Fragmenta Faunistica 68 (1): 71–77, 2025
PL ISSN 0015-9301 © Museum and Institute of Zoology PAS
DOI 10.3161/00159301FF2025.68.1.09

Cylindroiulus vulnerarius (Berlese, 1888) (Myriapoda: Diplopoda: Julida), a new addition to the Polish millipede fauna

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Abstract: We present the first record of *Cylindroiulus vulnerarius* (Berlese, 1888) from Poland, together with notes on possible pathways of its introduction, its habitat preferences, and its distribution in Europe. Species identification is additionally supported by DNA barcoding.

Key words: millipedes, Julidae, DNA barcoding, exotic species, urban areas, human activities

Introduction

Genus *Cylindroiulus* Verhoeff, 1894, comprises over 100 species, making it one of the largest in the family Julidae (Read 2007). So far, nine species of this genus have been recorded in Poland (Wytwer 2008): *Cylindroiulus arborum* Verhoeff, 1928, *Cylindroiulus britannicus* (Verhoeff, 1891), *Cylindroiulus burzenlandicus* Verhoeff, 1907, *Cylindroiulus caeruleocinctus* (Wood, 1864), *Cylindroiulus horvathi* (Verhoeff, 1897), *Cylindroiulus latestriatus* (Curtis, 1845), *Cylindroiulus parisiorum* (Brölemann & Verhoeff, 1896), *Cylindroiulus punctatus* (Leach, 1816), *Cylindroiulus truncorum* (Silvestri, 1896). Some members are found throughout the country, such as *C. caeruleocinctus*, which is very common at synanthropic sites in Poland, except the eastern and southern parts of the country. Some other representatives of the genus are rare and restricted to natural habitats in southern Poland, such as the Carpathian species *C. burzenlandicus*. There are also alien species, such as *C. truncorum* (Stojałowska 1961, Stojałowska & Staręga 1974). *Cylindroiulus vulnerarius* (Berlese, 1888) stands out due to its unusual appearance among Poland's fauna. It is a pale, eyeless julid millipede with an uncertain geographic provenance. It is speculated to have Italian origins; however, this is not entirely clear, as it has spread to many other European countries, where it is mainly found in urban and suburban areas (Kime & Enghoff 2017). Since it has been frequently recorded in Atlantic parts of Europe, such as caves in Belgium, it is possible that the species survived the glacial periods in these areas (Kime & Enghoff 2017, Gilgado 2020).

Molecular techniques such as DNA barcoding offer a reliable approach to identifying species that are often nearly indistinguishable morphologically (Hebert et al. 2003). In the case of myriapods, it is particularly useful, since females and juveniles are often impossible to identify based on morphology (Wesener et al. 2016). Here we report the first record of *C. vulnerarius* in Poland, supported by DNA barcode data.

Materials and Methods

The sampling took place in Żeromski Park in Szczecin (Poland) (Fig. 1) on February 19, 2023, during preliminary research on myriapod fauna in Szczecin. Żeromski Park was established in 1795 and currently serves as a public arboretum. It's the second-largest public park in Szczecin. It consists of both native tree species to the region, such as *Fagus sylvatica* L., *Tilia tomentosa* Moench, and *Quercus robur* L., as well as exotic trees from other parts of Europe and Asia, such as *Pterocarya fraxinifolia* (Lamb.). Spach, *Maclura pomifera* (Raf.) C.K. Schneid. and *Quercus cerris* L. (Portal Systemu Informacji Przestrzennej Miasta Szczecin 2025). The sampling of the park was performed using direct search and arthropods were collected directly by hand. The collected material (one female specimen) was preserved in 76% ethanol for further examination. The individual was identified using both morphological features and DNA barcoding. Later, we took a photo of specimen using a stereomicroscope LeicaM205 C and the LAS X software.

Figure 1. A - Szczecin, the locality where *Cylindroiulus vulnerarius* (Berlese, 1888) was found, B - location of Żeromski Park in the city of Szczecin, C - the exact location in Żeromski Park where the individual was collected (photo by Przemysław Piekarczyk).

To confirm the taxonomic identification of this female individual, PCR amplification, quality control, and Sanger sequencing of the DNA barcoding fragment were performed. The PCR reaction conditions and amplicon purification followed the procedures described by Querner et al. (2022). The Sanger sequencing of the purified PCR products with the forward primer was outsourced to Macrogen Europe BV. The resulting sequence was deposited and is publicly available via the Barcode of Life Datasystems (BOLD) (Ratnasingham & Hebert 2007) under the dx.doi.org/10.5883/DS--CYLEU (A specimen process ID for *C. vulnerarius* from Poland: MYRPL771-25), and in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (acc. num. PX894799-PX894847).

Sequences were analysed following the procedures outlined by Bienias et al. 2025; consisting in the alignment with MUSCLE (Edgar 2004) in Geneious 10.2.6 (Kearse et al. 2012), followed by manual curation of the alignment, and finally using a quality control for artifacts such as stop codons, double peaks, and frameshifts.

Analyses included the construction of a Neighbour-Joining tree (Saitou & Nei 1987) under the K2P model (Kimura 1980) with 500 bootstrap replicates (Felsenstein 1985), implemented in MEGA XI (Tamura et al. 2021). For the NJ tree, we used publicly available sequences of the following European species: *C. britannicus*, *C. caeruleocinctus, C. latestriatus*, *C. parisiorum*, *C. punctatus, and C. vulnerarius*. A single sequence of *Kryphioiulus occultus* (C. L. Koch, 1847) was included as the outgroup.

Results

A single, adult female specimen of millipede, characterised by a white head and pale greenish brown tergites (Fig. 2), was discovered under leaf litter and within the soil surrounding a growing tree in the northwestern part of the park (53.434498, 14.564900, 26 m a.s.l.) (Fig. 1C). Other myriapod taxa were recorded in the same area: *Cylindroiulus caeruleocinctus*, *Geophilus flavus* (De Geer, 1778), *Lithobius forficatus* (Linnaeus, 1758), *Stenotaenia linearis* (C.L. Koch, 1835) and *Stigmatogaster subterraneus* (Shaw, 1794). Additional searches for the millipede were conducted in November 2023 and 2024, and in April 2025. However, no further specimens were found. Because the collected specimen was female, gonopod-based identification was not possible. Moreover, although the vulvae were extracted, it was not analysed in detail for the purpose of this study. Nevertheless, even based on external morphology, several characteristics – including size (19.46 mm in length, 1.51 mm in width), the absence of eyes, the presence of a telson with a well-developed, slightly bent dorsal projection, the orange colouration of the ozopores (in alive animal), and the generally pale body – excluded all other millipede species known from the national fauna and pointed out to *Cylindroiulus vulnerarius*. Finally, specific taxonomical characters were used to separate *C. vulnerarius* from two similar European species: *Metaiulus pratensis* Blower & Rolfe, 1956, which is easily distinguished by the absence of a well-developed and pointed epiproct, and *Cylindroiulus salicivorus* Verhoeff, 1907, which possesses eyes (Blower & Rolfe 1956, Verhoeff 1908).

Figure 2. Habitus of *Cylindroiulus vulnerarius* (Berlese, 1888), female, lateral view (photo by Szymon Kaczmarek).

The taxonomical identification of the specimen was further confirmed using DNA barcoding. Our sequence clusters in the NJ tree with other *C. vulnerarius* specimens from the UK, forming a well-supported clade (Fig. 3), ,therefore confirming the previous morphological identification of the female specimen collected in Poland.

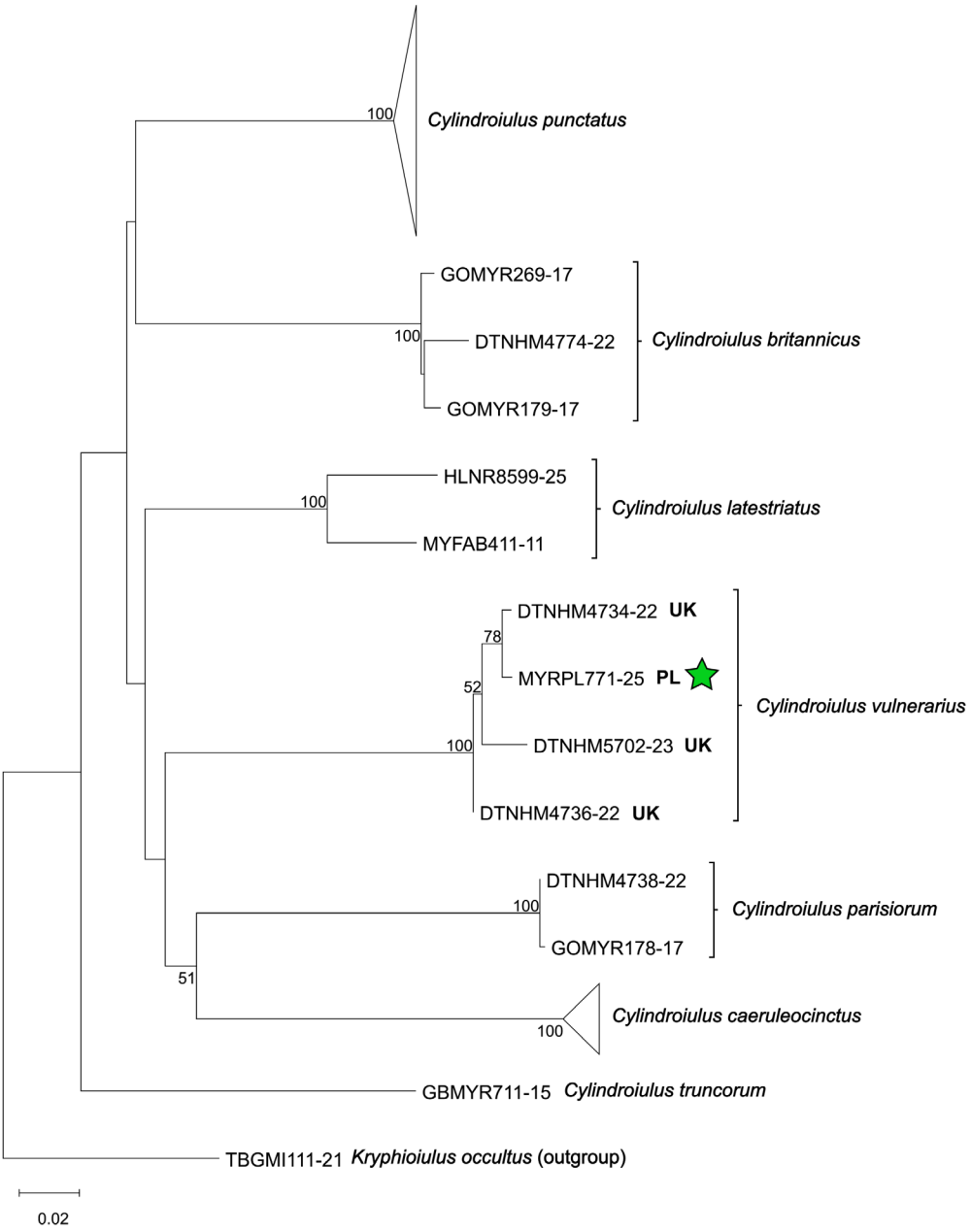

Figure 3. Neighbour-joining tree of COI sequences representing different *Cylindroiulus* species, including *C. vulnerarius*, with the Polish specimen marked by a green star.

Discussion

According to recent literature (see below), *C. vulnerarius* seems to be expanding its distribution into urban and suburban areas across Europe. However, it remains unclear whether the finding in Żeromski Park in Szczecin represents an isolated occurrence or indicates the presence of an established population. Given that only a single female specimen was found, it is possible that the individual was introduced accidentally, perhaps with plant material. The closest known occurrences of *C. vulnerarius* to Poland are in the neighbouring countries, i.e., Czechia, Germany, and Slovakia; where its presence is also linked to anthropogenic habitats like urban areas and greenhouses (Kocourek 2003, Decker et al. 2014, Kime & Enghoff 2017, Haľková et al. 2021). Given the proximity of Szczecin to the German border, it is likely that the specimen could be introduced from Mecklenburg-Vorpommern or Brandenburg – particularly considering records of this species in greenhouses at the Potsdam Botanical Garden, near Berlin (Decker et al. 2014) – a location well connected to Szczecin (approximately a two-hour drive).

Our NJ analysis showed that the Polish female of *C. vulnerarius* clustered with nearly identical COI sequences from the UK, forming a strongly supported clade. This highlights the reliability of DNA barcoding as a robust tool for confirming species identities in millipedes and other invertebrates. This is particularly important in cases where morphological determination - such as in females of millipedes - is often challenging. The minimal divergence observed among these sequences suggests low intraspecific variability and a genetically stable species. However, more comprehensive sampling across a broader European range, including population boundaries, is necessary to draw definitive conclusions about the species' genetic structure.

Acknowledgements

The study was done within the Polish Barcode of Life (PoLBOL, https://www.polbol.uni.lodz.pl/) framework. Special thanks to Prof. Małgorzata Leśniewska for her help in the identification of the specimen, as well as to Prof. Michał Grabowski for his help in editing the manuscript.

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Streszczenie

***Cylindroiulus vulnerarius* (Berlese, 1888) (Myriapoda: Diplopoda: Julida), nowy gatunek w faunie krocionogów Polski**

Prezentujemy pierwsze stwierdzenie *Cylindroiulus vulnerarius* (Berlese, 1888) w Polsce, omawiamy możliwe drogi jego introdukcji oraz wymagania siedliskowe i rozmieszczenie w Europie. Identyfikacja gatunku została dodatkowo potwierdzona za pomocą barkodingu DNA.

Received: 9 December 2025

Accepted: 29 December 2025