0-3.0) 1.4	-
Pristina proboscidea	(0.0-13.8) 6.8	-	-	-	(0.0-4.7) 1.7	(0.0-5.1) 1.9	(0.0-8.2) 2.8	(0.0-12.3) 4.3	-	-	-	(0.0-3.0) 0.8	-
Pristinella jenkinae	(3.7-10.5) 6.9	(0.0-11.3) 2.5	-	(0.0-3.1) 0.6	(3.2-11.0) 6.8	(0.0-29.1) 9.1	(7.8-17.8) 13.6	(0.0-12.2) 4.2	(0.0-2.6) 0.5	-	-	(0.0-8.8) 3.4	(0.0-5.4) 2.0
Slavina appendiculata	-	(0.0-4.5) 0.9	-	(0.0-6.7) 1.5	-	-	-	-	-	(0.0-8.6) 2.5	-	-	-
Spericaria josinae	-	(0.0-0.8) 0.2	-	(0.0-9.5) 3.0	-	(0.0-2.0) 0.4	(0.0-3.7) 1.4	-	-	-	-	(0.0-5.0) 2.3	-
Stylaria lacustris	(3.2-12.1) 8.2	(0.0-8.8) 4.6	-	(0.0-35.4) 13.3	(0.0-15.0) 6.6	(0.0-6.4) 1.3	(2.3-10.4) 6.9	(0.0-14.3) 5.1	(0.0-15.7) 6.8	-	(0-11.8) 5.33	(7.2-14.6) 10.5	(8.9-38.4) 19.5
Uncinais uncinata	(0.0-3.2) 1.8	(0.0-2.7) 0.5	-	(0.0-6.7) 3.1	-	-	-	-	(0.0-18.7) 6.9	-	(0-7.8) 2.85	(0.0-7.0) 1.7	(0.0-2.0) 0.7
Aulodrilus pigueti	(0.0-4.2) 1.9	-	-	-	-	-	-	-	-	-	-	-	-
Aulodrilus pluriseta	(0.0-5.1) 2.4	-	-	-	(0.0-8.9) 3.4	(0.0-6.4) 4.4	(0.0-7.0) 3.4	-	-	-	-	-	-
Haber speciosus	(0.0-2.8) 1.0	-	-	-	(0.0-0.9) 0.2	-	-	(0.0-23.6) 12.3	-	-	-	(0.0-2.0) 0.6	-
Limnodrilus claparedeianus	-	-	-	(0.0-11.9) 3.8	-	-	-	-	-	-	-	-	-
Limnodrilus hoffmeisteri	(0.0-19) 3.9	(0.0-12.0) 5.8	(26.1-74.0) 47.5	(3.2-27.0) 15.4	(0.0-2.8) 0.6	-	-	-	(0.0-11.6) 6.6	(28.6-76.8) 52.5	(12.7-35.4) 24.88	(0.0-8.6) 3.1	-
Limnodrilus udekemianus	-	(0.0-4.5) 1.3	(0.0-14.5) 9.4	(0.0-15.6) 6.0	-	-	-	-	(0.0-11.5) 4.0	(0.0-64.7) 26.5	(3.1-22.5) 12.52	(0.0-3.1) 0.6	-
Potamothrix bavaricus	(0.0-2.9) 0.8	-	-	-	-	-	(0.0-2.3) 0.5	-	-	-	-	(0.0-4.0) 0.8	-
Potamothrix bedoti	-	-	-	-	-	(0.0-4.7) 0.9	-	-	-	-	-	-	-
Potamothrix hammoniensis	(0.0-29.3) 7.1	(5.4-17.5) 12.0	(0.0-16.1) 6.1	(0.0-22.1) 10.5	(0.0-9.9) 4.1	(0.0-23.1) 6.2	(0.0-13.5) 6.6	-	(0.0-12.2) 6.9	(0.0-10.0) 3.1	(0.0-19.8) 9.66	(10.9-19.5) 14.3	(13.0-69.0) 29.9
Psammoryctides albicola	(3.2-7.4) 5.0	(4.1-28.5) 16.3	(0.0-2.3) 0.5	(0.0-9.5) 4.2	(7.0-20.4) 13.6	(11.8-37.2) 20.0	(10.3-26.5) 17.2	(0.0-3.7) 0.7	(0.0-10.3) 2.4	(0.0-5.7) 1.1	(0.0-5.8) 3.03	(10.8-23.9) 15.5	(0.0-20.7) 10.5
Psammoryctides barbatus	-	(0.0-5.4) 1.8	-	(0.0-1.3) 0.3	(0.0-1.2) 0.2	(0.0-7.7) 2.3	(0.0-2.5) 0.9	(0.0-22.6) 8.6	-	-	(0.0-0.8) 0.16	-	-
Psammoryctides moravicus	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhyacodrilus coccineus	-	(0.0-13.6) 6.6	(0.0-8.6) 2.6	(0.0-13.5) 5.9	-	-	(0.0-2.4) 0.5	-	(0.0-7.3) 2.6	-	(7.3-30.2) 15.69	-	-
Spirosperma ferox	-	(0.0-1.4) 0.3	-	-	-	(0.0-2.0) 0.4	(0.0-3.1) 0.8	-	-	-	(0.0-12.5) 2.98	-	-
Spirosperma velutinus	-	(0.0-2.3) 0.5	-	-	-	-	-	-	-	-	-	-	-
Stylodrilus parvus	-	-	-	-	(0.0-1.9) 0.4	-	-	-	-	-	-	(0.0-1.7) 0.3	-
Tubifex tubifex	(0.0-0.9) 0.2	(0.0-7.2) 3.1	(4.6-24.2) 14.3	(0.0-18.8) 8.1	-	-	-	-	(0.0-11.6) 3.9	-	(0.0-13.1) 7.36	(0.0-5.6) 1.7	(0.0-6.6) 1.8
Tubifex spp.	-	(0.0-2.3) 0.5	-	-	-	-	-	-	-	-	-	-	-
Quistadrilus multisetosus	-	(0.0-1.4) 0.3	-	-	(0.0-1.9) 0.4	-	-	-	-	-	-	-	-
Eisenella tetraedra	-	-	-	(0.0-2.5) 1.0	(0.0-2.1) 0.4	-	-	-	(0.0-1.7) 0.6	(0.0-5.7) 1.1	-	-	-

The highest EPT value in the basin were identified in two out of three stations located on Seydi Creek; at Station 5 (44.52 and 44.09) in the years 1995 and 2000, and at Station 6 (46.04, 36.72 and 24.72) in the remaining years (Table 3). EPT values at Station 7, which is another sampling point on Seydi Creek, are higher than the basin-wide values, although they are not as high as the values recorded at those two stations (Stations 5 and 6) (Table 3). The lowest EPT values were identified from samples collected from stations 4 and 10 on Porsuk Creek throughout the entire research period. Those two stations are the sampling areas in which the abundance of %Oligochaeta has the highest values, which is in parallel with %EPT results. Especially in the Station 10, Oligochaeta individuals constitute more than half of the zoo-benthic community. In parallel with the biotic indices, the highest BMWP values were recorded again stations 5, 6, and 7 except for the year 2015 (in 2015, no station was identified having BMWP value 1) (Table 3).

In total, 53 oligochaete species were present in samples collected from stations in the USRS study area; the highest taxonomic diversity (25 species) was reported for Station 6 (Table 2).

The lowest species diversity values were recorded at stations 3 and 10, where only 10 species of oligochaetes were recorded (Table 2). Throughout the study period, *Psammoryctides albicola* (collected from all the stations and *Potamothrix hammoniensis* (collected from 12 of the 13 stations) were the most widespread species. Three other species, *Stylaria lacustris, Nais pardalis,* and *N. communis* were collected from 11 of the 13 stations (Table 2). Except for semiaquatic Enchytraeidae species, *Lumbriculus* sp. (Station 7), *Chaetogaster diastrophus* and *Potamothrix bedoti* (Station 6), *Spirosperma velutinus* (Station 2) and *Aulodrilus pigueti* (Station 1) were detected only in 1 station (Table 2).

The dominance values of for oligochaetes present in the basin varied during this study, dependent primarily on the sampling year and the habitat conditions of the station. The species with the highest population density is as follows in 13 stations: 76.80% of the oligochaete fauna is composed of *Limnodrilus hoffmeisteri* (Station 10 in 2015), 69.00% of the fauna is composed of *Potamothrix hammoniensis* (Station 13 in 2015), 64.70% of the fauna is composed of *Limnodrilus udekemianus* (Station 10 in 2015), 48.90% of the fauna is composed of *Nais pardalis* (Station 8 in 2000), 38.40% of the fauna is composed of *Stylaria lacustris* (Station 5 in 1995), 37.20% of the fauna is composed of *Psammoryctides albicola* (Station 6 in 2005), 23.60% of the fauna is composed of *Haber speciosus* (Station 8 in 2010) (Table 2). Nevertheless, *Chaetogaster diaphanus* and *Chaetogaster langi* have a dense population at stations 1, 5, and 7 in the first 2 sampling periods, the population density decreased in time and no species was detected in the samplings carried out in 2015. The decrease in the population density of *Chaetogaster* species was also observed in two other species—*Aulodrilus pigueti* and *Aulodrilus pluriseta*. *A. pigueti* was only identified in samples from a single station (Station 1) and *A. pluriseta* was only identified in samples from four stations (stations 1, 5, 6, and 7) during the study period (Table 2). The existence and dominance of both species in samples collected from those stations decreased gradually and they were not identified in the last sampling campaign.

In general terms, the tubificine population increased while naidine population decreased in the stations in the basin between 1995 and 2015. The situation is highly dynamic at stations 3, 10, and 13. Naidine dominance was 29.75% in the Station 3 in 1995, however this value dropped to 7.53% in 2015 while it dropped from 61.24% to 24.42% at Station 13. Naidine rate which was 34.00% at Station 10 disappeared completely in 2015.

Minimum, maximum, mean and standard deviation values of several environmental parameters, measured at all 13 stations every five years during the 20-year period of this study, are presented in Table 4. The dominance variations of Naidine-Tubificine given for the above-mentioned stations support the water quality results in those stations. Stations 3, 10, and 13 had the lowest water quality of all sites in the basins. Among the water quality parameters, dissolved oxygen levels were low at Station 6, located on the Seydi River which forms the source of Sakarya River (known as Sakarbaşı). However low levels of DO are associated with the fact that the station is actually a crenal region. As presented in Table 4, BOD values of the same station are also low and it falls under the I. water quality class.

TABLE 3. Index values calculated for 13 stations during the research period (1995-2015) in Upper Sakarya River System in Turkey (Roman numbers indicate water quality for BMWP; H': Shannon Wiener Index, Marg: Margalef Index; EPT: Ephemeroptera-Plecoptera-Trichoptera groups' individual numbers; Olig: Oligochaeta group's individual numbers).

	St 1					**St 2**					**St 3**					**St 4**					**St 5**				
Index/Year	**1995**	**2000**	**2005**	**2010**	**2015**	**1995**	**2000**	**2005**	**2010**	**2015**	**1995**	**2000**	**2005**	**2010**	**2015**	**1995**	**2000**	**2005**	**2010**	**2015**	**1995**	**2000**	**2005**	**2010**	**2015**
%EPT	12.83	14.59	11.34	6.90	6.64	8.04	5.56	3.00	0.47	0.00	0.77	0.35	2.30	0.46	0.15	0.00	0.00	0.00	0.00	0.00	44.52	44.09	35.40	21.93	11.23
%Olig	6.36	10.03	19.27	26.74	8.47	26.43	50.90	51.59	26.64	34.20	36.59	75.13	21.67	26.95	25.04	14.37	16.48	16.52	44.83	39.53	15.96	15.18	12.70	25.03	37.97
BMWP	I	I	II	II	III	II	II	IV	IV	V	IV	IV	V	V	V	III	IV	III	IV	V	I	I	II	I	I
H'	2.35	2.31	2.58	2.4	2.53	2.34	1.55	1.67	1.954	1.59	1.04	0.74	1.72	1.835	1.65	1.70	1.42	1.86	1.549	1.75	2.22	1.95	2.20	2.282	2.01
Marg	4.45	3.41	4.42	3.76	3.97	3.53	2.46	2.86	2.42	1.75	1.97	1.10	1.75	1.761	1.75	2.42	2.01	2.42	2.201	2.20	3.98	2.72	3.98	3.751	3.52
	St 6					**St 7**					**St 8**					**St 9**					**St 10**				
Index/Year	**1995**	**2000**	**2005**	**2010**	**2015**	**1995**	**2000**	**2005**	**2010**	**2015**	**1995**	**2000**	**2005**	**2010**	**2015**	**1995**	**2000**	**2005**	**2010**	**2015**	**1995**	**2000**	**2005**	**2010**	**2015**
%EPT	31.60	29.67	46.04	36.72	24.72	24.10	27.95	7.97	11.14	8.98	8.78	31.16	17.67	21.33	16.17	39.64	26.13	34.20	7.52	11.09	0.00	0.00	0.00	0.00	0.00
%Olig	16.35	13.34	20.15	24.21	19.28	17.37	40.43	7.79	38.65	41.84	14.45	6.26	2.92	10.97	11.65	23.08	18.12	15.01	48.98	37.88	44.81	70.14	69.05	51.82	59.94
BMWP	I	I	I	I	II	I	I	II	II	II	I	I	I	I	I	II	II	II	III	III	V	V	V	V	V
H'	2.36	1.80	2.14	2.25	2.30	2.49	1.68	2.07	2.067	1.80	1.57	1.62	1.99	1.759	1.93	2.33	1.58	2.01	1.597	1.99	1.38	0.93	1.08	1.368	1.23
Marg	3.99	3.25	3.97	3.78	3.08	3.99	2.92	3.32	3.317	2.65	3.09	2.10	3.10	2.41	2.41	3.07	1.57	3.09	2.647	2.87	1.32	1.10	1.31	1.534	1.32
	St 11					**St 12**					**St 13**														
Index/Year	**1995**	**2000**	**2005**	**2010**	**2015**	**1995**	**2000**	**2005**	**2010**	**2015**	**1995**	**2000**	**2005**	**2010**	**2015**										
%EPT	12.08	13.90	1.17	0.00	0.00	25.76	16.67	4.99	7.17	8.60	37.90	20.77	19.85	35.71	15.85										
%Olig	56.38	51.34	36.26	43.88	40.32	45.00	41.04	53.91	32.78	47.96	39.69	30.10	35.11	17.97	36.19										
BMWP	III	III	IV	V	V	I	I	II	III	III	I	I	I	II	II										
H'	1.26	1.33	1.35	1.38	1.07	1.98	1.81	1.51	1.66	1.69	1.77	1.68	2.00	2.27	2.05										
Marg	1.09	0.97	1.03	1.09	0.87	3.78	2.93	3.54	3.089	2.65	3.09	2.08	2.63	2.63	3.09										

TABLE 4. Minimum-maximum, mean and standard deviation values of several parameters measured between 1995 and 2015 in the Upper Sakarya River System study area in Turkey (mean values are given in brackets. Min-Max(Mean) ± STD; D.O: dissolved oxygen; BOD: biological oxygen demand).

	Temparature (ºC)	***p*H**	**D.O (mg/l)**	**BOD (mg/l)**	**NO_3-N (mg/l)**	**NO_2-N (mg/l)**	**NH_4-N (mg/l)**	**SO_4 (mg/l)**
1	16.60-25.20 (19.80)±4.60	7.90-8.90 (8.37) ±35.10	3.00-16.00 (8.20) ±6.80	1.00-22.00 (11.50) ±14.80	0.10-0.80 (0.40) ±0.30	0.02-0.04 (0.03) ±0.01	0.07-0.10 (0.09) ±0.02	73.00-105.00 (85.33) ±17.20
2	15.90-24.50 (18.90) ±4.80	8.20-8.50 (8.30) ±0.15	8.80-11.30 (10.07) ±1.25	0.00-16.00 (8.30) ±8.02	0.37-1.40 (0.76) ±0.50	0.01-0.06 (0.03) ±0.02	0.07-0.19 (0.09) ±0.09	148.00-313.00 (204.30) ±94.10
3	14.40-23.00 (17.63) ±4.68	8.20-8.70 (8.37) ±0.29	10.10-11.20 (10.60) ±0.56	0.00-13.00 (7.00) ±6.50	0.70-1395.00 (466.10) ±804.30	0.01-0.10 (0.07) ±0.05	0.00-0.06 (0.06) ±0.00	196.00-267.00 (226.00) ±36.70
4	13.70-21.50 (16.90) ±4.08	8.00-8.20 (8.07) ±0.11	8.90-11.70 (10.10) ±1.40	0.00-6.00 (2.33) ±3.20	0.30-1.80 (1.14) ±0.70	0.01-0.20 (0.09) ±0.10	0.00-0.09 (0.02) ±0.03	95.00-250.00 (178.30) ±78.10
5	12.10-20.50 (15.10) ±4.60	7.90-8.28 (8.06) ±0.20	8.60-9.90 (9.40) ±0.70	1.00-8.00 (4.50) ±4.90	1.70-4.43 (2.80) ±1.40	0.02-0.10 (0.06) ±0.04	0.00-0.17 (0.06) ±0.09	68.00-189.00 (141.30) ±64.40
6	11.80-21.70 (15.30) ±5.50	7.60-7.80 (7.70) ±0.10	2.00-5.00 (3.60) ±1.50	0.00-1.00 (6.60) ±6.50	0.50-2.20 (1.40) ±0.80	0.02-0.06 (0.04) ±0.02	0.60-1.80 (1.20) ±0.62	59.00-159.00 (100.00) ±52.30
7	12.10-26.60 (17.00) ±8.30	8.40-8.70 (8.50) ±0.18	9.30-10.30 (9.90) ±0.50	0.00-5.00 (2.60) ±2.50	0.00-1.40 (0.06) ±0.70	0.01-0.19 (0.07) ±0.10	0.02-0.70 (0.20) ±0.40	87.00-213.00 (148.60) ±63.00
8	19.00-22.40 (20.10) ±1.30	8.10-8.60 (8.30) ±0.20	8.50-11.60 (10.30) ±1.60	0.00-9.00 (3.00) ±5.20	0.10-1.90 (0.90) ±0.90	0.00-0.03 (0.02) ±0.02	0.00-0.08 (0.03) ±0.04	39.00-98.00 (63.30) ±30.80
9	8.10-20.07 (14.20) ±6.30	7.40-8.20 (7.80) ±0.40	9.40-12.10 (10.60) ±1.30	0.00-8.00 (3.60) ±4.04	0.50-1.90 (1.20) ±0.90	0.01-0.02 (0.02) ±0.00	0.02-0.06 (0.05) ±0.01	12.00-132.00 (73.60) ±60.00
10	8.60-21.20 (14.10) ±6.45	7.80-8.10 (7.90) ±0.17	8.50-12.40 (10.03) ±2.08	1.00-7.00 (3.33) ±3.21	0.54-1.90 (1.25) ±0.92	0.01-0.01 (0.01) ±0.00	0.00-0.11 (0.07) ±0.06	48.00-87.00 (66.33) ±19.60
11	14.00-20.00 (17.60) ±3.20	7.90-8.50 (8.10) ±0.30	6.50-8.30 (7.17) ±0.90	0.00-4.00 (1.30) ±2.30	1.10-11.56 (386.30) ±666.50	0.03-0.08 (0.04) ±0.01	0.02-1466.00 (488.60) ±846.30	45.00-154.00 (99.50) ±77.00
12	9.00-16.40 (12.10) ±3.80	7.50-8.00 (7.80) ±0.26	6.30-10.80 (8.60) ±2.20	0.00-3.00 (2.00) ±1.70	0.08-2.10 (1.70) ±0.50	0.01-0.04 (0.04) ±0.01	0.02-0.04 (0.04) ±0.01	61.00-133.00 (107.30) ±40.20
13	14.90-19.70 (16.50) ±2.70	7.50-8.10 (7.80) ±0.30	7.20-9.60 (8.70) ±1.30	0.00-5.00 (2.30) ±2.50	0.08-2.20 (1.30) ±1.20	0.01-0.10 (0.09) ±0.05	0.02-0.10 (0.10) ±0.02	128.20-354.00 (244.50) ±126.50

Discussion and Conclusions

During this study in the Upper Sakarya River System in Turkey (1995–2015), 60 aquatic macroinvertebrate taxa were identified to class, order and family level; in addition, 53 oligochaete taxa were identified to species (Tables 1 and 2). When the zoobenthic community structure is analysed in the sampling years, it is realized that EPT rate, which is high in 1995, decreased each sampling period at Station 2 during this study, and was reduced to zero in samples collected in 2015 (Table 3). Station 2 is situated on the outlet of Porsuk Dam (Figure 1). One of the fundamental ways for providing resources for various purposes such as irrigation, electricity production and domestic use is to construct dams on rivers which are known as fluvial systems (Earthscan 2000). River systems can be physically altered by hydraulic infrastructures, which are regarded as significant components of fluvial habitat degradation (Almeida *et al.* 2013). Hydro morphological alterations such as dams and weirs constitute barriers for migration of aquatic fauna, and natural flow regimes are impacted by those alterations (Nilsson *et al.* 2005). As well, trophic resources of the fauna are disrupted and the abiotic environment is homogenized (Cortes *et al.* 1998). Aquatic ecosystems located upstream and downstream of hydraulic infrastructures such as dams are impacted by those infrastructures, affecting the aquatic ecosystem along the river (McAllister *et al.* 2001).

In 1972, Porsuk Dam was constructed on Porsuk Creek, a tributary of Sakarya River, for the purposes of irrigation, flood control, and municipal and industrial water supplies. The height of the dam from the river bed is 49.70 m. As presented in Table 3, the EPT value at Station 2 (located downstream of the Porsuk Dam)—which was 8.04% in 1995—diminished every five years, and was reduced to 0% in 2005. In addition, % Oligochaeta rate increased in parallel with the decrease in EPT. This rate which was 26.43% in the first sampling campaign was doubled in the years 2000 and 2005, and BMWP value was downgraded from quality level II to quality level V over the years. It is known that several species belonging to the insect orders Ephemeroptera, Trichoptera and Plecoptera are quite sensitive to changing environmental conditions, and that alfa-beta mesosaprobic species replace oligosaprobic species under decreasing environmental conditions, and when these environmental pressures persist, the faunal structure shifts towards dominance by polysaprobic species. Degradation of both habitat and environmental parameters negatively impacted the macroinvertebrate fauna this station. When oligochaete fauna of this station (Station 2) was analysed, the effect of negative change was also reflected in the population structure of species. For instance, the dominance value of *Dero furcatus* increased from 2.00% to 6.10% in 20 years. *Dero* spp. are known to occur in diverse environments—from swampy areas in ponds and rivers to slow moving marshy rivers. Distinctive features of *Dero furcatus* are branchial fossa, a pair of noncontractile palps and 3 pairs of gills—which allow this species to tolerate very low levels of dissolved oxygen concentrations (Brinkhurst & Jamieson 1971).

No EPT species were encountered in two stations (stations 4 and 10) during the whole study period in the basin. Station 10 is a good example of an "artificial or impoverished stream" which is located on Porsuk Creek near Alpu District of Eskişehir. In this region, irrigation water is supplied from Porsuk River by means of secondary ducts for agriculture in Alpu Plain. Running water systems are classified into five categories, namely crenal, rhithral, potamal, artificial or impoverished streams, and intermittent running waters—dependent on the geographical position, habitat structure, and natural or artificial nature of those systems. No taxa other than Oligochaeta, Gastropoda, Bivalvia, Chironomidae and Gammaridae were present in samples collected and identified from this station (Table 1). Relatively high levels of nitrate nitrogen, nitrite nitrogen, and ammonium nitrogen in water samples collected from station 10 during each 5-year sampling period might indicate the perennial presence fertilizers with high nitrogen content used in agricultural practices in this region. This conclusion suggests that this station is not an appropriate habitat for other aquatic macroinvertebrate taxa except for meso- and polysaprobic groups having wide tolerance interval in artificial water systems. This finding is also supported by oligochaete species identified in the same station. The tolerant *Limnodrilus hoffmeisteri* is dominant in this sampling area, followed by *Limnodrilus udekemianus, Dero furcatus, Nais elinguis* and *Dero digitata* (Table 2). Moreover, the naidine rate which was recorded as 34.3% in 1995 dropped to 0 in 2015, with tubificine taxa becoming completely dominant.

Station 4 is situated in a region in the vicinity of an industrial site and industrial facilities in Kütahya province. The river banks in this area are covered with stones, thus habitat structure is typical of a regulated river. Oligochaeta, Gastropoda and Chironomidae are the dominant taxonomic groups in this station. *Rhyacodrilus coccineus* and polysaprobic oligochaete species are dominant in this station, as is also the case at station 10.

Our results note that stations 5, 6, and 7 have the highest Shannon and EPT% values (Table 3). The common features of those stations are that they are positioned in areas which are relatively located some distance from settlements and thus have remained relatively undisturbed sites. Station 7 is an example of a hyporhithral region, and stations 5 and 6 are examples of eupotamal regions. All three stations (in addition to Station 2) are the areas in which the highest number of oligochaete species (23, 25 and 22 species, respectively) were recorded in the basin during this study (Table 2). The dominance rate of *Nais bretscheri, N. barbata* and *N. pardalis,* which are typical species of running waters, is high in every three station (Stations 5, 6, and 7) (Table 2). Moreover they are the stations in which *Haplotaxis gordioides* as well as *Lumbriculus variegatus, Lumbriculus* sp. and *Trichodrilus* sp. are recorded—taxa that have rarely if ever been reported previously, either from this basin or elsewhere in Turkey. Additionally, *Chaetogaster diastrophus* was only recorded from Station 6 in the basin (Table 2). Although *Chaetogaster diastrophus* was reported to cling to Odonata larvae and thus being transported passively (Corbi *et al.* 2004), and that it can occur even in saline waters (Erséus *et al.* 1999), there are only a few records of this species from surface waters in Turkey. Stations 5, 6, and 7 have the highest water quality values. Although water quality fluctuated during the study period, our results suggest that Station 5 is of quality class II., and that stations 6 and 7 in quality class II. until 2010, and later was upgraded to quality class III. in 2015.

The area associated with Station 8, which covers five different springs and their spring run drainages from which Sakarya River originates (known as Sakarbaşı), is the area having near similar taxonomic diversity and water quality (except for minor fluctuations) as that of station 8, and having a huge ponding area. As presented in Table 3, EPT rate which was 8.78% in 1995 did not decrease over the past 20 years in this station. On the contrary, the EPT rate in station 8 increased within past 20 years. Oligochaeta % rate can be projected to remain stable. BMWP value remained within the I. quality class during the entire study period. The two most important features of this site were that water temperature never drops below 16°C, even in winter, and the calcium concentration of the water remains high. Moreover, this area provided critical habitat for extant populations of the commercially important *Palaemonetes turcorum* (Holthuis, 1961) (Decapoda, Palemonidae)—a marine species that has adapted to living in freshwater habitats, and to date is endemic species to Sakarya River in Turkey. Despite the fact that the Sakarbaşı region accommodates picnic areas, restaurants and hotels and is used for recreational purposes, the area has remained the same with few alterations (other than minor changes that can be neglected) for over 20 years, largely because of the preservation activities of Çifteler Municipality of the district covering the area, and the conservation efforts of individuals and groups established for the region to raise the awareness of the public about water pollution and biological diversity.

To summarize, the zoobenthic community structure and water quality in the Upper Sakarya River System has declined, especially in Porsuk Creek—which flows through large settlements and which is used for irrigation purposes, and in which industrial facilities are densely located. Although EPT formed 40–45% of the total zoobenthic community in the years 1995, 2000 and (in part) in 2005, the dominance values for the oligochaete species *Nais bretscheri, Chaetogaster diastrophus, Chaetogaster langi, Pristinella jenkinae, Aulodrilus pigueti, Aulodrilus pluriseta, Potamothrix hammoniensis* and *Psammoryctides albicola* were high in several stations, the EPT rate decreased to 20–25% in the following years (and to zero in samples collected from several stations), and tubificine taxa replaced naidines. No substantial change was observed, either in water quality or in fauna structure in Seydi River (a tributary of the Sakarya River), during this study.

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Observations on the life cycles of aquatic Oligochaeta in aquaria

TARMO TIMM
Estonian University of Life Sciences, Centre for Limnology, Vehendi, Elva vald, Tartumaa, Estonia. Email: tarmo.timm@emu.ee

Abstract

Observations on the life cycles of aquatic oligochaetes were made in the period 1962–2017 at the Võrtsjärv Limnological Station (Estonia) using small aquaria with sieved profundal mud covered with unaerated water. The aquaria were mostly inseminated with 10 juvenile worms and checked four times a year, changing the mud and eliminating the progeny, until the natural death of the original worms. Besides, mass cultures were kept in bigger aquaria. Many individuals of *Tubifex tubifex, T. newaensis, Limnodrilus hoffmeisteri, L. udekemianus, Ilyodrilus templetoni, Psammoryctides barbatus, Spirosperma ferox, Potamothrix moldaviensis, P. vejdovskyi, P. bavaricus, Stylodrilus heringianus* and *Rhynchelmis tetratheca* survived for several years, reproduced repeatedly, and died out one by one during the observation period. In some cases, the most longevous individuals reached an age of up to 8 years (*I. templetoni*), 10–12 years (*T. tubifex*), 15–17 years (*L. hoffmeisteri, P. barbatus, S. heringianus*), or even more than 20 years (*L. udekemianus, S. ferox, T. newaensis*). *Criodrilus lacuum* did not reproduce in aquaria, although the oldest individual spent 46 years there. *Potamothrix hammoniensis, Lophochaeta ignota, Lamprodrilus isoporus*, most naidines and some others did not thrive in aquaria and usually died without reproducing. In a cellar, where temperature conditions imitated seasonal fluctuations in lakes, or when the aquaria were maintained at continuously low temperature, the lifetime of worms was often longer than at room temperature. At elevated temperatures (+25° to +35°C) *T. tubifex* and *L. hoffmeisteri* formed cocoons mostly with only 1–2 eggs while their life span was then shorter. Architomic clones of *Potamothrix bedoti, Bothrioneurum vejdovskyanum, Aulodrilus pluriseta* and *A. japonicus* survived and propagated for years. The architomic *Lumbriculus variegatus* was thriving only when fed, e.g., with yeast. Uniparental reproduction by parthenogenesis was observed in *T. tubifex, L. hoffmeisteri* and *S. heringianus* kept or reared single. Two special races(?) were noted both within *T. tubifex* and *L. udekemianus*.

Key words. Aquatic Oligochaeta, aquarial cultures, life cycle, life span, reproduction

Introduction

The life cycles of aquatic oligochaetes, mostly tubificids, have been studied on many occasions, both in the nature and as aquarial cultures. Researchers usually focused on the generation time in sexual reproduction, which was supposed to be mostly one year (e.g., Aston 1968; Bonacina *et al.* 1987), but for *Tubifex tubifex* (Müller, 1774) in aquarial cultures much shorter (e.g., Kosiorek 1974), by default presuming that they would die after forming cocoons with eggs. Sometimes also survival after reproduction, accompanied with the resorption of the reproductive system (e.g., Casellato 1984), or a „resting period" after reproduction, has been observed (e.g., Block *et al.* 1982).

A two-year reproduction cycle was suggested by Brinkhurst (1964) for *Tubifex costatus* (Claparède, 1864; = *Baltidrilus costatus*) and by Kennedy (1966a) for *Limnodrilus udekemianus* Claparède, 1862. Repeated reproduction in two subsequent years was noted by Jónasson & Thorhauge (1972) for *Potamothrix hammoniensis* (Michaelsen, 1901), and in 2–3 years, by Poddubnaja (1980) for *Limnodrilus newaensis*

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(Michaelsen, 1903; = *Tubifex newaensis*). Two reproduction periods per year were reported by Ladle & Bird (1984) for *Psammoryctides barbatus* (Grube, 1861), and by Lobo & Alves (2011) for *Branchiura sowerbyi* (Beddard, 1892). Kennedy (1966b) found that the reproductive cycle of *Limnodrilus hoffmeisteri* Claparède, 1962 and *Tubifex tubifex* can be longer or shorter depending on environmental conditions. A similar finding was reported by Poddubnaya (1980) who also proposed 2–3 years as the full life span of the two last species. Matsumoto & Yamamoto (1966) established a reproduction frequency of twice per year and an expected life span of 4–6 years for *T. hattai* Nomura, 1926 (= *T. tubifex*). Until now, nobody has attempted to establish the maximum life span of these worms, which can last many years and sometimes exceed their fertile period.

I have been most fortunate to have worked consistenly at the same institution (Centre for Limnology, former Limnological Station, at Lake Võrtsjärv in Estonia) for almost 60 years. In the 1960s, a project was launched on to test local freshwater invertebrates as possible objects for mass cultivation of live food for fish fry. Aquaria with various animals, including oligochaetes, were set up. I continued to keep and supplement them as an „Oligochaete Zoo" also after the end of this project. These aquarium cultures gave me a chance to follow the individual life span of many oligochaete species, mostly tubificids and lumbriculids, until their natural death. Some individuals became outright my personal acquintances. Some preliminary data of my work were published more than 30 years ago (Timm 1972, 1984, 1987). Here I will undertake a survey of the main results of my recorded observations during a 55-year period, 1962–2017.

Methods and materials

Most observations were made in small cylindrical glass aquaria (crystallizers), diameter 10 cm, height 5.5 cm, volume 300 ml, filled up to ¼–¾ with sieved mud from the profundal of Lake Võrtsjärv, and the remaining space, with artesian well water (Fig. 1).

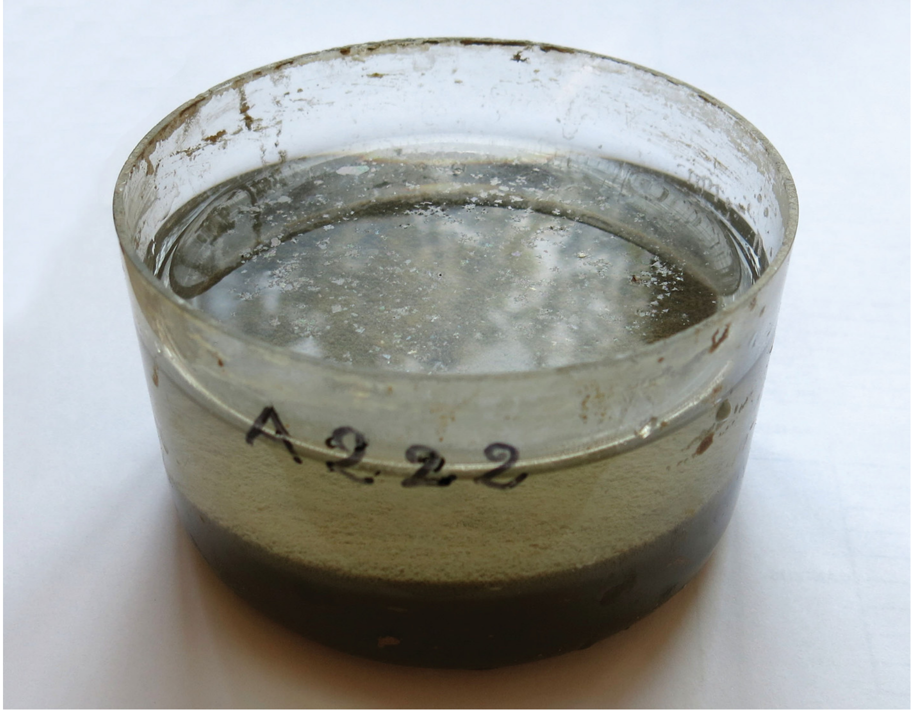

FIGURE 1. A standard aquarium used in long-term observations on aquatic Oligochaeta. Photo by Henn Timm.

The aquaria were maintained either at unregulated, often highly variable, room temperature, or in a cellar with seasonally fluctuating temperatures (about +4°C or less in winter, and about +15°C in summer), seldom also at regulated higher (+20°C, +25°C, +30°C and +35°C) or lower (about +5°) temperatures. The devices for temperature regulation were not reliable on a many-year scale. Usually, a batch of oligochaetes was initiated in each small aquarium with 10 conspecific newborn or young individuals when available, or with adults taken from natural waters, that were expected to produce progeny. The contents of aquaria were usually sieved four times each year, with a few aquaria sieved five or six times per year. Each time the number and the sexual condition of worms was registered, the original (old) individuals were returned to the aquarium with fresh mud and water while young worms and the egg cocoons were either discarded or used for starting a new culture. This procedure was repeated until the natural death of the last individual in the original cohort. Young worms were usually distinguishable from their parents, even when they have reached similar size, owing to the lighter colour of the chloragogen tissue on their intestine. No aeration was used in the aquaria, but a certain amount of water was added considering evaporation. As a rule, the worms subsisted on the muddy substrate only, without any additional food; when tested, small amounts of yeast or fish food pellets accelerated their growth. Besides the small aquaria, bigger ones were used for maintaining mass cultures, and enameled pans or wooden boxes were used for mass cultivation of *Tubifex tubifex*, a topic not discussed further in this paper.

The total number of aquaria and larger containers used during the years 1962 through 2017 was as large as 810, half of them containing *Tubifex tubifex*. In total, 45 species were tested in the above-described conditions but only about 17 tubificids and lumbriculids survived and reproduced during many years. Most species were originally collected from Estonian fresh waters, with the exception of three species: *Criodrilus lacuum*—collected from the Daugava River in Latvia, a strain of *Limnodrilus udekemianus*—collected from irrigation ditches in Kyrgyzstan, and a culture of *Aulodrilus pluriseta*—donated by Dr. N.R. Arhipova (Borok at the Rybinsk Reservoir, Russia). Preliminary results of the above observations were published previously in Timm (1974).

Results

The most common tubificid in Estonian lakes, *Potamothrix hammoniensis* (Michaelsen, 1901) was a disappointment during these studies: although surviving for some years in aquaria and even maturing, it rarely if ever reproduced (Fig. 2). Several other tubificid and lumbriculid species also failed to adapt (e.g., *Lophochaeta ignota* Štolc, 1886 and *Lamprodrilus isoporus* Michaelsen, 1901), as well as the Naididae *s.s.,* Enchytraeidae, *Propappus* and the lumbricid *Eiseniella tetraedra* (Savigny, 1826). The most successful species for cultivation—in both in aquaria and in bigger containers—was the well-known opportunistic pioneer species *Tubifex tubifex* (Müller, 1774). *Limnodrilus hoffmeisteri* Claparède, 1862 may be as suitable; however, its body is 'stickier' and often covered with a slimy mud envelope, making manipulations with it inconvenient.

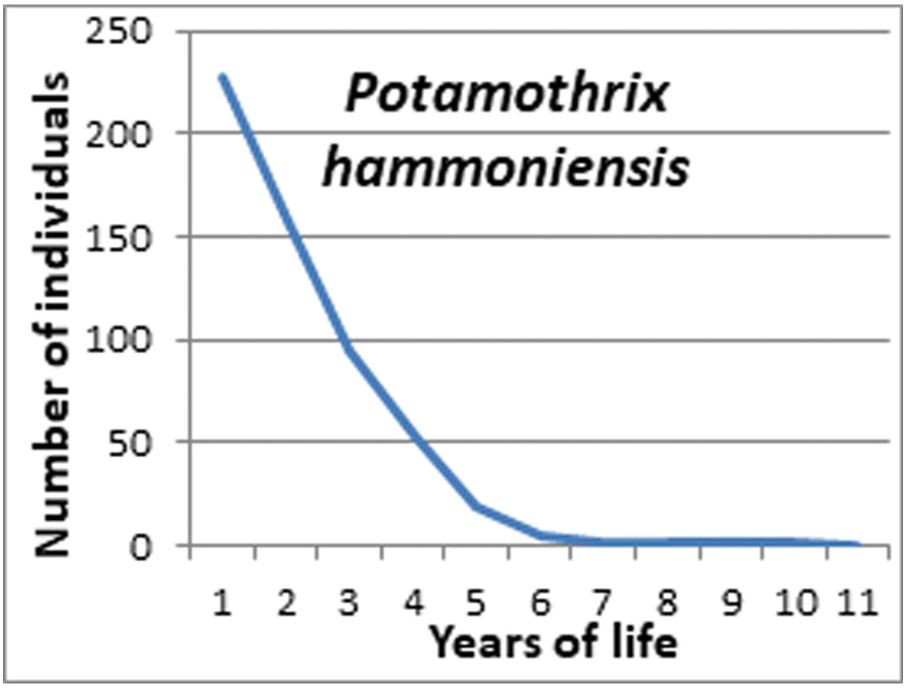

FIGURE 2. Survival of *Potamothrix hammoniensis* in aquaria, at room and seasonally changing temperatures, combined.

Besides *T. tubifex* and *L. hoffmeisteri*, several other tubificids—*L. udekemianus* Claparède, 1862, *Ilyodrilus templetoni* (Southern, 1909), *Psammoryctides barbatus* (Grube, 1861), *Spirosperma ferox* Eisen, 1879, *Potamothrix moldaviensis* Vejdovský et Mrázek, 1903, *P. vejdovskyi* Hrabě, 1941, *P. bavaricus* (Oschmann, 1913), and *Tubifex newaensis* (Michaelsen, 1903), as well as the lumbriculids *Stylodrilus heringianus* Claparède, 1862, and *Rhynchelmis tetratheca* Michaelsen, 1920—survived for years and laid numerous cocoons in the aquaria.

Architomic species—which seldom mature but reproduce well in an asexual way, by fragmentation—were similarly successful in the aquaria: these included the tubificids *Potamothrix bedoti* (Piguet, 1913), *Bothrioneurum vejdovskyanum* (Štolc, 1886), *Aulodrilus pluriseta* (Piguet, 1906), and *Aulodrilus japonicus* Yamaguchi, 1953 (although observations of *A. japonicus* were limited). The lumbriculid, *Lumbriculus variegatus* (Müller, 1774), thrived well only when it was supplied with additional food. Under the paratomic Naididae *s.s.*, only the clones of *Nais communis* Piguet, 1906, and *Nais elinguis* Müller, 1774 managed to survive in the mud for some time (with *N. communis* thriving for up to four years).

Finally, several individuals of the „aquatic earthworm“ *Criodrilus lacuum* Hoffmeister, 1845 lived for many years in the aquaria without reproduction (a single but viable cocoon was laid during the long observation period).

In nature only a few oligochaetes may reach at least the first reproduction period, with the majority of them having perished (mostly consumed as prey?) much earlier. In the conditions of a „Zoo“, in the absence of predation, some individuals also die at a young age (because of diseases?), but most of them perish, one by one, later over the course of several years. They can mature and lay eggs repeatedly, usually once a year (preferably in the spring season), with a „rejuvenation“ [referred to as *ananeosis* by Dózsa-Farkas (1973)]—resorption and renewal of the genital organs (with the exception of the gonads)—after each reproduction period. Many aquatic species can reach an age of up to 10 or even more than 20 years. The most senile worms cease reproducing during their last few years of life, decreasing in size and with their chloragogen tissue turning black.

Figs 3 and 4 demonstrate the gradual decrease of individuals in some comparable micropopulations of *Tubifex tubifex*, at two different temperature regimes.

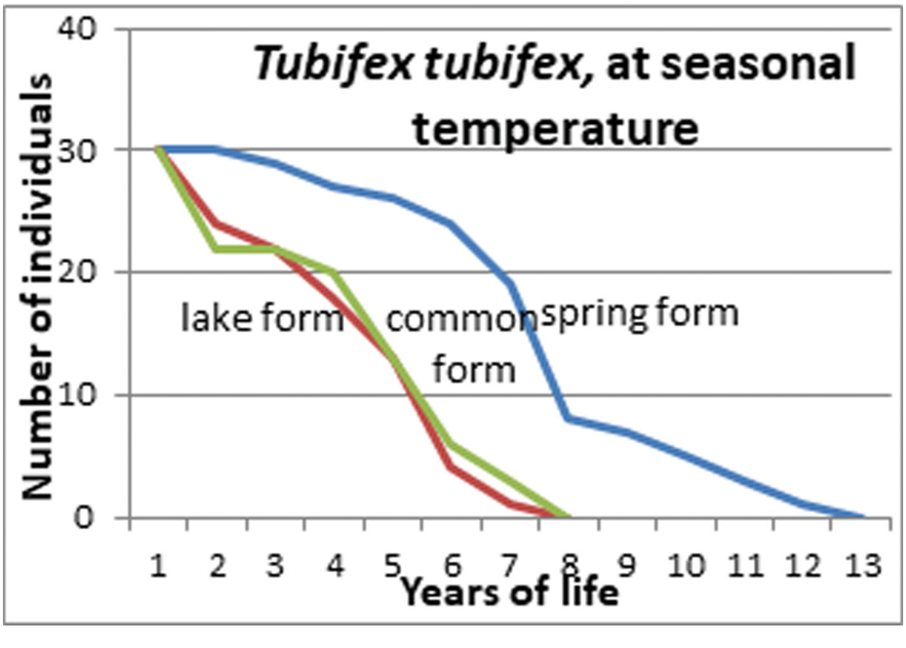

FIGURE 3. Survival of three forms of *Tubifex tubifex* of different origin in aquaria, at seasonally changing temperatures.

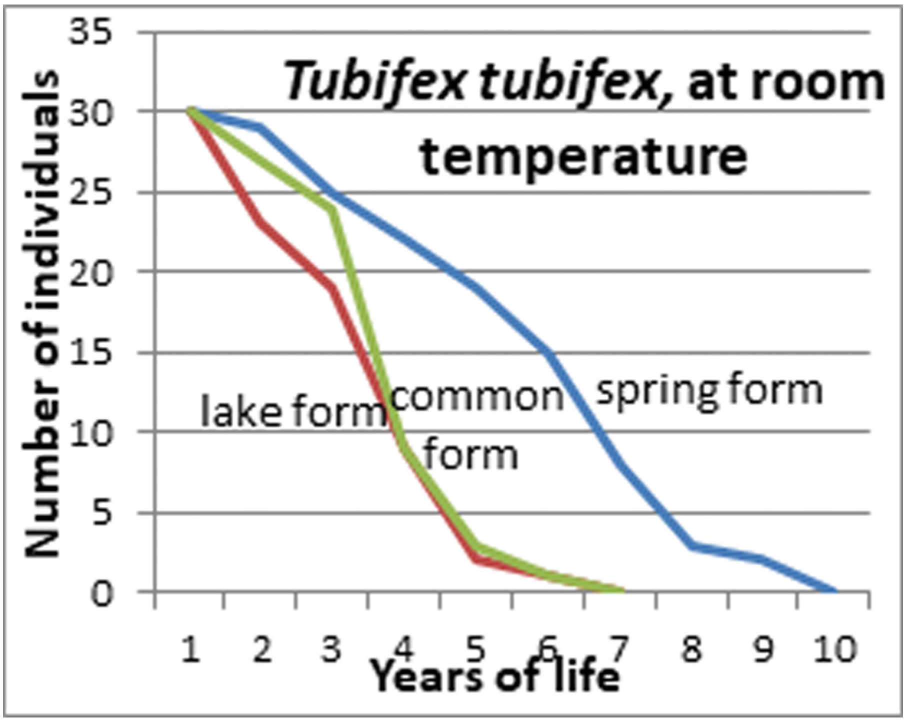

FIGURE 4. Survival of three forms of *Tubifex tubifex* of different origin in aquaria, at room temperature.

Half of the worms survived for 6–8 years in the „spring form“ and 3–5 years in two other forms, living in the cellar with cool winters for about two years longer than at those kept room temperature. One individual of the „spring form“ reached the maximum age of 12 years, in cellar. The „common form“ in the figures represents the widely distributed opportunistic populations. The „lake form“ of *T. tubifex* originated from the dwarf individuals living in the profundal of moderately eutrophic lakes together with the dominating *Potamothrix hammoniensis*, and may have been suppressed by it (Timm 1996). Their successors in the aquaria did not differ from the „common form“, either in size or in the life cycle. The „spring form“ originated from a large and cool (+5°C) karst spring of Roosna-Alliku in Central Estonia. It was always much larger (live weight 6–9 mg instead of 1.5–4 mg) when adult, laid larger cocoons with more eggs, and lived longer both at room and seasonal temperatures (but less at +25°C) compared with the others. It may represent a genetically different form (so far not proved). *T. tubifex* from some other Estonian springs resembled genetically „common“ individuals (Marotta *et al.* 2014).

At consistently elevated temperatures (+25°C) the life processes of all three forms of *T. tubifex* accelerated: the worms matured already during a few months and produced several generations a year, without any seasonal resting period. The cocoons were smaller and contained only 1–3 eggs instead of the usual 4–6. The total lifetime decreased nearly by 50%. *Tubifex tubifex* was able to form odd cocoons even at +30°C, while at +35°C the worms died before long. The accelerated reproduction of *T. tubifex* without hibernation at cooler conditions, may have been possible owing to parthenogenesis which excluded the need for lower temperatures for spermatogenesis. Presence of parthenogenesis was confirmed when singly reared *T. tubifex* individuals of all three forms repeatedly produced numerous cocoons with viable eggs. The second generation was also capable of uniparental reproduction and survived for 2–5 years while the third generation left behind no offspring.

Ilyodrilus templetoni, a small tubificid externally similar to *T. tubifex*, also lived and reproduced in various temperature conditions (most effectively at +25°C!), but on an average for only 2–3 years; the oldest individual survived in the cellar for 8 years (Fig. 5). Parthenogenesis can be suspected; unfortunately, no uniparental cultures were founded with this species.

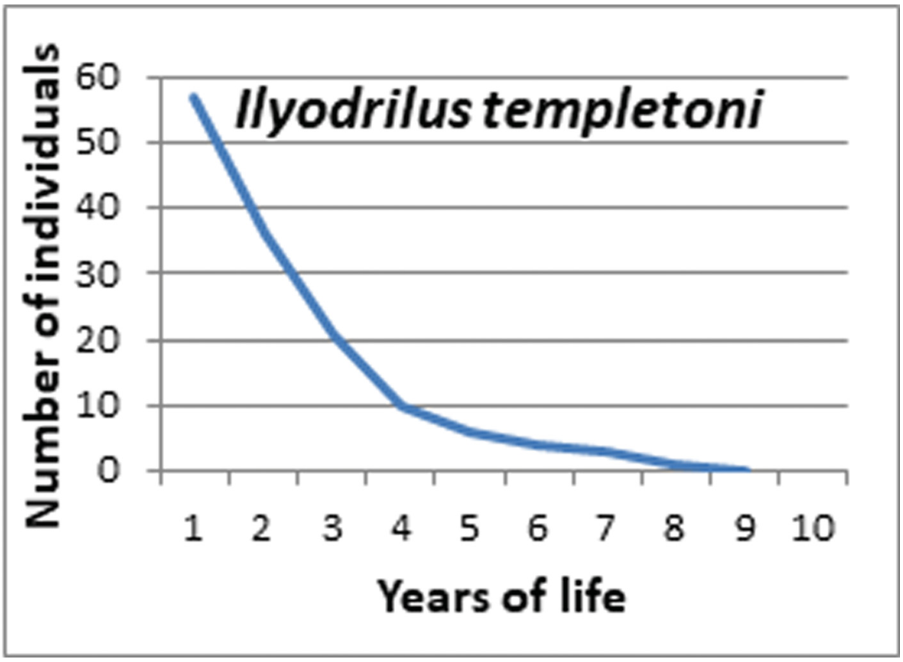

FIGURE 5. Survival of *Ilyodrilus templetoni* in aquaria, at room and seasonally changing temperatures, combined .

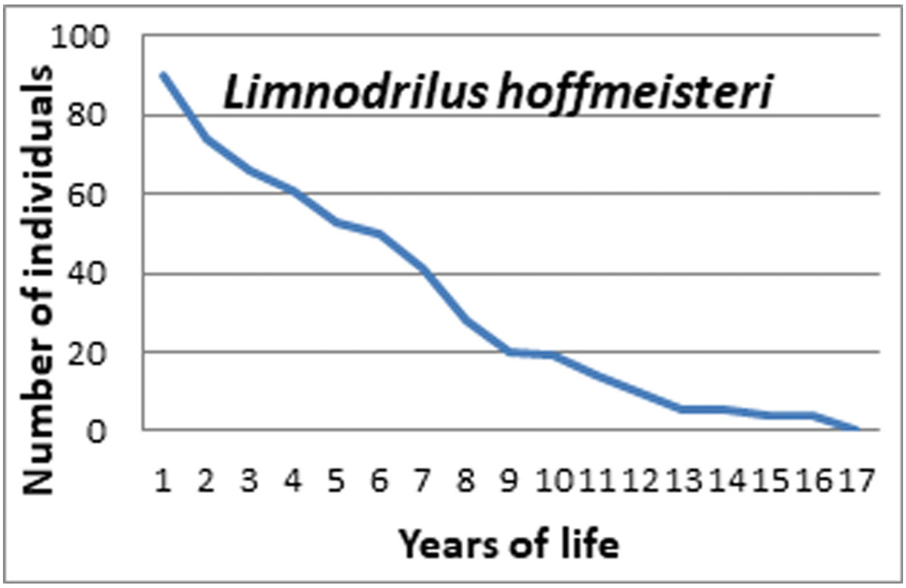

FIGURE 6. Survival of *Limnodrilus hoffmeisteri* in aquaria, at room and seasonally changing temperatures, combined.

Limnodrilus hoffmeisteri survived for an average of 5–6 years, maximum 16–17 years, in the aquaria (Fig. 6). It appeared to be the most heat-resistant tubificid, producing cocoons even at +35°C (then mostly with a single egg) and perishing only at +40°C. At elevated temperatures, several generations per year ocurred, as was the case with *Tubifex tubifex*. In the last case, individuals maturing already in the first summer of life were often relatively small.

Uniparental, probably parthenogenetic, reproduction of single-reared individuals was confirmed, too; however, the second generation was usually infertile in the „Zoo".

Cultures of *Limnodrilus udekemianus* were represented by two forms of different origins in aquaria (Figs 7 and 8). The worms from Estonian waters were very large, less fertile but long-lived (usually 5–6, maximum 28 years). Those by the parents collected from irrigation ditches of Kyrgyzstan were smaller, more productive, and died at the age of about 2–3 years, maximum 19 years. These cultures from two separate regional areas may represent two different races or cryptic taxa.

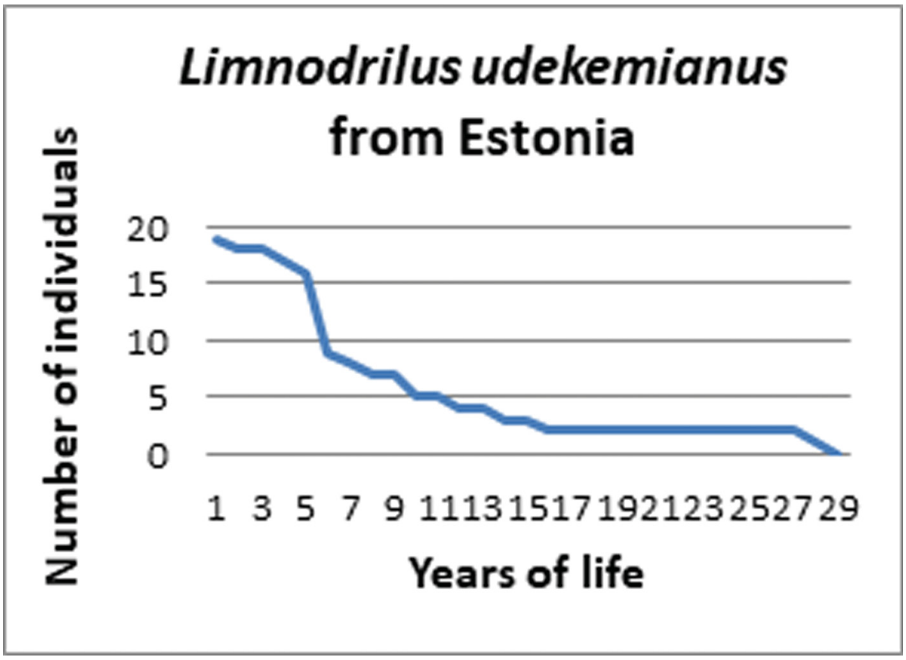

FIGURE 7. Survival of *Limnodrilus udekemianus* from Estonia in aquaria, at room and seasonally changing temperatures, combined.

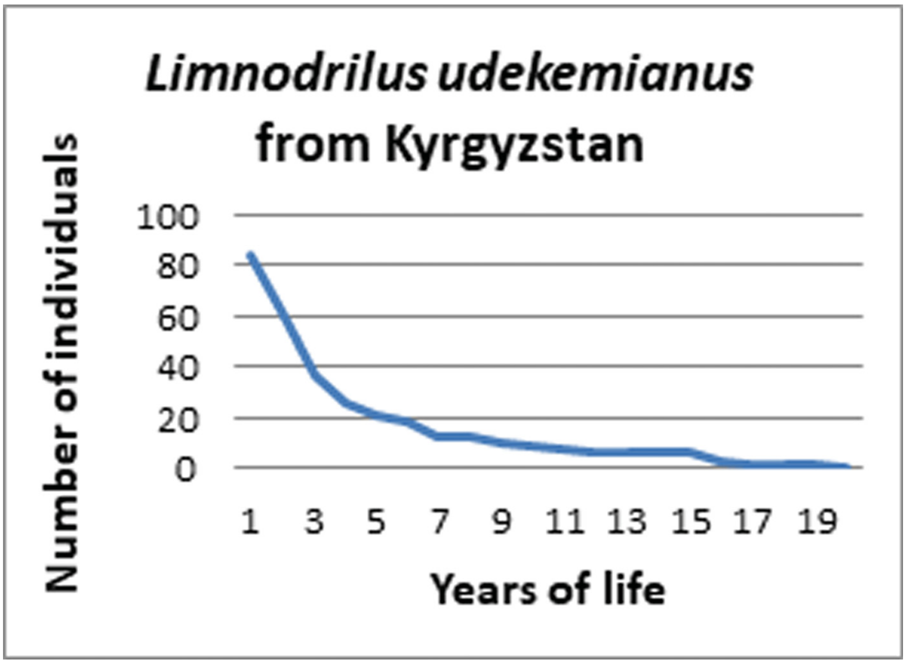

FIGURE 8. Survival of *Limnodrilus udekemianus* from Kyrgyzstan in aquaria, at room and seasonally changing temperatures, combined.

Psammoryctides barbatus survived at room temperature for an average of 3–4 years, maximum 9 years (Fig. 9), forming cocoons once a year. In the case of seasonal temperatures (in the cellar), one individual reached the age of 15 years. Reproduction was suppressed at continuously elevated temperatures (+20°C to +30°C), and the worms soon died when cultures attained temperatures of at +35°C. Uniparentally laid eggs did not develop.

Spirosperma ferox, sluggish in all activities, reproduced for the first time in the aquaria only when approaching the age two years. At unregulated room temperatures, half the individuals survived for about 7 years, the oldest reaching the age of 14 years. At seasonal temperature (in the cellar), the average age reached

15 years while the two oldest individuals, aged 25 years (Fig. 10), perished accidentally after producing a cocoon still at the age of 24 years. Some individuals survived for up to 10 years at +20°C, and for 9 years at +25°C, but with very scarce reproduction.

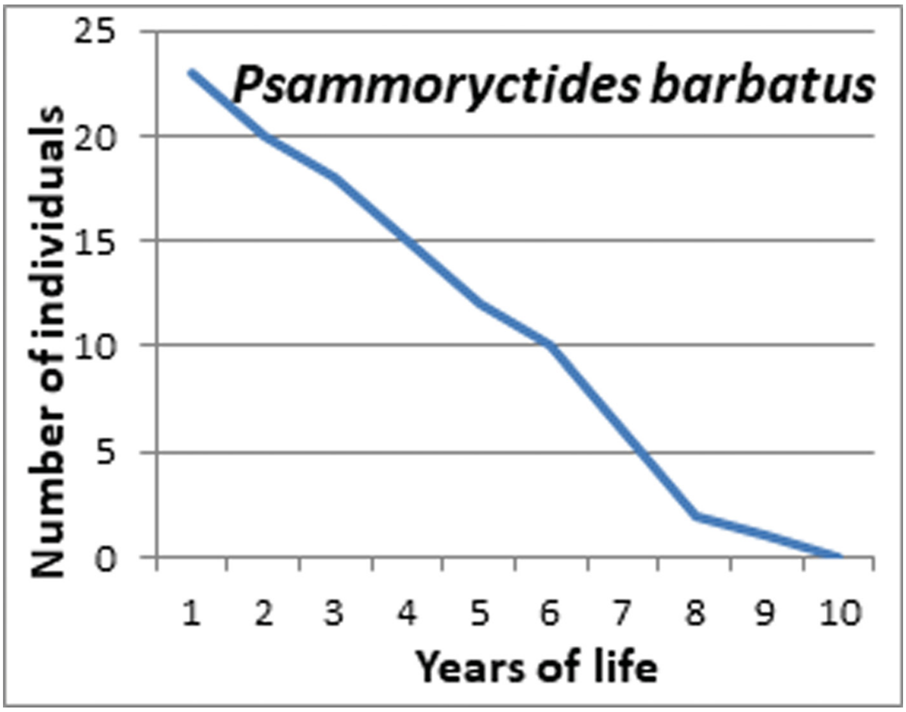

FIGURE 9. Survival of *Psammoryctides barbatus* in aquaria, at room temperature.

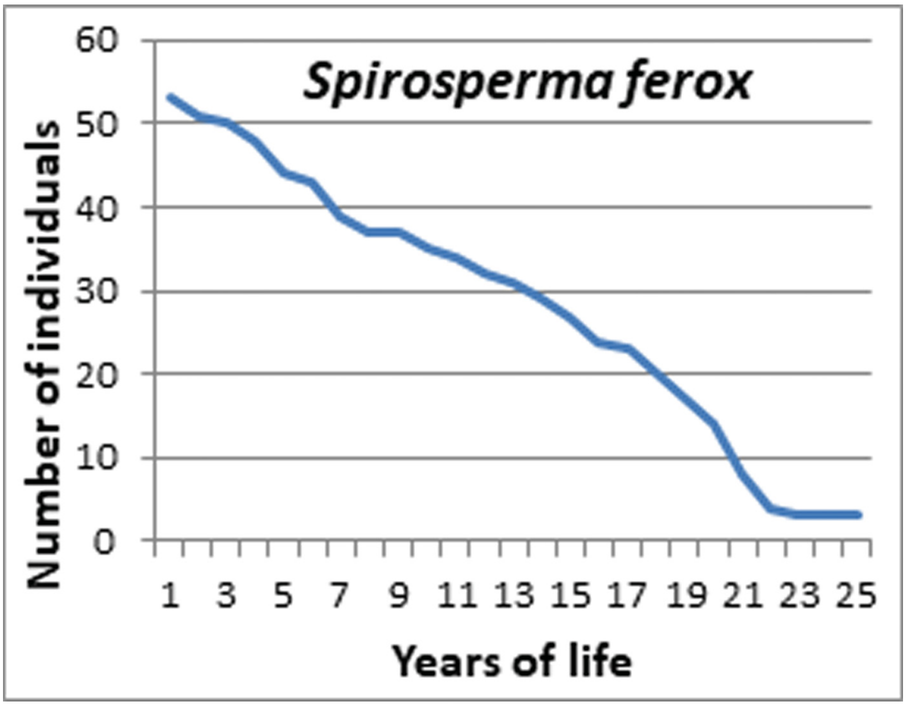

FIGURE 10. Survival of *Spirosperma ferox* in aquaria, at seasonally changing temperature.

Tubifex newaensis, a giant tubificid known mostly from rivers, survived in the standing water of the aquaria at room temperature for up to 8 years and even matured, although without producing any eggs. It also persisted for up to 3 years at +20°C, and for up to 2 years at +25°C. At the seasonally alternating temperature regime (in the cellar, Fig. 11), its average age reached 5–6 years, and maximum age, 22 years. It produced cocoons only at seasonal temperatures, with viable eggs in the first years but with only rotten eggs later on.

Three species of *Potamothrix*: *P. vejdovskyi, P. moldaviensis* and *P. bavaricus* survived in the aquaria for up to 9, 7 and 4 years, respectively (Figs 12–14) and successfully reproduced sexually, unlike their congener *P. hammoniensis*—which did not thrive in aquaria and usually died without reproducing (as noted above).

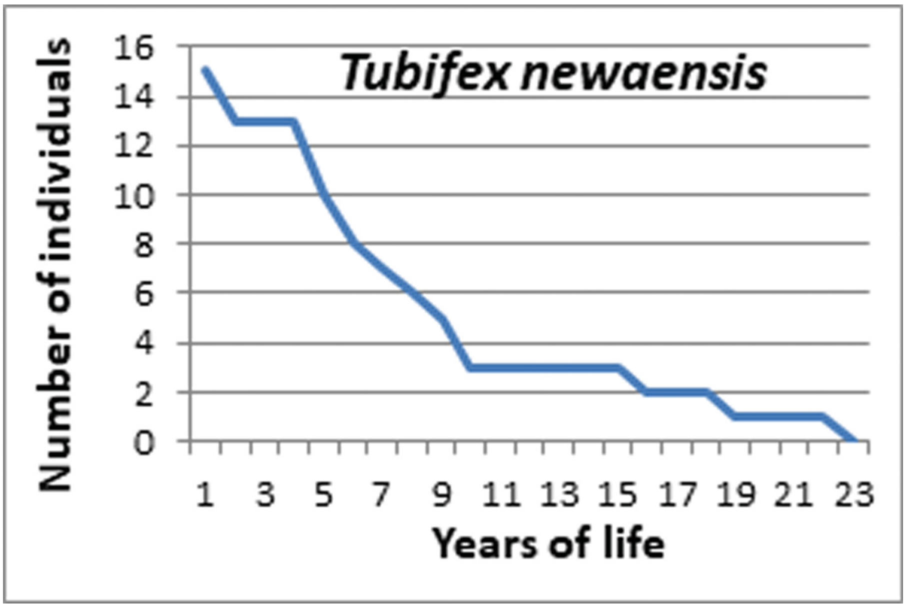

FIGURE 11. Survival of *Tubifex newaensis* in aquaria, at seasonally changing temperature.

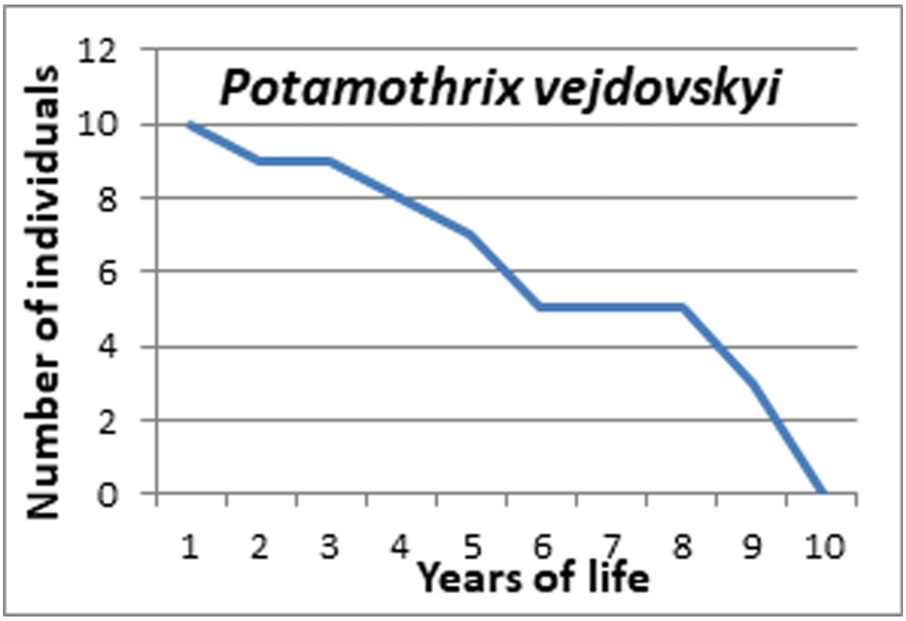

FIGURE 12. Survival of *Potamothrix vejdovskyi* in aquaria, at seasonally changing temperature.

The morphologically tubificid-like lumbriculid *Stylodrilus heringianus*, known as an oxyphilous animal in nature, survived and reproduced in a sexual way both at room and seasonal temperatures surprisingly well, with an average age about 5 years and maximum age of 17 years (Fig. 15). Even at +25°C, some individuals survived for up to 5 years. Single-reared *S. heringianus* reproduced well parthenogenetically, and so did their successors who survived for up to 12 years.

Rhynchelmis tetratheca, another oxyphilous lumbriculid, was also surprisingly well capable to live and reproduce sexually in standing water in the cellar (not tested at room temperature). Half of the individuals survived here for 4 years, with one individual lasting for nearly 13 years (Fig. 16).

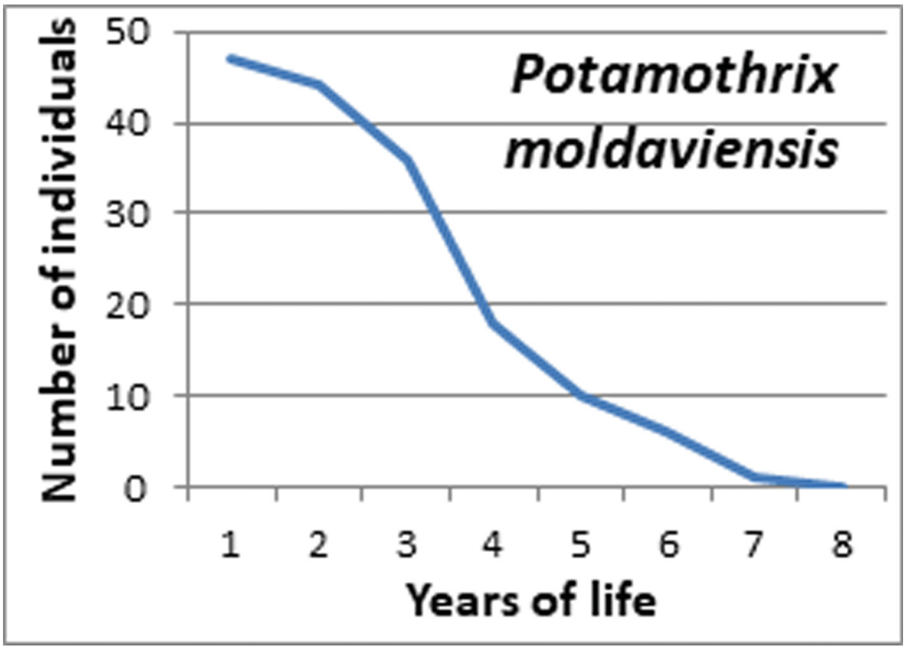

FIGURE 13. Survival of *P. moldaviensis* in aquaria, at room and seasonally changing temperatures, combined.

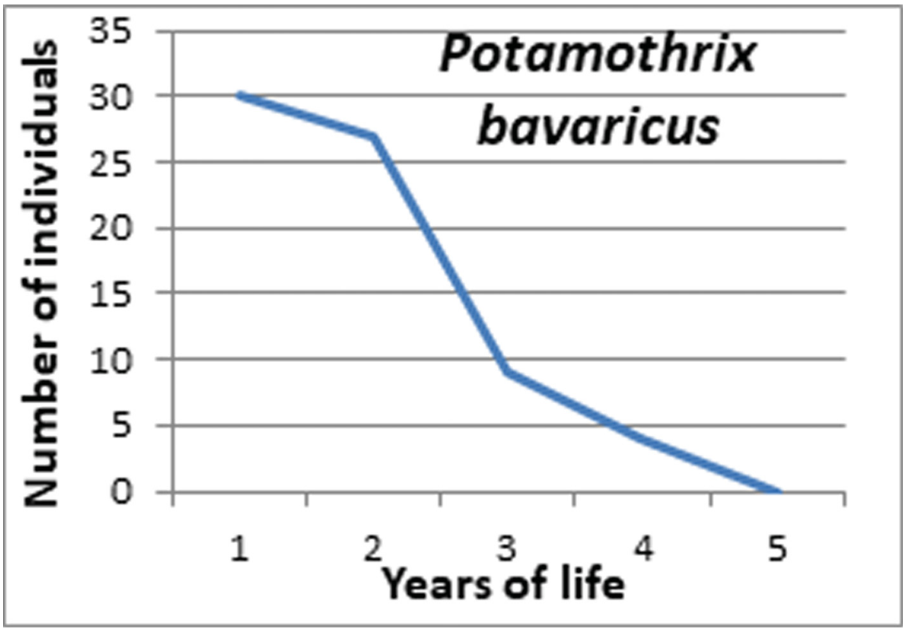

FIGURE 14. Survival of *P. bavaricus* in aquaria, at room and seasonally changing temperatures, combined.

The small architomic (fragmenting) tubificid *Potamothrix bedoti* can survive as a clone for unlimited time. A maximum of 5–10 young per parent were produced asexually during the first three months in the aquarium. When I tried to save only the individuals with darker chloragogen tissue (presumably old ones) at each inspection, the corresponding micropopulations persisted for 4–18 years. One individual, conspicuous because of the presence of a pair of teratological outgrowths in the genital region, was observed during 6 successive years. The clones were able to tolerate, at least temporarily, temperatures reaching +25°C, and some persisted for a short time when temperatures reached +35°C. Although sexually mature individuals of *P. bedoti* forming cocoons and viable eggs were observed mostly at cooler temperatures, a few were even observed when aquaria were maintained at room or seasonal temperatures. At consistently low temperatures, +1°C to +7°C, *P. bedoti* specimens reached considerable length—fragmentation had ceased, and maturing individuals were becoming more common. Of note here: *P. bedoti* in aquaria were repeatedly observed to be infested with plerocercoids of the cestode *Caryophyllaeus laticeps* (Pallas, 1781)—parasites that were close to the same size as their small hosts!

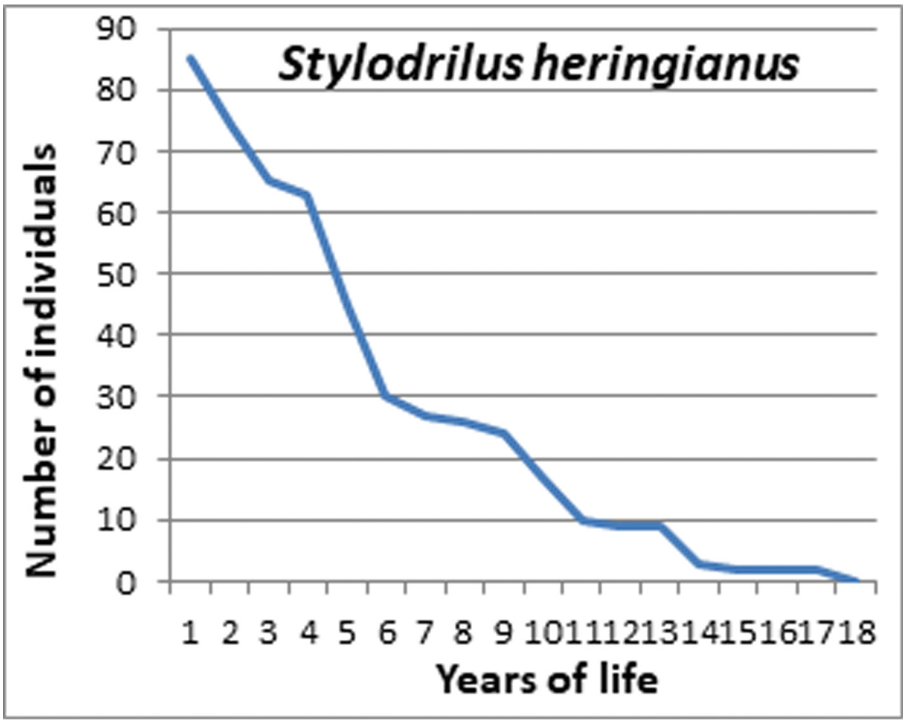

FIGURE 15. Survival of *Stylodrilus heringianus* in aquaria, at room and seasonally changing temperatures, combined.

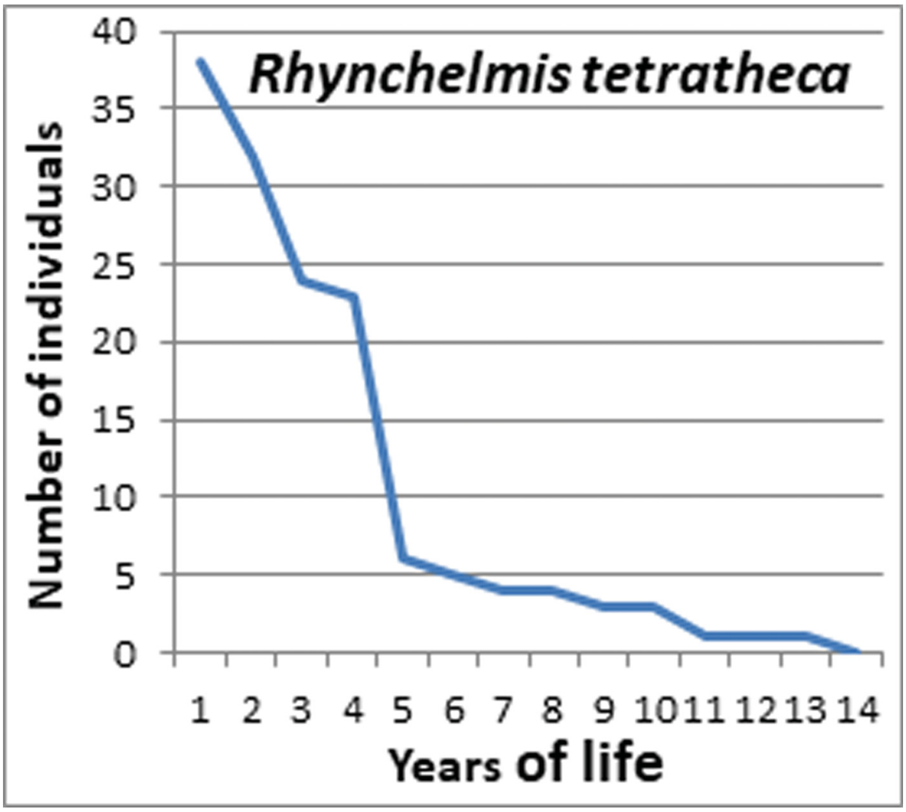

FIGURE 16. Survival of *Rhynchelmis tetratheca* in aquaria, at seasonally changing temperature.

Fragmenting clones of *Aulodrilus pluriseta* survived in the cellar, at room and elevated (+25°C) temperatures for at least 16–18 years and were very productive, usually with 10–30 young produced, per parent, in a three-month period. Some cocoons were occasionally observed in some years, after periods of cooler temperatures. The single micropopulation of *Aulodrilus japonicus* was as productive as that of *A. pluriseta*, but survived for only 5 years. Two mass cultures of *A. japonicus* perished at room temperatures—one culture after one year, and the second after two years—both for unknown reasons.

Populations of *Bothrioneurum vejdovskyanum*—observed to be almost exclusively architomic in Estonian water bodies, expanded the same in aquaria. Several clones lasted for at least 21–30 years, with some perishing as the result of casualties rather than because of senescence. The number of progeny fluctuated between 1–20 per parent during three months. Sexually mature individuals that had copulated (with external spermatophores) were observed, although seldomly. Cocoons occurred in mass cultures but not in the small aquaria.

Lumbriculus variegatus, a widely known architomic laboratory animal and object of mass cultivation, did not grow in the initial sieved mud substrate in aquaria—requiring some additional food (yeast, microalgae, fish food pellets, or presence of live plants like *Elodea*). The number of offspring produced by one *L. variegatus* parent during three months was low, usually 0–2, seldom up to 17. However, some micropopulations survived for 20–30 years. Sexual maturation was never observed.

Criodrilus lacuum, a large aquatic „earthworm“, barely reproduced in the aquaria (only a single cocoon was observed), despite the presence of the external male pores in all full-grown individuals. The numerous individuals taken as adults from the Daugava River in Latvia, usually survived in the aquaria for 5–6 years; some individuals survived for longer than 20 years, with the oldest in captivity observed for 46 years (Fig. 17). They diminished gradually in size and turned black in their last years of life.

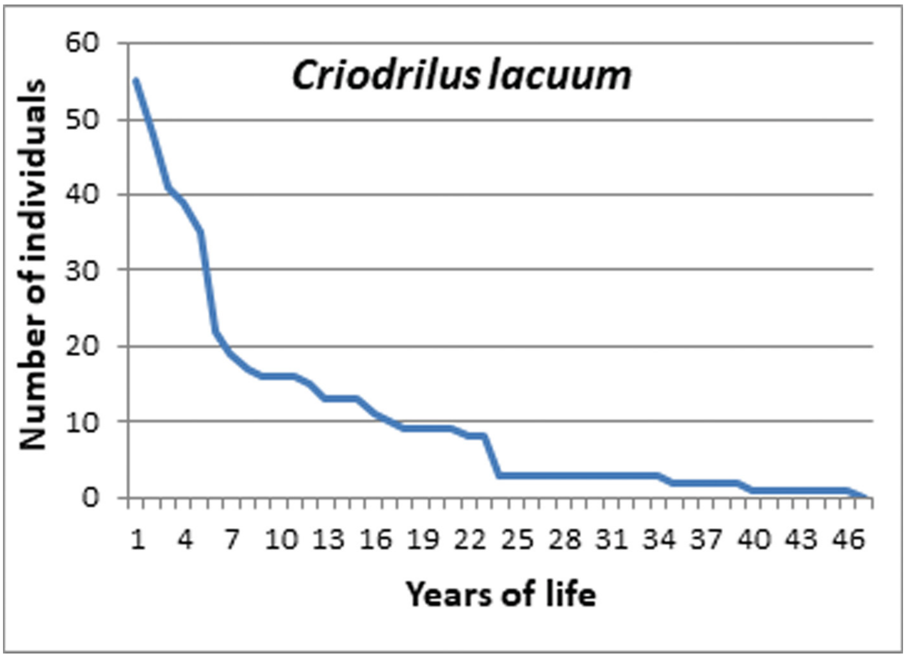

FIGURE 17. Survival of *Criodrilus lacuum* in aquaria, at room and seasonally changing temperatures, combined.

Abnormalities were noted in some individuals of the oligochaetes in aquaria. In the case of sexual maturation in architomic clones of *P. bedoti* and *B. vejdovskyanum*, the entire genital apparatus was always shifted forward, with the male pores in segment IX instead of XI. Individuals with a forked tail (and less often, those with a forked anterior end), were observed in both architomic (e.g., *B. vejdovskyanum*, *P. bedoti*) as well as in sexually reproducing species (e.g., *T. tubifex,* once even with a triple tail; *L. hoffmeisteri, S. ferox,* and *P. hammoniensis*)—persisting sometimes longer than one year. One individual of *P. bedoti* with a forked forebody had developed a reproductive apparatus in each of the two anterior branches. Four short-living „siamese twins“ with a double head and double tail were observed (in two cases) among the newborn *T. tubifex*. An individual of *T. tubifex* regularly produced dumbbell-shaped cocoons, with one shell containing eggs and the other, empty, partially enclosing it. The last inhabitant of the „Zoo" was the posterior half of *L. hoffmeisteri*, with a healed but not regenerated anterior end (Fig. 18). It survived for 2½ years, 2015–2017, mouthless and with an empty intestine, probably assimilating some nutrients osmotically through its body wall.

FIGURE 18. Mouthless individual of *Limnodrilus hoffmeisteri*. Bar = 1 cm. Photo by Henn Timm.

Observations on pure cultures provided opportunities to find and identify cocoons of several tubificid and lumbriculid species. Because descriptions and images of cocoons are scarce in the literature (e.g., Ditlevsen 1904, Penners 1933, Bonacina *et al.* 1986, Arhipova 1983, 1989, Timm 1970, 1972, 2006), a selection of the cocoons found in the aquarium cultures during this study are presented in Figs 19–20 (partially taken from Timm 1970, 1972). One can see that, besides the „traditional", naked and thin-walled, cocoons as in *T. tubifex* and many other species, there can also occur cocoons that are armoured with adhered sand or detritus (e.g., *L. hoffmeisteri, L. isoporus, P. moldaviensis*); their shell can be double (*L. udekemianus*), thickened (*A. pluriseta, B. vejdovskyanum*) or even stone-like and brittle (*Spirosperma ferox*); cocoons can firmly adhere to the substratum either by their unarmoured side (*P. moldaviensis*), or by the terminal stalk (*R. tetratheca*).

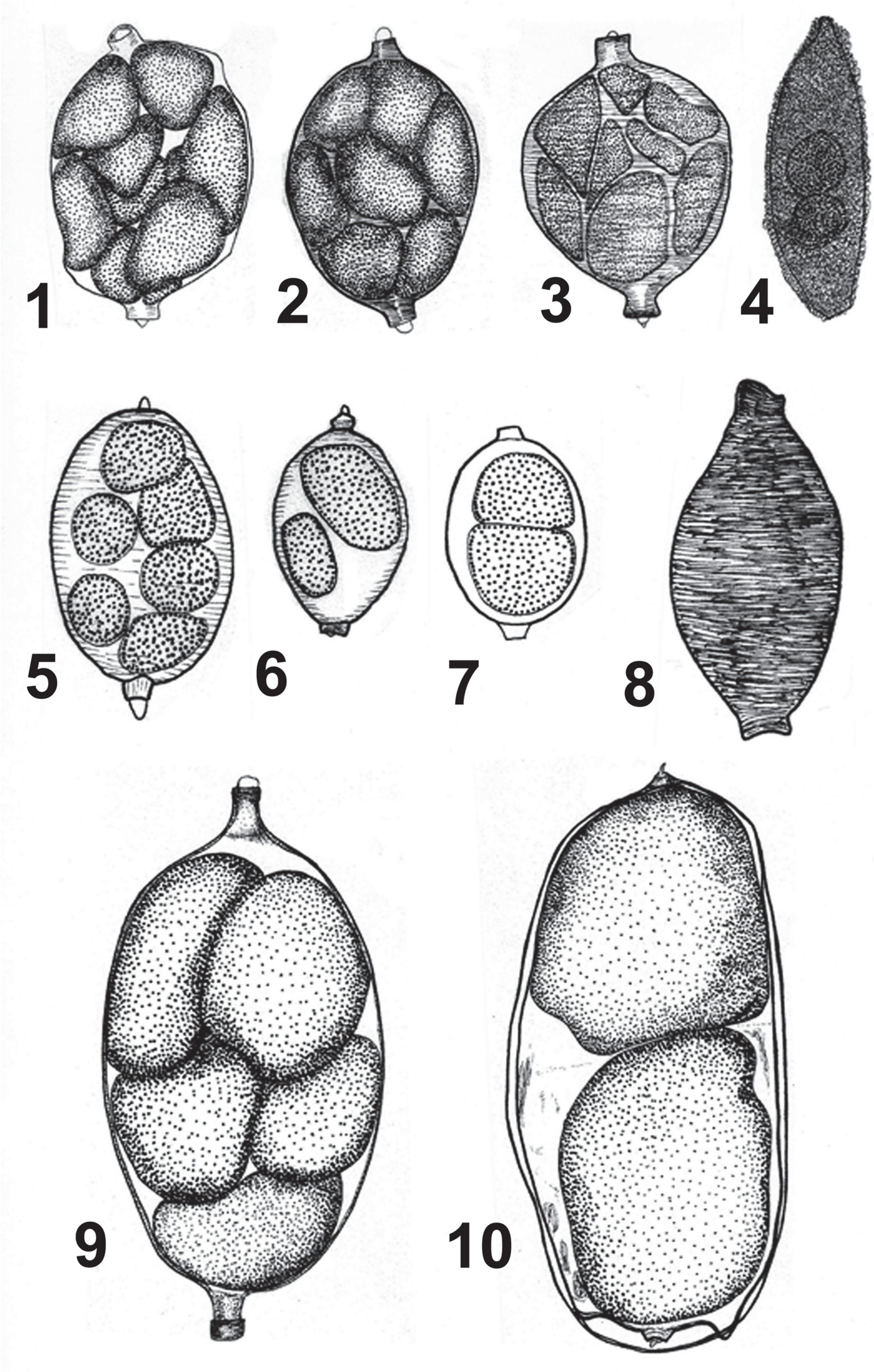

FIGURE 19. Cocoons of some tubificids (drawn without scale). 1—*Tubifex tubifex*; 2—*Potamothrix hammoniensis*; 3—*Psammoryctides barbatus*; 4—*Bothrioneurum vejdovskyanum*; 5—*Potamothrix vejdovskyi*; 6—*Potamothrix bedoti*; 7—*Ilyodrilus templetoni*; 8—*Spirosperma ferox*; 9—*Tubifex newaensis*; 10—*Limnodrilus udekemianus*.

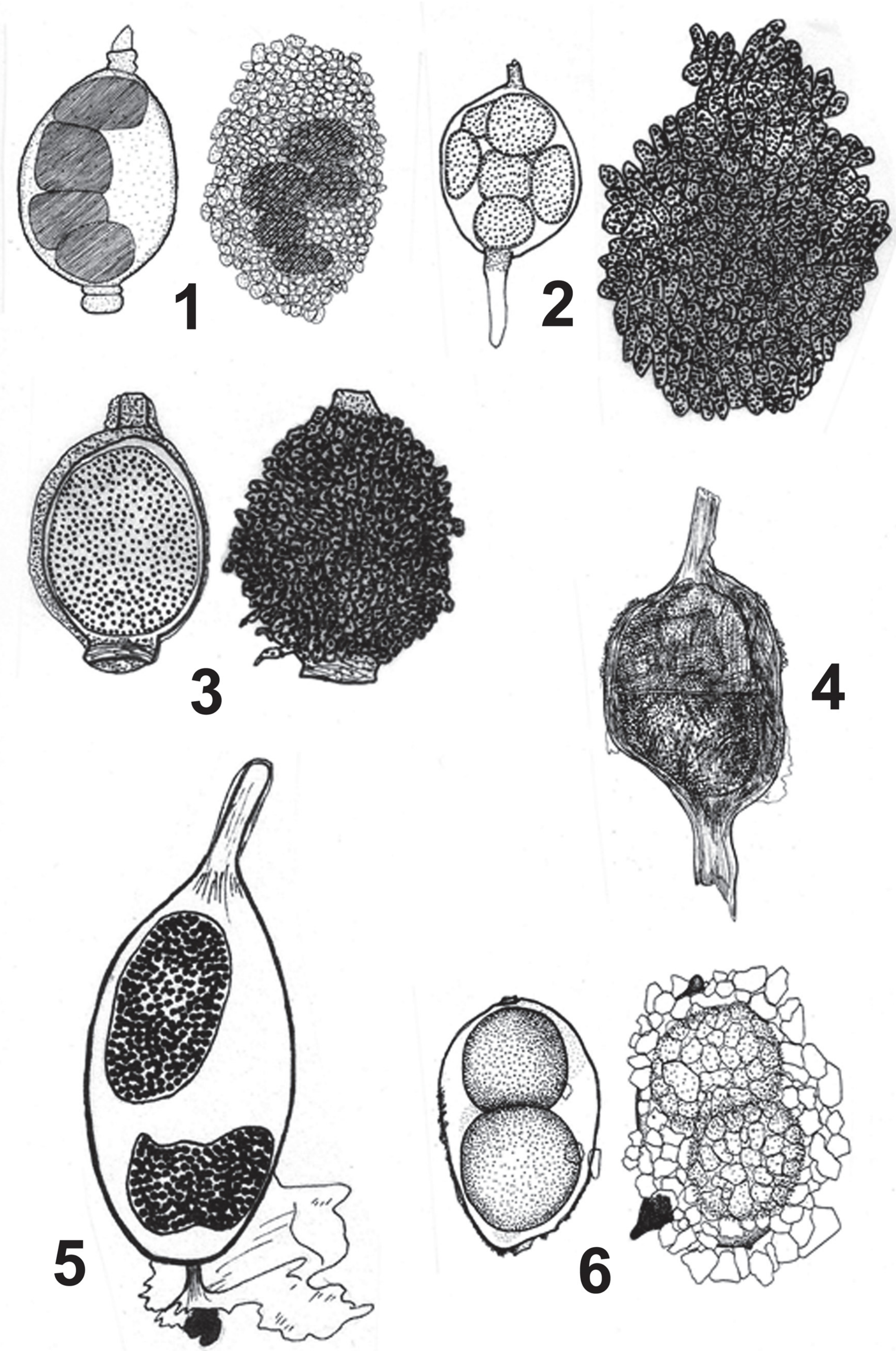

FIGURE 20. Cocoons of some tubificids and lumbriculids (drawn without scale): 1—*Limnodrilus hoffmeisteri*; 2—*Potamothrix moldaviensis*; 3—*Aulodrilus pluriseta*; 4—*Stylodrilus heringianus*; 5—*Rhynchelmis tetratheca*; 6—*Lamprodrilus isoporus* (from sand bottom of Lake Peipsi). Left images in 1–3 and 6 are devoid of ’armour’ of detritus and sand; right images with attached ’armour’ of detritus and sand.

Discussion

The new observations confirmed the experience of several authors (e. g., Block *et al.* 1982; Kaster 1982; Poddubnaya 1971, 1980) that freshwater tubificids and lumbricids resorb their reproductive apparatus (except for the gonads) after oviposition, and restore it in the subsequent reproduction period(s). Observations in the „Zoo“ demonstrated an unexpectedly long potential life span in most species, up to 10–20 years, with many annual reproduction periods, but with the number of individuals decreasing gradually every year. Maximum age for an oligochaete (*Criodrilus lacuum*) kept in an aquarium was at least 46 years, for a tubificid *Limnodrilus udekemianus* 28 years, and for *Tubifex tubifex* 12 years. This exceeds considerably the maximum expected life span of 4–6 years suggested by Matsumoto & Yamamoto (1966) for *T. tubifex*. Several authors, e. g. Monti (1986), Arhipova (1976, 1980) and Poddubnaya (1980) had suggested that the lifetime of *T. tubifex, T. newaensis, L. hoffmeisteri, L. claparedianus* and *P. hammoniensis* is likely limited to 1–3 years.

Most aquatic oligochaetes are known to reproduce once a year, usually in spring or early summer, with the first observable reproduction occurring in the year following their hatching. The first reproduction at the age of two years was suggested by Brinkhurst (1964) for *T. costatus* (= *Baltidrilus costatus*), and by Thorhauge (1976) for a deep-water population of *Potamothrix hammoniensis* in Lake Esrom, Denmark. In the „Zoo“, the first reproduction at the age of two years was recorded only in *Spirosperma ferox*. Accelerated reproduction in *T. tubifex* and *L. hoffmeisteri*, with several generations per year in suitable temperature conditions, sometimes associated with parthenogenesis (as was the case in the „Zoo“), had been reported previously by several authors (e.g., Arhipova 1976, Poddubnaya 1980, Moroz 1983).

After many years of observing cultures of oligochaetes in the „Zoo“, I believe that many past researchers (e. g., Čekanovskaja 1962, Hrabě 1981) may have often misidentified „neotenic“ *Limnodrilus hoffmeisteri* individuals—those not yet fully grown during their first summer of life but already sexually mature—as either *L. parvus* Southern, 1909 or *L. hoffmeisteri* f. *parvus*. The latter taxon had also been synonymized with *L. hoffmeisteri* by Brinkhurst (1971).

Phenotypic heterogeneity in the size and reproduction of the nominal species *T. tubifex* and *L. udekemianus* was observed in the aquaria, but was not tested using genetic methods. At the same time, the dwarf *T. tubifex* from the lacustrine profundal (Timm 1996) appeared to be a simple ecological form. In the literature, some phenotypic variability in *T. tubifex* has been described by Bonacina *et al.* (1987), and phenotypically identical cryptic species have been described by Beauchamp *et al.* (2001) and Marotta *et al.* (2014). Matsumoto (1983) described a co-existence of two forms of *T. hattai* (= *T. tubifex*) in cultures—one large and one small, although it is possible that these two forms were in fact representatives of two different age classes.

Uniparental reproduction was first described in *T. tubifex* by Černosvitov (1927) who explained it with possible self-fertilization. Self-fertilization was suggested also by Gavrilov (1935) who obtained offspring from single-reared *L. udekemianus, L. hoffmeisteri* and *L. claparedianus*, although the spermatozeugmata in spermathecae were present only in *L. udekemianus*—where self-fertilization is theoretically possible due to its short penis sheaths in comparison to those of its congeners. Purdy (1945) also associated the uniparental reproduction of *Tubifex* and *Limnodrilus* in aquaria with putative self-fertilization. Gavrilov (1955) later verified parthenogenesis during at least three generations in the tubificid *Paranadrilus descolei* (Gavrilov, 1955). Poddubnaya (1984) explained uniparental reproduction in the tubificids with parthenogenesis. Morev (1983) obtained 3–4 subsequent parthenogenetic generations of putative *L. udekemianus* (which could have been confused with *L. hoffmeisteri* by Morev) and *T. tubifex* reared single at 22–23°C.

Observations in the „Zoo“ confirmed that parthenogenesis occurs in several tubificid species as well as in the lumbriculid *S. heringianus*. Parthenogenesis (the laying of viable diploid eggs without fertilization) seems to appear in the case of unavailability of the partner’s sperm [due to absence of the spermathecae in some individuals, or running out of spermatozeugmata (see Poddubnaya 1984); lack of copulation partners; and/or elevated temperature—which inhibits spermatogenesis (see Timm, 1987)].

Parthenogenesis can support the colonization of new habitats by single individuals, or reproduction during periods temporarily affected by elevated temperatures. However, parthenogenesis may not be sustainable for longer periods of time, since parthenogenically formed eggs do not develop after a few generations (Poddubnaya 1984, and new data in this study). This can be a plausible explanation for the decline of some mass cultures of *T. tubifex* kept at room temperature, in the „Zoo“. Baldo & Ferraguti (2005) were faced with a mysterious situation where sexually mature and copulated *T. tubifex* laid only parthenogenetic eggs. The cause of this can be the relatively high temperature, +21°C, during the experiment. The relative unsustainability of parthenogenesis can indirectly explain the absence of *T. tubifex* in permanently hot tropical regions, e.g., the Tamil Nadu (India) lowlands, although the species inhabits the nearby Ooty Hills with a cool winter (Naveed 2012). The wide circumtropical distribution of *L. hoffmeisteri*, with presumably sexual reproduction, can be explained by the possible adaptation of spermatogenesis of this species [or one of the cryptic forms of this polytypic species separated by Liu *et al.* (2017)] to higher temperatures, avoiding the need for a cooler period.

Architomic clones of some tubificids can survive for many years without any symptoms of degeneration; hovewer, rare maturation and sexual reproduction of some individuals in cooler periods can support their sustainability. Certainly also the posterior fragments are physiologically younger then the most anterior „mother" fragment. In a situation where only the oldest individuals (with darker chloragogen tissue) were maintained, a micropopulation of *Potamothrix bedoti* in the „Zoo" tended to cease after some years.

The records of *P. bedoti* mainly from cool springs in Estonia (Timm 1972) are associated with its sexual maturing at relatively low temperatures, as I have confirmed now in aquaria. It is possible that *P. bedoti* is more common in lakes and rivers than has been previously estimated, given that it is often confused with small, immature specimens of *P. hammoniensis*. The results of this study also verify the regular forward shift of the reproductive system in *P. bedoti* and in other architomical oligochaetes occurring after the regeneration of the anterior end—as previously described by Hrabě (1935, 1981) and Sokol'skaja (1968).

The long survival of a mouthless posterior half of *L. hoffmeisteri*, without any possibility to ingest food, seems to support the possibility of osmotic assimilation of dissolved nutrients from surrounding waters by tubificids, as observed by Annabaeva *et al.* (1977), Hipp *et al.* (1985), and Sedlmeier & Hoffman (1989).

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North American Branchiobdellida (Annelida: Clitellata) or Crayfish Worms in France: the most diverse distribution of these exotic ectosymbionts in Europe

JEAN-FRANÇOIS PARPET[1] & STUART R. GELDER[2,*]

[1]*Royal Belgian Institute of Natural Sciences, Operational Directorate Taxonomy and Phylogeny, Vautier Street 29, B-1000 Brussels, Belgium.*

[2]*University of Maine at Presque Isle, 181 Main Street, Presque Isle, Maine 04769, USA. E-Mail: stuart.gelder@maine.edu*

**Corresponding author: stuart.gelder@maine.edu*

Abstract

Crayfishes and other invertebrates were collected during 2010 to 2016 from 519 sites located in the river basins of the Adour, Charente, Dordogne, Garonne, Loire, Seine, Rhône and Sélune in France. North American species included *Pacifastacus leniusculus* at 255 sites, *Faxonius limosus* at 206 sites, *Procambarus clarkii* at 56 sites, and the endemic *Austropotamobius pallipes* at two sites. However, branchiobdellidans were only recorded from 100 sites with 23 of these being sampled more than once, resulting in a total of 127 collections.

The widely distributed western North American, *P. leniusculus* carried four of its endemic branchiobdellidan species: *Cambarincola gracilis*, *C. okadai*, *Triannulata magna* and *Xironogiton victoriensis*. *X. victoriensis* was found at the majority of sites, with *C. okadai*, *C. gracilis*, and *T. magna* at fewer locations. Although *F. limosus* was the second most numerous crayfish species collected, it did not carry any of its endemic North American branchiobdellidan species. However, it was found cohabiting with *P. leniusculus* at four sites but carried *X. victoriensis* at only one of these. European *A. pallipes* only occurred at two sites with individuals harboring *X. victoriensis*, although no cohabiting exotic crayfish were found. Crayfish were also absent from three sites where free-living *X. victoriensis* were recovered from substrate samples. *Procambarus clarkii* appeared at sites scattered across the country, while specimens with *C. mesochoreus* were restricted to the Adour drainage.

This study has shown the widespread distribution of exotic branchiobdellidans in the wild and the virtual extinction of endemic species in France. In addition, we have provided the first European record of *T. magna*, and the first record of *C. mesochoreus* in France; the latter being only the second recording in Europe. With this comprehensive survey of their crayfish hosts in France, monitoring future exotic range expansions and endemic contraction or extinction can be traced. These data will be available to authorities for future planning in maintaining healthy freshwater bodies by reducing the damaging effects caused by exotic crayfishes.

Keywords: Ectosymbiosis, French distribution, *Triannulata magna, Cambarincola mesochoreus, Cambarincola gracilis, Cambarincola okadai, Xironogiton victoriensis*, North American crayfish, *Pacifastacus leniusculus*, *Procambarus clarkii*, *Faxonius limosus*, *Austropotamobius pallipes*

Introduction

The French naturalist, Alphonse Odier, discovered specimens of *Branchiobdella astaci* Odier, on *Astacus astacus* (Linnaeus) in 1819 and this became the second species to be described in the Branchiobdellida (Annelida: Clitellata) or crayfish worms. Unfortunately reports of this and subsequent finds of *B. astaci*, *Branchiobdella hexadonta* Gruber, *Branchiobdella parasita* (Braun) and *Branchiobdella pentadonta* Whitman in the country were not accompanied by any detailed collection site information (Odier 1823;

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Subchev 2014 & 2016). As a result, French distribution records on branchiobdellidans are very limited, consisting only of *Austropotamobius pallipes* (Lereboullet) carrying *B. astaci* at Grand Lac Laffrey, south of Grenoble, from the Druyes river, a tributary of the Yonne river, southeast of Paris (Subchev 2008), *A. pallipes* carrying *B. hexadonta* from an unidentified stream in an unlocated locality called "La Roche" (Subchev 2008), *A. pallipes* supporting *B. astaci* and *B. pentadonta* from an unidentified stream at Saint-Quentin-sur-Coole, Marne Department (Subchev 2016), an unknown crayfish host supporting *B. pentadonta* in an unknown stream at Strasbourg, Bas-Rhin Department (Subchev 2008) and finally with *A. astacus* supporting *B. astaci* in ponds at Thonnance-les-Joinvilles, Haute-Marne Department (J.-F. Parpet, unpub. data). Interest increased when exotic, North American branchiobdellidans were found on their translocated crayfish hosts. The first record in France of *Pacifastacus leniusculus* (Dana) carrying *Xironogiton victoriensis* Gelder and Hall, came from the River Lot, when a fisherman brought specimens to Laurent (2007) for identification; no details of location, date etc. were reported. Similarly, Subchev (2008) found the same association on a specimen from the River Dourbie, a tributary of the Tarn River, in the crayfish collection of the French National Museum of Natural History (Paris); again, collection details had not been recorded. However, collections of *P. leniusculus* from 11 recorded sites in the Rivers Lot, Tarn and Mayenne, recovered three branchiobdellidan species: *Cambarincola gracilis* Robinson, *Cambarincola okadai* Yamaguchi, and *X. victoriensis* (Gelder *et al.* 2012). Subsequently, Lecaplain and Noël (2015) reported *P. leniusculus* with the same three branchiobdellidans in Northwest France, probably from the Mayenne and Sélune River drainages. Following publication, they realized the report of *C. gracilis* in this region was an error (B. Lecaplain, pers. comm.). These imported crayfish were destined for human consumption, and the presence of branchiobdellidans mistakenly identified as parasitic leeches by consumers, reduced the public's interest for their hosts (J.-F. Parpet, unpub. obs.). In reality, most branchiobdellidans feed on epibiota attached to the crayfish's exoskeleton so they actually perform a beneficial host cleaning function thus forming a mutualistic association. However, the branchiobdellidan/ host relationship can vary, with periods of mutualism, commensalism or parasitism, depending on the species and habitat conditions (Skelton *et al.* 2013; Ames *et al.* 2015).

The three most important commercial North American crayfishes translocated to Europe are: *P. leniusculus*, *Procambarus clarkii* (Girard) and *Faxonius limosus* (Rafinesque), and all of them have been reported in France (Holdich *et al.* 2009; Kouba *et al.* 2015). Details of *P. leniusculus* translocations from the USA's northwestern states and subsequent multiple stockings in Europe, France in particular, were presented in Gelder *et al.* (2012). The European distribution of *P. clarkii* favors southwestern Europe which includes south and southwest France, while *F. limosus* extends from France in a wide band to Poland (Kouba *et al.* 2015). Recently these crayfishes were joined in northeastern France by *Faxonius juvenilis* (Hagen) in 2005, and *Faxonius immunis* (Hagen) in 2010 (Kouba *et al.* 2014), however, no branchiobdellidans have been reported on these new arrivals. Gelder (2004) listed the endemic branchiobdellidan species that were known on the three major commercial North American crayfishes and predicted their likely appearance in the importing countries. Currently four of these North American branchiobdellidan species have been recorded in Europe. The first one to be reported was *X. victoriensis* (= *X. instabilis* under various spellings) in Sweden by Franzén (1962), and subsequently in Finland (Kirjavainen & Westman 1999), Austria (Nesemann & Neubert 1999), Spain (Gelder 1999), Italy (Quaglio *et al.* 2002), Germany (Martens *et al.* 2006), Hungary (Kovács & Juhász 2007), France (Laurent 2007), Wales (James *et al.* 2015), Croatia (Dražina *et al.* 2018), Luxembourg (Dr. D. Templeman, pers. comm.) and Switzerland (R. Krieg, pers. comm.). This species has since been joined by *C. gracilis*, and *C. okadai*, in France (Gelder *et al.* 2012), with the latter species also appearing in Wales (James *et al.* 2015). Although *P. clarkii* is widespread in southwestern Europe, it is surprising there has been only one report of it carrying an endemic branchiobdellidan, and that was *Cambarincola mesochoreus* Hoffman, in northern Italy (Gelder *et al.* 1994). Continued translocations and natural range expansions are introducing these exotic crayfishes, and in some cases their branchiobdellidans, into new European freshwater habitats.

The present study in France was designed to extend the preliminary survey reported in Gelder *et al.* (2012) by sampling new sites and resampling previous sites at different times of year. These data will provide a detailed National baseline for local and state authorities to address the negative impact of exotic species on their freshwater habitats. Fortunately, these dangers to France's endemic freshwater fauna have already been recognized resulting in many state and local authorities increasing their monitoring programs as a prelude to future conservation legislation.

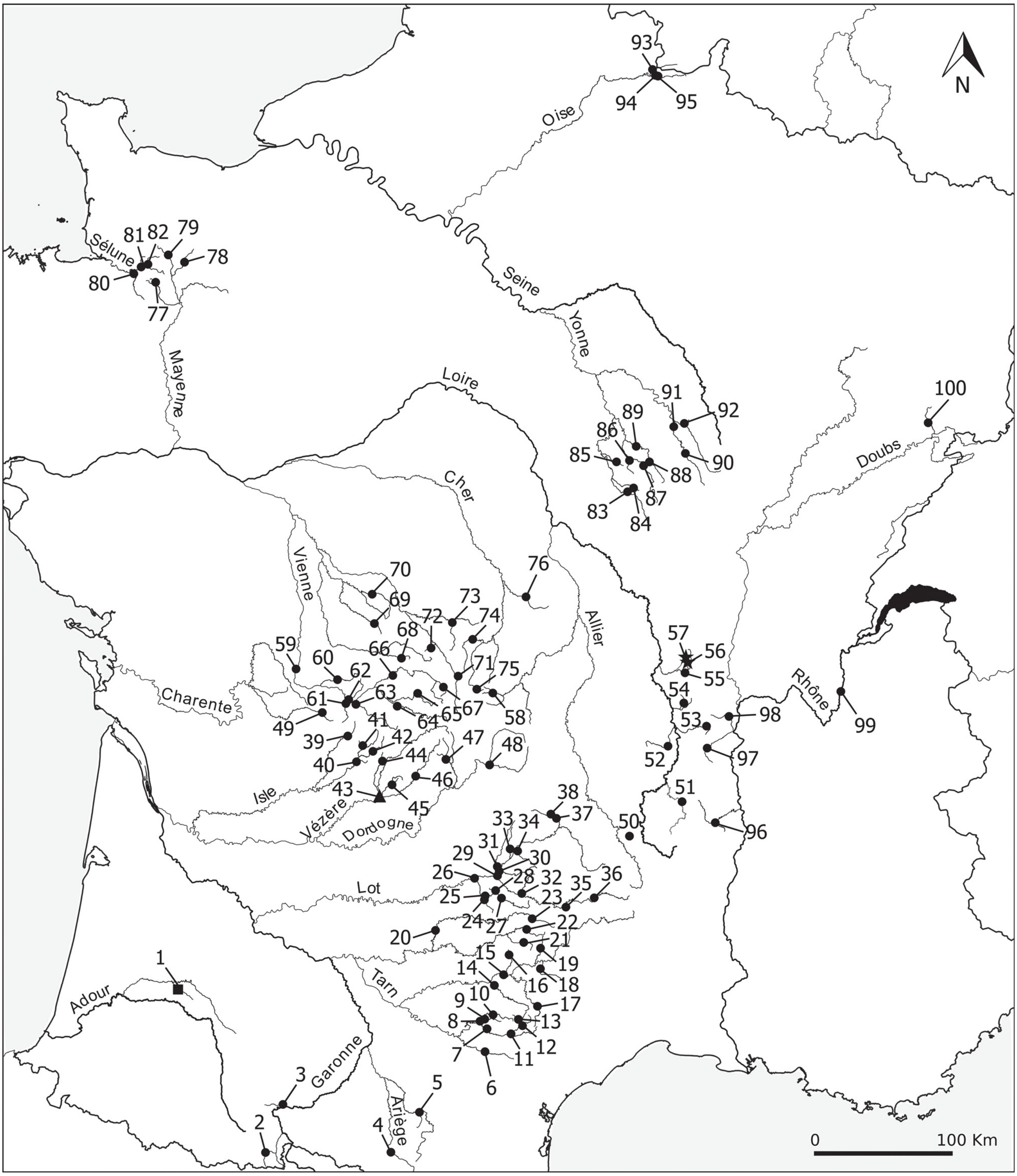

FIGURE 1. Map of France showing numbered sites where branchiobdellidans were collected, while the other crayfish sites have been omitted for clarity: discs, *Pacifastacus leniusculus*; square, *Procambarus clarkii*; triangle, *Faxonius limosus* and stars, *Austropotamobius pallipes*.

Material and methods

Five hundred and nineteen collection sites across France were located in the river basins of the Seine (Oise and Yonne drainages), Loire (Rhins, Allier, Cher, Vienne and Mayenne drainages), Dordogne (Isle and Vézère drainages), Garonne (Lot, Tarn and Ariège drainages), Rhône (Doubs) as well as the Sélune, Charente and

Adour basins (Table 1 and Fig. 1). Collections of crayfishes and their branchiobdellidan ectosymbionts were usually made along with other invertebrates as part of surface-water quality monitoring for French Water Agencies Adour-Garonne, Loire-Bretagne and Seine-Normandie during 2010-2016. Crayfishes were also captured during fish population monitoring in rivers for the Office National de l'Eau et des Milieux Aquatiques (ONEMA, today AFB, Agence Française pour la Biodiversité) in 2013-2016 (data access: http://www.naiades.eaufrance.fr/acces-donnees#/hydrobiologie). The majority of these collections were conducted during contract work by Asconit Consultants, Lyon, France, with additional collections being made by sport fishing enthusiasts. Specimens of the endemic *A. pallipes* were caught alive in the Drioule and Mardoret rivers (Rhône Department) during the springs of 2014 and 2016 as part of the fish and crayfish monitoring program of the Rhin's drainage (Loire basin) by the Fédération Départementale du Rhône et de la Métropole de Lyon pour la Pêche et la Protection du Milieu Aquatique, La Tour-de-Salvagny, France.

Exotic crayfishes, *P. leniusculus*, *P. clarkii* and *F. limosus*, were captured using one of four methods. Usually a Surber macro invertebrate stream net ("S" in Table 1) was used following the protocol described in Gelder *et al.* (2012), then captured crayfish were preserved in a 10% formalin solution for examination. Crayfishes immobilized by an electro-fishing protocol ("E" in Table 1) were transferred to holding tanks with dip nets. A collapsible Lift net or Balance method (Kozák *et al.* 2015) was used in streams with large crayfish populations ("B" in Table 1). A benthic nylon gill net ("G" in Table 1) was set between 1.7 and 2 m deep for about 12 hs overnight in Lake Bouchet, a crater lake at 1200 m altitude (site 55, Table 1 and Fig. 1), and on retrieving the net, crayfish were found eating any trapped fish. Crayfish caught by electro-fishing, balance and gill net, were preserved in 70% ethanol. Specimens of *A. pallipes* are protected and were hand collected ("H" in Table 1) alive at night with flashlights, marked and their body dimensions measured. Only their exposed surface was examined and any branchiobdellidans observed were removed before the crayfish were carefully returned to the river, thus preventing any bodily damage and minimizing handling stress.

In the laboratory all exotic crayfishes were identified, and their exposed surface and branchial chambers examined for branchiobdellidans; the worms found were transferred into 70% ethanol-containing tubes. Branchiobdellidans from each collection site were separated into groups based on body shape and size, and up to ten specimens were selected from each group. These were then dehydrated in a graded ethanol series from 70% to 99%, cleared in oil of wintergreen, infiltrated with Canada balsam, and mounted individually on a microscope slide under a cover-glass (Gelder & Williams 2015). Specimens were examined using an Olympus BX53F with Differential Interference Contrast (DIC) illumination and photographed with a Nikon 5100 camera mounted adapter lens #6144 providing 2.5X and 4X additional magnification. Unmounted branchiobdellidans were photographed using a Leica IC80 HD digital camera mounted on a Leica MZ95 zoom stereomicroscope with Leica CLS 150 X cold light source illumination. Morphological terminology used in the brief descriptions follows that in Gelder and Williams (2015). The results obtained were largely qualitative and therefore unsuitable for the statistical analyses usually applied to studies of branchiobdellidan-crayfish associations.

Results

Crayfishes were collected from a total of 519 sites with the exotic *Pacifastacus leniusculus* being present at 255 sites, *Faxonius limosus* at 206 sites, *Procambarus clarkii* at 56 sites, and the endemic *Austropotamobius pallipes* at two sites. However, branchiobdellidans were only recorded from 100 sites (Table 1) and consisted of five North American species: *Cambarincola gracilis*, *C. mesochoreus*, *C. okadai*, *Triannulata magna* Goodnight, and *X. victoriensis*. Although collections were made at 100 numbered sites (Fig. 1), 23 of which were visited twice on different dates and two received three visits (Table 1), resulting in 127 collections. The multiple site visits provided an indication of the stability of species incidence.

Infected *P. leniusculus* were found in upland, cool waters mainly in the north facing Pyrénées, Massif Central, Plateau de Millevaches, Massifs Morvan and Jura, with an isolated group in southwestern Normandie and another near Saint-Michel, Aisne Department in northeast France (Fig. 1). These crayfish carried *X. victoriensis* at 95 sites, either as the only species at 58 sites or part of a cohabiting group at 37 others. Where *X. victoriensis* was the only species, 10 sites received more than one visit, usually separated by a year. These data establish *X. victoriensis* as the most widespread branchiobdellidan species in France with an abundance ranging from one, e.g., at site 15, to 784 individuals at site 97.

TABLE 1. Collection sites and numbers of alien Branchiobdellidans with their host crayfish species, total numbers, collectors, hosts and worms species identifiers, and catching method (CM) for each site by drainages. S = Surber net, G = Gill net, B = "Balance" net, E = Electrofishing, H = Hand. NC = No data communicated. (* See Gelder *et al*., 2012) Branchiobdellidans: *Cg = Cambarincola gracilis*, *Co = Cambarincola okadai*, *Cm = Cambarincola mesochoreus*, *Tm = Triannulata magna*, *Xv = Xironogiton victoriensis*. Crayfishies: *Ap = Austropotamobius pallipes*, *Fl = Faxonius limosus*, *Pc = Procambarus clarkii*, *Pl = Pacifastacus leniusculus*.

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Adour Drainage :										
01	26/03/2014	Pujo-le-Plan	Landes	Aff. du Barrouquet	N43.848604 W0.338082	*Cm* (53)	*Pc* (14)	J. Rimour	J.-F. Parpet	B
Garonne Drainage :										
02	27/08/2012	Ôo	Haute-Garonne	Neste d'Ôo	N42.795583 E0.505650	*Xv* (1)	None	C. Rougé	R. Rudel	S
03	23/09/2014	Villeneuve-de-Rivière	Haute-Garonne	Lavet	N43.115995 E0.648545	*Xv* (96)	*Pl* (5)	S. Charansol	S. Charansol	S
Ariège Drainage:										
04	08/10/2013	Ornolac	Ariège	Ariège	N42.817577 E1.627303	*Xv* (168)	*Pl* (2)	L. Lopez	M. Bach	S
	29/09/2014	Ornolac	Ariège	Ariège	N42.816433 E1.628651	*Xv* (736)	*Pl* (1)	J. Revaud	R. Imbert	S
05	04/09/2013	Mirepoix	Ariège	Countirou	N43.084203 E1.877737	*Xv* (9)	*Pl* (1)	C. Blanco	Y. Duprat	S
Tarn Drainage:										
06	11/09/2012	Saint-Amans-Soult	Tarn	Thoré	N43.488106 E2.463361	*Co* (3) *Xv* (21)	*Pl* (1)	J. Rimour	O. Maingot	S
07	12/09/2012	Bez	Tarn	Agout	N43.639183 E2.480218	*Cg* (4) *Co* (10) *Xv* (10)	*Pl* (2)	J. Rimour	O. Maingot	S
08	12/09/2012	Vabre	Tarn	Gijou	N43.688927 E2.415895	*Xv* (14)	*Pl* (2)	J. Rimour	A. Morel	S
09	12/09/2012	Vabre	Tarn	Bertou	N43.702622 E2.458969	*Xv* (1)	*Pl* (2)	J. Rimour	A. Morel	S
	15/11/2013	Vabre	Tarn	Bertou	N43.702610 E2.458975	*Xv* (27)	*Pl* (2)	B. Vallée	J.-F. Parpet	E
10	13/09/2012	Lacaze	Tarn	Gijou	N43.732430 E2.534562	*Xv* (74)	*Pl* (2)	J. Rimour	O. Maingot	S
11*	13/09/2011	Salvetat-sur-Agout	Hérault	Agout	N43.606684 E2.698347	*Cg* (2) *Co* (1) *Xv* (3)	*Pl* (1)	M. Bach	J. Rimour	S
	14/09/2012	Salvetat-sur-Agout	Hérault	Agout	N43.606695 E2.698340	*Co* (14) *Xv* (15)	*Pl* (5)	J. Rimour	A. Meunier	S
12	13/09/2012	Murat-sur-Vèbre	Tarn	Vèbre	N43.660584 E2.802265	*Xv* (1)	None	J. Rimour	O. Maingot	S
13*	14/09/2011	Lacaune	Tarn	Caunaise	N43.702322 E2.764630	*Co* (6) *Xv* (12)	*Pl* (4)	M. Bach	C. Rougé	S
	13/09/2012	Lacaune	Tarn	Caunaise	N43.702333 E2.764624	*Cg* (27) *Co* (18) *Xv* (262)	*Pl* (2)	J. Rimour	A. Morel	S
14*	21/09/2011	Curvalle	Tarn	Rance	N43.926207 E2.544494	*Xv* (30)	*Pl* (3)	D. Bouché	J.-F. Parpet	S
	09/10/2013	Curvalle	Tarn	Rance	N43.926205 E2.544495	*Co* (2) *Xv* (433)	*Pl* (3)	S. Charansol	J.-F. Parpet	E
15*	01/08/2011	Brousse-le-Château	Aveyron	Alrance	N43.996508 E2.628392	*Co* (1)	*Pl* (3)	M. Daprey	F. David	S
	31/07/2012	Brousse-le-Château	Aveyron	Alrance	N43.996525 E2.628382	*Xv* (1)	*Pl* (3)	C. Rougé	C. Rougé	S

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TABLE 1. (Continued)

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Tarn Drainage end:										
16*	02/08/2011	Alrance	Aveyron	Alrance	N44.127860 E2.677678	*Xv* (1)	*Pl* (2)	M. Daprey	F. David	S
17*	01/08/2011	Brusque	Aveyron	Dourdou de Camarès	N43.789027 E2.938024	*Xv* (32)	*Pl* (1)	M. Daprey	A. Burgnies	S
18	01/08/2012	Saint-Rome-de-Cernon	Aveyron	Cernon	N44.036835 E2.964661	*Co* (1)	*Pl* (2)	C. Rougé	J. Rimour	S
19*	29/07/2011	Saint-Beauzély	Aveyron	Muze	N44.172432 E2.965483	*Xv* (12)	*Pl* (2)	M. Daprey	A. Burgnies	S
	02/08/2012	Saint-Beauzély	Aveyron	Muze	N44.172457 E2.965463	*Xv* (2)	*Pl* (1)	C. Rougé	J. Cayrou	S
20	03/09/2012	Monteils	Aveyron	Aveyron	N44.285585 E2.001088	*Xv* (14)	*Pl* (1)	J. Rimour	O. Maingot	S
21	10/10/2013	Curan	Aveyron	Vioulou	N44.210162 E2.811396	*Co* (57) *Xv* (768)	*Pl* (5)	A. Burgnies	J.-F. Parpet	E
22	24/07/2012	Ségur	Aveyron	Viaur	N44.296369 E2.838236	*Co* (1)	*Pl* (1)	C. Rougé	J. Cayrou	S
23	10/10/2013	Lugans	Aveyron	Aveyron	N44.366787 E2.888619	*Xv* (816)	*Pl* (2)	A. Burgnies	J.-F. Parpet	E
Lot Drainage :										
24*	18/08/2010	Nauviale	Aveyron	Créneau	N44.490380 E2.448379	*Xv* (12)	*Pl* (2)	M. Bach	M. Bach	S
	20/05/2015	Nauviale	Aveyron	Créneau	N44.490266 E2.448386	*Xv* (193) on *Pl*	*Pl* (2) & *Fl* (1)	E. Fievet	J.-F. Parpet	B
25	20/05/2015	Nauviale	Aveyron	Dourdou de Conques	N44.516353 E2.455228	*Xv* (2)	*Pl* (2)	E. Fievet	J.-F. Parpet	B
26	05/09/2012	Grand Vabre	Aveyron	Dourdou de Conques	N44.631991 E2.355842	*Xv* (8)	*Pl* (1)	J. Rimour	O. Maingot	S
27	19/05/2015	Rodelle	Aveyron	Dourdou de Conques	N44.502292 E2.605606	*Xv* (106)	*Pl* (1)	E. Fievet	J.-F. Parpet	B
	18/08/2015	Rodelle	Aveyron	Dourdou de Conques	N44.502292 E2.605606	*Xv* (76)	*Pl* (2)	Y. Pons	J.-F. Parpet	B
28*	17/08/2010	Saint-Félix-de-Lunel	Aveyron	Rau de Servan	N44.551966 E2.552527	*Xv* (4	*Pl* (4)	M. Bach	C. Rougé	S
29	20/10/2015	Entraygues-sur-Truyère	Aveyron	Truyère	N44.649800 E2.567268	*Co* (2) *Xv* (105)	*Pl* (1)	C. Roide	J.-F. Parpet	B
30	21/08/2014	Montpeyroux	Aveyron	Selves	N44.679893 E2.583438	*Xv* (28)	*Pl* (1)	C. Roide	J.-F. Parpet	B
	16/09/2014	Montpeyroux	Aveyron	Selves	N44.679893 E2.583438	*Co* (6) *Xv* (61)	*Pl* (1)	C. Roide	J.-F. Parpet	B
	20/05/2015	Montpeyroux	Aveyron	Selves	N44.679893 E2.583438	*Co* (51) *Xv* (1307)	*Pl* (5)	C. Roide	J.-F. Parpet	B
31	13/05/2014	Saint-Hippolyte	Aveyron	Goul	N44.710404 E2.565347	*Co* (2) *Xv* (108)	*Pl* (1)	N. Boidin	J.-F. Parpet	B
	19/08/2014	Saint-Hippolyte	Aveyron	Goul	N44.710411 E2.565349	*Co* (5) *Xv* (158)	*Pl* (1)	C. Roide	J.-F. Parpet	B
32	21/07/2015	Saint-Côme-d'Olt	Aveyron	Boralde Flaujaguèse	N44.533796 E2.792361	*Co* (1) *Xv* (147)	*Pl* (1)	C. Roide	J.-F. Parpet	B
33	20/05/2015	Brommat	Aveyron	Bromme	N44.827211 E2.683195	*Xv* (163)	*Pl* (1)	C. Roide	J.-F. Parpet	B
34	21/07/2015	Ste-Geneviève-sur-Argence	Aveyron	Argence Vive	N44.813568 E2.753040	*Xv* (489)	*Pl* (1)	C. Roide	J.-F. Parpet	B
35	19/07/2012	Banassac	Lozère	Lot	N44.443365 E3.198968	*Xv* (1)	*Pl* (5)	C. Blanco	J.-F. Parpet	S
36*	20/07/2011	Balsièges	Lozère	Lot	N44.503892 E3.460192	*Xv* (9)	*Pl* (1)	C. Blanco	M. Rossignol	S
	18/07/2012	Balsièges	Lozère	Lot	N44.503897 E3.460191	*Xv* (1)	*Pl* (1)	C. Blanco	C. Blanco	S

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TABLE 1. (Continued)

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Lot Drainage end:										
37	21/05/2015	Saint-George	Cantal	Lander	N45.029645 E3.112954	*Xv* (1474)	*Pl* (3)	C. Roide	J.-F. Parpet	B
	19/08/2015	Saint-George	Cantal	Lander	N45.029645 E3.112954	*Xv* (38)	*Pl* (1)	Y. Pons	J.-F. Parpet	B
38	17/07/2014	Andelat	Cantal	Lander	N45.057361 E3.061141	*Xv* (144)	*Pl* (6)	O. Maingot	J.-F. Parpet	E
	19/08/2015	Andelat	Cantal	Lander	N45.057361 E3.061141	*Co* (59) *Xv* (487)	*Pl* (1)	Y. Pons	J.-F. Parpet	B
Isle Drainage:										
39	19/08/2013	Le Chalard	Haute-Vienne	Isle	N45.558120 E1.154582	*Co* (3) *Xv* (2)	*Pl* (1)	C. Rougé	R. Imbert	S
	17/06/2014	Le Chalard	Haute-Vienne	Isle	N45.558170 E1.154675	*Co* (2) *Xv* (61)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
40	20/08/2013	Payzac	Dordogne	Rau des Belles-Dames	N45.389256 E1.241430	*Xv* (1)	*Pl* (12)	C. Rougé	S. Marty	S
41	09/07/2013	Coussac-Bonneval	Haute-Vienne	Rau de Marcognac	N45.497117 E1.296632	*Xv* (4)	*Pl* (4)	C. Blanco	C. Blanco	S
42	21/08/2013	Lubersac	Corrèze	Auvézère	N45.460608 E1.393369	*Co* (3)	*Pl* (3)	C. Rougé	S. Charansol	S
Vézère Drainage:										
43	26/09/2013	Saint-Pantaléon-de-Larche	Corrèze	Corrèze	N45.167843 E1.463142	*Xv* (3) on *Fl*	*Pl* (1) & *Fl* (1)	J. Rimour	J. Rimour	S
44	22/08/2013	Troche	Corrèze	Loyre	N45.397322 E1.484695	*Co* (1) *Xv* (3)	*Pl* (6)	C. Rougé	C. Rougé	S
	09/07/2014	Troche	Corrèze	Loyre	N45.397468 E1.485017	*Co* (1)	*Pl* (1)	C. Blanco	Y. Duprat	S
45	17/09/2014	Sainte-Féréole	Corrèze	Maumont Noir	N45.242352 E1.578193	*Co* (4) *Xv* (3)	*Pl* (4)	C. Blanco	S. Charansol	S
46	09/09/2013	Les Angles-sur-Corrèze	Corrèze	Corrèze	N45.301877 E1.797793	*Cg* (9) *Co* (8) *Xv* (118)	*Pl* (6)	J. Rimour	J. Rimour	S
	25/06/2015	Les Angles-sur-Corrèze	Corrèze	Corrèze	N45.301566 E1.797724	*Cg* (9) *Co* (4) *Xv* (133)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
Dordogne Drainage:										
47	08/10/2013	Egletons	Corrèze	Rau d'Egletons/Millet	N45.415133 E2.078524	*Co* (28) *Xv* (452)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
48	13/08/2014	Bort-les-Ogues	Corrèze	Rhue	N45.380969 E2.487629	*Cg* (25) *Co* (92) *Xv* (236)	*Pl* (6)	O. Maingot	J.-F. Parpet	E
Charente Drainage:										
49	06/08/2015	Champagnac-la-Rivière	Haute-Vienne	Tardoire	N45.708720 E0.909530	*Cg* (21) *Co* (3) *Xv* (133)	*Pl* (1)	C. Roide	J.-F. Parpet	B
Loire Drainage:										
50	22/08/2013	Cayres	Haute-Loire	Lac du Bouchet (L)	N44.909301 E3.790820	*Co* (23) *Xv* (657)	*Pl* (2)	A. Bijon	J.-F. Parpet	G
51	25/06/2014	Tence	Haute-Loire	Lignon du Velay	N45.132275 E4.284021	*Co* (42) *Xv* (43)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
	27/07/2015	Tence	Haute-Loire	Lignon du Velay	N45.132275 E4.284021	*Xv* (137)	*Pl* (1)	O. Maingot	J.-F. Parpet	E

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TABLE 1. (Continued)

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Loire Drainage End:										
52	27/08/2014	Saint-Marcelin-en-Forez	Loire	Mare	N45.500300 E4.160510	*Xv* (275)	*Pl* (3)	N. Boidin	J.-F. Parpet	E
53	25/08/2014	Larajasse	Rhône	Coise	N45.627998 E4.525517	*Xv* (792)	*Pl* (1)	N. Boidin	J.-F. Parpet	E
54	01/07/2014	Panisières	Loire	Charpasonne	N45.783705 E4.315772	*Xv* (584)	*Pl* (14)	N. Boidin	J.-F. Parpet	E
	30/10/2015	Panisières	Loire	Charpasonne	N45.783705 E4.315772	*Xv* (31)	*Pl* (1)	Y. Pons	J.-F. Parpet	E
	16/06/2016	Panisières	Loire	Charpasonne	N45.783705 E4.315772	*Xv* (31)	*Pl* (1)	C. Roide	J.-F. Parpet	E
Rhins Drainage :										
55	07/08/2015	Amplepuis	Rhône	Rhins	N45.982623 E4.332064	*Xv* (157) on *Pl*	*Pl* (1) & *Fl* (1)	Y. Pons	J.-F. Parpet	E
56	07/08/2014	NC	Loire	Drioule	NC	*Xv* (51)	*Ap* (1)	J.-P. Faure	J.-F. Parpet	H
57	26/07/2016	NC	Loire	Mardoret	NC	*Xv* (12)	*Ap* (1)	J.-P. Faure	J.-F. Parpet	H
Allier Drainage:										
58	31/07/2015	Saint-Avit	Puy de Dôme	Ru de Letrade	N45.856011 E2.513899	*Xv* (58)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
Vienne Drainage:										
59	28/08/2014	Ansac-Sur-Vienne	Charente	Vienne	N45.991501 E0.649968	*Xv* (1)	None	N. Gouneau	J.-F. Parpet	S
60	23/06/2015	Oradour-sur-Glane	Haute-Vienne	Glane	N45.928387 E1.044815	*Co* (1) *Xv* (74)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
61	19/06/2014	Aixe-Sur-Vienne	Haute-Vienne	Aixette	N45.772382 E1.129334	*Xv* (278)	*Pl* (2)	O. Maingot	J.-F. Parpet	E
	24/06/2015	Aixe-Sur-Vienne	Haute-Vienne	Aixette	N45.772375 E1.129324	*Xv* (11)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
62	12/06/2014	Aixe-Sur-Vienne	Haute-Vienne	Aurence	N45.798651 E1.154958	*Xv* (1)	*Pl* (1)	C. Gerret	S. Estevenon	S
	18/06/2014	Aixe-Sur-Vienne	Haute-Vienne	Aurence	N45.798655 E1.154954	*Co* (3) *Xv* (2)	*Pl* (17)	O. Maingot	J.-F. Parpet	E
63	24/06/2015	Condat-sur-Vienne	Haute-Vienne	Briance	N45.768548 E1.224145	*Cg* (9) *Co* (18) *Xv* (105)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
64	08/09/2016	Neuvic-Entier	Haute-Vienne	Ru de Vergnas	N45.761578 E1.613662	*Cg* (3) *Co* (23) *Xv* (34)	*Pl*(3)	J. Cayrou	M. Rossignol	S
65	17/06/2014	Saint-Martin-Château	Haute-Vienne	Maulde	N45.848667 E1.804524	*Xv* (8)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
	21/08/2014	Saint-Martin-Château	Haute-Vienne	Maulde	N45.848654 E1.804522	*Xv* (1)	*Pl* (4)	O. Maingot	O. Maingot	S
66	23/06/2015	Saint-Martin-Ste-Catherine	Haute-Vienne	Taurion	N45.963863 E1.566920	*Xv* (87)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
67	21/08/2014	Vallières	Creuse	Banize	N45.892132 E2.046535	*Xv* (29)	*Pl* (4)	O. Maingot	O. Maingot	S
68	28/10/2014	Mourioux-Vieilleville	Creuse	Ardour	N46.078932 E1.645914	*Co* (5)	*Pl* (2)	C. Gerret	M. Daprey	S
69	27/10/2014	Saint-Sulpice-les-Feuilles	Haute-Vienne	Benaize	N46.304217 E1.382553	*Xv* (19)	*Pl* (2)	C. Gerret	S. Charansol	S
70	19/05/2014	Sacierges-Saint-Martin	Indre	Abloux	N46.497754 E1.355734	*Xv* (60)	*Pl* (1)	A. Burgnies	A. Burgnies	S
71	22/08/2014	Aubusson	Creuse	Rau d'Aubusson	N45.965823 E2.184793	*Xv* (2)	*Pl* (4)	O. Maingot	O. Maingot	S

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TABLE 1. (Continued)

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Vienne Drainage End:										
72	22/06/2015	Sainte-Feyre	Creuse	Ru de la Pisciculture	N46.149065 E1.925434	*Xv* (202)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
73	22/06/2015	Domeyrot	Creuse	Verraux	N46.319225 E2.124189	*Xv* (108)	*Pl* (1)	O. Maingot	J.-F. Parpet	E
Cher Drainage:										
74	09/09/2014	Lussat	Creuse	Voueize	N46.209530 E2.319701	*Co* (1)	*Pl* (1)	O. Maingot	O. Maingot	S
75	13/06/2014	Saint-Oradoux-près-Crocq	Creuse	Tardes	N45.880548 E2.362425	*Xv* (10)	*Pl* (2)	C. Gerret	S. Estevenon	S
76	30/08/2013	Cosne-d'Allier	Allier	Aumance	N46.491960 E2.825308	*Co* (13) *Xv* (15)	*Pl* (3)	O. Maingot	J.-F. Parpet	S
Mayenne Drainage:										
77*	24/06/2011	Heussé	Orne	Rau de Longueves	N48.506092 W0.856354	*Xv* (1)	*Pl* (1)	J. Martin	M. Rossignol	S
78	27/08/2013	Saint-Bômer-les-Forges	Orne	Varenne	N48.647277 W0.579295	*Co* (8) *Xv* (6)	*Pl* (6)	X. Jaladon	J. Rimour	S
79	15/07/2015	Beauchène	Orne	Égrenne	N48.690112 W0.744126	*Co* (14) *Xv* (204)	*Pl* (12)	F. Ogier	J. Rimour	S
Sélune Drainage :										
80	26/07/2012	Les Loges-Marchis	Manche	Airon	N48.555000 W1.077222	*Co* (1) *Xv* (4)	*Pl* (1)	F. Martignac	J. Cayrou	S
81	24/07/2013	Milly	Manche	Gueuche	N48.602002 W1.007757	*Co* (1) *Xv* (1)	*Pl* (1)	S. Bouron	C. Blanco	S
82	25/07/2012	Romagny	Manche	Cance	N48.621666 W0.941388	*Xv* (14)	*Pl* (1)	F. Martignac	C. Blanco	S
Yonne Drainage :										
83	16/06/2014	Montreuillon	Nièvre	Yonne	N47.183043 E3.805829	*Xv* (112)	*Pl* (1)	L. Baraillé	J.-F. Parpet	E
84	11/07/2012	Mhère	Nièvre	Aff. de l'Anguison	N47.207784 E3.864033	*Xv* (176)	*Pl* (1)	A. Morel	R. Rudel	S
85	10/09/2015	Nuars	Nièvre	Armance	N47.381277 E3.701388	*Xv* (33)	*Pl* (1)	C. Roide	J.-F. Parpet	B
86	16/07/2012	Saint-André-en-Morvan	Nièvre	Rau de la Brinjame	N47.389479 E3.827269	*Xv* (43)	*Pl* (4)	A. Meunier	A. Burgnies	S
	17/07/2013	Saint-André-en-Morvan	Nièvre	Rau de la Brinjame	N47.389548 E3.828473	*Xv* (20)	*Pl* (1)	O. Maingot	A. Burgnies	S
87	29/08/2016	Marigny-L'Église	Nièvre	Cure	N47.354071 E3.963813	*Cg* (8) *Xv* (66)	*Pl* (1)	C. Henry	J.-F. Parpet	B
88	11/09/2015	Saint-Léger-Vauban	Yonne	Trinquelin/Cousin	N47.378440 E4.020953	*Cg* (27) *Co* (8) *Xv* (137)	*Pl* (1)	C. Roide	J.-F. Parpet	B
89	16/07/2012	Avallon	Yonne	Cousin	N47.482590 E3.894428	*Xv* (1)	*Pl* (1)	O. Maingot	A. Burgnies	S
90	02/07/2015	Montigny-sur-Armançon	Côte-d'Or	Armançon	N47.431230 E4.371562	*Xv* (2)	*Pl* (3)	A. Bijon	J.-F. Parpet	B
91	02/07/2015	Quincy-le-Vicomte	Côte-d'Or	Armançon	N47.608609 E4.262646	*Xv* (23)	*Pl* (2)	A. Bijon	J.-F. Parpet	B
92	17/06/2014	Marmagne	Côte-d'Or	Rau de Fontenay	N47.629237 E4.366302	*Xv* (93)	*Pl* (4)	A. Bijon	J.-F. Parpet	S

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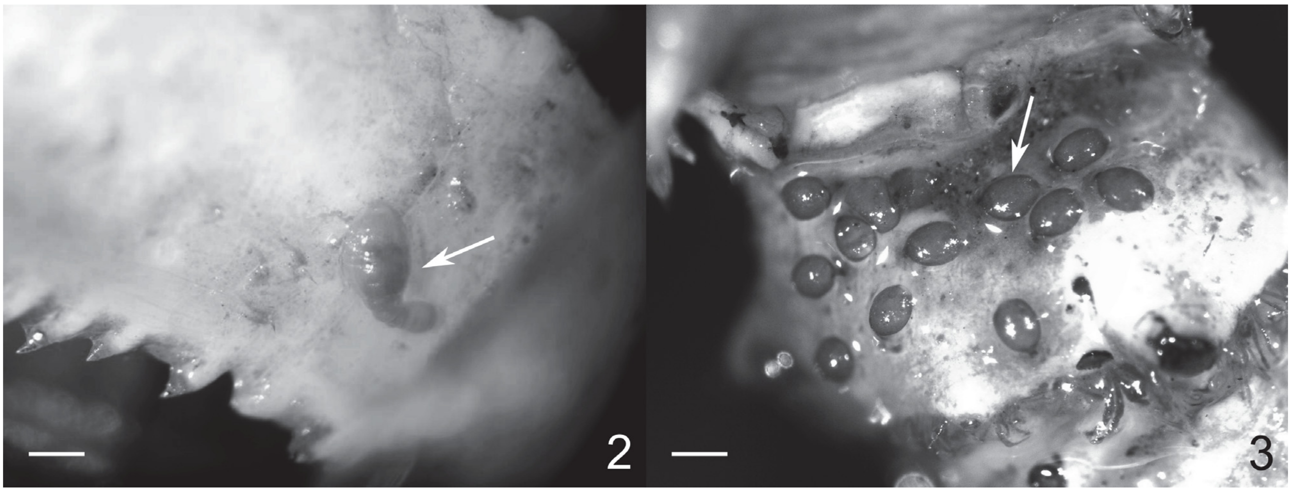
TABLE 1. (Continued)

Site no.	Date Sampled	Site name	Department	River / Lake (L)	Latitude/Longitude degrees	Worm species (Number.)	Host species (Number.)	Host Collectors	Host/Worms Identifiers	CM
Oise Drainage:										
93	31/07/2013	Hirson	Aisne	Oise	N49.965933 E4.104384	*Co* (9) *Xv* (33)	*Pl* (10)	E. Golembecki	J. Rimour	S
	28/07/2014	Hirson	Aisne	Oise	N49.965712 E4.104116	*Co* (4) *Xv* (9)	*Pl* (6)	E. Golembecki	J.-F. Parpet	S
94	16/07/2014	Saint-Michel	Aisne	Gland	N49.927497 E4.133415	*Cg* (67) *Co* (66) *Xv* (261)	*Pl* (4)	T. Mattioni	J.-F. Parpet	B
95	28/07/2014	Saint-Michel	Aisne	Gland	N49.922060 E4.159413	*Cg* (3) *Co* (3) *Tm* (2) *Xv* (48)	*Pl* (1)	E. Golembecki	J.-F. Parpet	S
	02/07/2015	Saint-Michel	Aisne	Gland	N49.921970 E4.159182	*Cg* (1) *Co* (8) *Tm* (7) *Xv* (38)	*Pl* (1)	E. Golembecki	J.-F. Parpet	S
Rhône Drainage:										
96	31/03/2015	Lamastre	Ardèche	Grozon	N44.989567 E4.589638	*Xv* (142)	*Pl* (1)	B. Vallée	J.-F. Parpet	B
97	19/08/2015	Saint-Chamond	Loire	Gier	N45.483054 E4.527758	*Xv* (784)	*Pl* (1)	B. Vallée	J.-F. Parpet	E
98	19/06/2015	Auberge du Garon	Rhône	Garon	N45.689119 E4.738934	*Xv* (1267)	*Pl* (2)	J.-P. Faure	J.-F. Parpet	E
99	27/10/2015	Culoz	Savoie	Rhône	N45.832643 E5.799751	*Xv* (305)	*Pl* (1)	N. Hendrick	J.-F. Parpet	E
Doubs Drainage:										
100	10/08/2016	Héricourt	Haute-Saône	Lizaine	N47.581416 E6.743084	*Xv* (857) on *Pl*	*Pl* (1) & *Fl* (1)	C. Roide	J.-F. Parpet	B

Although *C. okadai* was the sole species on *P. leniusculus* at sites 18, 22, 68 and 74, it was more usual to find it cohabiting along with *X. victoriensis* as reported at sites 45, 47, 50, 60, 76, 78, 79, 80, 81 and 87. In addition, two collections about a year apart were made at sites 31, 32, 39 and 93, indicating the stability of the cohabitating populations. In contrast, only *C. okadai* was recorded at site 15 in 2011, followed by only *X. victoriensis* in 2012. While at other site examinations separated by about a year, *X. victoriensis* was found initially and then both *C. okadai* and *X. victoriensis* at sites 30, 38 and 62, with the reverse occurring at sites 44 and 51 with *C. okadai* initially then *C. okadai* and *X. victoriensis*. A triple species cohabitation of *C. gracilis*, *C. okadai*, and *X. victoriensis* was identified at eight sites (7, 46, 48, 49, 63, 64, 88 and 94), while one of the double collections at sites 11 and 13, lacked *C. gracilis*. Site 95 near Saint-Michel in northeast France was unique in that two collections, one in 2014 and the other in 2015, both captured a single crayfish each time carrying four species, *C. gracilis*, *C. okadai*, *T. magna* and *X. victoriensis*.

Although site 43 was cohabited by *P. leniusculus* and *F. limosus*, *X. victoriensis* was only present on the latter host with adult worms occupying their usual position on the chelae and cocoons on the protected inner surface (Figs 2 and 3). A more unexpected adoption was found at sites 56 and 57 where the North American *X. victoriensis* had populated the European indigenous white-clawed crayfish, *A. pallipes*. In addition to monitoring invertebrates, which included crayfishes, samples of substrate were also collected from many sites for mesofaunal examination. Although no crayfish were captured from sites 2, 12 and 59, specimens of *X. victoriensis* were recovered from the substrate collections. *Procambarus clarkii* was only found at site 1 in the central Adour basin, southwest France (Fig. 1, Table 1) where it carried *Cambarincola mesochoreus*, making this the first record of *C. mesochoreus* in France.

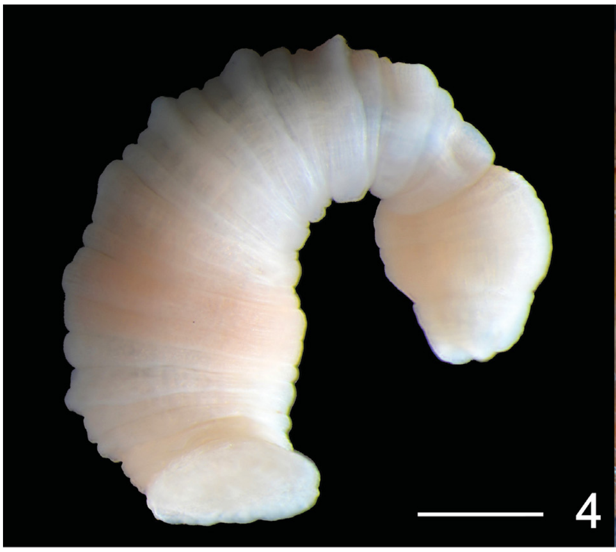


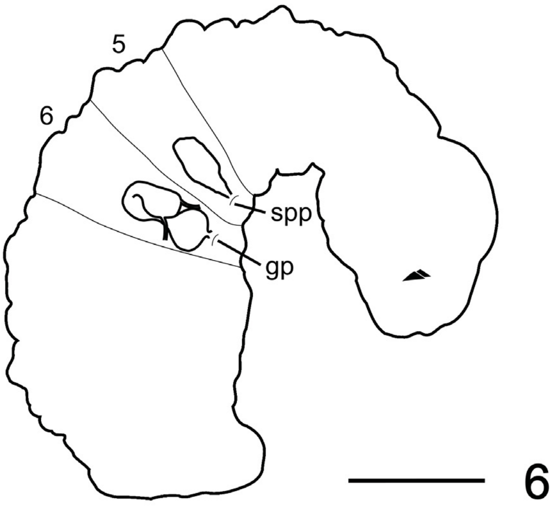
FIGURES 2-3. *Xironogiton victoriensis*. **Fig. 2.** Adult in dorsal aspect on the external surface of *Faxonius limosus* cheliped's merus, bar = 1mm. **Fig. 3.** Cocoons on *F. limosus* internal surface of cheliped's carpus, bar = 40µm.

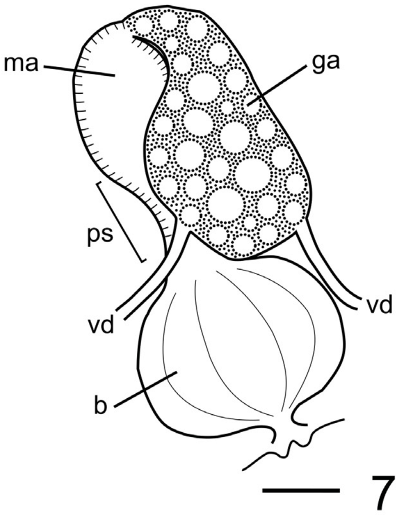
Brief descriptions and observations of the two newly reported exotic North American branchiobdellidan species

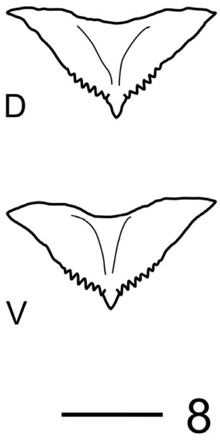
Previously Gelder *et al.* (2012) provided diagnostic descriptions for *C. gracilis* and *C. okadai* reported in France, and so similar information are presented here for *T. magna* and *C. mesochoreus*. As juvenile worms lack male organs, they can only be tentatively identified by their jaws when collected along with adult specimens; cocoons are similarly recognized by association and inference. Reference specimens of the five exotic North American branchiobdellidan species collected in France were submitted to Professor Jean-Loup Justine, Curator of the Annelida Collection at the Museum National d'Histoires Naturelles (MNHN), Paris, France, on April 25th, 2017. The museum's registration number of a specimen is followed in parentheses by its site number taken from Table 1: *Cambarincola gracilis,* HEL647 (site 11), HEL651 (site 7) and HEL652 (site 95); *C. mesochoreus*, HEL655 (site 1); *C. okadai*, HEL648 (site 11), HEL653 (site 7) and HEL654 (site 95); *Triannulata magna*, HEL649 (site 95) and HEL656 (site 95) and *Xironogiton victoriensis,* HEL650 (site 36) and HEL657 (site 95).

Triannulata magna Goodnight, 1940: Adults have a large triangular head and elongated ovoid body, measuring 3.5 to 5.5mm long, with three annuli per segment and no transverse segmental ridges (Figs 4 and 6). The dorsal peristomial lip is without lobes and the jaws have a dental formula of 8-1-8/8-1-8 (Figs 6 and 8). Both dorsal and ventral jaws are triangular with a large median tooth and eight pairs of very small lateral teeth (Fig. 8), however, the lateral teeth may not be visible resulting in an apparent dental formula of 1/1. Spermatozoa occur in segments 5 and 6, while the male organs consist of a sac-shaped glandular atrium (ga), a muscular atrium of similar length and spherical bursa containing an eversible penis in segment 6 (Figs 6 and 7) (emended from Holt 1974).





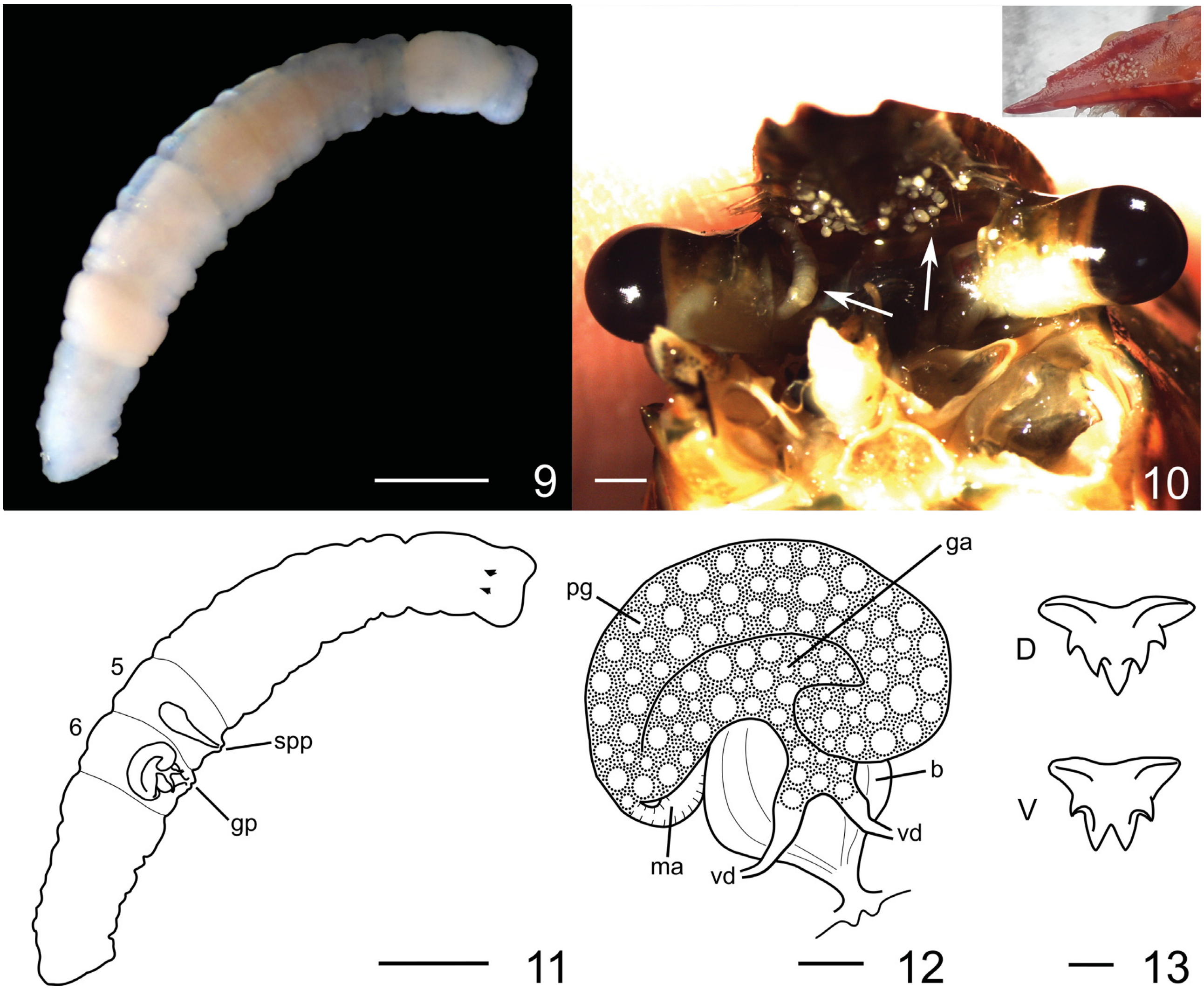




FIGURES 4-8. *Triannulata magna*. **Fig. 4.** Adult in latero-ventral view (MNHN-HEL656), bar = 1.0mm. **Fig. 5.** Adults (right arrows) near cocoons (left arrows) on ventral abdomen of *Pacifastacus leniusculus*, bar = 2.0mm. **Fig. 6.** Diagram of an adult in latero-ventral view showing position of jaws, spermatheca in segment 5 and major male organs in segment 6: gp, genital pore, spp, spermatheca pore, bar = 1.0mm. **Fig. 7.** Diagram of adult male organs in lateral view: b, bursa; ga, glandular atrium; ma, muscular atrium; ps, penial sheath; vd, vas deferens, bar = 100µm. **Fig. 8.** Juvenile jaws in ventral view with teeth pointing posteriorly: D, dorsal jaw; V, ventral jaw, bar = 50µm.

Triannulata magna is endemic on *P. leniusculus* in the Pacific Northwest, USA, and this is not only the first record of *T. magna* in Europe, but also in the Palearctic region. Specimens were found in the lateral depressions along the ventral abdominal segments (Fig. 5) and are consistent with its reported ventral body microhabitat. Close to adult *T. magna* were three cocoons (Fig. 5) with a length, including stalk, of 2.8 to 3.0mm and containing 8 to 15 embryos. In one cocoon an embryo was visible and measured about 0.8mm long, with a well-formed triangular head, triple body annulations, and triangular jaws with crenelated sides and large medium tooth (Fig. 8). These observed morphological characters provide a virtual assurance the cocoons were *T. magna*. Without seeing such details in an embryo, it could have been possible that other cohabitants, *C. gracilis* and *C. okadai*, had deposited their cocoons in the area.

Cambarincola mesochoreus Hoffman, 1963: Adults have a distinct head and a rod-shaped body (Figs 9 and 11), measuring 2.5 to 4.2mm long, with two annuli per segment and no transverse segmental ridges. There are four small lobes on the dorsal peristomial lip, which may not always be visible due to preservation effects, and jaws with a dental formula of 5/4 (Fig. 13). The dorsal jaw is triangular with a large median tooth and two pairs of slightly smaller lateral teeth, while the ventral jaw is trapezoidal and has two pairs of teeth that interdigitate with those on the dorsal. Spermatozoa occur in segments 5 and 6, with the spermatheca located in segment 5 and male organs in 6 (Fig. 11). The male organs consist of a curved glandular atrium (ga) and a longer reflexed prostate gland (Fig. 12, pg). These organs are shown overlaying a muscular atrium and bursa containing a protrusible penis.



FIGURES 9-13. *Cambarincola mesochoreus*, for abbreviations see Figs. 4-8**. Fig. 9.** An adult in lateral view, bar = 0.5mm. **Fig. 10.** In foreground, an adult (left arrow) with cocoons (right arrow) under the rostrum of *Procambarus clarkii* (the antennae and antennules were removed), bar = 1mm. Thumbnail in top right, cocoons on the rostrum of *P. clarkii*, picture with Julien Rimour used permission. **Fig. 11.** Diagram of an adult in lateral view showing jaws, spermatheca in segment 5 and major male organs in segment 6, bar = 0.5mm. **Fig. 12.** Diagram of male organs in lateral view: pg, prostate gland, bar = 50µm. **Fig. 13.** Jaws in ventral aspect with teeth pointing posteriorly, bar = 20µm.

Cambarincola mesochoreus is endemic on *P. clarkii* in the southeast USA and adjacent Mexico, and this is the first record of the association in France, although the crayfish are much more widely distributed in the country. Branchiobdellidans were observed over most of the exposed body surface, but the rostrum area appeared to be a favored microhabitat for both individuals and cocoons (Fig. 10: arrows and top right thumbnail).

Discussion

The spread of exotic species and establishment of breeding populations through commercial and accidental translocations is resulting in the reduction or extinction of endemic species and consequent modification or destruction of traditional habitats (Keith & Allardi 1997; Lévêque 1997). This is particularly noticeable in Europe where large scale commercial introductions of North American crayfishes started about 1960 and have continued to date (Holdich *et al.* 2009; Kouba *et al.* 2015). Such translocations of crayfish are always accompanied by their endemic pathogenic and non-pathogenic symbionts (Longshaw 2011). While ectosymbiotic branchiobdellidans are generally non-pathogenic mutualists, a few species have a mainly parasitic association with their host (Gelder & Williams 2016).

Branchiobdellidans are found in most areas of North America (Gelder 2016) and the majority on crayfish species. However, only a few of these hosts are of commercial interest; Gelder (2004) listed a total of 24 endemic branchiobdellidan species reported on the three most popular commercial crayfishes: *P. leniusculus* (15), *P. clarkii* (6) and *F. limosus* (4). Since then, additional exotic crayfishes have been reported in Europe (Kouba *et al.* 2015), principally *F. immunis F. juvenilis*, and *Faxonius virilis* (Hagen) with at least 10 more ornate species imported by the pet trade (Holdich *et al.* 2009), some of which also harbor branchiobdellidans. A revision of the endemic branchiobdellidans recorded on the newer crayfish imports has increased the total potential number of North American worms from 24 to about 50 (S.R. Gelder, unpub. data). Some of these are less than 1.5mm in length and will only be seen during a careful host examination using a magnification of x20 or greater. Up to the 1950s, branchiobdellidans found wild in Europe were assuredly members of the *Branchiobdella*, but as large-scale translocations of exotic North American crayfishes started, their endemic branchiobdellidans began to be observed on the continent (Franzén 1962). Identification of these exotic branchiobdellidans presented researchers with a challenge as some species had not been described at the time of their collection. For example, Franzén (1962) used the name of an eastern North American species, but he actually studied *X. victoriensis* which was only described 28 years later by Gelder and Hall (1990). Although being aware of this background, some reviewers still cite the original name, and this perpetuates the confusion in the literature. The taxonomic situation of North America branchiobdellidans has greatly improved with the availability of a recently published key to Nearctic species (Gelder 2016). Brief descriptions of the commonly occurring exotic European branchiobdellidans were included here for *C. mesochoreus* and *T. magna*, and in Gelder *et al.* (2012), to assist in the accurate identification and recognition of these species as their ranges expand.

During this freshwater faunal survey, four species of crayfish – *P. leniusculus*, *F. limosus*, *P. clarkii* and *A. pallipes* – were found carrying North American branchiobdellidans at 105 sites in France. *Pacifastacus leniusculus* tends to be found in colder, upper and headwaters of the Massif Central, Plateau de Millevaches, Massif Morvan, Basse Normandie and near Saint-Michel in northeast France. The three sites near Saint-Michel in northeast France are quite close together and each contains a multiple species cohabitation but with differing compositions: site 93 – *C. okadai* and *X. victoriensis*, site 94 – *C. gracilis*, *C. okadai* and *X. victoriensis*, and site 95 – *C. gracilis*, *C. okadai*, *T. magna* and *X. victoriensis*. In addition, sites 93 and 95 were sampled twice and the same species composition was found. Site 95 is unique as it is the first exotic, quadruple cohabitation of branchiobdellidans on *P. leniusculus* to be found in Europe, and also contains the first record of *T. magna* in the Palaearctic realm. It is probable that *P. leniusculus* and its four branchiobdellidan species were introduced to these sites at the same time, but why one and two species were lost at sites 93 and 94 respectively, is unknown. Similarly, the disparate distribution of triple species cohabitations at sites 7, 11, 13, 46, 48, 49, 63, 64, and 88, with two or single species at nearby sites is most likely the result of the same combination of unknown factors. One approach that might explain these variations in worm species on *P. leniusculus* is to trace the host's original locations back in the Pacific Northwest, USA. Unfortunately, the endemic distribution of *P. leniusculus* is complex and not fully understood (Larson and Williams 2016) and this is compounded as wild crayfish were collected from a number of waterbodies and held for subsequent translocation, thus resulting in both a taxonomic and geographic mixing. Finally, after the initial translocation into Europe, many secondary introductions, both legal and illegal occurred, not only from the USA but also from breeding stocks in Europe. An attempt at tracking these movements in Europe was attempted using fragments of the mitochondrial gene for cytochrome *c* oxidase subunit I (COI) by Petrusek *et al.* (2017), but no clear distribution of haplotypes was found that corresponded to the various introductions. Therefore, any possibility of tracing the observed branchiobdellidan species combinations found in this study to their origins in the Pacific Northwest USA was discounted.

Following *P. clarkii*'s Spanish introduction in 1973 from Louisiana, USA, it has spread across the Iberian Peninsula, while subsequent introductions of *P. leniusculus* onto the Peninsula tended to extend across the northern half of the region (Kouba *et al.* 2015). *Xironogiton victoriensis* on *P. leniusculus* was first reported in the Basque Country (Gelder 1999) and later in the Ebro Basin, northeastern Spain, by Oscoz *et al.* (2010). Although the two crayfish species typically live in different habitats, both have shown an adaptability which has allowed them to cohabit in certain areas. Vedia *et al.* (2014) reported such an area in the River Piedra, Zaragoza, Spain, where *X. victoriensis* were found on both crayfishes. Iván Vedia (pers. comm.) also observed that this branchiobdellidan occupied the same microhabitat on the chelae on the adopted *P. clarkii*. This find is consistent with our observation on the exotic *F. limosus* and the endemic *A. pallipes* and that of the first published record of such an association on this last crayfish recently described in Teruel, eastern Spain, by Martín-Torrijos *et al.* (2018).

It is most likely that some illegal introductions *P. clarkii* into France came from Spain (Holdich *et al.* 2009; Kouba *et al.* 2015) and were probably supplemented by translocations from Louisiana, USA. Our study indicates *P. clarkii* are predominantly found in southern and southwestern France, with scattered records across the rest of the country (Kouba *et al.* 2015), but only at site 1 in the Adour drainages was it found to carry *C. mesochoreus* (Table 1). Subsequent collections in the same southwest area have shown this is not an isolated occurrence (J.-F. Parpet, unpub obs.). Although this crayfish has an almost worldwide distribution (Kouba *et al.* 2015), endemic branchiobdellidans on translocated stocks are surprisingly rare with the only other European record being in northern Italy (Gelder *et al.* 1994), and a recent one in Japan (Ohtaka *et al.* 2017). A subsequent study of the northern Italian population of *P. clarkii* (Gelder *at al.* 1999) found the association unchanged, but in a second population a short distance away, the *C. mesochoreus* population had been replaced by European *Branchiobdella italica* Canegallo and *B. parasita*. Unfortunately, events leading to this replacement were not observed, but it became the first report of *Branchiobdella* species adopting *P. clarkii*. Although *C. mesochoreus* is the most common species found on *P. clarkii*, it is not the only one, and so specimens need to be carefully examined for sympatric *Cambarincola barbarae* Holt and *Cambarincola pamelae* Holt. All three species have similar body shapes and a dental formula of 5/4, but each has differing shaped jaws which can be only recognized if the specimens are correctly orientated on a microscope slide. Hence an examination should also include the male reproductive system to confirm the species identification and determine if a multi-species cohabitation has been found (Holt & Opell 1993).

The first European introduction of *F. limosus* from the USA was made into Poland in 1890, followed by an unsuccessful attempt into northern France in 1896. A subsequent translocation of stock from Germany to France resulted in a breeding population being established shortly after (Kouba *et al.* 2015). To date, none of its endemic branchiobdellidans have been found on *F. limosus* in Europe, even though it was the second most numerous species to be collected in our survey. Twenty-one sites were found with *F. limosus* and *P. leniusculus* cohabiting, while at four of these sites (24, 55 and 100), *X. victoriensis* only populated *P. leniusculus*, in contrast with site 43, where only *F. limosus* carried *X. victoriensis*. This observation of *X. victoriensis* transferring to another host species could be explained if the *P. leniusculus* was unhealthy or molting. Unfortunately, no record of its condition was made, but whatever the reason, this is the first record of the western North American *X. victoriensis* being found on an exotic eastern *F. limosus*. This crayfish's acceptability also extends to endemic *Branchiobdella* species when it cohabits waters with European crayfishes (Bláha *et al.* 2017; Ďuriš *et al.* 2006; Dr. W. Struzynski, unpub. obs.; Vogt 1999). The reverse is also true as *X. victoriensis* has adopted the European *A. pallipes* at two closely located sites (56 and 57) making it the first report of such an ectosymbiotic combination in France and follows that in Spain (Martin-Torrijos *et al.* 2018). This association appears to be stable, as it was observed in samples taken at the sites two years apart. Although no exotic crayfishes were captured at sites 56 and 57, at site 55 in an adjacent tributary, *F. limosus* and *P. leniusculus* were found, with *X. victoriensis* on the latter species. Whether this reflected a host preference is difficult to say. However, it should be remembered that crayfish can travel overland from one freshwater sources to another, e.g., ponds, streams, seeps, and as demonstrated experimentally (Hunt *et al.* 2018) are capable of carrying viable branchiobdellidans with them.

These observations demonstrate conclusively that most branchiobdellidans are not species specific to a particular crayfish host, or even to one from its endemic region. Therefore, endemic associations once restricted to a region can no longer be assumed, as uncontrolled and new crayfish translocations continue to occur. The usual method of branchiobdellidan transfer from one host to another is during direct contact (Hunt *et al.* 2018). Alternatively, branchiobdellidans will leave the host following molting, bodily damage and death, to live on the substratum until another crayfish comes in contact with them, whether endemic or acceptable exotic. Such a temporary free-living situation was reported at sites 2, 12 and 59, where *X. victoriensis* was found in substrate samples, but no crayfish were captured. It is most probable crayfish were present, but they eluded capture during the collection period. Although free-living branchiobdellidans have been recorded in similar situations (Holt 1973; Timm 1991) and experimentally by Hunt et al (2018), other extensive examinations of substrate samples have not found these worms (James *et al.* 2017, Gelder unpub. data). As the majority of branchiobdellidans have a diet of micro-flora and -fauna, food would not be a limiting factor while waiting for a host to make contact with them. Indeed *in vitro* observations of some branchiobdellidan species have shown they can be maintained alive for months (Gelder and Williams, 2015). However, it is most likely survival time is both species and conditions dependent. James *et al.* (2017) observed that *X. victoriensis* survived for over 21 weeks and *C. okadai* for less than two weeks while cohabiting on *P. leniusculus*, but when kept separately *in vitro* without a host, both ectosymbionts lasted for about the same amount of time (14 weeks). This free-living period would be adequate under most conditions for them to survive while awaiting contact with a new host. Such habitat adaptability and capacity to adopt either local endemic or exotic astacoidean crayfish favors the survival and range expansion of branchiobdellidans in Europe.

A consideration not so far discussed is the potential for branchiobdellidans in Europe to adopt commercially imported Australian crayfish. Yabbys, or *Cherax destructor* Clark were imported into Spain in 1983 from a farm in California, USA, and Redclaws, *Cherax quadricarinatus* (von Martens), primarily into Italy (Kouba *et al.* 2015). Although Australian crayfishes do not have endemic branchiobdellidans, they do carry flatworm temnocephalidans which fill the same niche (Gelder 1999). This ectosymbiotic association has already been reported three times in Europe, with *Temnosewellia minor* Haswell (synonym *Temnocephala minor*) on *C. destructor* in Italy (Quaglio *et al.* 1999; Scalici *et al.* 2009; Chiesa *et al.* 2015), but it is unlikely that these are the only examples of the association in Europe. Whether endemic or exotic branchiobdellidans are capable of adopting the southern parastacoidean crayfish is not known at this time. However, Vayssière (1898) did find branchiobdellidans and temnocephalidans cohabiting a specimen of *Procambarus digueti* Bouvier in Mexico. This demonstrates that *Temnocephala* sp. have found a northern cambarid crayfish an acceptable host, therefore it is predicted that branchiobdellidans may well adopt *Cherax* species if the opportunity arises.

The demand for crayfish is being driven by increased human consumption and the pet trade, and as a result the business of illegally translocating crayfishes is thriving. *Pacifastacus leniusculus* and *P. clarkii* have been known in Britain since 1970 (Holdich & Lowery 1988) and extensive studies have followed (Holdich *et al.* 2014); therefore, if exotic branchiobdellidans were present they would have been reported. As the first report of *P. leniusculus* carrying both *C. okadai* and *X. victoriensis* only occurred recently in south Wales (James *et al.* 2015), the population must have been a very recent translocation from outside the UK. This assessment is further supported as extensive sampling of Welsh rivers over the previous 35 years have yielded no branchiobdellidans (James *et al.* 2017). One explanation for these south Wales populations of infected *P. leniusculus* is that specimens were acquired for gastronomic reasons, probably from France, and then some individuals were either released or escaped into local waterbodies to form a starter population. This hypothesis is certainly supported by our reported presence of *C. okadai* and *X. victoriensis* on *P. leniusculus* in numerous areas of France. In response to the public demand in France, particularly at holiday times, fish markets offer both endemic and exotic live crayfish under the label “European crayfish” (J.-F. Parpet, unpub. obs.). Technically this would be true if the exotic species were raised in Europe, and so the chances they were imported into the country are high. However, a recent decree bans the import, transport, sale and purchase of live specimens of *F. limosus*, *P. leniusculus* and *P. clarkii* in metropolitan France (decree of February 14, 2018).

Although most European countries have stringent legislation banning uncontrolled importation and transportation of exotic crayfish species for human consumption and the pet trade, public demand appears to be greater than current enforcement efforts. In areas not yet invaded by the exotic crayfishes, active enforcement of regulations has a chance to stop further range expansions. However, such measures need to be accompanied by a coordinated public education program on the environmental damage and financial costs these invasives cause to recreational areas and natural resources. Exotic crayfishes continue to be introduced for commercial reasons, but the hidden costs resulting from such biological invasions, e.g., the ancillary introduction of "crayfish plague", have resulted in much higher indirect damages than is generally recognized.

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An ongoing saga: Endemic branchiobdellidans (Annelida: Clitellata) on translocated commercial North American crayfish

STUART R. GELDER
Department of Biology, University of Maine at Presque Isle, 181 Main Street, Presque Isle, Maine, USA.
E-mail: stuart.gelder@maine.edu

Abstract

Over the last 130 years demand for crayfish has exceeded regional supplies around the world, so stocks, primarily from North America, have been imported to satisfy this need. These demands are human based and include gastronomy, sport fishing bait, food for rearing animals, educational aides, ornamentation in waterbodies, and more recently a significant increase in pet crayfish sales. The three most common commercial species from North America are *Pacifastacus leniusculus, Procambarus clarkii* and *Orconectes limosus*, although four other species are increasing in importance as they become more widely distributed. All of these crayfish in their endemic range have been reported to carry branchiobdellidan annelids. Therefore, when the crayfish are translocated, their ectosymbionts likely accompany them. Eighty-six potentially transportable branchiobdellidans are recognized in this paper, along with the distribution of six species known to have been translocated. Moreover, branchiobdellidans endemic to the translocation regions have adopted introduced crustacean hosts, which demonstrates that branchiobdellidan host species specificity is not as restrictive as many researchers believe. On the evidence to date, these translocated branchiobdellidans appear to have the same relationship and cause the same amount of damage, if any, as those on their endemic crayfish hosts. The geographical distribution of endemic branchiobdellidan—crayfish species associations are unique to each of four disjunct regions as defined by Bănărescu: Euro-Mediterranean, East Asian, western North American and eastern North American; the latter includes eastward drainages from Canada to Costa Rica.

Key words: Illegal and legal introductions, *Pacifastacus leniusculus, Procambarus clarkii, Orconectes limosus*, pet trade

Introduction

Humans have been translocating fauna and flora for hundreds of years to construct zoological gardens, and for personal and economic reasons (Hulme 2009). Among these many species, freshwater crayfish have figured in the last two categories. Clavero *et al.* (2016) presented convincing evidence that *Austropotamobius italicus* (part of the *A. pallipes* complex) was introduced into Spain in 1588 from NW Italy. The authors also showed that *A. pallipes* is common to France, England, Wales and Ireland; Grandjean *et al.* (2006) suggested it was introduced from France, possibly as early as the 12th century with subsequent translocations occurring. Currently, there are no known records of comparable movements of crayfish from one country to another or from area to area within East Asia or North America that long ago. The era of commercial intercontinental translocations of North American crayfish began in the mid-19th century with the arrival of unknown species into Italy along with crayfish plague or *Aphanomyces astaci* Schikora (Ackefors 1999). After a pause, *Orconectes immunis* (Rafinesque) was introduced into Poland in 1890, where it established a viable population (Henttonen & Huner 1999). This was followed by more translocations, legal and otherwise, of various North American species.

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The export of crayfish has always been based on human demand, but in the new era additional uses have been found which include: bait in commercial and sport fishing, food for rearing animals, educational aids, and ornamentation in waterbodies, although more recently their importance in the pet trade has increased significantly (Holdich 2002; Policar & Kozák 2015; Patoka *et al.* 2017). Many of these demands have been met by the procurement of selected North American crayfish species, which has resulted in their translocation to most parts of the world, except Australia and New Zealand. Some of the exotic crayfish species show morphological intraspecific variations that can sometimes make a positive identification difficult. This becomes more problematic when molecular sequences reveal significant differences within a morphologically described species, thus raising the possibility of a species complex or a group of cryptic species. However, as most crayfish host identifications recorded in the literature are based on older morphological keys, these are the names used in this work unless otherwise stated.

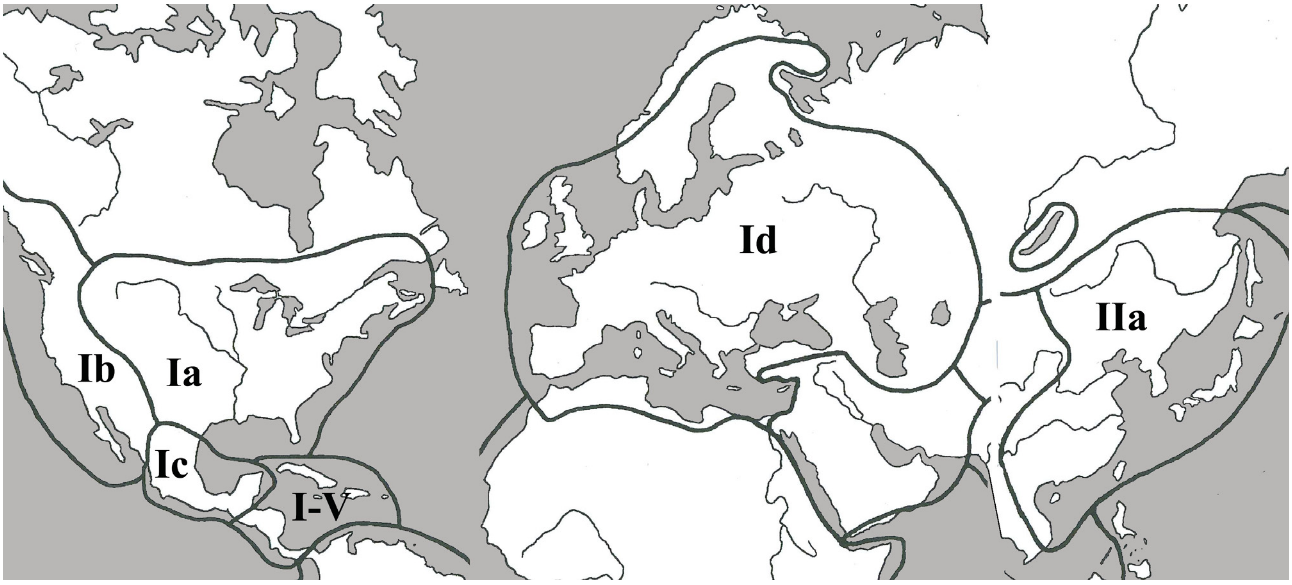

FIGURE 1. Faunal regions (the Atlantic Ocean and Central Asia are omitted) with endemic branchiobdellidans being found in the Holarctic: Ia, eastern North American subregion; Ib, western North American subregion; Ic, central Mexican subregion; Id Euro-Mediterranean subregion; I–V, Middle American/ Antillean transitional region; IIa, East Asian subregion (redrawn and modified from Bănărescu 1990: 526).

The ectosymbiotic association of branchiobdellidan annelids and their crustacean hosts was reviewed by Gelder and Williams (2015; 2016). The worms have a disjunct Holarctic distribution, with 8 genera in the Palaearctic (Gelder 2019) and 16 in the Nearctic and northern Neotropical realms (Gelder 2016, in press). Geographical and political regional descriptors used by authors are often well suited for a regional study, e.g., Central America, but become less consistent when dealing with a worldwide distribution. Therefore, this work uses the zoogeographical freshwater faunal regions and subregions proposed by Bănărescu (1990: 526) (Fig. 1). As branchiobdellidans range from France to the Ponto-Caspian Basin, "European" does not accurately reflect this distribution, but Bănărescu's Euro-Mediterranean subregion Id does. The term East Asia has been interpreted in many ways while East Asian subregion IIa encompasses all the known locations of branchiobdellidans in that area. In North America, 13 of the 16 genera live exclusively either east or west of the continental divide, with the remaining genera having species on one or other sides of the divide. While Bănărescu's western North American subregion Ib reflects the western branchiobdellidan taxa range, eastern taxa extend from Canada through the USA (Ia) and Mexico (Ic) to Costa Rica (I–V) (Fig. 1). Although these eastern components form a single sector of branchiobdellidan taxa, their hosts exhibit a transition from being almost exclusively crayfish in subregion Ia, with both crayfish and other crustaceans in subregion Ic, to non-crayfish crustaceans in region I–V. Techniques for preparing branchiobdellidans for identification are given in Gelder & Williams (2015; 2016) along with keys to the genus level. Now keys to all described species and their respective distributions are available in the multi-volume "Thorp and Covich's Freshwater Invertebrates" according to their faunal realm: Volume II, Keys to Nearctic Fauna (Gelder 2016), Volume IV, Keys to

Palaearctic Fauna, (Gelder 2019), and Volume V, Keys to Neotropical and Antarctic Fauna" (Gelder, in press). However, the reader will find an inconsistency in the taxon's rank in this series which needs to be explained. The order Branchiobdellida (Holt 1965) has been the accepted rank for the last 50 plus years and it continues to provide a pragmatic arrangement for taxonomic studies, hence the chapter in "Thorp and Covich's Freshwater Invertebrates, Ecology and General Biology", volume I was titled Branchiobdellida (Gelder & Williams 2015). In subsequent volumes editorial policy required the taxon to be raised to a subclass, Branchiobdellidea, to maintain consistency with the other clitellate taxa. However, in volume IV the subclass in the key (Thorp & Lovell 2019: 364) and chapter title (Gelder 2019: 483) is given in error as the ordinal Branchiobdellida.

Members of the Gondwanian crayfish superfamily Parastacoidea do not carry endemic branchiobdellidans, but some Australian species of *Cherax* have been introduced and cultivated in both Euro-Mediterranean and East Asian regions (Policar & Kozák 2015). To date, there are no reports of their being adopted as alternative hosts by branchiobdellidans; however, they should be considered potential hosts and a careful watch maintained for their presence. The Gondwanian analog of branchiobdellidans are temnocephalidan rhabdocoels and they are known to adopt Holarctic crayfish. Vayssière (1898) reported *Temnocephala mexicana* Vayssière, on *Procambarus digueti* Bouvier, in Mexico and they were cohabiting a host with an unidentified branchiobdellidan. More recently, Xylander (1997) collected *Temnocephala minor* Haswell, from *Astacus leptodactylus* Eschscholtz, provided by a pet reptile supply company, Exo Terra, Germany, who had in turn obtained the crayfish from Turkey. This was followed by Cuellar *et al.* (2002) reporting small numbers of *Temnocephala* sp. between March and October on *P. leniusculus* that also supported a large population of *Xironogiton victoriensis* Gelder and Hall, year-round at an aquaculture site in Spain. Cuellar *et al.* (2002) offered no explanation as to where this small temporary population of temnocephalidans could have come from, even though *Cherax destructor* Clark, had first been introduced into Spain in 1983 (Kouba *et al.* 2015: 132). Contrary to expectations, the initial translocated stock did not come from Australia, but a breeding facility in California, USA, which coincidentally is in the endemic area of the *P. leniusculus* and *X. victoriensis* association. Whether these facts are part of a fragmented story of introduction or disconnected coincidences on the ectosymbionts origins remains speculative. However, potential temnocephalidan interactions with the northern ectosymbiotic associations are not considered further in this work.

The terminology used to describe the origins and movements of symbionts and hosts in the literature has evolved, particularly over the last 50 years, to produce a number of alternate names. However, Gherardi and Holdich (1999) provided definitions and a list of synonyms that have reduced previous misunderstandings. **Endemic** (= native, indigenous and autochthonous) refers to a taxon originating and found in a particular geographical area; **exotic** (= alien, allochthonous, non-native and non-indigenous) refers to an organism introduced into an area in which it had not naturally occurred in historic times; and **translocation**, refers to the deliberate transfer of organisms for the purposes of introduction, reintroduction, or stocking.

Having covered essential background areas and terminology, this paper focuses on the actual and potential distribution of ectosymbiotic branchiobdellidan annelids through the commercial translocation of their astacoidean crayfish hosts (Gelder 2004; Gelder & Williams 2016). The histories and current distributions of crayfish involved in commercial translocations outside their endemic regions has been well documented in recent reviews (Holdich *et al.* 2009; Kawai *et al.* 2016; Kozák *et al.* 2015), so only pertinent details relevant to their branchiobdellidan ectosymbionts will be given here.

Commercial crayfish species translocated outside their endemic region

All commercial exotic crayfish species carrying branchiobdellidans currently originate from North America, with *Pacifastacus leniusculus* (Dana), from the western subregion Ib, and the remainder from the eastern sector, primarily subregion Ia (Fig. 1). The three most widespread, exotic crayfish species are, *Orconectes limosus* (Rafinesque), *P. leniusculus* and *Procambarus clarkii* (Girard), but four others are important although not so widely distributed: *Procambarus* cf. *acutus*, white river crayfish; *Orconectes immunis* (Hagen), calico crayfish; *Orconectes juvenilis* (Hagen), Kentucky river crayfish and *Orconectes virilis* (Hagen), virile crayfish (see Kouba *et al.* 2015). All of these species have been reported in Europe (Holdich *et al.* 2009), while *P. clarkii* and *P. leniusculus* account for the majority of introductions in East Asia. Initially *Orconectes rusticus*

(Girard) was report in France, but the population was later identified as *O. juvenilis* (Holdich *et al.* 2009); however, *O. rusticus* has been found in the Pacific Northwest (subregion Ib). These hosts and their reported endemic branchiobdellidans are listed in Table 1. Two other species, *Orconectes neglectus* (Faxon) and *Orconectes sanbornii* (Faxon), have also been reported from the Pacific Northwest but no mention of any branchiobdellidans was made. However, in their endemic eastern localities *Pterodrilus annulatus* Gelder, and *Pterodrilus mexicanus* Ellis, were found on *O. neglectus* and *Pterodrilus alcicornus* Moore, on *O. sanbornii*. Although many of these species are kept as pets, there are more ornate and colorful crayfish available through aquarist suppliers, some legal and others not. A web search in 2018 of North American and European aquarist catalogs (Gelder unpub. data) provided an additional 15 species of which four (*Procambarus alleni* (Faxon), *Procambarus fallax* f. *virginalis* (see Scholtz 2016: 5) *Orconectes luteus* Creaser, *Cambarus montezumae* = *Cambarellus montezumae* (Saussure)) have been reported to carry branchiobdellidans (Table 2). This number of species is increased significantly if supplies from Australia, SE Asia and the Pacific Islands are included, but as these do not have endemic branchiobdellidans, they are not considered further.

TABLE 1. Endemic branchiobdellidans recorded on commercial North America crayfish species translocated to the Palaearctic; with those already reported in Europe (▲) and East Asia (●).

Order Branchiobdellida	**Superfamily Astacoidea**								**Ref. nos**
Family Branchiobdellidae	*Pacifastacus*	*Procambarus*		*Orconectes*					
	leniusculus	*acutus*	*clarkii*	*immunis*	*juvenilis*	*limosus*	*rusticus*	*virilis*	
Branchiobdellinae									
Ankyrodrilus legaeus				**o**	**o**		**x**		**14**
Xironogiton instabilis				**x**	**x**	**x**			**17**
X. kittitasi	**x**								**17**
X. occidentalis	**x**								**10, 17**
X. victoriensis ▲●	**x**								**7, 30**
Bdellodrilinae									
Bdellodrilus illuminatus				**x**				**x**	**2, 9, 10**
Uglukodrilus hemophagus	**x**								**19, 27**
Cambarincolinae									
Cambarincola barbarae			**x**						**3, 23**
C. chirocephalus				**x**			**x**	**x**	**1, 2, 12**
C. desmissus					**x**				**12**
C. fallax			**x**					**x**	**3, 23, 28**
C. gracilis ▲	**x**								**23, 32**

...Continued on the next page

TABLE 1. (Continued)

Family Branchiobdellidae	*Pacifastacus*	*Procambarus*		*Orconectes*					
	leniusculus	*acutus*	*clarkii*	*immunis*	*juvenilis*	*limosus*	*rusticus*	*virilis*	
C. holti							**x**	**x**	**5**
C. illinoisensis								**x**	**24**
C. jamapaenesis		**x***							**16**
C. macrodontus		**x**	**x**	**x**				**x**	**1, 2, 28**
C. mesochoreus ▲ ●		**x**	**x**	**x**		**x**		**x**	**3, 9, 23**
C. okadai ▲ ●	**x**								**8, 17, 30**
C. olmecus		**x***							**16**
C. pamelae			**x**						**3, 25**
C. philadelphicus				**x**		**x**		**x**	**1, 9**
C. susanae		**x***							**16**
C. vitreus			**x**	**x**	**o**			**x**	**2, 12, 30**
Ellisodrilus clitellatus					**x**				**13**
E. durbini								**x**	**13**
Oedipodrilus anisognathus					**x**				**25**
O. macbaini				**x**			**x**	**x**	**25**
Pterodrilus alcicornus					**x**		**x**		**15**
P. cedrus					**x**		**x**	**x**	**15**
P. distichus				**x**	**x**		**x**	**o**	**15**
P. hobbsi					**x**		**x**		**4, 15**
P. missouriensis						**x**			**9**
Sathodrilus attenuatus ●	**x**								**22, 30**
S. chehalisae	**x**								**22**
S. dorfus	**x**								**20**
S. elevatus				**x**			**x**	**x**	**21**
S. inversus	**x**								**22**
S. lobatus	**x**								**20**
S. norbyi	**x**								**20**
S. prostates		**x***							**16**
S. shastae	**x**								**6, 22**
S. wardinus	**x**								**22**
Triannulata magna ▲	**x**								**10, 18**

...Continued on the next page

TABLE 1. (Continued)

Family Branchiobdellidae	*Pacifastacus*	*Procambarus*		*Orconectes*					
	leniusculus	*acutus*	*clarkii*	*immunis*	*juvenilis*	*limosus*	*rusticus*	*virilis*	
Xironodrilinae									
Xironodrilus formosus				**x**			**x**	**x**	**1, 10**
No. of species 78	**15**	**6**	**6**	**12**	**10**	**4**	**10**	**15**	

Legend: Each published association is shown by "x" and its reference(s) is given as a number with the respective citations being, (1) Ellis 1919, (2) Evans 1939, (3) Gelder 1991, (4) 1996a, (5) 1996b, (6) Gelder & Ferraguti 2001, (7) Gelder & Hall 1990, (8) Gelder & Ohtaka 2000, (9) Gelder *et al.* 2001, (10) Goodnight 1940, (12) Hoffman 1963, (13) Holt 1960, (14) 1965, (15) 1968, (16) 1973a, (17) 1974a, (18) 1974b, (19) 1977a, (20) 1977b, (21) 1978, (22) 1981a, (23) 1981b, (24) 1982, (25) 1984, (26) 1988, (27) 1989, (28) Keller 1992, (29) Lahser 1975, (30) Ohtaka *et al.* 2005, (31) Penn 1959, (32) Robinson 1954. Records from the Catalog of the National Museum of Natural History, Smithsonian Institution, Washington DC, are shown by "o". "x*" indicates these species have been recorded on *Procambarus acutus cuevachicae* Hobbs.

TABLE 2. Endemic branchiobdellidans on decorative North American crayfish listed as available through European aquarist catalogs.

Crayfish	Branchiobdellidans	References
Cambarellus montezumae	*Bdellodrilus illuminatus*	Holt, 1973a
Orconectes luteus	*Pterodrilus mexicanus*	Holt, 1968
	Pterodrilus missouriensis	Holt, 1968
	Xironodrilus formosus	Goodnight, 1940
Procambarus alleni	*Cambarincola manni*	Holt, 1973b
Procambarus fallax	*Cambarincola goodnighti*	Holt, 1973b
	Cambarincola manni	Holt, 1973b

Large scale commercial harvesting of endemic crayfish pays little or no attention to species composition. For example, populations of *P. clarkii* sometimes include small numbers of cohabiting *Procambarus acutus* (Girard). An examination of *P. acutus* by Crandall (2010a; b) found this species was composed of two different taxa that he called *P. a. acutus* and *Procambarus zonangulus* Hobbs and Hobbs. Although specimens of these two species rarely establish breeding populations when released, exceptions have been found with two populations of *P. a. acutus* (or *P. acutus*) reported in the Netherlands in 2005 (Kouba *et al.* 2015), and one at an isolated farm near Windsor in England (Almeida *et al.* 2014). Having demonstrated they can survive translocation and produce viable populations, the closely related, ornate *Procambarus acutus cuevachicae* Hobbs, from Mexico is included under *P. acutus* in Table 1. This subspecies is available through some pet trade outlets and is known to host four species of branchiobdellidans in its endemic area.

Another problematic crayfish is *Procambarus fallax* (Hagen) f. *virginalis*, the Marbled crayfish or Marmorkrebs, which was the subject of a multi-disciplinary review entitled, Section 1: Marble Crayfish—A New Model Organism for Biology, in Kawai *et al.* (2016). Although of North American origin, this new parthenogenetic form was first recognized and exploited in the mid-1990s by the aquarist trade in Germany and Austria (Souty-Grosset *et al.* 2006). Because of its attractive appearance, ability for prolific reproduction and hardiness, it is now probably the most popular pet crayfish in the world (Pârvulescu *et al.* 2017). Even though this form has been reported wild in numerous countries in Europe, Madagascar and Japan (Feria & Faulkes 2016, Kawai 2017a), none mention harboring any branchiobdellidans. Souty-Grosset *et al.* (2006: 96) stated that molecular sequences indicated *P. f.* f. *virginalis* to be closely related to *P. fallax*, the deceitful crayfish, and

Procambarus alleni (Faxon), the Florida crayfish. Both of these species are natives of Florida and Georgia, USA, where they carry branchiobdellidans, and *P. alleni* has been recorded recently in France (Table 2).

The number of crayfish species and their endemic branchiobdellidans represents a formidable list of potential invasive ectosymbionts. However, only six exotic North American branchiobdellidan species have been reported off the continent, four of them in Japan and five in Europe (Table 1). There are many reasons for branchiobdellidans dying in transit, but it appears the six species reported so far are either more resilient than most or were transported under favorable conditions. As the demand by aquarists for ornate crayfish increases, these pet species enjoy a better level of care during transportation as many suppliers guarantee them to be alive on delivery.

1. Distribution of exotic branchiobdellidans and crayfish in Eastern North America (subregion Ia, Ic and region I–V)

Although crayfish species in this sector have discrete endemic ranges, many of them have been translocated around the sector for commercial reasons. However, tracing such movements in the absence of adequate documentation is probably impossible and, fortunately, beyond the scope of this paper. No records of *Pacifastacus* species have been found to indicate their translocation into the eastern sector, and neither have there been any records of crayfish from Europe or East Asia establishing wild populations. This does not rule out the possibility that small populations of these exotic pets exist in the wild where they potentially carry any endemic branchiobdellidans or are available as alternative hosts. Although selling exotic species in aquarist shops is no longer legal in many States in the USA and Provinces in Canada, the worldwide trade in these pets continues to flourish via mail order suppliers.

2. Distribution of exotic branchiobdellidans and crayfish in Western North America (subregion Ib)

Procambarus clarkii was introduced into southern California in the 1920's (Hobbs *et al.* 1989), but an even earlier date has been suggested. A verifiable translocation of *P. clarkii* as a food source for frogs was made in 1932 near Lakeside, San Diego County, California (Riegel 1959: 46). His survey showed the crayfish populations existed from the Mexican border to north of Sacramento. Additional references of *P. clarkii* translocations into Arizona, Nevada, California, Oregon, Idaho and Utah, USA and adjacent Baja California and Sonora, Mexico, are given in Huner and Barr (1991). Although British Columbia, Canada, was not included in this range, rumors of illegal consignments from unknown locations for aquaculture were circulating in the 1990's (Gelder, unpubl. data). A survey and review of invasive crayfish in the Pacific Northwest by Larsen and Olden (2011) recorded, *O. neglectus, O. rusticus*, *O. sanbornii*, *O. virilis*, *P. acutus* and *P. clarkii* (see Table 1). In their endemic ranges, *O. neglectus* hosts *Pterodrilus annulatus* Gelder, and *P. mexicanus* Ellis, with *P. alcicornis* Ellis on *O. sanbornii* (Hobbs & Fitzpatrick), but neither crayfish have been reported carrying branchiobdellidans in this subregion; endemic or adopted.

Holt (1981a) identified *C. mesochoreus* on *P. clarkii* collected from Merced, Santa Barbara and Sonoma Counties in California, and subsequently (Holt 1984) transferred some of the specimens to a new species, *Cambarincola pamelae* Holt, while adding Stanislaus County to their range. A multi-species association of *C. gracilis* and *C. okadai* (= *C. montanus*) together with *C. barbarae* and *C. pamelae* was found on *P. clarkii* collected in 1960 at Solvang, Santa Barbara County, California (Holt 1981a: 683). The presence of western and eastern species indicates that *P. clarkii* had made contact with *P. leniusculus* in the recent past. Given the aggressive behavior of both species, any contact almost certainly would result in a fight and affording ample opportunity for worms to transfer to the victor to initiate a starter population. Although Holt (1981a: 680) identified *C. fallax* from one *P. clarkii* in Sonoma County, he expressed reservations about this species being in California. Gelder and Hall (1990) found *C. fallax* with *C. gracilis* and *C. okadai* cohabiting preserved *P. leniusculus* in the Royal British Columbia Museum, Victoria, Canada, that had been collected by G. C. Carl from Burdette Creek near Vancouver in 1942. Whether these specimens identified as *C. fallax* originated from an eastern host translocation or represents a very similar but new western species requires further study.

Orconectes was first recorded in California at Chic, Butte County, between 1939 and 1941 where *O. virilis* was used in the biology laboratory at Chico State College (Riegel 1959); this is about 150km north of Sacramento. Later records include Daniels (1980) finding it in the middle Pitt River around the Britton Reservoir in 1978; however, its introduction as sport fishing bait dates back to the 1960's (Maria J. Ellis unpub. data). Further north, *O. neglectus* (= *O. transfuga*) was released into the Rogue River, Jackson County, Oregon (Hobbs *et al.* 1989) probably in the 1960's. Larson and Olden (2008) collected *O. virilis* from three lakes along Puget Sound's eastern border, Washington State. The identification of crayfish in Big Lake near Mt. Vernon was subsequently corrected (Larson *et al.* 2010) to *O. sanbornii* which is endemic to the Ohio River Basin, eastern USA. Additional locations of *O. virilis* were reported at six sites on the Franklin D. Roosevelt Lake and in adjacent tributaries at Patterson, Wapato, and Moses Lakes. *Orconectes virilis*, *O. rusticus* and *P. clarkii* were found being used extensively for educational studies in Washington State schools, so finding an escaped population of *O. rusticus* is only a matter of time. In the neighboring state of Oregon, a very dense population was found in a stretch of the John Day River in the vicinity of John Day, (Olden *et al.* 2009). *Orconectes neglectus* was widespread in the Rogue River system by 1977 and believed to have been introduced following sport fishing activities (Larson & Olden 2011). To date no branchiobdellidans (Table. 1) have been reported on these exotic crayfish, but whether they failed to survive translocation, or were unable to adapt to the new habitat conditions, remain to be discovered is unknown.

3. Distribution of exotic branchiobdellidans and crayfish in the Euro-Mediterranean (subregion Id)

Europe has received the largest commercial translocation of the three main North American crayfish species along with some of their endemic branchiobdellidans (Table 1). In some areas, these exotic crayfish have been adopted successfully by local endemic branchiobdellidans showing that host specificity is less limited than many researchers believe. These two situations are dealt with separately: first, (3a) the introduced endemic associations, and second, (3b) those associations where endemic branchiobdellidans have adopted exotic hosts.

Stocks of *P. leniusculus* from the Sacramento area, California, USA, were shipped to Sweden in 1959 and released the following year. Later large-scale exportations occurred from Lakes Hennessy and Tahoe to Finland in 1967–8, and from Lake Tahoe to Sweden in 1969 (Svärdson 1995; Ackefors 1999). The latter breeding stocks were highly successful and translocations, both legally and illegally account for current populations in many western European countries including Cyprus (Holdich *et al.* 2009; Kouba *et al.* 2015). The high genetic variation found in these European populations of *P. leniusculus* is consistent with the original heterogeneous stock population created commercially in the USA, followed by multiple introductions and secondary translocations on the continent (Petrusek *et al.* 2017). Although not yet recorded from Russia, *P. leniusculus* is extending its range eastward and has already entered Slovenia and northern Croatia (Hudina *et al.* 2012).

The first introduction of *P. clarkii* into Europe consisted of shipments from Louisiana, USA, to Spain in 1973, and its subsequent history is reviewed by Kouba *et al.* (2015). These starter populations rapidly became established and thrived, and in turn loads were translocated, both legally and illegally to other areas and countries. France received its first *P. clarkii* in 1976 from translocated East African commercial stocks before being supplemented by Spanish imports in 1978 (Laurent *et al.* 1991). The species is now reported to extend from Portugal to Germany and Italy, and it is only a matter of time before its eastward expansion reaches the Caspian basin. Additional introductions within the Euro-Mediterranean region have been made into southern England, the Azores, Canaries, Sardinia, Corsica, Sicily, Cyprus (Holdich *et al.* 2009), Egypt (El Zein 2005) and Israel (Wizen *et al.* 2008). Currently, the distribution of this species can be assumed to be much greater.

In spite of *O. limosus* being introduced repeatedly into Europe over the last 120 years, there are no reports of any individuals carrying any of its endemic North American branchiobdellidans. It is included here because the species has been adopted by a number of *Branchiobdella* species. This crayfish was first introduced into Poland in 1890 with a consignment from the Delaware River, Pennsylvania, USA. Following an unsuccessful introduction into France in 1895, success followed in 1911–1913 with crayfish from New York, USA (Henttonen & Huner 1999). Records of where these crayfish were collected or how many were subsequently shipped are incomplete (Kouba *et al.* 2015; Souty-Grosset *et al.* 2006: 102). Although initially introduced for human consumption, *O. limosus* soon became an important bait for sport and commercial fishing, and accounts for its current distribution from the Pyrenees Mountains to Poland and south to Serbia and Italy; England also has viable populations of the species. Its range is enlarging as breeding populations spread eastwards along central Europe's major river systems towards the Black Sea.

a. Introduced branchiobdellidans on their endemic hosts: *Pacifastacus leniusculus* has the largest number of potential branchiobdellidan species for translocation (Table 1), with *Xironogiton victoriensis* being the most numerous and found across this host's native range (Gelder & Hall 1990; Larson & Williams 2016). The first record and study of a xironogitonid in Europe occurred on individuals from a crayfish rearing site in Drottningholm, near Stockholm, Sweden (Franzén 1962). These were identified as the eastern North American *X. instabilis* based on information then available. However, a detailed study of specimens from British Columbia, Canada, by Gelder and Hall (1990) resulted in a description of a new species, *X. victoriensis*, thereby separating it from the eastern *X. instabilis*. Although the two species are very similar morphologically, Martens *et al.* (2006) supported the validity of *X. victoriensis* and molecular sequencing has confirmed it (Williams *et al.* 2013). Without realizing this background, some researchers continue to cite the earlier incorrect name. *Cambarincola* sp. and "*X. instabilis*" were reported by Fürst (1984) in the first shipment of *P. leniusculus* to Finland from California, USA, in 1967. On termination of a 25-year study on translocated crayfish development in a small Finnish Lake, Kirjavainen and Westman (1999) found branchiobdellidans being, "either *Xironogiton instabilius* Moore or *Cambarincola* sp.". Cuellar *et al.* (2002) added a further level of confusion by calling *X. victoriensis* by its type name which Moore (1894) had placed in a different genus. Two important review publications (Minelli *et al.* 2015: 11, Longshaw 2016: 211) unfortunately ignored the corrected name of *X. victoriensis* which had already been validated as a separate species by Martens *et al.* (2006) and Williams *et al.* (2013). Additionally, and puzzling but not explained, is why Longshaw (2016) combined *X. instabilis* and *X. victoriensis*, making the former name a junior synonym contrary to the Principle of Priority as presented in Art. 23.1 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 2000). Despite these nomenclatural aberrations, *X. victoriensis* has been reported at crayfish culture sites and in the wild at: "Kőszeg: Kálvária-hegy, Gyöngyös", Hungary (Kovács & Juhász 2007); in Carinthia, Austria (Nesemann & Neubert 1999); Germany (Martens *et al.* 2006; Martens & Roos 2015), Auenbachl Creek, Bolzano Province, (Morolli & Quaglio 2002; Oberkofler *et al.* 2002; Quaglio *et al.* 2002), and Lake Brugneto, Liguria Province (Capurro *et al.* 2007), Italy; tributary streams of the Altube Erreka, Basque Province (Gelder 1999) and Ebro Basin (Oscoz *et al.* 2010; Vedia *et al.* 2014; 2016), Spain; eastern Garonne and Mayenne Basins, France (Laurent 2007; Subchev 2008; Gelder *et al.* 2012; Parpet & Gelder 2020); Wales (James *et al.* 2015); Croatia (Dražina *et al.* 2018); plus Luxembourg and Switzerland (Parpet & Gelder 2020).

Xironogiton victoriensis has been reported cohabiting with *C. gracilis* and *C. okadai* in France (Gelder *et al.* 2012; Parpet & Gelder 2020), but only with *C. okadai* in Wales (James *et al.* 2015). Initially these three species were collected from the Tarn River and its tributaries in France (Gelder *et al.* 2012) which provided the first confirmed report of a western North American *Cambarincola* species on *P. leniusculus* in Europe. Subsequent and expanded collections of *P. leniusculus* have yielded the first record of *Triannulata magna* Goodnight, in the River Seine basin in northern France (Parpet & Gelder 2020). Records of *P. leniusculus* translocations into France are sketchy but include shipments from Sweden in 1972, Lakes Tahoe and Donner, USA in 1974 (Arrignon 1996), and an unknown number of illegal shipments directly from Oregon, USA (Holdich *et al.* 2009). Which of these accounts for the three exotic branchiobdellidans being introduced will never be known. Of James *et al.*'s (2015) collections of *P. leniusculus* from mid- and south Wales, only those from the latter area yielded *C. okadai* and *X. victoriensis*, making this the first record of exotic branchiobdellidans in the UK. As this combination of exotic branchiobdellidans has only been reported in France, and the source of these crayfish was not known, it is possible that they were imported from France for culinary reasons. Support for this comes from Parpet (pers. obs.) who observed live "European crayfish", which included exotic species from unknown locations, in a French fish market. An example of host choice flexibility was demonstrated when specimens of *X. victoriensis* were observed on cohabiting *P. leniusculus* and *P. clarkii* collected from the Piedra River at Cimballa, Zaragoza, Spain. Meanwhile a population of *P. clarkii* in the Zadorra River, at Villodas, Álava, Spain, also supported *X. victoriensis* (Vedia *et al.* 2014).

Currently only one, *Cambarincola mesochoreus* Hoffman, of the six endemic branchiobdellidans reported on *P. clarkii* have been found in Europe. The first association was observed in a stream at Carmagnola, near Torino, northern Italy (Gelder *et al.* 1994), and it was rumored locally that the crayfish had been brought in illegally from France. While a recent survey (Parpet & Gelder 2020) discovered the association at a site in Southwest France.

b. Branchiobdellidans adopting exotic hosts: In spite of *O. limosus* being introduced repeatedly into Europe over the last 120+ years, there have been no reports of their carrying any of their endemic branchiobdellidans. However, it appears they are acceptable alternate hosts for some European species of *Branchiobdella*. Sympatric populations of *O. limosus* and the endemic *Au. torrentium* in a tributary to the

Steinbeck Creek, Hesse, Germany, were both found to support populations of *B. parasita* and *B. pentadonta* (Vogt 1999). *Branchiobdella* sp. and cocoons were observed in 2001 on *O. limosus* from the Świętokrzyska uplands in Poland (Dr. W. Struzynski unpub. obs.). Local anglers had told him the waterbody had contained *A. astacus* and *A. leptodactylus* a few years earlier, but it is unlikely that these anglers would have been aware of any surviving relict populations of *Astacus* species and their branchiobdellidans. Another adoption of *O. limosus* by *B. balcanica, B. hexadonta, B. parasita* and *B. pentadonta* was reported, together with their cocoons, on specimens from the River Elbe (Labe) in Obřistvi near Mělnik, Czech Republic (Czechia) (Ďuriš *et al.* 2006). After these observations were made in 2001, major flooding in the Elbe and Vltava watersheds prevented collections in 2002, and in 2003 only a few *O. limosus* were found with one carrying five *B. parasita*. Sampling at 30 sites in 2003–4 revealed some *O. limosus* but no branchiobdellidans. Bláha *et al.* (2017) collected large numbers of crayfish from two sites in the Czech Republic approximately 60km apart; a brook south of Prague and a pond west of Brno. The upper part of the brook contained *A. astacus* and the lower *O. limosus* with a narrow overlap where they cohabited. Specimens of *O. limosus* carried *B. parasita*, but those of *A. astacus* were not examined. The pond site had contained cohabiting *A. astacus* and *P. leniusculus* for over 20 years, and although the latter supported *B. parasita* and *B. pentadonta*, again specimens of *A. astacus* were not examined. Even so, this was the first report of *B. parasita* and *B. pentadonta* adopting *P. leniusculus* (Bláha *et al.* 2017). A similar displacement occurred in Estonia. In August 2018, Margo Hurt (Estonian University of Life Science, Institute of Veterinary Medicine and Animal Sciences, Tartu, Estonia; pers. com.), collected *P. leniusculus* from the Riksu Stream on Saaremaa Island, Estonia, which carried small numbers of *B. pentadonta* (identified by S. R. Gelder) on the chelae. Prior to the illegal stocking of *P. leniusculus*, Riksu Stream had a healthy population of *Astacus astacus*, but these succumbed to crayfish plague following the introduction of the exotic crayfish (M. Hurt, per. com.).

Although *C. mesochoreus* had been reported on *P. clarkii* in a stream near Torino, northern Italy (Gelder *et al.* 1994), a subsequent collection yielded *P. clarkii* without *C. mesochoreus* but populated by endemic *B. italica* and *B. parasita* from *Au. pallipes* that cohabited streams in the area (Gelder *et al.* 1999). Unfortunately, no observations were made during the interim to provide an explanation of how the change in branchiobdellidan species occurred.

4. Distribution of exotic branchiobdellidans and crayfish in East Asia (subregion IIa)

Until the early 20th century, the demand for crayfish in East Asia appears to have been met with endemic species from local suppliers. As the demand increased in Japan, individuals arranged for the North American crayfish, *P. leniusculus* and *P. clarkii* to be imported. Records show that five shipments of *P. l. leniusculus* and *P. l. trowbridgii* from Oregon, USA, between October 1926 and July 1930, were released into farm ponds across Japan (Kamita 1970). However, most introductions are believed to have survived for only a short time and no further translocations were allowed after 1930. Therefore, recent records of *P. leniusculus* in country are those that survived and spread through natural range expansions, and by deliberate or unintentional translocations (Usio *et al.* 2016). Yamaguchi (1933) stated there were no other crayfish in the area when *P. leniusculus* were released into Lake Chuzenji (=Chunzenji), Tochigi Prefecture, Honshu. More recent records show populations in the Tankai Reservoir, Shiga Prefecture, Lake Onogawa, Fukushima Prefecture (Kawai *et al.* 2004), and Akita, Gunma, Ishikawa, Nagano, Shiga and Tochigi Prefectures, all on Honshu Island, based on preserved *P. leniusculus* deposited in various academic institutions (Ohtaka *et al.* 2005). The most recent find being on an island in the Tone drainage, Chiba Prefecture, Honshu (Nakata *et al.* 2010). Specimens of *P. leniusculus* on Hokkaido Island were reported in Lake Mashu (Kawai *et al.* 2004) and are now at 12 sites indicating the crayfish's expanding range (Ohtaka *et al.* 2005; Kawai 2017a).

From the original crayfish translocation into Lake Chuzenji, a branchiobdellidan was described, *Cambarincola okadai* Yamaguchi. Unfortunately, only its external features and jaws were drawn and described, but no type specimens designated, therefore the species status was deemed *nomen inquirendum* by Holt and Opell (1993). Specimens from Washington State, USA, were subsequently described and named *Triannulata montanus* Goodnight, then Holt (1974b) transferred the species to the *Cambarincola*, where it became *C. montanus*. Professor A. Ohtaka found Yamaguchi's slide collection (Gelder & Ohtaka 2000; Ohtaka *et al.* 2020) and they recognized the original specimen from his figure, which enabled them to designate a lectotype and paralectotypes for *C. okadai*. In addition to publishing a detailed species description from the newly found specimens, *T. montanus* and *C. montanus* were designated junior synonyms.

Neseumann and Neubert (1999: 20) reported an imported *Cambarincola* in Japan but gave no details. Examinations of preserved *P. leniusculus* from various Institutions in Japan (Ohtaka *et al.* 2005) revealed *X. victoriensis* in a stream in Akashina, Nagano Prefecture and *Sathodrilus attenuatus* Holt, at Tachihiraki, Shika, Ishikawa Prefecture, both on Honshu Island; the latter species being widespread across Hokkaido.

In 1927, about 100 *P. clarkii* were shipped in a beer barrel from Louisiana, USA, to Japan (Kawai 2017b). Only 20 survived the journey and these were release into a pond to feed bullfrogs near Kamakura City, Kanagawa Prefecture (about 30km south of Tokyo). The population grew rapidly and within 20 years crayfish had been translocated to every major prefecture in Japan (Kawai & Kobayaski 2006), mainly as family aquarium pets (Hobbs *et al.* 1989). This made subsequent importations unnecessary and so after 1930 any importations made were illegal. Japanese specimens were translocated to Nanjing, China, in 1929 and started *P. clarkii*'s expansion through China, Taiwan, the Korean peninsula (Kawai & Kobayashi 2006) and to the rest of Southeast Asia (Hobbs *et al.* 1989); these were supplemented by many illegal introductions. Given the extensive legal and illegal translocations of *P. clarkii* around East Asia, it is interesting that neither *C. mesochoreus* nor any of its other endemic branchiobdellidans have been reported. However, the origin of *C. mesochoreus* on *P. clarkii* collected at two sites in suburban Tokyo (Ohtaka *et al.* 2017) is strongly suspected to be the result of escaped or released domestic pets that were recently imported into the country. It should be anticipated that other American ornate crayfish pet species will be discovered in East Asia, possibly carrying exotic branchiobdellidans.

As noted earlier, Marbled crayfish are probably the most popular pet crayfish in the world (Chucholl 2016; Pârvulescu *et al.* 2017). Eight established populations have been identified in Germany, The Netherlands, Czechia and Hungary, with 12 additional single reports extending from central Italy to western Sweden (Feria & Faulkes 2016), to which can be added the discovery of specimens in a gravel pit lake near the Drava River in Croatia (Samardžić *et al.* 2014) and the Narva River, Estonia, in 2018 (T. Timm, pers. com.). The recent record of *P. fallax* f. *virginalis* on Hokkaido and Shikoku islands in Japan is a clear reminder that exotic species continue to be introduced in spite of legislation to protect endemic species (reviewed in Kawai 2017a). Although there is little reason to believe *P. fallax* f. *virginalis* has any endemic branchiobdellidans, the chances of its being adopted by endemic worms from the introduced regions does exist. An unusual, but creative diplomatic gift of pet crayfish occurred when approximately 70 live *Cambarellus montezume patzcuarensis* Villalobos, or "Acocil", were presented to Emperor Hirohito by President Luis Echeverria of Mexican in 1972 (Kamita 1973). Kawai (2017a) provided additional details of this gift, reporting Dr. Luis Kasuga Osaka brought the crayfish to Japan where some were maintained in an aquarium in the Imperial Palace in Tokyo. The remaining specimens were divided among three aquaria and a private laboratory where the latter conducted rearing experiments; however, none of the crayfish were released into the wild.

In closing

There are only two commercial considerations when branchiobdellidans are visible on crayfish, first, live worms are considered leeches and it is "well known" by the populous that all leeches are parasites. Therefore, they must damage the crayfish and this misconception results in lowered prices at the fish market. Second, any unhealthy-looking or damage crayfish are automatically assumed to have been attacked by branchiobdellidans, even though the condition is usually due to microorganism infections or environmental conditions; however, seller and buyer visual perceptions are paramount. In reality, there have been only a few reports of a North American branchiobdellidan being an intermediate hosts for a metazoan parasite (Gelder & Williams 2015) and there is no evidence they act as vectors or paratenic hosts in translocating endemic pathogenic microorganisms. Therefore, the translocated branchiobdellidans identified so far probably have the same relationship and cause the same amount of damage, if any, as those on their endemic crayfish hosts.

In spite of the undesirability of exotic or alien branchiobdellidans and their hosts, tracking range expansions and future introductions of the worms is dependent on the alertness of a wide range of personnel in wildlife conservation, food inspection, biology teachers, and aquaculturists, as well as crustacean researchers. Keys are available (Gelder 2016, 2019, in press) for all the described species of branchiobdellidans; however, the challenge now is the accurate identifications of these ectosymbionts when found. The old approach of identifying branchiobdellidans based on host species, geographic location of capture, body size and jaw morphology is no longer acceptable as the exotic specimens are likely in a key for another realm. In addition, exotic branchiobdellidan species currently identified have been easy to recognize on their host, as they were often active and over 2.0mm long. In contrast, many of the additional, potential species listed in Tables 1 and 2 are less than 2.0mm long and about half average less than 1.0mm. Therefore, a new level of thoroughness in host examination is required that involves using a dissecting microscope, preferably while the host is still alive. An examination of the removed worms under a compound microscope will then yield information on the morphological characters, primarily the male reproductive system, required by the identification keys (Gelder & Williams 2015, 2016). Although some future reports of exotic branchiobdellidans are predictable as the host continues its range expansion, other will depend on keen eyes and serendipity.

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A catalog and assessment of Prof. Hideji Yamaguchi's slide collection of branchiobdellidans (Annelida: Clitellata) with the identification of syntypes

AKIFUMI OHTAKA[1], STUART R. GELDER[2] & SUSAN M. PETERSON[2]
[1] *Faculty of Education, Hirosaki University, Hirosaki 036-8560, Japan.*
[2] *Department of Science, University of Maine at Presque Isle, Maine 04769, USA.*
Email: ohtaka@hirosaki-u.ac.jp

Abstract

The branchiobdellidan slide collection of Professor Hideji Yamaguchi was rediscovered in 1995; however, all of the written records associated with it appear to have been lost. The slide collection has been examined, cataloged and deposited in the Invertebrate Collection of the Hokkaido University Museum, Sapporo, Japan (ICHAM). The collection was found to contain whole mounted specimens of all 17 species described by Prof. Yamaguchi, together with specimens of three other Oriental species described after his death. In addition, mounted specimens of some of these species were designated syntypes. Many sectioned and stained specimens were also cataloged, but those were not identified to species at this time.

Key Words: Annelida, Clitellata, Branchiobdellida, taxonomy, catalog, East Asian distribution

Introduction

Branchiobdellidans, or crayfish worms, are ectosymbiotic clitellate annelids that live primarily on freshwater Holarctic astaciodian crayfishes. Based on Bănărescu's (1990) zoogeographic organization, they have an endemic, disjunct distribution in the East Asian, Euro-Mediterranean, and eastern and western North American regions (Gelder 1999; Gelder & Williams 2016). The order Branchiobdellida Holt, 1965 is monophyletic (Williams *et al.* 2013) consisting of one family, Branchiobdellidae Grube, 1851 which is divided into five subfamilies (Brinkhurst & Gelder 2001) with about 150 species in 22 genera (Gelder 1996, 2011).

A detailed historical review of branchiobdellidans in Eastern Asia was given in Gelder & Ohtaka (2002) with subsequent studies being conducted by Gelder (2010), Niwa *et al.* (2005), Fujita *et al.* (2010), Ohtaka (2010), Ohtaka & Chen (2010), Ohtaka *et al.* (2012), Ohtaka & Gelder (2015), Ahn & Min (2016), Tanaka *et al.* (2016) and Kitayabu *et al.* (2016). Early studies of East Asian worms were based on poorly preserved specimens (Pierantoni 1912); however, Professor Hideji Yamaguchi (1932a, b, c, 1933, 1934) ensured that his material was free from such artifacts, thus resulting in 17 new species and some redescriptions that, together, formed the first comprehensive investigation of branchiobdellidans in East Asia. Unfortunately, he did not designate any type series for these species, nor state in which institution his material had been deposited. After Yamaguchi's death in 1970, it was believed that both his slides and his fluid-preserved specimen collection of branchiobdellidans had been lost and probably destroyed, until one of us (A.O.) found the slide collection in 1995 (Gelder & Ohtaka 2002).

A preliminary study of the slides revealed they consisted of whole mount and sectioned specimens with some having annotation. Unfortunately, no catalogs or recorded information accompanied the collection. Although many of the whole specimens could be identified to species, others labeled "unidentified" may still be named following further study. Yamaguchi's sections were of a high standard, but given the limited conditions at the time and without labels, these specimens had to be set aside for future study. Consequently,

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the preliminary report (Gelder & Ohtaka 2002) contained a list of species thus far identified by the authors and a summary of other details then available. Additional details obtained since 2002 are included in this present paper: the collection—and particularly the sectioned material—remains to be fully investigated. To this end, the details included in our Table 1 were designed to assist future researchers by ensuring all the information currently available is not lost.

TABLE 1. Microscope slides of branchiobdellidans in the Prof. H. Yamaguchi collection. Each slide is arranged by its original box/slide number, assigned museum number, original slide markings, specimen(s) status, species identification and type status followed by the Japanese common name.

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
1. 2	ICHUM-1398	c.d.l. 3	sect.	-		-
1. 4	ICHUM-1399	E. 16 / Sp.E / D.1	WM	*Cirrodrilus nipponicus* (Yamaguchi, 1932)	syntype	Nippon-zariganimimizu
1. 6	ICHUM-1400	A. 10 / A. / 107	WM 1	*Cirrodrilus cirratus* Pierantoni, 1905	non-type	Kamuri-zariganimimizu
"	ICHUM-1401	"	WM 2	*Cirrodrilus cirratus* Pierantoni, 1905	non-type	Kamuri-zariganimimizu
1. 8 *	ICHUM-1402	H. 4 **	WM	*Cirrodrilus makinoi* (Yamaguchi, 1934)	syntype	Hime-zariganimimizu
1. 9	ICHUM-1403	H. 5 / c.m.b.	sect.	-		-
1. 14	ICHUM-1404	H. 3 **	WM	*Cirrodrilus makinoi* (Yamaguchi, 1934)	syntype	Hime-zariganimimizu
1. 17	ICHUM-1405	H. - 14 **	WM 1	*Cirrodrilus makinoi* (Yamaguchi, 1934)	syntype	Hime-zariganimimizu
"	ICHUM-1406	"	WM 2	*Cirrodrilus makinoi* (Yamaguchi, 1934)	syntype	Hime-zariganimimizu
1. 19	ICHUM-1407	E. 11 / 2 / E. 2	sect.	-		-
1. 20*	ICHUM-1408	20	WM 1	*Cirrodrilus makinoi* (Yamaguchi, 1934)	syntype	Hime-zariganimimizu
"	ICHUM-1409	"	WM 2	*Cirrodrilus makinoi* (Yamaguchi, 1934)	syntype	Hime-zariganimimizu
1. 23	ICHUM-1410	Sp. E. 8 - 2	sect.	-		-
1. 25	ICHUM-1411	Sp. E. 9 - 2	sect.	-		-
1. 26	ICHUM-1412	Sp. E. 9 - 1	sect.	-		-
1. 27	ICHUM-1413	Sp. E. 8 - 4	sect.	-		-
1. 28	ICHUM-1414	Sp. E. 8 - 3	sect.	-		-
1. 29	ICHUM-1415	Sp. E. 8 - 1	sect.	-		-
1. 30	ICHUM-1416	Sp. E. 7 - 2	sect.	-		-

...Continued on the next page

TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
1. 31	ICHUM-1417	Sp. E. 7 - 1	sect.	-		-
1. 32	ICHUM-1418	Sp. E. 6 / (APF?) **	sect.	-		-
1. 33	ICHUM-1419	E. 5	sect.	-		-
1. 34	ICHUM-1420	E. 4	sect.	-		-
1. 35	ICHUM-1421	Sp. E 3	sect.	-		-
1. 36	ICHUM-1422	E. 2	sect.	-		-
1. 37	ICHUM-1423	E. 1 - 2	sect.	-		-
1. 38	ICHUM-1424	Sp E 1	sect.	-		-
1. 39	ICHUM-1425	c.m.b. 19 / 19	sect.	-		-
1. 40	ICHUM-1426	c.m.b 20 / 1 / 12-1	sect.	-		-
1. 41	ICHUM-1427	c.m.b.-20 / 2 / 12-2	sect.	-		-
1. 42	ICHUM-1428	c.m.b 20 - 3 / 12-3	sect.	-		-
1. 43	ICHUM-1429	c.m.b 21 / 10	sect.	-		-
1. 44	ICHUM-1430	K. 6 - 2	sect.	-		-
1. 46 *	ICHUM-1431	K 6 / 6	WM	*Hidejiodrilus koreanus* (Pierantoni, 1912)	non-type	Yotsuyubi-zariganimimizu
1. 47	ICHUM-1432	K. 6 / 5	sect.	-		-
1. 48	ICHUM-1433	K. 6 / 4	sect.	-		-
1. 49	ICHUM-1434	K. 6 / 3 - 2	sect.	-		-
1. 50	ICHUM-1435	K. 6 / 3 - 1	sect.	-		-
1. 51	ICHUM-1436	F 3 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
1. 52 *	ICHUM-1437	F 4 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
1. 54	ICHUM-1438	F 6 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932) ?	syntype ?	Uchida-zariganimimizu ?
1. 56	ICHUM-1439	Sp. E / D.3 / E 15	WM	*Cirrodrilus nipponicus* (Yamaguchi, 1932)	syntype ?	Nippon-zariganimimizu
1. 58	ICHUM-1440	H. 9 - 2 / H. K. 1 - 2	sect.	-		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
1. 59	ICHUM-1441	E. 19	WM	*Cirrodrilus nipponicus* (Yamaguchi, 1932)	syntype ?	Nippon-zariganimimizu
1. 60	ICHUM-1442	c.d l. / 1 / c.d l	sect.	-		-
1. 61	ICHUM-1443	c.s. - 1	sect.	-		-
1. 62	ICHUM-1444	c.s. - 2	sect.	-		-
1. 63	ICHUM-1445	c.s. - 3	sect.	-		-
1. 64	ICHUM-1446	c.s. - 18	sect.	-		-
1. 65	ICHUM-1447	Sp. c.s. 4 - 2	sect.	-		-
1. 66	ICHUM-1448	Sp. c.s. 6 - 1	sect.	-		-
1. 67	ICHUM-1449	Sp. c.s. 6 - 2 **	sect.	-		-
1. 68	ICHUM-1450	Sp. c.s. 7	sect.	-		-
1. 69	ICHUM-1451	c.s. - 15	sect.	-		-
1. 70	ICHUM-1452	c.s. 9	sect.	-		-
1. 71	ICHUM-1453	c.s. - 10	sect.	-		-
1. 72	ICHUM-1454	c.s. - 11	sect.	-		-
1. 73	ICHUM-1455	c.s. - 12	sect.	-		-
1. 74	ICHUM-1456	c.s. - 14	sect.	-		-
1. 75	ICHUM-1457	c.s. - 13	sect.	-		-
1. 76	ICHUM-1458	Sp. c.s. - 5	sect.	-		-
1. 77	ICHUM-1459	c.s. - 17	sect.	-		-
1. 78	ICHUM-1460	E. 10	sect.	-		-
1. 79	ICHUM-1461	H. 20 - 2	sect.	-		-
1. 83	ICHUM-1462	H 9 - 1 / H 21 - 1	sect.	-		-
1. 86	ICHUM-1463	E 21	WM	*Cirrodrilus nipponicus* (Yamaguchi, 1932)	syntype ?	Nippon-zariganimimizu
1. 88 *	ICHUM-1464	E 20	WM	*Cirrodrilus nipponicus* (Yamaguchi, 1932)	syntype ?	Nippon-zariganimimizu
1. 92	ICHUM-1465	H. 10 - 1	sect.	-		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
1. 99	ICHUM-1466	K 6 / 7	WM	*Hidejiodrilus koreanus* (Pierantoni, 1912)	non-type	Yotsuyubi-zariganimimizu
2. 4	ICHUM-1348	4	WM	*Cambarincola okadai* Yamaguchi, 1933	paralectotype	Yadorimimizu
2. 6	ICHUM-1467	am 2 - 10	sect.	-		-
2. 8	ICHUM-1468	A 8	sect.	-		-
2. 9	USNM 186575	9	WM	*Cambarincola okadai* Yamaguchi, 1933	syntype	Yadorimimizu
2. 10 *	ICHUM-1349	10	WM	*Cambarincola okadai* Yamaguchi, 1933	paralectotype	Yadorimimizu
2. 12	ICHUM-1469	D. 11 - 1	sect.	-		-
2. 13	ICHUM-1470	D 12 / 1 / 4	sect.	-		-
2. 14	ICHUM-1471	D. 10 - 2	sect.	-		-
2. 15 *	ICHUM-1472	c.s 33 **	WM 1	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
"	ICHUM-1473	"	WM 2	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
"	ICHUM-1474	"	WM 3	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
"	ICHUM-1475	"	WM 4	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
2. 16	ICHUM-1476	D. 10 - 1	sect.	-		-
2. 18	ICHUM-1477	18 / 2 - 9	sect.	-		-
2. 20 *	ICHUM-1347	20	WM	*Cambarincola okadai* Yamaguchi, 1933	lectotype	Yadorimimizu
2. 21	ICHUM-1478	A. 7 / H	sect.	-		-
2. 22	ICHUM-1479	D. 9 - 2 / 3	sect.	-		-
2. 23	ICHUM-1480	c.m.b. 7 - 2	sect.	-		-
2. 26 *	ICHUM-1481	26	WM	*Cirrodrilus nipponicus* (Yamaguchi, 1932)	syntype ?	Nippon-zariganimimizu

...Continued on the next page

TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
2. 29	ICHUM-1482	5 - 2	sect.	-		-
2. 32	ICHUM-1483	A 6 - 2	sect.	-		-
2. 34	ICHUM-1484	III / c.d.s 16	sect.	-		-
2. 35	ICHUM-1485	II / c.d.s 11	sect.	-		-
2. 36	ICHUM-1486	I / c.d.s 16	sect.	-		-
2. 40	ICHUM-1487	c. d. s. / (?) / c d s / 14	sect.	-		-
2. 41	ICHUM-1488	8	sect.	-		-
2. 43	ICHUM-1489	43	WM	*Cirrodrilus cirratus* Pierantoni, 1905	non-type	Kamuri-zariganimimizu
2. 44	ICHUM-1490	10 / D 5	sect.	-		-
2. 46	ICHUM-1491	8	sect.	-		-
2. 48	ICHUM-1492	D / 2. 2 - 3 / D.- 8 - 3	sect.	-		-
2. 49	ICHUM-1493	49	WM	unidentified		-
2. 50	ICHUM-1494	Sp.cmb / D.1	WM 1	*Cirrodrilus homodontus* (Yamaguchi, 1932)	syntype ?	Hoso-zariganimimizu
"	ICHUM-1495	"	WM 2	unidentified		-
"	ICHUM-1496	"	WM 3	unidentified		-
2. 54	ICHUM-1497	54 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
2. 55	ICHUM-1498	3 / D 5	sect.	-		-
2. 56	ICHUM-1499	c.m.b. 2	sect.	-		-
2. 57	ICHUM-1500	D. 9 - 1	sect.	-		-
2. 58	ICHUM-1501	D. 6 - 6	sect.	-		-
2. 59	ICHUM-1502	D. 6 - 5	sect.	-		-
2. 60 *	ICHUM-1503	59	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
2. 61	ICHUM-1504	61 **	-	-		-
2. 62	ICHUM-1505	c.m.b. / 62	sect.	-		-
2. 63	ICHUM-1506	F 2 **	sect.	-		-

...*Continued on the next page*

TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
2. 64	ICHUM-1507	D. 9 - 3	sect.	-		-
2. 65	ICHUM-1508	D.12 / 2 / 3	sect.	-		-
2. 66	ICHUM-1509	D. 11 - 2 / 2	sect.	-		-
2. 67	ICHUM-1510	D. 11. 4	sect.	-		-
2. 68	ICHUM-1511	D. 6 - 4	sect.	-		-
2. 69	ICHUM-1512	D.12 / 3 / 2	sect.	-		-
2. 70	ICHUM-1513	7 - 7	sect.	-		-
2. 71	ICHUM-1514	D. 6 - 7	sect.	-		-
2. 72	ICHUM-1515	7 - 1	sect.	-		-
2. 74	ICHUM-1516	D.12 / 4 / D	sect.	-		-
2. 77	ICHUM-1517	1 - 4	sect.	-		-
2. 78	ICHUM-1518	am / 1 - 2	sect.	-		-
2. 80	ICHUM-1519	2 - 7	sect.	-		-
2. 82	ICHUM-1520	am 2 - 1	sect.	destroyed		-
2. 84	ICHUM-1521	2 - 4	sect.	-		-
2. 86	ICHUM-1522	am / 2 - 2	sect.	-		-
2. 87	ICHUM-1523	2 - 3	sect.	-		-
2. 89	ICHUM-1524	am / 2 - 5	sect.	-		-
2. 90	ICHUM-1525	2 - 6	sect.	-		-
2. 91	ICHUM-1526	2 - 8	sect.	-		-
2. 92	ICHUM-1527	a.m 7 - 5	sect.	-		-
2. 93	ICHUM-1528	1 - 3	sect.	-		-
2. 95	ICHUM-1529	D. 6 - 2	sect.	-		-
2. 96	ICHUM-1530	D. 6 - 1	sect.	-		-
2. 97	ICHUM-1531	ext	sect.	destroyed		-
2. 98	ICHUM-1532	13 / D - 5	sect.	-		-
2. 99	ICHUM-1533	D. 6 - 3 A	sect.	-		-
2. 100	ICHUM-1534	D. 6 - 3 B	sect.	-		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
3. 1	ICHUM-1535	D. 5 / 2	sect.	-		-
3. 2	ICHUM-1536	D 4 / 7	sect.	-		-
3. 3	ICHUM-1537	D. 5 / 1	sect.	-		-
3. 4	ICHUM-1538	D 4 / 6	sect.	-		-
3. 5	ICHUM-1539	D 4 / 8	sect.	-		-
3. 6	ICHUM-1540	D 4 / 5	sect.	-		-
3. 7	ICHUM-1541	D 4 / 4	sect.	-		-
3. 8	ICHUM-1542	D 4 / 3	sect.	-		-
3. 9	ICHUM-1543	D 4 / 2	sect.	-		-
3. 10	ICHUM-1544	10 **	sect.	-		-
3. 11	ICHUM-1545	D 3 / 35	sect.	-		-
3. 12	ICHUM-1546	D 3 / 34	sect.	-		-
3. 13	ICHUM-1547	D. 3 / 33	sect.	-		-
3. 14	ICHUM-1548	D 3 / 32	sect.	-		-
3. 15	ICHUM-1549	D 3 / 31	sect.	-		-
3. 16	ICHUM-1550	D. 3 / 30	sect.	-		-
3. 17	ICHUM-1551	D. 3 / 29	sect.	-		-
3. 18	ICHUM-1552	D. 3 / 28	sect.	-		-
3. 19	ICHUM-1553	D. 3 / 27	sect.	-		-
3. 20 *	ICHUM-1554	D. 14	WM	*Cirrodrilus sapporensis* (Pierantoni, 1906)	non-type	Zariganimimizu
3. 21	ICHUM-1555	D. 15	WM 1	*Cirrodrilus sapporensis* (Pierantoni, 1906)	non-type	Zariganimimizu
"	ICHUM-1556	"	WM 2	*Cirrodrilus sapporensis* (Pierantoni, 1906)	non-type	Zariganimimizu
3. 22	ICHUM-1557	D. 16	WM	*Cirrodrilus sapporensis* (Pierantoni, 1906)	non-type	Zariganimimizu
3. 23	ICHUM-1558	D. 17	WM	unidentified		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
3. 25	ICHUM-1559	D. 19	WM	*Cirrodrilus sapporensis* (Pierantoni, 1906)	non-type	Zariganimimizu
3. 26 *	ICHUM-1560	D 21	WM	destroyed		-
3. 28 *	ICHUM-1561	D. 22	WM	*Cirrodrilus sapporensis* (Pierantoni, 1906)	non-type	Zariganimimizu
3. 29 *	ICHUM-1562	D. 23	WM 1	*Cirrodrilus sapporensis* (Pierantoni, 1906)	non-type	Zariganimimizu
"	ICHUM-1563	"	WM 2	*Cirrodrilus sapporensis* (Pierantoni, 1906)	non-type	Zariganimimizu
3. 32 *	ICHUM-1564	D. 24	WM	*Cirrodrilus sapporensis* (Pierantoni, 1906)	non-type	Zariganimimizu
3. 34	ICHUM-1565	D. 1 - 1	sect.	-		-
3. 35	ICHUM-1566	D. 1 - 2	sect.	-		-
3. 36	ICHUM-1567	D. 1 - 4	sect.	-		-
3. 38	ICHUM-1568	D. 1 - 5	sect.	-		-
3. 39	ICHUM-1569	D. 1 - 6	sect.	-		-
3. 40	ICHUM-1570	D. 1 - 7	sect.	-		-
3. 41	ICHUM-1571	D. - 2 - 1	sect.	-		-
3. 42	ICHUM-1572	D. 2 - 2	sect.	-		-
3. 43	ICHUM-1573	D. 2 - 3	sect.	-		-
3. 44	ICHUM-1574	D. 2 - 4	sect.	-		-
3. 45	ICHUM-1575	D. 2 - 5	sect.	-		-
3. 46	ICHUM-1576	D. 2 - 6	sect.	-		-
3. 47	ICHUM-1577	D. 2 - 7	sect.	-		-
3. 48	ICHUM-1578	D. 3 - 1 / 1	sect.	-		-
3. 49	ICHUM-1579	D. 3 - 2 / 2	sect.	-		-
3. 50	ICHUM-1580	D. 3 - 3 / 3	sect.	-		-
3. 51	ICHUM-1581	D. 3 / 4 / 4	sect.	-		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
3. 52	ICHUM-1582	D. 3 / 5 / 5	sect.	-		-
3. 53	ICHUM-1583	D. 3 / 6 / 6	sect.	-		-
3. 54	ICHUM-1584	67	sect.	-		-
3. 55 *	ICHUM-1585	55	WM 1	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
"	ICHUM-1586	"	WM 2	unidentified		-
3. 56	ICHUM-1587	56	WM	unidentified		-
3. 57 *	ICHUM-1588	57	WM 1	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
"	ICHUM-1589	"	WM 2	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
3. 58	ICHUM-1590	58	WM 1	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
"	ICHUM-1591	"	WM 2	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
3. 59	ICHUM-1592	59	WM 1	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
"	ICHUM-1593	"	WM 2	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
3. 60	ICHUM-1594	60	WM	unidentified		-
3. 61	ICHUM-1595	61	WM	unidentified		-
3. 62	ICHUM-1596	D. 3 / 26 / 26	sect.	-		-
3. 63	ICHUM-1597	D. 3 / 25 / 25	sect.	-		-
3. 64	ICHUM-1598	D. 3 / 24 / 24	sect.	-		-
3. 65	ICHUM-1599	D. 3 / 23 / 23	sect.	-		-
3. 66	ICHUM-1600	D. 3 / 22 / 22	sect.	-		-
3. 67	ICHUM-1601	D. 3 / 21 / 21	sect.	destroyed		-
3. 68	ICHUM-1602	D. 3 / 20 / 20	sect.	-		-
3. 69	ICHUM-1603	D. 3 / 19 / 19	sect.	-		-
3. 70	ICHUM-1604	D. 3 / 18 / 18	sect.	-		-
3. 71	ICHUM-1605	D. 3 / 17 / 17	sect.	-		-
3. 72	ICHUM-1606	D. 3 / 16 / 16	sect.	-		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
3. 73	ICHUM-1607	D. 3 / 15 / 15	sect.	-		-
3. 74	ICHUM-1608	D. 3 / 14 / 14	sect.	-		-
3. 75	ICHUM-1609	D. 3 / 13 / 13	sect.	-		-
3. 76	ICHUM-1610	D. 3 / 12 / 12	sect.	-		-
3. 77	ICHUM-1611	D. 3 / 11 / 11	sect.	-		-
3. 78	ICHUM-1612	D. 3 / 10 / 10	sect.	-		-
3. 79	ICHUM-1613	D. 3 / 9 / 9	sect.	-		-
3. 80	ICHUM-1614	D. 3 / 8 / 8	sect.	-		-
3. 81	ICHUM-1615	D. 3 / 7 / 7	sect.	-		-
3. 82 *	ICHUM-1616	F 6 **	WM	*Cirrodrilus suzukii* (Yamaguchi, 1934)	syntype	Suzuki-zariganimimizu
3. 83	ICHUM-1617	F 5 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932) ?	syntype ?	Uchida-zariganimimizu ?
3. 84	ICHUM-1618	F 4 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 85	ICHUM-1619	F 3 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 86 *	ICHUM-1620	F 2 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 87	ICHUM-1621	G. 4	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 88	ICHUM-1622	G. 5 **	WM	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
3. 89	ICHUM-1623	B. 11 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 90	ICHUM-1624	B. 6	WM 1	unidentified		-
"	ICHUM-1625	"	WM 2	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 91	ICHUM-1626	B. 5	WM 1	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
"	ICHUM-1627	"	WM 2	unidentified		-
"	ICHUM-1628	"	WM 3	unidentified		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
3. 92	ICHUM-1629	B. 10 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 93 *	ICHUM-1630	F 7 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 94 *	ICHUM-1631	G. 1 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 95	ICHUM-1632	G. 3 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 96	ICHUM-1633	D. 1 - 3	sect.	-		-
3. 97 *	ICHUM-1634	A' 35 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 98	ICHUM-1635	A' 34 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932) ?	syntype ?	Uchida-zariganimimizu ?
3. 99	ICHUM-1636	A 33 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932)?	syntype ?	Uchida-zariganimimizu
3. 100	ICHUM-1637	A 32 **	WM	*Cirrodrilus uchidai* (Yamaguchi, 1932) ?	syntype ?	Uchida-zariganimimizu ?
4. 2 *	ICHUM-1638	215 / 2	WM 1	*Branchiobdella kobayashii* Yamaguchi, 1934	syntype ?	Kobayashi-hirumimizu
"	ICHUM-1639	"	WM 2	*Branchiobdella kobayashii* Yamaguchi, 1934	syntype ?	Kobayashi-hirumimizu
4. 3	ICHUM-1640	215 / 3	WM	unidentified		-
4. 4	ICHUM-1641	215 / 4	WM	*Cirrodrilus inukaii* (Yamaguchi, 1934) ?	syntype	Inukai-zariganimimizu ?
4. 5 *	ICHUM-1642	215 / 5	WM	*Cirrodrilus kawamurai* (Yamaguchi, 1934)	syntype ?	Kawamura-zariganimimizu
4. 6	ICHUM-1643	215 / 6	WM	unidentified		-
4. 7	ICHUM-1644	215 / 7	WM	unidentified		-
4. 8 *	ICHUM-1645	215 / 8	WM	*Branchiobdella monodontus* Liu & Zhang, 1983	non-type	-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
4. 9 *	ICHUM-1646	215 / 9	WM	*Branchiobdella monodontus* Liu & Zhang, 1983	non-type	-
4. 12 *	ICHUM-1647	215 / 12	WM	*Branchiobdella kobayashii* Yamaguchi, 1934	syntype ?	Kobayashi-hirumimizu
4. 13	ICHUM-1648	215 / 13	WM	unidentified		-
4. 14 *	ICHUM-1649	215 / 14	WM	*Holtodrilus truncatus* (Liang, 1963)	non-type	Ebi-yadorimimizu
4. 15 *	ICHUM-1650	215 / 15	WM	*Holtodrilus truncatus* (Liang, 1963)	non-type	Ebi-yadorimimizu
4. 16 *	ICHUM-1651	215 / 16	WM	*Branchiobdella kobayashii* Yamaguchi, 1934	syntype ?	Kobayashi-hirumimizu
4. 18	ICHUM-1652	215 / 18	WM	*Holtodrilus truncatus* (Liang, 1963)	non-type	Ebi-yadorimimizu
4. 19	ICHUM-1653	230	WM	oligochaete		-
4. 21 *	ICHUM-1654	21	WM 1-7	unidentified		-
4. 31	ICHUM-1655	c.s. 25	WM 1	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu
"	ICHUM-1656	"	WM 2	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu
"	ICHUM-1657	"	WM 3	unidentified		-
4. 34	ICHUM-1658	34	WM	unidentified		-
4. 36	ICHUM-1659	c s. - 25/ sp. c.s. /D 3	WM	*Cirrodrilus inukaii* (Yamaguchi, 1934) ?	syntype	Inukai-zariganimimizu ?
4. 38	ICHUM-1660	c.s - 23/ sp. c.s /D. 5	WM 1	*Cirrodrilus inukaii* (Yamaguchi, 1934) ?	syntype	Inukai-zariganimimizu ?
"	ICHUM-1661	"	WM 2	*Cirrodrilus inukaii* (Yamaguchi, 1934) ?	syntype	Inukai-zariganimimizu ?
"	ICHUM-1662	"	WM 3	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
4. 40	ICHUM-1663	c. - 27	WM	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
4. 42	ICHUM-1664	c s. - 20/ sp. c.s /D 1	WM 1-3	unidentified		-
4. 43	ICHUM-1665	c.s - 21/ Sp. c.s /D 8	WM 1	*Cirrodrilus inukaii* (Yamaguchi, 1934) ?	syntype	Inukai-zariganimimizu ?
"	ICHUM-1666	"	WM 2	*Cirrodrilus ezoensis* (Yamaguchi, 1934)	syntype	Ezo-zariganimimizu
"	ICHUM-1667	"	WM 3	*Cirrodrilus inukaii* (Yamaguchi, 1934) ?	syntype	Inukai-zariganimimizu ?
4. 45	ICHUM-1668	45	sect.	-		-
4. 46	ICHUM-1669	8	sect.	-		-
4. 48	ICHUM-1670	K. 3 2	WM	destroyed		-
4. 49	ICHUM-1671	12	WM	unidentified		-
4. 69	ICHUM-1672	69	WM	unidentified		-
4. 71	ICHUM-1673	71	WM	unidentified		-
4. 73	ICHUM-1674	73	sect.	-		-
4. 74	ICHUM-1675	10	sect.	-		-
4. 76	ICHUM-1676	76	sect.	destroyed		-
4. 77	ICHUM-1677	14	sect.	-		-
4. 78	ICHUM-1678	78	sect.	-		-
4. 82	ICHUM-1679	11	sect.	-		-
4. 86	ICHUM-1680	86	sect.	-		-
4. 92	ICHUM-1681	13	sect.	-		-
4. 97	ICHUM-1682	97	sect.	-		-
5. 1	ICHUM-1683	2 / Branch. Pentadonta	sect.	-		-
5. 2	ICHUM-1684	3 / Branch. Pentadonta	sect.	-		-
5. 3	ICHUM-1685	3 A / Branch. Pentadonta	sect.	-		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
5. 4	ICHUM-1686	K. 1 / 6	WM	*Branchiobdella macroperistomium* Liu & Zhang, 1983 ?	non-type	-
5. 8	ICHUM-1687	K. 1. 6 / K 1	sect.	-		-
5. 11	ICHUM-1688	K. 1. 8	sect.	-		-
5. 13	ICHUM-1689	K. 1. 9	sect.	-		-
5. 14	ICHUM-1690	K. 1. 10	sect.	-		-
5. 15 *	ICHUM-1691	K. 1 / 11	WM	*Branchiobdella kobayashii* Yamaguchi, 1934	syntype ?	Kobayashi-hirumimizu
5. 16 *	ICHUM-1692	K. 1. 12	WM	*Branchiobdella kobayashii* Yamaguchi, 1934	syntype ?	Kobayashi-hirumimizu
5. 18 *	ICHUM-1693	K. 1. 14 / K. 1 / D	WM	*Branchiobdella macroperistomium* Liu & Zhang, 1983 ?	non-type	-
5. 19	ICHUM-1694	K 1. / 15 / K 1. / D	WM	*Branchiobdella kobayashii* Yamaguchi, 1934	syntype ?	Kobayashi-hirumimizu
5. 20	ICHUM-1695	K. 1. / 13	WM	*Branchiobdella kobayashii* Yamaguchi, 1934 ?	syntype ?	Kobayashi-hirumimizu ?
5. 21	ICHUM-1696	K 1. 4 / K 1	sect.	-		-
5. 24	ICHUM-1697	K. 1 / 19	sect.	-		-
5. 28	ICHUM-1698	K. 2 - 4	sect.	-		-
5. 31	ICHUM-1699	K 2/ 2 /K. 2 - 5 - 2	sect.	-		-
5. 33	ICHUM-1700	K 2/ 1 /K. 2 - 5 - 1	sect.	-		-
5. 34	ICHUM-1701	K 2 / 17	sect.	-		-
5. 37	ICHUM-1702	K. 2	sect.	-		-
5. 40	ICHUM-1703	K 3 / 17 - 2	sect.	-		-
5. 42	ICHUM-1704	K 3 / 17 - 1	sect.	-		-
5. 44	ICHUM-1705	K. 3 / 8	WM	*Cirrodrilus kawamurai* (Yamaguchi, 1934)	syntype ?	Kawamura-zariganimimizu

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
5. 45	ICHUM-1706	K. 3/ 10 /K. 3 / D	WM	*Cirrodrilus kawamurai* (Yamaguchi, 1934)	syntype ?	Kawamura-zariganimimizu
5. 46	ICHUM-1707	K 3/ 9 / K 3 / D	WM	*Cirrodrilus kawamurai* (Yamaguchi, 1934)	syntype ?	Kawamura-zariganimimizu
5. 47 *	ICHUM-1708	K. 3/ 11 /K 3 / D	WM	*Cirrodrilus kawamurai* (Yamaguchi, 1934)	syntype ?	Kawamura-zariganimimizu
5. 48 *	ICHUM-1709	K 3 / 2	WM	*Cirrodrilus kawamurai* (Yamaguchi, 1934)	syntype ?	Kawamura-zariganimimizu
5. 49	ICHUM-1710	K. 3 / 12	WM	*Cirrodrilus kawamurai* (Yamaguchi, 1934)	syntype ?	Kawamura-zariganimimizu
5. 50	ICHUM-1711	K. 3 / 13 / K 3 / D	WM	*Cirrodrilus kawamurai* (Yamaguchi, 1934)	syntype ?	Kawamura-zariganimimizu
5. 51 *	ICHUM-1712	K. 3 / 14	WM	*Cirrodrilus kawamurai* (Yamaguchi, 1934)	syntype ?	Kawamura-zariganimimizu
5. 52	ICHUM-1713	K. 3 / 15	WM	*Cirrodrilus kawamurai* (Yamaguchi, 1934)	syntype ?	Kawamura-zariganimimizu
5. 58	ICHUM-1714	K. 6	sect.	-		-
5. 59	ICHUM-1715	K. 5 - 4 - 2	sect.	-		-
5. 60	ICHUM-1716	K. 5 - 4 - 3/ K 5 /1 - 3	sect.	-		-
5. 61	ICHUM-1717	K. 4 -10 - 3	sect.	-		-
5. 62	ICHUM-1718	K. 4 - 6	sect.	-		-
5. 63	ICHUM-1719	K. 3 - 6 - 4 / K 3	sect.	-		-
5. 64	ICHUM-1720	K. 3 / 5	sect.	-		-
5. 65	ICHUM-1721	K. 4 - 5	sect.	-		-
5. 66	ICHUM-1722	K 4 / 4	WM	unidentified		-
5. 67	ICHUM-1723	K 4 / 8	WM	unidentified		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
5. 68	ICHUM-1724	K. 4 / 9	WM	*Branchiobdella orientalis* Yamaguchi, 1934	syntype ?	Oo-hirumimizu
5. 69	ICHUM-1725	K. 4 / 10	WM	*Branchiobdella orientalis* Yamaguchi, 1934	syntype ?	Oo-hirumimizu
5. 70	ICHUM-1726	K 3 / 16 -1	sect.	-		-
5. 72	ICHUM-1727	K. 2. - 3	sect.	-		-
5. 73	ICHUM-1728	1./ Branchio. Pentadonta	sect.	-		-
5. 75	ICHUM-1729	K. 4. 4 - 2	sect.	-		-
5. 76	ICHUM-1730	K 3 / K 3 - 6 - 3	sect.	-		-
5. 77	ICHUM-1731	K 3. 16 - 2	sect.	-		-
5. 78	ICHUM-1732	K. 4. 4 - 1 / 1 - 1	sect.	-		-
5. 79	ICHUM-1733	K. 4 / 2. - 3	sect.	-		-
5. 80	ICHUM-1734	K. 4 / 2. 2 - 3	sect.	-		-
5. 81	ICHUM-1735	K 5 / K. 5 / 5	sect.	-		-
5. 82	ICHUM-1736	K 3 / K. 3 - 6 - 1	sect.	-		-
5. 83	ICHUM-1737	K 3 / K 3. - 6 - 2	sect.	-		-
5. 85	ICHUM-1738	K. 4 -11- 1	sect.	-		-
5. 86	ICHUM-1739	K. 4 -11- 2	sect.	-		-
5. 87	ICHUM-1740	K. 5. 13 - 2	sect.	-		-
5. 88	ICHUM-1741	K 5 / 13 - 4	sect.	-		-
5. 89	ICHUM-1742	K. 5 - 13 - 1	sect.	-		-
5. 90	ICHUM-1743	K 4 / 2. 1 - 2	sect.	-		-
5. 91	ICHUM-1744	K 4 / 3. 1 - 1 / 2. 1	sect.	-		-
5. 93	ICHUM-1745	K 5. - 4 - 1	sect.	-		-
5. 94	ICHUM-1746	K. 4. - 11 - 3	sect.	-		-
5. 95	ICHUM-1747	K. 5 / 5	sect.	-		-
5. 96 *	ICHUM-1748	K 2 / 6	WM 1	*Cirrodrilus chosen* (Yamaguchi, 1934)	syntype	Chosen-zariganimimizu

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
"	ICHUM-1749	"	WM 2	*Cirrodrilus chosen* (Yamaguchi, 1934)	syntype	Chosen-zariganimimizu
"	ICHUM-1750	"	WM 3	*Cirrodrilus chosen* (Yamaguchi, 1934)	syntype	Chosen-zariganimimizu
5. 97	ICHUM-1751	K 5 / 7	WM	*Cirrodrilus suzukii* (Yamaguchi, 1934)	syntype ?	Suzuki-zariganimimizu
5. 98 *	ICHUM-1752	K. 5 / 8	WM	*Cirrodrilus chosen* (Yamaguchi, 1934)	syntype	Chosen-zariganimimizu
5. 99 *	ICHUM-1753	K 5 / 9	WM	*Cirrodrilus suzukii* (Yamaguchi, 1934)	syntype ?	Suzuki-zariganimimizu
5. 100	ICHUM-1754	K. 5 / 11	WM	*Cirrodrilus suzukii* (Yamaguchi, 1934)	syntype ?	Suzuki-zariganimimizu
6. 1	ICHUM-1755	am A / 2	sect.	-		-
6. 2	ICHUM-1756	am B / 4	sect.	-		-
6. 3	ICHUM-1757	am A / 1	sect.	-		-
6. 4	ICHUM-1758	am A / 5	sect.	-		-
6. 5	ICHUM-1759	am A / 6	sect.	-		-
6. 6	ICHUM-1760	am A / 4	sect.	-		-
6. 7	ICHUM-1761	am. B / 5	sect.	-		-
6. 8	ICHUM-1762	am A / 3	sect.	-		-
6. 9	ICHUM-1763	am B / 1	sect.	-		-
6. 10	ICHUM-1764	am. B / 2	sect.	-		-
6. 11	ICHUM-1765	am B / 3	sect.	-		-
6. 12	ICHUM-1766	am A / 7	sect.	-		-
6. 13	ICHUM-1767	am B / 6	sect.	-		-
6. 21	ICHUM-1768	AO / 1	WM	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu
6. 24	ICHUM-1769	AO / 2	WM	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu
6. 25	ICHUM-1770	AO / 3	WM	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
6. 26 *	ICHUM-1771	AO / 4	WM 1	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu
"	ICHUM-1772	"	WM 2	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu
6. 27 *	ICHUM-1773	27	WM 1	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
"	ICHUM-1774	"	WM 2	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
"	ICHUM-1775	"	WM 3	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu
"	ICHUM-1776	"	WM 4	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu
"	ICHUM-1777	"	WM 5	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu
"	ICHUM-1778	"	WM 6	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu
6. 28	ICHUM-1779	AO / A 2	WM	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
6. 29	ICHUM-1780	AO / 5	WM	*Cirrodrilus aomorensis* (Yamaguchi, 1934)	syntype	Aomori-zariganimimizu
6. 31	ICHUM-1781	C - 8	WM 1	*Cirrodrilus suzukii* (Yamaguchi, 1934)	syntype ?	Suzuki-zariganimimizu
"	ICHUM-1782	"	WM 2	unidentified		-
"	ICHUM-1783	"	WM 3	unidentified		-
"	ICHUM-1784	"	WM 4	*Cirrodrilus megalodentatus* (Yamaguchi, 1934)	syntype	Ooago-zariganimimizu
6. 32	ICHUM-1785	K 1. / 5 / 51 / K 1	sect.	-		-
6. 33	ICHUM-1786	c.d.s. 20 / c.d.s.	sect.	-		-
6. 34	ICHUM-1787	c.d.s / c.d s / 18	sect.	-		-
6. 35	ICHUM-1788	c d s. 19 / c.d.s 5	sect.	-		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
6. 37 *	ICHUM-1789	Sp.c d l / D 1 /c.d s 17	WM	*Cirrodrilus megalodentatus* (Yamaguchi, 1934)	syntype	Ooago-zariganimimizu
6. 38	ICHUM-1790	c.d.s. 21	sect.	-		-
6. 39	ICHUM-1791	39	WM	unidentified		-
6. 40	ICHUM-1792	40	WM	unidentified		-
6. 41	ICHUM-1793	41	WM 1-2	unidentified		-
6. 42	ICHUM-1794	c.s - 22/ Sp. c.s. /D 2	WM 1	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
"	ICHUM-1795	"	WM 2	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
"	ICHUM-1796	"	WM 3	*Cirrodrilus inukaii* (Yamaguchi, 1934)	syntype	Inukai-zariganimimizu
6. 43	ICHUM-1797	c. - 7	WM 1	unidentified		-
"	ICHUM-1798	"	WM 2	*Cirrodrilus megalodentatus* (Yamaguchi, 1934)	syntype	Ooago-zariganimimizu
"	ICHUM-1799	"	WM 3	*Cirrodrilus ezoensis* (Yamaguchi, 1934)	syntype	Ezo-zariganimimizu
"	ICHUM-1800	"	WM 4	*Cirrodrilus megalodentatus* (Yamaguchi, 1934)	syntype	Ooago-zariganimimizu
"	ICHUM-1801	"	WM 5	*Cirrodrilus ezoensis* (Yamaguchi, 1934)	syntype	Ezo-zariganimimizu
"	ICHUM-1802	"	WM 6	*Cirrodrilus ezoensis* (Yamaguchi, 1934)	syntype	Ezo-zariganimimizu
"	ICHUM-1803	"	WM 7	unidentified		-
"	ICHUM-1804	"	WM 8	*Cirrodrilus megalodentatus* (Yamaguchi, 1934)	syntype	Ooago-zariganimimizu
"	ICHUM-1805	"	WM 9	unidentified		-
"	ICHUM-1806	"	WM 10	*Cirrodrilus ezoensis* (Yamaguchi, 1934)	syntype	Ezo-zariganimimizu
6. 44	ICHUM-1807	c - 9	WM 1	*Cirrodrilus ezoensis* (Yamaguchi, 1934)	syntype	Ezo-zariganimimizu
"	ICHUM-1808	"	WM 2	unidentified		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
"	ICHUM-1809	"	WM 3	*Cirrodrilus ezoensis* (Yamaguchi, 1934)	syntype	Ezo-zariganimimizu
"	ICHUM-1810	"	WM 4	unidentified		-
6. 46 *	ICHUM-1811	c.- 4/c.d.s /c.d.l./ Soranuma	WM 1	*Cirrodrilus ezoensis* (Yamaguchi, 1934)	syntype	Ezo-zariganimimizu
"	ICHUM-1812	"	WM 2	*Cirrodrilus megalodentatus* (Yamaguchi, 1934)	syntype	Ooago-zariganimimizu
6. 47	ICHUM-1813	c. - 6	WM 1	unidentified		-
"	ICHUM-1814	"	WM 2	*Cirrodrilus megalodentatus* (Yamaguchi, 1934)	syntype	Ooago-zariganimimizu
"	ICHUM-1815	"	WM 3	*Cirrodrilus ezoensis* (Yamaguchi, 1934)	syntype	Ezo-zariganimimizu
"	ICHUM-1816	"	WM 4	unidentified		-
"	ICHUM-1817	"	WM 5	*Cirrodrilus megalodentatus* (Yamaguchi, 1934)	syntype	Ooago-zariganimimizu
"	ICHUM-1818	"	WM 6	unidentified		-
6. 48	ICHUM-1819	c. - 5	WM 1	unidentified		-
"	ICHUM-1820	"	WM 2	unidentified		-
"	ICHUM-1821	"	WM 3	*Cirrodrilus ezoensis* (Yamaguchi, 1934)	syntype	Ezo-zariganimimizu
"	ICHUM-1822	"	WM 4	unidentified		-
"	ICHUM-1823	"	WM 5	unidentified		-
"	ICHUM-1824	"	WM 6	unidentified		-
6. 50	ICHUM-1825	c. - 3 / Soranuma	WM 1	*Cirrodrilus megalodentatus* (Yamaguchi, 1934)	syntype	Ooago-zariganimimizu
"	ICHUM-1826	"	WM 2	unidentified		-
"	ICHUM-1827	"	WM 3	unidentified		-
"	ICHUM-1828	"	WM 4	unidentified		-
6. 51	ICHUM-1829	c.m.b / 9	sect.	-		-
6. 52	ICHUM-1830	c.m.b. - 3 / A - 1 **	sect.	-		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
6. 53	ICHUM-1831	c m b - 3 / A - 2 **	sect.	-		-
6. 54	ICHUM-1832	c m b - 4 / B - 1 **	sect.	-		-
6. 55	ICHUM-1833	c.m.b - 4 / B - 2 **	sect.	-		-
6. 56	ICHUM-1834	c m b. / 6 **	sect.	-		-
6. 57	ICHUM-1835	c m b / 7 / 7 **	sect.	-		-
6. 58 *	ICHUM-1836	c m b 2 **	-	original slide		-
6. 59	ICHUM-1837	Box 6, Slide 58 A / cmb 2	WM	*Cirrodrilus homodontus* (Yamaguchi, 1932)	syntype ?	Hoso-zariganimimizu
6. 60	ICHUM-1838	Box 6, Slide 58 B / cmb 2	WM	*Cirrodrilus homodontus* (Yamaguchi, 1932)	syntype ?	Hoso-zariganimimizu
6. 61	ICHUM-1839	Box 6, Slide 58 C / cmb 2	WM	*Cirrodrilus homodontus* (Yamaguchi, 1932)	syntype ?	Hoso-zariganimimizu
6. 62	ICHUM-1840	Box 6, Slide 58 D / cmb 2	WM	*Cirrodrilus homodontus* (Yamaguchi, 1932)	syntype ?	Hoso-zariganimimizu
6. 63	ICHUM-1841	c.m.b / 8 / 8	sect.	-		-
6. 64	ICHUM-1842	c.m.b. / 12 / c	WM	*Cirrodrilus homodontus* (Yamaguchi, 1932)	syntype ?	Hoso-zariganimimizu
6. 65	ICHUM-1843	c m b / 13	WM 1	unidentified		-
"	ICHUM-1844	"	WM 2	unidentified		-
"	ICHUM-1845	"	WM 3	*Cirrodrilus homodontus* (Yamaguchi, 1932)	syntype ?	Hoso-zariganimimizu
"	ICHUM-1846	"	WM 4	*Cirrodrilus homodontus* (Yamaguchi, 1932)	syntype ?	Hoso-zariganimimizu
6. 67	ICHUM-1847	c m b. / 14	WM	unidentified		-
6. 68	ICHUM-1848	c m b / 15	WM	unidentified		-
6. 69	ICHUM-1849	c m b /16 / c m b /9 -	sect.	-		-
6. 74	ICHUM-1850	c m b / 17 / 4	sect.	-		-

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
6. 80	ICHUM-1851	c m b /18 / c m b /3	sect.	-		-
6. 83	ICHUM-1852	13 - 1	sect.	-		-
6. 85	ICHUM-1853	13 - 2	sect.	-		-
6. 87	ICHUM-1854	c m b / 6 - 2	sect.	-		-
6. 88	ICHUM-1855	6 - 2	sect.	-		-
6. 89	ICHUM-1856	c m b / 13 - 3	sect.	-		-
6. 90	ICHUM-1857	90	sect.	-		-
6. 91	ICHUM-1858	91	sect.	-		-
6. 92	ICHUM-1859	c.d l. 10 - 3	sect.	-		-
6. 93	ICHUM-1860	c.d l. 10 - 2	sect.	-		-
6. 94	ICHUM-1861	c.d l. 10 - 1	sect.	-		-
6. 95	ICHUM-1862	c.d.l. 9 - 2	sect.	-		-
6. 96	ICHUM-1863	c.d l 9 - 1 / 1	sect.	-		-
6. 98	ICHUM-1864	c.d l. 8 /Sp. c.d.l. / D 2	WM	*Cirrodrilus megalodentatus* (Yamaguchi, 1934)	syntype	Ooago-zariganimimizu
6. 99	ICHUM-1865	c.d.l./ 7 /Sp. c d l /D 3	WM	*Cirrodrilus megalodentatus* (Yamaguchi, 1934)	syntype	Ooago-zariganimimizu
-	-	100 / A3	sect.	-		-
-	-	101 / A4	sect.	-		-
-	-	103 / A6-1	sect.	-		-
-	-	106 / A A7	WM	*Cirrodrilus cirratus* Pierantoni, 1905	non-type	Kamuri-zariganimimizu
-	-	108	WM	*Cirrodrilus cirratus* Pierantoni, 1905	non-type	Kamuri-zariganimimizu
-	-	113 / A16	WM	*Cirrodrilus cirratus* Pierantoni, 1905	non-type	Kamuri-zariganimimizu
-	-	114 / A' 37-1A' 37-2	sect.	-		-
-	-	119 / 32-2	sect.	*Cirrodrilus cirratus* Pierantoni, 1905	non-type	Kamuri-zariganimimizu

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TABLE 1. (Continued)

Box / Slide No.	Catalog No.	Slide Markings	Specimen(s) Status	Species Ident.	Type Status	Japanese common name
-	-	120	WM	*Cirrodrilus cirratus* Pierantoni, 1905	non-type	Kamuri-zariganimimizu
-	-	123	sect.	-		-
-	-	124 / Tot E	WM	*Cirrodrilus nipponicus* (Yamaguchi, 1932)	syntype	Nippon-zariganimimizu
-	-	125 / E18	WM	*Cirrodrilus nipponicus* (Yamaguchi, 1932)	syntype	Nippon-zariganimimizu
-	-	131 / F10	sect.	-		-
-	-	133 / F12	sect.	-		-

Abbreviations according to column: * in Box / Slide No. column, specimen was remounted.
Catalog No. ICHAM, Invertebrate Collection of the Hokkaido University Museum, Sapporo, Japan, previously the Zoological Institute, Hokkaido University (ZIHU); USNM, National Museum of Natural History, Smithsonian Institution, Washington D.C., U.S.A.
** in Slide Markings column, Japanese characters present on slide; WM / sect., specimens whole mounted (WM) or sectioned (sect.); ?, tentative identification or "unidentified" species with specimens; -, no sections were identified.

Slide collection organization and examination

As already noted, Prof. Yamaguchi's records and slide catalog have not been found to date, but there is no evidence to indicate they have been destroyed. Therefore it is vital that the slides be kept in the original order in their boxes so that details in the catalog, when found, can be unambiguously assigned to a slide. Therefore, each slide was given a reference number based on its slide box and slot number; as an example, the slide in box 2, slot 46, is referred to as 2.46 (Table 1). There is no information about the slides on the box lids, but many slides carry abbreviations in Roman letters and Arabic numbers (Table 1, Slide Markings), plus occasional names or characters; the latter being indicated by ** in Table 1. Although some of these letters have an obvious interpretation, e.g., "c.s." being cross section (corroborated by viewing the sections), other letters and numbers are meaningless without the catalog.

Prior to depositing the collection, Prof. Ohtaka arranged for slides to be assigned sequential catalog numbers from the museum in the Zoological Institute, Hokkaido University, Japan, using the prefix ZIHU. Subsequently, the museum had a name change and it is now called the Invertebrate Collection of the Hokkaido University Museum, Sapporo, Japan, and so holdings now have the prefix ICHUM although catalog numbers remain the same. It is important to clearly state this change as the slides referred to in Gelder & Ohtaka (2002) carry the old ZIHU prefix, while their ICHUM designation is used in this article.

As the morphological characters of the jaws and peristomial appendages in the serial, stained sections could not be easily recognized during the preliminary study, no attempt was made to identify the species. While the sections would not allow the jaws to be reconstructed, the arrangement of peristomial tentacles and other surficial features can be determined—including the taxonomically important positions of the anterior nephidial pores and the male reproductive organs. As these organs are important features in determining a species in many branchiobdellidans, identification of this material is probable. However, the male system has only been described in detail in recent new species from Japan, although Yamaguchi (1934: Fig. 4) did provide such information for *Cirrodrilus inukaii* and Gelder (1987) for *C. cirratus* and *C. uchidai*. Therefore, for the male reproductive system to be fully utilized in an identification, the morphology needs to be described in newly collected specimens of the remaining Japanese species.

The whole mounted specimens were examined with a Nikon research microscope using bright-field, dark-field, phase contrast, and differential interference contrast (DIC) illuminations. Slides with a broken coverglass, or with air-bubbles touching a specimen were remounted and examined.

Whole-mounted specimens were identified and the name of the species was written on the slide label; if the identification was equivocal, a question mark was added. Some slides had multiple specimens on them and so each specimen was assigned a number, written on the coverglass in ink. As an example, slide 6.43 (ICHUM-1797) had seven specimens on it; these are designated WM 1 to WM 7 becoming (ICHUM-1797 to ICHUM-1806) with an identification given for each, which were indicated both on the slide label and in Table 1. Where the mounting medium was damaged the specimens were remounted and this is indicated by an asterisk (*) in Table 1. In one case, the four specimens on slide 6.58 (ICHUM-1836) were remounted on new, separate slides and cataloged as 6.59 to 6.62 (ICHUM-1837 to ICHUM-1840), and the original slide (6.58) with its written characters was returned to its original slot. Six slides (box/slot nos. 2.82, 2.97, 3.26, 3.67, 4.48, 4.76) had either been broken into many pieces and/or the specimens had cracked into small pieces, and were beyond restoration. These destroyed slides were not returned to the original slide box slots, but they were assigned museum catalog numbers so their fate could be traced when the original laboratory records are found.

In 2018, ten slides with branchiobdellidans were found scattered among Prof. Yamaguchi's oligochaete collection in Hakodate University. The specimens were in poor condition, and have not been entered into the museum's register, but placed at the end of Table 1 for completeness.

Results and Discussion

The rediscovered slide collection was found to contain specimens of all 17 species described by Prof. Yamaguchi, together with specimens of three other species described after his death. These latter species included *Holtodrilus truncatus* (Liang, 1963) (slides 4.14 and 4.15), *Branchiobdella macroperistomium* Liu & Zhang, 1983 (slides 5.4 and 5.18), and *Branchiobdella monodontus* Liu & Zhang, 1983 (slides 4.8 and 4.9). Yamaguchi (1934:177, 178) obtained specimens of the European *Branchiobdella pentadonta* Whitman, 1882, from Prof. W. Michaelsen of Hamburg, Germany, for comparison with his Oriental material. Whole mounts on slides 5.1 (ICHUM-1683), 5.2 (ICHUM-1684), 5.3 (ICHUM-1685), 5.73 (ICHUM-1728) carry the name *B. pentadonta* (Table 1). It is probable that some of the other specimens supplied by Michaelson were sectioned by Yamaguchi and placed in the collection. Such material could be identified in the future as the male reproductive organs and number of paired testes in *Branchiobdella* differs from those in *Cirrodrilus*.

The type description of *Cirrodrilus cirratus* Pierantoni, 1905 was based on poorly preserved and deformed specimens; the type having its body rotated 180º in relation to its head, hence dorsal appendages were called "ventral cirri" (Pierantoni 1905: fig. 1). Yamaguchi (1932a) made a detailed redescription of *C. cirratus,* and as a result was able to recognize a different but very similar species, *Cirrodrilus uchidai* (Yamaguchi, 1932). Although he studied their reproductive systems, little information was included in his paper. Subsequent studies of *C. uchidai* specimens showed that some morphological characters—body length, peristomial tentacle length and the presence and number of dorsal segmental appendages—were quite variable (Yamaguchi 1934: 212, fig. 15 A, B, C, and D). Whether such variablity is intraspecific or an indication of a species complex is unknown; however, the specimens examined by Yamaguchi—and recently by us as discussed in this paper—are cataloged in the ICHUM collection for future investigation.

Pierantoni (1906) described a second Japanese species, but because it lacked segmental "cirri" he placed it in a new genus, *Stephanodrilus*. He (Pierantoni 1912) then described another two species and placed them in the same genus. In subsequent works these species were assigned to various genera, but Yamaguchi (1934) reverted back to using *Stephanodrilus* (Gelder & Ohtaka 2002). Professor Yamaguchi gave a few of his specimens to Prof. P.C. Holt, and this allowed Holt (1967: 2–3), using the Principle of Priority as presented in Art. 23.1 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 2000), to state that *Cirrodrilus* has historical priority and is therefore the correct name for the genus.

The jaws provide a major diagnostic character for *Cirrodrilus nipponicus* (Yamaguchi, 1932c). The ends of the ridge supporting the small teeth on the anterior-facing surface of the jaws curve inwards toward their respective bases. This curling of the lateral ends is not always easy to distinguish, but it is clearly shown in slides 1.4 (ICHUM-1399), 1.86 (ICHUM-1463), and 1.88 (ICHUM-1464).

Yamaguchi (1934: 198, 199) recognized that only three character states separate *Cirrodrilus ezoensis* (Yamaguchi, 1934) from *Cirrodrilus megalodentatus* (Yamaguchi, 1934), as the two species are identical in all other respects. These characters are: the position of the anterior nephridiopores on segment 3, and the difference in size and number of small teeth on the dorsal jaws. In the specimens available, the position of the anterior nephridiopores could not be determined. The number of small teeth on the dorsal jaw of *C. ezoensis* is eight and for *C. megalodentatus* is six (Yamaguchi 1934:197, 198); however, these numbers were observed to vary by plus or minus two. The difference in size of the dorsal jaw in the two species is significant, and this was verified by measuring the dimensions of the dorsal jaws from both species. The dorsal jaw in ten specimens of *C. ezoensis* was measured (width of jaw base, 15.0 to 15.2 mm; height of median tooth from base to apex, 15.8 to 20.5 mm) and compared to measurements made on 11 specimens of *C. megalodentatus* (width of jaw base, 25.3 to 30.0 m; height of median tooth from base to apex, 26.8 to 31.6 m). When the jaws are in a direct dorso-ventral orientation, the sides of the central, dorsal tooth appear straight in *C. ezoensis* but convex in *C. megalodentatus*. Although shown by Yamaguchi (1934:197, fig.7B, and 198, fig. 8B), this feature was not easy to recognize until the syntype specimens were found for reference (Table 1).

Two specimens of *Hidejiodrilus koreanus* (Pierantoni, 1912) were identified (slides 1.46 (ICHUM-1431) and 1.99 (ICHUM-1466) based on the characteristic appearance of the jaws, with all of them having a 3-1-3/ 3-1-3 dental formula. This is an important discovery as the type material no longer exists in the Hamburg Museum, Germany (Gelder 1987). Gelder & Brinkhurst (1990) recognized this species was not a member of the *Cirrodrilus* and transferred it to the new monotypic *Hidejiodrilus* based on the type description. As this did not include any details of the male reproductive system, the new genus could not be assigned to a family and was designated *incertae sedis* by Gelder (1996). After receiving specimens from Korea, Gelder (2010) redescribed the species, including the male reproductive system, which allowed the genus to be placed in the subfamily Bdellodrilinae. The examined specimens were then designated as a neotype and two neoparatypes.

Signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), from Oregon, U.S.A., were introduced into Japan five times between 1926 to 1930 (Kamita 1970; Kawai 2017), and they carried branchiobdellidans which were named, *Cambarincola okadai* Yamaguchi, 1933. The type description only included body shape and jaw structure, and these were similar to the subsequently described *Triannulata montanus* Goodnight, 1940 from Washington State, USA. Holt (1974) recognized the latter species had been placed incorrectly in the *Triannulata*, and transferred it to *Cambarincola montanus* (Goodnight, 1940). By this time the Yamaguchi collection was presumed lost, and so Holt and Opell (1993: 253) considered *C. okadai* to be *nomen inquirendum*. During the examination of the rediscovered collection, four specimens of *C. okadai* were identified, including the individual drawn in Yamaguchi's (1933: fig. 1) type description. This resulted in a redescription of the species, the names *Triannulata montanus* and *Cambarincola montanus* reduced to junior synonyms, and the four specimens being designated as syntypes (Gelder & Ohtaka 2000b). Three of these specimens were deposited in the Invertebrate Collection of the Hokkaido University Museum, Sapporo (ICHAM)—slide 2.20 is designated the lectotype (ICHUM-1347) and slides 2.4 and 2.10 become paralectotypes (ICHUM-1348, ICHUM-1349). The fourth specimen—a third paralectotype, slide 2.9 (USNM 186575)—was placed in the National Museum of Natural History—Smithsonian Institution, Washington D.C., U.S.A.

Information from a series of taxonomical studies by Yamaguchi, plus the history of Prof. Yamaguchi's research (Mikami 1963) and an interview with Prof. Kamihira by Prof. Ohtaka, has enabled the status of some identified specimens to be determined. Yamaguchi's (1934) collection and descriptions of six species, *Cirrodrilus inukaii, C. aomorensis, C. ezoensis, C. megalodentatus, C. makinoi* and *C. chosen*, are only found in that publication, hence these specimens were designated syntypes. In contrast, additional specimens were used to supplement the original descriptions for three Japanese species (*Cirrodrilus uchidai, C. homodontus* and *C. nipponicus*) and four mainland species (*Branchiobdella orientalis, B. kobayashii, C. kawamurai* and *C. suzukii*), therefore these slides in the collection probably contain both original (syntype) and additional (non-type) specimens; hence they are labeled as "syntype ?" in Table 1.

The Yamaguchi slide collection provides an invaluable resource for future research on East Asian branchiobdellidans, and this review of current information will greatly assist researchers, particularly those investigating the sectioned material in the collection.

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Citations for the published proceedings of our 14 International Symposia on Aquatic Oligochaetes (1979–2018)

MARK J. WETZEL
General Secretary, ISAO. Illinois Natural History Survey, Champaign, IL 61801 USA

These special publications, below, present overviews of and papers presented during our triennial international symposia on aquatic oligochaete biology. Three similar names for our symposia have been used since we began as a group in 1979: International Symposium on Aquatic Oligochaete Biology (ISAOB), International Symposium on Aquatic Oligochaetes (ISAO), and International Symposium on Aquatic Oligochaeta (ISAO).

The proceedings of the first symposium were published in a hard-bound book (Brinkhurst and Cook 1980). The proceedings of the second through ninth symposia were published as part of the regular series of the journal, *Hydrobiologia*, and were also reprinted and bound for symposium participants (and the scientific community) in a regular series entitled *Developments in Hydrobiology*. The proceedings of the 10th symposium were published in a supplement to volume 31 of the journal, *Acta Hydrobiologica Sinica*; the proceedings of the 11th symposium were published in the *Turkish Journal of Zoology*; the proceedings of the 12th symposium were published in *Zoosymposia*; the proceedings of the 13th symposium were published in a special volume of the journal, *Biologia*, and the proceedings of the 14th symposium are presented in this current volume of *Zoosymposia*.

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