

Rapid Communication**Overlooked expansion? The case of the millipede *Polydesmus angustus* Latzel, 1884 in Poland**Jakub Bienias¹, Karin Voigtländer², Łukasz Trębicki¹, Julia Kościelniak¹ and Michał Grabowski¹¹University of Łódź, Faculty of Biology and Environmental Protection, Department of Hydrobiology and Invertebrate Zoology, Banacha 12/16, 90-237 Łódź, Poland²Senckenberg Museum of Natural History, Am Museum 1, 02826 Görlitz, GermanyCorresponding author: Jakub Bienias (jakub.bienias1802@wp.pl)

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OPEN ACCESS**Abstract**

The expansion of myriapod species outside their native range is a well-known phenomenon, although well-studied only in the western part of Europe. Our study reports a recent rapid eastward range extension of *Polydesmus angustus* Latzel, 1884, from Germany to Poland, where its new eastern limit of distribution is probably the Vistula River. We confirm that the species occupies disturbed, anthropogenic habitats, supporting its human-mediated dispersal scenario. We also consider the possibility of competition between this species and its closest relative, *Polydesmus complanatus* (Linnaeus, 1761). We also point out that in the case of taxonomic groups containing externally similar species, the verification of citizen science-based records by specialists examining morphological details of specimens and, if possible, by DNA barcodes, is necessary for reliable insight into the range extension of alien species.

Key words: Diplopoda, range extension, anthropogenic habitats, DNA barcoding, citizen science**Introduction**

The expansion of myriapod species outside their native range is a well-known phenomenon. The available data are too limited to unequivocally determine their impact on colonised ecosystems, although there are reports that some species may cause severe damage to growing crops both in fields and greenhouses (Stoev et al. 2010). In Europe, one of the most spectacular examples of colonisation by non-native species is Iceland, where the majority of myriapod fauna descends from continental Europe and was unintentionally introduced with imported goods (Eason 1970). There are also several exotic myriapod species that have successfully established in Europe, such as the millipede *Oxidus gracilis* (C.L. Koch, 1847) or the centipede *Lamyctes emarginatus* (Newport, 1844) (Stoev et al. 2010). The spread of such species is often strongly associated with the transportation of habitat material, such as soil or ornamental plants (Faulkner et al. 2020, for an example see Bienias et al. 2021) or even with military vehicles (Decker et al. 2017). The genera

Polydesmus Latreille, 1802 and *Brachydesmus* Heller, 1858 are the most species-rich ones in the mainly Holarctic family Polydesmidae (Kime and Enghoff 2011). The first genus includes over 200 described species, most of which occur in Europe and the Mediterranean region. However, a small number of them are also known from Asia, with few reaching the southern parts of the continent (Nguyen 2009; Nguyen et al. 2024). So far, eight species of this genus have been recorded from Poland (Stojałowska 1961; Stojałowska and Starega 1974; Wytwer 2008). The most common and widely distributed one is *Polydesmus complanatus* (Linnaeus, 1761). It occurs predominantly in natural habitats, being often one of the most dominant millipedes (Wytwer and Tracz 2003). On the other hand, in synanthropic sites, it is often completely replaced by other congeneric and confamiliar diplopods, namely *Polydesmus inconstans* Latzel, 1884, *Polydesmus denticulatus* C. L. Koch, 1847 and *Brachydesmus superus* Latzel, 1884 (Stojałowska 1961). The other species, i.e., *Polydesmus polonicus* Latzel, 1884, *Polydesmus montanus* Daday, 1889, *Polydesmus tatranus* Latzel, 1882, *Polydesmus burzenlandicus* Verhoeff, 1925 and *Polydesmus komareki* Gulička, 1962, have much more restricted ranges and habitat preferences (Jędrzycki 1979; Stojałowska 1961).

Two decades ago, DNA barcoding was proposed as a handy, molecular identification tool for biomonitoring, especially for morphologically similar or indistinguishable species (Hebert et al. 2003) and, since then, has proven useful also in the detection of alien species and elucidating invasion processes (Comtet et al. 2015). In the case of myriapods in Europe, the method has been successfully used to characterise the local Central European fauna (Spelda et al. 2011), to explore cryptic diversity (Wesener et al. 2016), and also to confirm the identity of alien species (Decker et al. 2017).

Our paper aims to provide the first-ever records of *P. angustus* in Poland, supported by photographs of specimens and their gonopods. Given the morphological resemblance of *P. angustus* to other congeneric species, we confirm the identity of individuals collected in Poland by DNA barcodes. In addition, we analyse the publicly available online records from the iNaturalist citizen science platform, regarding their reliability and discuss the history and further perspectives for range expansion of the species in Central Europe and the associated risks. Finally, we point out habitats that are particularly vulnerable to colonisation and should be surveyed for the presence of these myriapods.

Materials and methods

Collection sites

All our records come from eight localities in Poland, west of the Vistula River (Table 1, Figure 1).

The first record of *P. angustus* in Poland comes from Kliczków, Lower Silesia, and was extracted from the GBIF information system for soil zoology

Table 1. Records of *Polydesmus angustus* in Poland.

Location (Coordinates)	Habitat type	Date	No. indiv. (Total/♂/♀/juv./ barcoded)	Co-occurring myriapod species
Gajków (51.052611, 17.190000)	Edge of riparian forest adjacent to Odra river	04.04.2024	<u>1</u> /0/1/0/0	<i>Geophilus flavus</i> (De Geer, 1778) <i>Julus scandinavicus</i> (Latzel, 1884) <i>Lithobius forficatus</i> (Linnaeus, 1758) <i>Lithobius microps</i> Meinert, 1868 <i>Lithobius mutabilis</i> L. Koch, 1862 <i>Lithobius tenebrosus</i> Meinert, 1872 <i>Nemasoma varicorne</i> C.L. Koch, 1847 <i>Ommatoiulus sabulosus</i> (Linnaeus, 1758) <i>Polyzonium germanicum</i> Brandt, 1837 <i>Proteroiulus fuscus</i> (Am Stein, 1857) <i>Schendyla nemorensis</i> (C.L. Koch, 1837) <i>Strigamia crassipes complex</i> (sensu Bonato et al. 2023)
Kraków (50.08660, 19.87695)	Garden	12.09.2021 10.10.2021	<u>16</u> /1/3/12/0	<i>Geophilus flavus</i> (De Geer, 1778) <i>Lithobius forficatus</i> (Linnaeus, 1758) <i>Proteroiulus fuscus</i> (Am Stein, 1857) <i>Stenotaenia linearis</i> (C.L. Koch, 1835) <i>Unciger foetidus</i> (C.L. Koch, 1838)
Kliczków (51.3392908, 15.4367425)	Mixed deciduous forest, heavily anthropogenically influenced	11.11.2017	<u>1</u> /0/1/0/0	<i>Craspedosoma rawlinsii</i> Leach, 1816 <i>Glomeris hexasticha</i> Brandt, 1833 <i>Leptoiulus proximus</i> (Němec, 1896) <i>Lithobius agilis</i> C.L. Koch, 1847 <i>Lithobius forficatus</i> (Linnaeus, 1758) <i>Lithobius microps</i> Meinert, 1868 <i>Lithobius tenebrosus</i> Meinert, 1872 <i>Mastigona bosniensis</i> (Verhoeff, 1897) <i>Nemasoma varicorne</i> C.L. Koch, 1847 <i>Polyzonium germanicum</i> Brandt, 1837 <i>Schendyla nemorensis</i> (C.L. Koch, 1837)
Sopot (54.463784, 18.560718)	City park with semi-natural forest, near Swelina river	16.06.2023 17.06.2023	<u>28</u> /5/14/9/6	<i>Cylindroiulus caeruleocinctus</i> (Wood, 1864) <i>Cylindroiulus punctatus</i> (Leach, 1816) <i>Kryphioiulus occultus</i> (C.L. Koch, 1847) <i>Leptoiulus proximus</i> (Němec, 1896) <i>Lithobius forficatus</i> (Linnaeus, 1758) <i>Lithobius microps</i> Meinert, 1868 <i>Ommatoiulus sabulosus</i> (Linnaeus, 1758) <i>Polydesmus denticulaus</i> C.L. Koch, 1847 <i>Polyzonium germanicum</i> Brandt, 1837 <i>Schendyla nemorensis</i> (C.L. Koch, 1837) <i>Unciger foetidus</i> (C.L. Koch, 1838)
Szczecin (53.452287, 14.518601)	Botanical Garden	30.05.2024	<u>13</u> /5/3/5/0	<i>Cylindroiulus caeruleocinctus</i> (Wood, 1864) <i>Kryphioiulus occultus</i> (C. L. Koch, 1847) <i>Lithobius forficatus</i> (Linnaeus, 1758)
Warszawa (52.165, 21.0525)	Skarpa Ursynowska Nature reserve	04.2024- 09.2024	<u>29</u> /-/-/0	<i>Allajulus nitidus</i> (Verhoeff, 1891) <i>Brachydesmus superus</i> Latzel, 1884 <i>Brachyiulus pusillus</i> (Leach, 1816) <i>Choneiulus palmatus</i> (Němec, 1895) <i>Cryptops hortensis</i> (Donovan, 1810) <i>Cylindroiulus caeruleocinctus</i> (Wood, 1864) <i>Geophilus flavus</i> (De Geer, 1778) <i>Geophilus truncorum</i> Bergsøe & Meinert, 1866 <i>Kryphioiulus occultus</i> (C.L. Koch, 1847) <i>Leptoiulus proximus</i> (Němec, 1896) <i>Lithobius forficatus</i> (Linnaeus, 1758) <i>Lithobius microps</i> Meinert, 1868 <i>Lithobius mutabilis</i> L. Koch, 1862 <i>Megaphyllum projectum kochi</i> (Verhoeff, 1907) <i>Melogona voigtii</i> (Verhoeff, 1899) <i>Ommatoiulus sabulosus</i> (Linnaeus, 1758) <i>Polydesmus complanatus</i> (Linnaeus, 1761) <i>Polydesmus inconstans</i> Latzel, 1884 <i>Proteroiulus fuscus</i> (Am Stein, 1857) <i>Rossiulus vilnensis</i> (Jawłowski, 1925) <i>Schendyla nemorensis</i> (C.L. Koch, 1837) <i>Strigamia acuminata</i> (Leach, 1815) <i>Unciger foetidus</i> (C.L. Koch, 1838)

Table 1. (continued).

Wrocław (51.105330, 17.139300)	Las Strachociński, Small oak forest, very close to the edge of city	02.04.2024	<u>1</u> /1/0/0/0	<i>Geophilus flavus</i> (De Geer, 1778) <i>Julus scandinavicus</i> (Latzel, 1884) <i>Lithobius crassipes</i> L. Koch, 1862 <i>Lithobius forficatus</i> (Linnaeus, 1758) <i>Lithobius microps</i> Meinert, 1868 <i>Lithobius mutabilis</i> L. Koch, 1862 <i>Ophiulus pilosus</i> (Newport, 1843) <i>Pachymerium ferrugineum</i> (C.L. Koch, 1835) <i>Polydesmus inconstans</i> Latzel, 1884 <i>Proteroiulus fuscus</i> (Am Stein, 1857) <i>Schendyla nemorensis</i> (C.L. Koch, 1837) <i>Strigamia crassipes complex</i> (sensu Bonato et al. 2023)
Żabie Doły (Bytom) (50.331859 18.956487)	Post-industrial reclaimed area with bodies of water, forested and open areas	28.09.2023 01.10.2023 20.10.2023 26.12.2023	<u>24</u> /1/4/19/4	<i>Blaniulus guttulatus</i> (Fabricius, 1798) <i>Cryptops parisi</i> Brölemann, 1920 <i>Cylindroiulus caeruleocinctus</i> (Wood, 1864) <i>Geophilus flavus</i> (De Geer, 1778) <i>Lithobius forficatus</i> (Linnaeus, 1758) <i>Lithobius melanops</i> Newport, 1845 <i>Lithobius microps</i> Meinert, 1868 <i>Melogona voighti</i> (Verhoeff, 1899) <i>Schendyla nemorensis</i> (C.L. Koch, 1837)

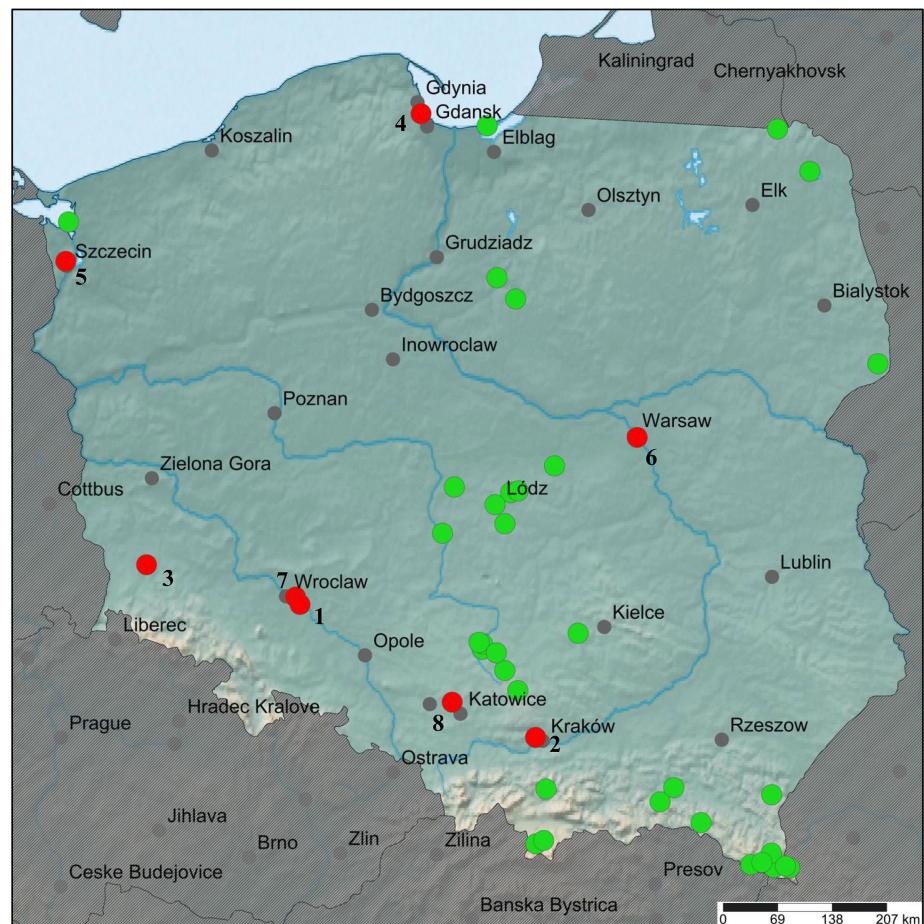


Figure 1. Records of *Polydesmus angustus* (red). 1 – Gajków, 2 – Kraków, 3 – Kliczków, 4 – Sopot, 5 – Szczecin, 6 – Warszawa, 7 – Wrocław, 8 – Żabie Doły (Bytom). Locations recently sampled for myriapods with no *P. angustus* recorded (green).

i.e., the Edaphobase (www.edaphobase.org, Burkhardt et al. 2014). Material from Bytom, Gajków, Kraków, Sopot, Szczecin, and Wrocław was collected by hand or using an entomological sieve, then, in most cases, preserved in 96% ethanol. Specimens from Warsaw were sampled using pitfall traps, soil

samples and by hand, then preserved in 99% ethanol. Photos of habitats were taken with smartphone Samsung Galaxy M31s and Apple iPhone 8. Photos of specimens and gonopods were taken with LeicaMDG41 and LAS X software.

Additionally, we analysed all of the *P. angustus* records from Poland that appeared on the iNaturalist platform (2024).

DNA barcoding

Isolation

The DNA barcode sequences provided for this study were obtained from ten individuals of *Polydesmus angustus* collected in Poland (Table 1). Total genomic DNA was extracted from multiple legs of each specimen, using the Chelex-based method (Casquet et al. 2012). The samples were PCR-amplified for different sequencing approaches: the traditional Sanger sequencing method (six individuals), and ONT (Oxford Nanopore Technologies) sequencing using the Flongle flow cell system (four individuals).

Sanger sequencing

The COI gene fragment was PCR-amplified with the primer pairs LCO1490-JJ/HCO2198-JJ (Astrin and Stüben 2008). The PCR reaction conditions as well as the amplicon purification followed the procedures described by Querner et al. (2022). The Sanger sequencing of the purified PCR products with forward primer was outsourced to Macrogen Europe BV.

ONT Sequencing

The DNA barcode sequencing on the ONT platform (Srivathsan et al. 2021) was done with the following procedure. To amplify the COI DNA barcode fragment for nanopore sequencing, we used the PCR primer pair of LCO1490-JJ/HCO2198-JJ (Astrin and Stüben 2008), tagged with 9 base pair index sequences on both the forward and reverse primer for each individual specimen. Indexed primers allowed for the unambiguous assignment of each sample during demultiplexing of nanopore sequencing reads. Primer tags were designed using Barcode Generator (Comai and Howell 2012), following Srivathsan et al. (2019).

The PCR cocktail in a total volume of 11 μ L per sample contained: 6.5 μ L Dream Taq polymerase (ThermoFisher), 1 μ L (5 μ M) of each, both indexed LCO1490-JJ and HCO2198- JJ primers, 3.5 μ L ddH₂O, and 1 μ L of DNA extract. The PCR program was as follows: initial denaturation of 5 min at 95 °C, five cycles of 50 s at 95 °C, 50 s at 45 °C, 60 s at 72 °C, then 35 cycles of 50 s at 95 °C, 50 s at 51 °C, 60 s at 72 °C; and final 2 min extension at 72 °C. A subset of the PCR products was run on a 1% agarose gel to ensure the PCR succeeded. We pooled 5 μ L of each of the PCR products into a single 1.5 μ L Eppendorf tube. This pool was then cleaned up with Sera-Mag™ Select (Cytiva LiveScience), cleaned pool was run on a 1% agarose gel to

ensure that the PCR product purification was successful. The final concentration of the pool was measured with a Qubit 4 Fluorometer using the Qubit dsDNA HS Assay Kit (Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA). The sequencing library was prepared using the Oxford Nanopore Ligation Sequencing kit version V14 (SQK-LSK114), following the manufacture protocols and sequenced on the Flongle (R10.4.1; FLO-FLG114) flow cell. In one Nanopore run, four *Polydesmus angustus* specimens were sequenced out of a total of 288 samples.

Base-calling of ONT Reads

Raw ONT data (fast5 format) were base-called with the latest version of Dorado (<https://github.com/nanoporetech/dorado>), under the base-calling model dna_r10.4.1_e8.2_400bps_sup@v4.3.0, on the Athena server (<https://www.plgrid.pl> accessed 4.04.2024). For demultiplexing of nanopore sequencing data and generating individual consensus barcodes we used ONTbarcoder (mismatch 0), under the default settings (Srivathsan et al. 2021)

Sequence analysis

The sequences were aligned in Geneious 10.2.6 (Biomatters Ltd.) package (Kearse et al. 2012), using MUSCLE (Edgar 2004) with default parameters. The resulting alignment was edited and trimmed of primers, and checked for the absence of stop codons, double peaks (in case of sequences generated in Macrogen) and frameshifts using Geneious 10.2.6 (Biomatters Ltd.). All the DNA barcode sequences have been deposited in BOLD (<https://www.boldsystems.org/>) and GenBank (<https://www.ncbi.nlm.nih.gov/>), along with relevant voucher information, taxonomic identification, and photos. The molecular data is publicly accessible through the dataset DOI dataset: <https://doi.org/10.5883/DS-PANGU>, in BOLD (www.boldsystems.org) (Ratnasingham and Hebert 2007), and in GenBank (accession numbers: PP695030–PP695039). The dataset also includes all publicly available COI sequences from BOLD identified as belonging to *Polydesmus* Latreille, 1802. In addition, we found three COI records for the genus in GeneBank, from which two were not considered in our analysis due to the presence of stop codons. The remaining COI fragment was extracted from the first published complete mitochondrial genome for the genus (Zuo et al. 2022). Sequences were clustered in BOLD into the molecular operational taxonomic units known as Barcode Index Numbers (BINs). The Neighbour-Joining distance tree (Saitou and Nei 1987) was constructed based on the K2P distance (Kimura 1980); bootstrap values were calculated with 500 replicates (Felsenstein 1985) as implemented in MEGA XI (Tamura et al. 2021). Estimates of inter- and intraspecific divergence and the corresponding standard errors were obtained under the MEGA XI (Tamura et al. 2021).

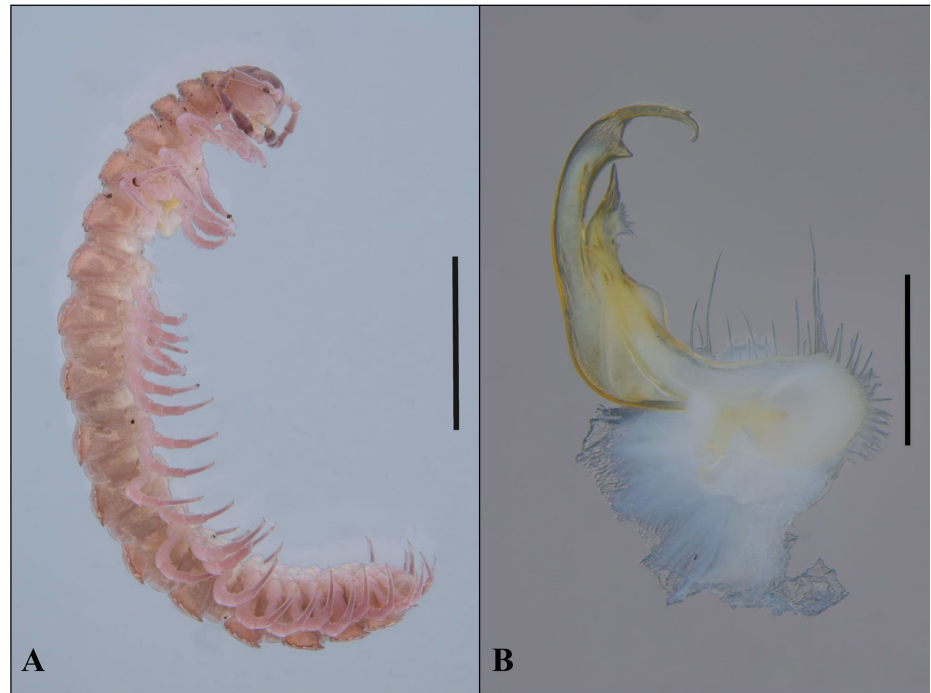


Figure 2. Habitus of *Polydesmus angustus*, ♂ (A), Gonopods (B). Scale bars: A = 0.5 cm; B = 0.5 mm. Photomicrographs by Jakub Bienias and Łukasz Trębicki



Figure 3. Habitats of *Polydesmus angustus* in Sopot (A) and Żabie Doły (B).

Results

Records

In total, we recorded 113 specimens (42 males, 26 females and 45 juveniles) of *P. angustus* (Figure 2A, Table 1). Twenty-eight individuals were collected in Sopot; a copulating couple in the compost pile on the IO PAN premises and the remaining 26 in the leaf litter of the less cultivated area (semi-natural forest) of the Park Północny, near the Swelina River (Figure 3A). In Warsaw, 29 individuals were collected in the semi-natural forest park on the western margin of the Vistula River.

During the investigation in Żabie Doły, we found 21 adults and juveniles of *P. angustus*. Most of the material was collected in forest patches, with small trees and shrubs, overgrowing rubble mixed with leaf litter (Figure 3B). Some records (Table 1) also come from more natural habitats like the ones from forests in Kliczków, Gajków or Wrocław. However, these sites are located in the immediate vicinity of urbanised areas, so that an anthropogenic influence cannot be ruled out. In Kraków, 16 individuals were found in the garden located in the city. Finally, 13 individuals were found in the Botanical Garden in Szczecin, under leaves and a fallen tree.

Regarding the records from iNaturalist, there are only a few observations claimed to represent *P. angustus* from Poland between 2013 and 2023. After examining the associated photos, we conclude that it is not possible to say unambiguously whether they represent *P. angustus*. Most of the specimens resemble adult individuals of *P. complanatus* or they are juveniles or smaller species, which cannot be reliably identified from the pictures alone. Therefore, we do not consider these records valid. Thus, we corrected their taxonomic assignments directly on the website.

DNA barcoding results

The final alignment comprised 65 COI barcoding sequences, each representing a single specimen, and had a length of 658 bp. The DNA sequences could be translated into amino acid sequences without any stop codons; the transition to transversion ratio amounted to 1.57. For most of the species and BINs analysed, the interspecific distances ranged from 9.84% to 17.73%, (Supplementary material Table S1). *Polydesmus angustus* clustered into the 7 BINs divergent by 4.07–4.64%. The closest species for *P. angustus* was *P. complanatus* with 9.84% of distance between the two (Table S1). All the sequences of *P. angustus* from Poland, were clustered with maximum branch support with the already published *P. angustus* sequences belonging to one haplotype of the BIN: BOLD-AAF1373, which was found before in other parts of Europe (France, Germany, Norway), but also in New York in the United States (Figure 4).

Discussion

The vicariant species pair *P. angustus* and *P. complanatus* used to show almost parapatric distribution in Central Europe. The main range of *P. angustus* included western Europe and the western half of Germany, while the eastern neighbouring range of *P. complanatus* extended far into eastern Russia. A century ago, the border between the ranges of the two species ran across Germany, with few exceptions, along a line perpendicular to the Harz Mountains, through Thuringia and Bavaria (Figure 5, Schubart 1934). From then on, there was a rapid spread of the western species *P. angustus* eastwards, as far as eastern Saxony, even with finds on the German side of the river Lusatian

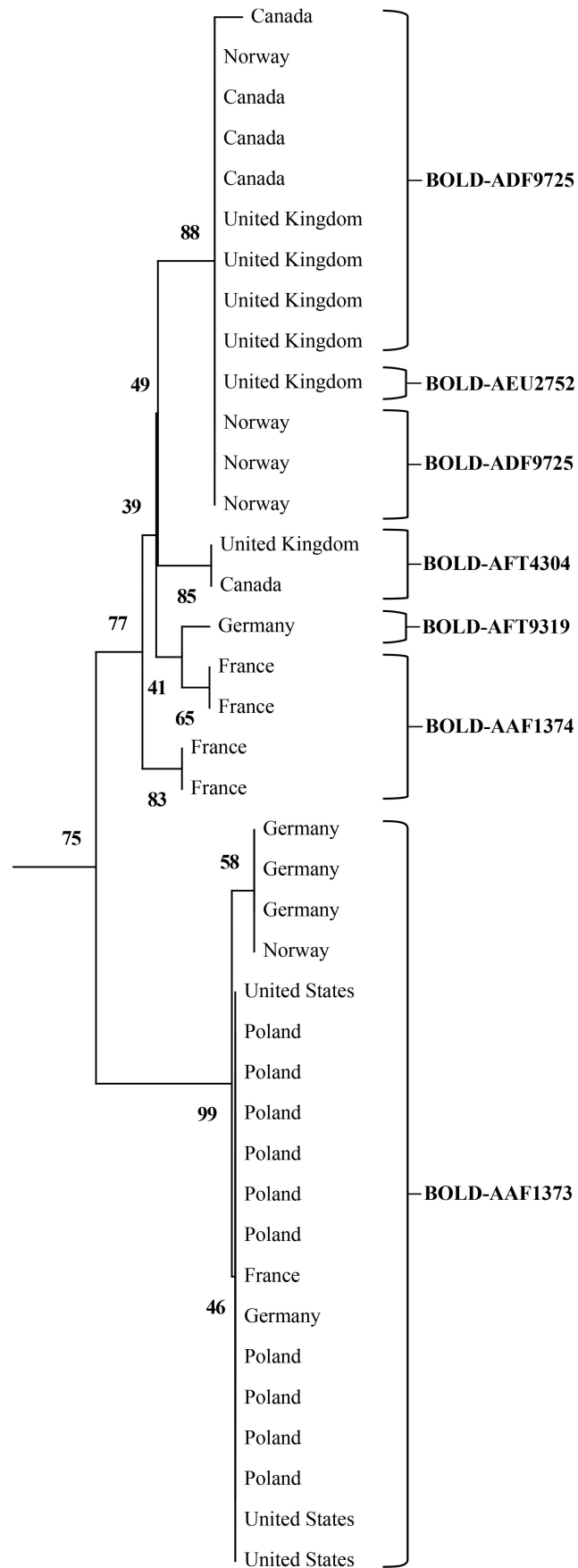


Figure 4. Neighbour-joining tree of the COI sequences representing 7BINs species of *Polydesmus angustus*. Numbers next to nodes are bootstrap values. Outgroup not shown (full version of the tree available in supplementary materials).

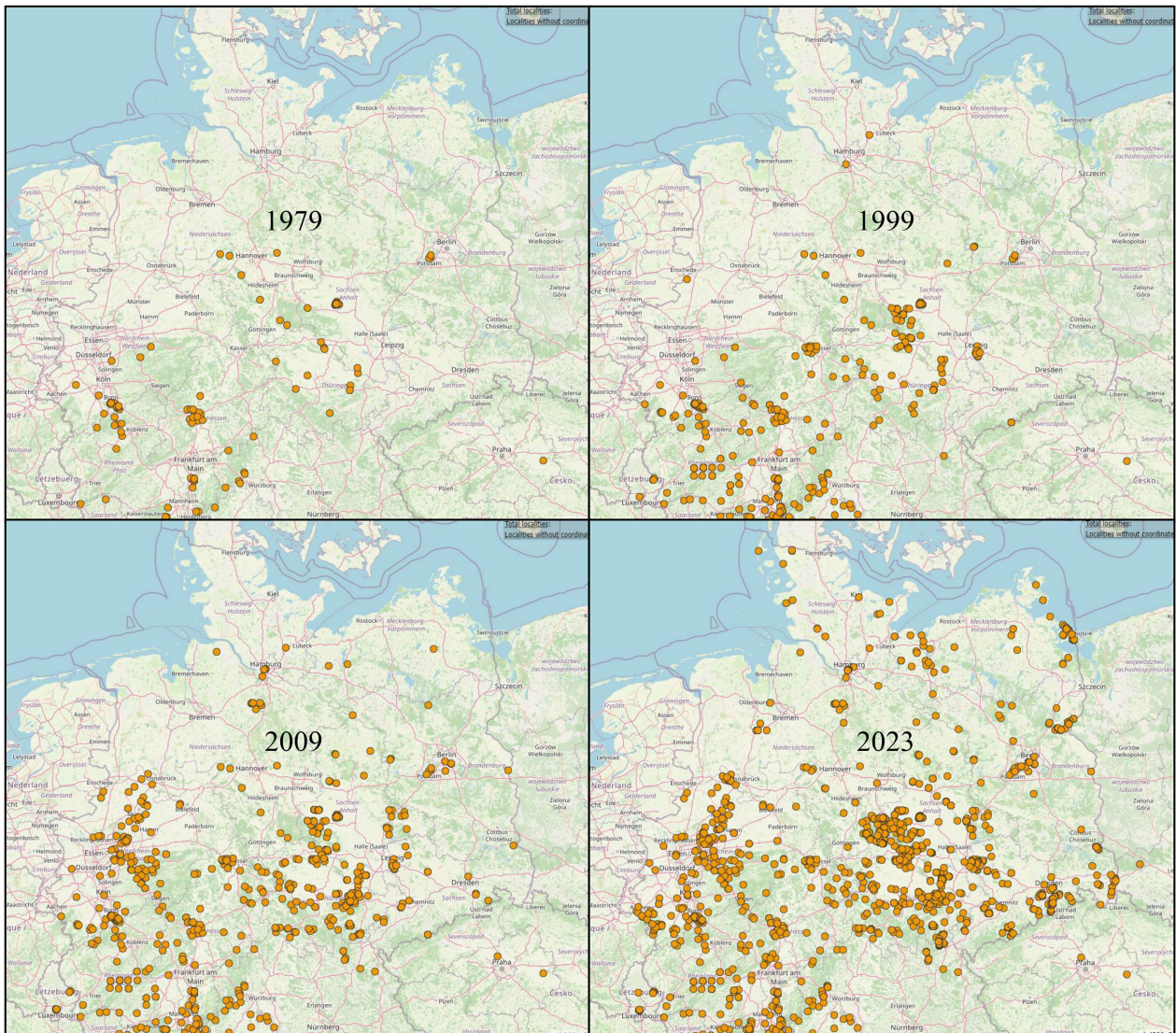


Figure 5. Expansion of *Polydesmus angustus* in Germany. Source: <https://portal.edaphobase.org/>.

Neisse (Nysa Łużycka in Polish), which marks the border with Poland. The species was found on the Polish side of the Lusatian Neisse in 2017, when it was recorded from a parking place near the Kliczków Castle, ca. 30 km from the German-Polish border. The range extension of *P. angustus* in the Czech Republic was similar. The first two records occurred in 1971 and 1997, near the Czech-German border, in the Ore (Rudawy) Mountains (2 individuals on the Vejprty railway and 1x under a bridge of the Polava brook) and from Kolín-Borky, near the Elbe River in a suburban park on the edge of a sports facility. From 2000 onwards, findings of this species have been more frequent in the Czech Republic (see Kocourek et al. 2023). In some colonised habitats there, such as the semi-cultivated countryside, it is even a nearly as abundant as *P. complanatus* (Kocourek 2003).

All the previous eastern records suggest that the spread of this species did not occur through spontaneous and active immigration from the West. This is advocated by the rapid process, the long distance covered and the

habitats from which the first records of *P. angustus* come. In Germany, the first records in Saxony originate from human settlements or from their immediate vicinity (e.g. Leipzig, Glauchau, Dresden) or are located along riverbanks (Oder, Mulde, Weiße Elster, Neisse, coll. SMNG and priv. collection Norman Lindner). The first records from the Czech Republic also indicate spread along rivers (Elbe) as well as in urban and suburban areas. After the political changes of 1989, a lot of plant material from the west came to eastern gardens, which explains the increased occurrence of the species in gardens and parks from 1990 onwards. For example, some finds in Upper Lusatia clearly indicate that the species was carried over with garden waste and then spread massively into neighbouring woodland (Decker and Voigtländer 2012). In eastern Germany, there are clear signs that *P. angustus* is the more competitive species displacing the autochthonous species *P. complanatus* (*personal observations*, based on data from Edaphobase system).

However, the dispersal capacity of *P. angustus* is not limited to the expansion of its range to the east, but also extends to the north and north-east, although the process appears to have started much earlier here than in Middle Europe. The first records for (western) Norway were made in the surroundings of Bergen already 1967 (Meidell 1967). Since then, the species has been found in other areas of Norway (Meidell and Enghoff 1993; Andersson et al. 2005). In Denmark (Jutland), *P. angustus* was found in 1992 for the first time (possibly introduced, Meidell and Enghoff 1993) and has since spread over a large part of the country (Andersson et al. 2005). In 2009, the species was found for the first time in Sweden in a suburban area in Gothenburg (Jonsson 2010).

The role of global warming in the expansion of *P. angustus* is questionable. In laboratory conditions, the life history parameters of *P. angustus* were positively affected by temperature rise (David and Gillon 2009). On the other hand, in Upper Lusatia, it occurs also in very humid deep-slope forests on river banks (e.g. Neißetal near Ostritz), which have not experienced any climatic changes that would have affected the composition of the myriapod fauna (Bowler et al. 2017). Thus, we can suspect that climate warming is not a prerequisite for the expansion of this species.

Polydesmus angustus is a rather eurytopic species most abundant in forests, where it can be found under logs, dead tree bark or in the woodland litter, however, it is also found in gardens, arable lands and other anthropogenic habitats. Occasionally it can be a crop pest as it has been recorded eating strawberries (Kime and Enghoff 2011). *Polydesmus angustus* seems to have expanded its range across Poland quite quickly, probably mostly via man-modified habitats as well as with garden soil transport. Given that, despite recent collection efforts, the species has still not been found in eastern Poland (Jakub Bienias *unpubl. data*), it is possible that the

current distribution limit of this species runs somewhere through the central part of the country, possibly along the course of the Vistula River. The reason for that can be the generally harsher winter condition in Poland, east of the Vistula River, as indicated by the climatic hardiness zones (Wulf and Bouillon 2024). However, it needs to be further investigated and confirmed. The status of this species should depend on which mode of expansion dominates, natural or artificial, which is currently unknown and requires further study. Until then, it should be treated as cryptogenic regarding its provenance in Poland, expanding and possibly non-native within some areas in Europe.

In external appearance, *P. angustus* is seemingly very similar to its close relative *P. complanatus* (Spelda et al. 2011). Thus, to avoid confusion it is best to examine male gonopods of the collected specimens (Figure 2C). In females, the epigyne must be carefully examined (see figures in Blower 1985: 195 and Hauser and Voigtländer 2019: plate 3). Subadult and juvenile specimens are problematic and should not be taken into account. Additionally, finding individuals superficially similar to *P. complanatus* should raise doubts if they are encountered in highly cultivated habitats, such as heavily managed gardens, which are not typical for this species; however, such records should be treated with caution.

The DNA barcode data definitively supports our identification of *P. angustus* from Poland. The relatively low level of intraspecific between-BIN divergence in *P. angustus* compared to interspecific divergence within *Polydesmus* corresponds to values obtained for other millipedes (Reip and Wesener 2018; Spelda et al. 2011) and does not suggest a presence of cryptic diversity within this morphospecies (Table S1). On the other hand, the existence of multiple, yet weakly divergent, BINs of *P. angustus* may indicate some faint genetic population structure in Europe. However, based on the very limited data we have collected so far, no geographic pattern can be observed, and deriving any phylogeographic conclusions is beyond the scope of our study. Further research involving thorough sampling at a much larger geographic scale will be required to explore this issue. Finally, a very wide geographic distribution of the BIN recorded in Poland, and the fact that all our barcoded individuals represent just one haplotype present also in other parts of Europe hints at a rapid expansion of the species in Poland from the west.

Conclusion

Our study shows the fast recent eastward range extension of *P. angustus*, from Germany to Poland, where its new eastern limit of distribution species is probably the Vistula River course. We also confirm that the species occupies disturbed, anthropogenic habitats, advocating its human-mediated dispersal scenario. We also point out that in the case of taxonomic

groups containing externally similar species, the verification of citizen science-based records by specialists examining morphological details of specimens and, if possible, by DNA barcodes, is necessary for reliable insight into the range extension of non-native taxa. In addition, we expand the public DNA barcode library for *P. angustus*.

Authors' contribution

JB: research conceptualization, investigation and data collection, data analysis and interpretation, funding provision, writing – original draft; KV: research conceptualization, investigation and data collection, data analysis and interpretation, writing – original draft; ŁT: investigation and data collection, data analysis and interpretation, writing – original draft; JK: investigation and data collection; MG: research conceptualization, data analysis and interpretation, funding provision, writing – original draft.

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Supplementary material

The following supplementary material is available for this article:

Table S1. The mean intraspecific and interspecific genetic distances (K2P %) of 5 *Polydesmus* species and 5 specimens of *P. angustus*.

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2025/Supplements/BIR_2025_Bienias_etal_SupplementaryMaterial.pdf