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PENSOFT

Cognettia sphagnetorum (Vejdovský, 1878) (Annelida, Clitellata, Enchytraeidae) in North America, confirmed with molecular support

Will K. Reeves[1*], Jeremy R. Shaw[2], Mark J. Wetzel[3]

1 C. P. Gillette Museum of Arthropod Diversity, Fort Collins, Colorado, USA • wkreeves@gmail.com

2 Department of Forest and Rangeland Stewardship, Colorado State University, Fort Collins, Colorado, USA • jeremy.shaw@colostate.edu

3 Illinois Natural History Survey, Prairie Research Institute at the University of Illinois at Urbana-Champaign, Illinois, USA • mjwetzel@illinois.edu

*Corresponding author

Abstract

Cognettia sphagnetorum (Vejdovský, 1878), a common inhabitant of forest soils and bogs in northern Europe, is a model organism in soil biology. We report the first documented occurrence of *C. sphagnetorum* in North America, based on DNA sequencing from a *Sphagnum* bog in western Washington, USA. Sequences were identical to that of worms from Sweden and the Czech Republic.

Keywords

Chamaedrilus, microdriles, potworms, *Sphagnum* bog, Washington, COI DNA barcodes

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Introduction

Potworms are oligochaetes in the family Enchytraeidae (Annelida, Clitellata) that are common and often dominant inhabitants of soils and other substrates throughout the world. While most enchytraeid species are terrestrial and recorded in high abundance in acidic soils with high organic matter content, many species are found in moist semiterrestrial habitats (including streams, wetlands, springs, fens, bogs, other surfaces) and subsurface freshwater, estuarine, marine, and limicolous habitats, and glaciers. Their abundance in boreal and northern temperate zones and sensitivity to drought have been suggested as evidence of an Arctic origin. *Cognettia sphagnetorum* (Vejdovský, 1878), a common inhabitant of forest soils and bogs in northern Europe, has been a model organism in soil biology research (Martinsson and Erséus 2014).

During the course of peat monolith extraction as part of a peatland paleoecological study, we observed microdrile oligochaete worm densities of 10^3 to 10^4 individuals/m^3 throughout a raised hummock within a *Sphagnum* L. bog in western Washington, USA. A bulk peat sample containing live worms was collected for identification. Specimens of enchytraeids were discovered that resembled species in the *Cognettia sphagnetorum* complex. The recent revision of *Cognettia* as *Chamaedrilus* Friend, 1913 by Martinsson et al. (2015a) showed that morphologically identical species were in the *C. sphagnetorum* complex and we chose to use molecular tools to identify the worms.

The oligochaete genus *Cognettia* Nielsen & Christensen, 1959 (Enchytraeidae) was established by Nielsen and Christensen (1959), with *Pachydrilus sphagnetorum* Vejdovský, 1878 as its type species and including four other species, each previously described in other genera: *Cognettia glandulosa* (Michaelsen, 1888), *Cognettia paxi* (Moszyński, 1938), *Cognettia anomala* (Černosvitov, 1928), and *Cognettia cognettii* (Issel, 1905). Molecular studies published by Martinsson and Erséus (2014) concluded that *Cognettia sphagnetorum* (Vejdovskýi, 1878), the morphotaxon by Nielsen and Christensen (1959), is a complex of several species that do not form a monophyletic group. *Cognettia sphagnetorum* s. str. is the type species and was differentiated from other morphotaxa by molecular tools by Martinsson et al. (2015a). Martinsson (2019) recently published a morphological key to the *Cognettia* species of the world, recognizing 20 nominal species in the genus; subsequent to that paper, a 21st species in this genus, *Cognettia koreana* Felföldi, Dózsa-Farkas, Nagy & Hong, 2020, was described from Korea (Felföldi et al. (2020).

Healy (1989, 1996) tentatively reported *C. sphagnetorum* from western Florida, although based on just a few juvenile specimens, and Wetzel et al. (2021) included these records. Schlaghamerský (2013a) documented what is believed to be the first record (based on the morphology of live specimens) of *C. sphagnetorum* in North America, summarized from his faunistic and ecological studies of enchytraeid assemblages in old-growth forests in the upper peninsula of the state of Michigan. Martin et al. (2017) noted seven aquatic or semiaquatic enchytraeid genera as occurring in North America, but without listing those genera by name. Publications discussing aquatic and terrestrial Enchytraeidae in North America are presented in Schlaghamerský and Wetzel (2021). To date, the reported distribution of *C. sphagnetorum* includes Denmark, Czech Republic, Germany, Iceland, Ireland, Isle of Man, Finland, Italy, Norway, Poland, Portugal, Romania, Russia, Scotland, Serbia, Slovakia, Spain, Sweden, Switzerland, USA, Wales, and the Antarctic region (South Georgia) (Vejdovský 1878; Block and Christensen 1985; Healy 1989, 1996; Martinsson and Erséus 2014; Martinsson et al. 2015a, 2015b; Cui et al. 2015; Martinsson et al. 2017; Martinsson 2019; Nurminen 1965 Schenková et al. 2018; Schlaghamerský 2013a; Schmelz and Collado 2010).

We report the range extension of *C. sphagnetorum* based on morphology and DNA sequencing from a *Sphagnum* bog in western Washington, USA.

Methods

Specimens were collected from a forested *Sphagnum* bog in King County, Washington, USA, during the course of extracting hummock monoliths as part of a paleoecological study (Fig. 1). The site is a 10 ha peatland composed of *Sphagnum* spp. and *Pleurozium schreberi* (Brid.) Mitten (Red-stemmed Feather Moss) with a *Rhododendron groenlandicum* (Oeder) Kron & Judd (Bog Labrador Tea), and *Kalmia microphylla* (Hook.) A.Heller (Western Bog Laurel) understory, and an overstory dominated by *Tsuga heterophylla* (Raf.) Sarg. (Western Hemlock).

A 100 cm tall peat hummock was dissected using pruning shears and a serrated knife to allow complete

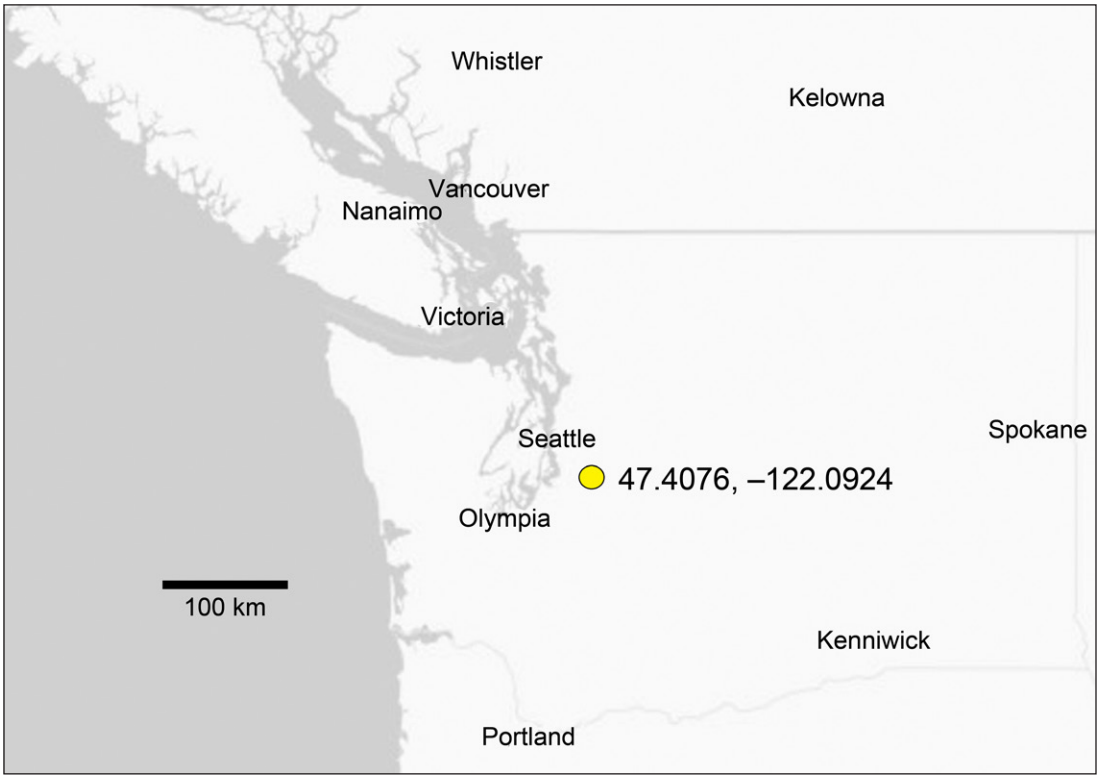

Figure 1. *Cognettia sphagnetorum* (Vejdovský, 1878). Yellow dot denotes the collection site in Washington, USA.

viewing of the upper peat profile and monolith extraction. The worms were present at between 40 and 100 cm above the water table. Approximately 20 live individuals were collected on 7 January 2019 by removing a ~250 cm^3 bulk peat sample from the hummock, which was stored in a plastic bag and refrigerated until examined on 24 January 2019.

Live worms were removed from wet peat in the laboratory with forceps when sorting small portions of the sample under a dissecting microscope. Worms were initially morphologically identified as Enchytraeidae and resembled *C. sphagnetorum*, which was later confirmed by microscopy. They were preserved in 75% ethanol and later transferred to 99% ethanol for storage. Worms were then washed in ethanol to remove soil, with an intact worm selected for DNA extraction. DNA was extracted with an UltraClean Tissue and Cells DNA Isolation Kit (Qiagen, Germantown, Maryland) following the manufacturer's protocols for invertebrates as described by Colton et al. (2019). A fragment of the cytochrome oxidase I (COI) gene was amplified by PCR using primers LCO1490 and HCO2198 following the original protocols by Folmer et al. (1994) and later techniques presented by Reynolds et al. (2020) when studying parasites of annelids. As positive and negative controls, we verified that PCR primers worked on a different enchytraeid from Colorado, USA, extracted in the same way, and we used distilled water as a negative control.

PCR products were sequenced using a DNA Clean and Concentrator Kit (Zymo, Irvine, California), and Sanger sequencing was performed by GENEWIZ (South Plainfield, New Jersey). The primer sequences were deleted by hand and the remaining sequence were aligned and assembled with ClustalW (Kyoto University Bioinformatics Center, Japan) and compared to sequences in GenBank using the BLAST program (NCBI, Bethesda, Maryland). A 658 bp COI sequence was submitted to GenBank. Whole, unmounted morphologically identified voucher specimens of *C. sphagnetorum* were accessioned and deposited in the Illinois Natural History Survey Annelida Collection, Champaign, Illinois, USA (n = 3) and the C.P. Gillet Museum, Fort Collins, Colorado, USA (n = 1).

Results

DNA sequencing was successful and after assembly produced a clean 658 bp COI barcode, which was a 100% match to COI sequences of *C. sphagnetorum* from Gothenburg, Sweden, and the Czech Republic (GenBank KM874811 and KM874810.1) that was part of the study by Martinsson et al. (2015a). The positive control was successfully amplified as a *Fridericia* sp. (GenBank MT356951.1). The negative control produced no amplicon.

New record. USA • Washington, King County, Shadow Lake Nature Preserve, 3.8 km WNW of the town of Maple Valley; 47.4076, −122.0924; elevation 180 m (Fig. 1: inset); 7 January 2019; Jeremy R. Shaw leg.; [20 worms]; GenBank MK618581.1; INHS AnnColl 10436-549; collection database SerialFJID 6422.

Identification. Worms were identified to family by gross morphology and then by DNA sequencing as described in the methods. The COI DNA sequences were 100% identical to other *C. sphagnetorum* s. str. and species in the group are currently morphologically indistinguishable.

Discussion

Our genetic analysis provides the first conclusive evidence that *C. sphagnetorum* s. str. occurs in North America. Martinsson et al. (2015a) described several morphologically identical species in the *C. sphagnetorum* complex based on molecular data but our molecular data matches that of their *C. sphagnetorum* s. str. As noted previously by Schlaghamerský (2013a), this species was reported in Michigan, USA, but the high diversity of cryptic species in the *sphagnetorum* complex (Martinsson et al. 2015a) hampers morphological determination and added some doubt to those records. That report, and earlier tentative reports from Florida, USA (Healy 1989, 1996) were based on sexually immature individuals and fragments, which precludes definitive identification from morphological characteristics.

The DNA sequence from our sample being an identical match to recently published European sequences of *C. sphagnetorum* (Fig. 2) from Sweden and the Czech Republic by Martinsson et al. (2015a) indicates a possible accidental introduction to the vicinity of our study site may have resulted from global horticultural trade, as have the numerous introductions of megadrile oligochaetes from Europe and Asia. It is possible that *C. sphagnetorum* were in imported *Sphagnum* peat that is used in growing media or in containerized plants, although we are not aware of any such reports. Shadow Lake Bog is in an exurban area with several private

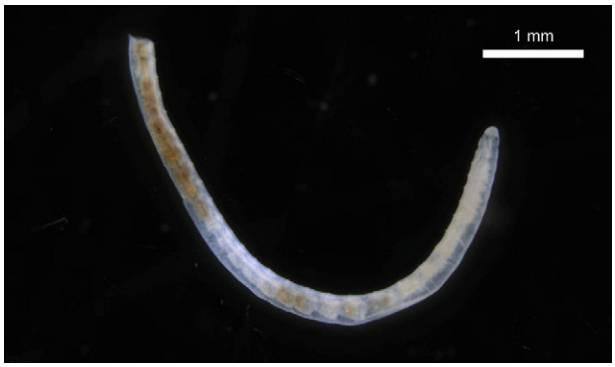

Figure 2. *Cognettia sphagnetorum* (Vejdovský, 1878). Photomicrograph CE18922, courtesy Márten Klinth (born Eriksson), University of Gothenburg, Sweden; note absence of posterior end of specimen, removed for sequencing. The photo is reproduced from BOLD under Creative Commons Attribution NonCommercial ShareAlike (CC BY-NC-SA 3.0).

residences within 2 km of the peatland perimeter, so it is plausible that ornamental plantings were the source of this population.

Emphasizing the discussions in Martinsson and Erséus (2014, 2018, 2021), Martinsson et al. (2015), and Martinsson et al. (2018), cryptic species—those that are morphologically indistinguishable from one another or have been considered so similar that they had been classified as the same species (see also Bickford et al. 2007)—are common among the enchytraeids and other clitellate oligochaetes. In their overview of cryptic species in the Enchytraeidae, Schmelz et al. (2017) presented recommendations for the study and description of cryptic species in the family. Recently, Martinsson and Erséus (2021) presented a historical review of data and methods that have been used to delimit clitellate oligochaetes, followed by a discussion on taxonomic treatment of cryptic species, and recommendations supporting the description and naming of cryptic species whenever possible.

We reiterate the importance of proper collecting, processing, and preservation methodologies, and the use of molecular identification techniques during taxonomic and systematic studies of enchytraeids and other cryptic clitellate taxa.

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Authors' Contributions

JRS collected worms, initially identified them, wrote and revised drafts of the manuscript. WKR identified worms with microscopy and molecular biology and wrote and revised drafts of the manuscript. MJW identified worms with microscopy, curated museum specimens, conducted literature reviews, and wrote and revised drafts of the manuscript.

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