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SHORT COMMUNICATION‡

FIRST RECORD OF INVASIVE SLUG *KRYNICKILLUS MELANOCEPHALUS* KALENICZENKO, 1851 (GASTROPODA: EUPULMONATA: AGRIOLIMACIDAE) IN POLAND

JAROSŁAW J. MAĆKIEWICZ[1]*, OLSZA BORYS[2]

[1] Łupkowa 1, 91-528 Łódź (e-mail: malakofil@gmail.com)
[2] Faculty of Biology, University of Warsaw, Ilji Miecznikowa 1, 02-096 Warszawa, Poland (e-mail: d.borys2@student.uw.edu.pl)
* corresponding author

ABSTRACT: During amateur nature observations in Kabaty Woods Nature Reserve in Warsaw, a slug of family Agriolimacidae was sighted. Photos with localisation tags have been submitted to iNaturalist website, where the algorithm suggested an identification of *Krynickillus melanocephalus* Kaleniczenko, 1851. Following specialists' interests, three specimens were collected from the original location. Anatomical examination confirmed their identification. It is therefore the first documented record of *K. melanocephalus* in Poland.

KEY WORDS: invasive species; *Krynickillus melanocephalus*; protected area; slugs

Several distincive-looking slugs were spotted by the second author while examining a decaying log during amateur mycological observations in Kabaty Woods Nature Reserve in Warsaw, Poland. The slugs, measuring around 45 mm, were noticed for their bluish grey body with a characteristic deep black head and tentacles (Figs 1–4). The specimens were found on 13th November 2022, in an oak-hornbeam forest next to a Kanał Grabowski ditch and a recreational hiking trail used commonly by residents (52°08'00.2"N, 21°02'16.8"E).

The author took photos and a video of one of the slugs and posted them on the iNaturalist website (https://www.inaturalist.org/observations/141913696, accessed on 5 April 2023). The app is a citizen science network that allows users to post observations, identify them with the aid of a Machine Learning algorithm, and cross-verify them by other users, including expert scientists. The slug observation was automatically suggested to be *Krynickillus melanocephalus* (Agriolimacidae), which was noticed by the (first) author. Igor Balashov, an app user and a malacologist from Ukraine, was asked to verify the identification. Although he generally agreed, he also recommended detailed anatomical analysis to distinguish it from similarly looking *Deroceras caucasicum* Simroth, 1902 (Agriolimacidae, likewise). Neither species had previously been reported in Poland.

Two days later, on 15th November 2022, two specimens were collected at the same spot for further identification. Another few living individuals were found there and left under the log. Additionally, a similarly looking slug was found and collected from a location on a hiking trail several dozen meters away (52°08'05.3"N, 21°02'06.7"E).

The identification was subsequently confirmed on an anatomical basis (Figs 5–7). In particular, collected specimens have cylindrical penises, without any external appendages. For every individual, the length

‡ Preliminary report was presented during the 37th Polish Malacological Seminar in Katowice (see Folia Malacologica 31(4), p. 251, https://doi.org/10.12657/folmal.031.023).

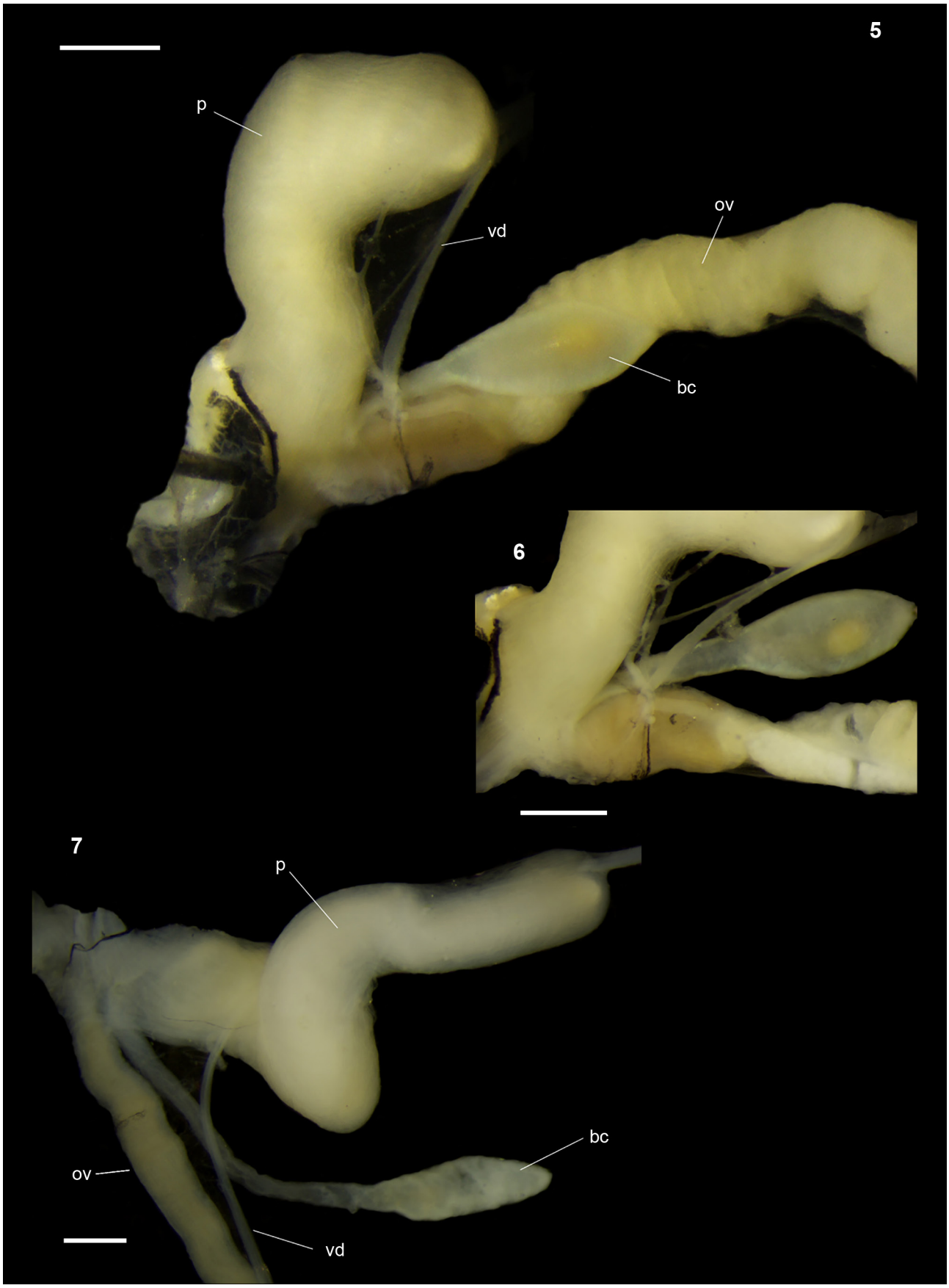
Figs 1–4. *Krynickillus melanocephalus*: 1 – external appearance of individual collected in Warsaw on 15 November 2022; 2–4 – cooled slug before preservation in ethanol. Scale bars 1 mm. Photos: ANNA SULIKOWSKA-DROZD

of the penis is similar to spermatheca with its duct. These traits agree with the species description provided by WIKTOR (2000). Reporting invasive specimens from Hungary, TURÓCI et al. (2020) noted that the *K. melanocephalus* penis consists of a thinner basal (distal) and a swollen apical portion. The specimens from Warsaw do not show this trait, having the penis of constant width, in accordance with WIKTOR's (2020) description.

The initial identification of *K. melanocephalus* collected in Warsaw was also corroborated by mitochondrial cytochrome c oxidase (COI) DNA barcode. The sequences have been deposited in GenBank (accession numbers OQ728907, OQ728908). BLASTn analysis (ALTSCHUL et al. 1990) showed that the sequences are near-identical with the only one other sequence of *K. melanocephalus* available in GenBank (ID: MT827967). Both scored 100% sequence identity on 98% query cover, meaning that all three sequences are virtually indistinguishable, up to the trimming of their edges. Additionally, all other result alignments were less than 87% identical, which supports the anatomical identification. Maximum Likelihood tree of these three COI sequences and several other of agriolimacid species, obtained from GenBank (Fig. 8, Table 1) showed a well separated cluster of *K. melanocephalus* sequences, confirming species identification.

Krynickillus melanocephalus is a land slug of the family Agriolimacidae. It was first described by Kaleniczenko in 1851 based on the population from the Caucasus around the city of Stavropol, Russia (KALENICZENKO 1851) (Fig. 9). The natural range of the slug is the Caucasus, Crimea, Turkey and northern Iran (WIKTOR 2000). In its native range it is mainly a forest species, occurring chiefly in the neighbourhood of water. During dry periods it hides in soil cracks (KALENICZENKO 1851), leaf litter, or under rocks and logs (LIKHAREV & WIKTOR 1980). Its body is dirty whitish, sometimes bluish grey, darker on the back. The front part of the body is covered by a mantle with brighter-rimmed breathing pore (pneumostome) on its rear end. Its characteristic trait is deep black head, tentacles, and nape (also the section under the mantle) (WIKTOR 2000).

Since the 1990s, the species has been reported outside its native range: Germany (MENG &



Figs 5–7. *Krynickillus melanocephalus*: genitals of slugs collected in Warsaw on 15 November 2022. Abbreviations: p – penis, vd – vas deferens, ov – oviductus, bc – bursa copulatix. Scale bars 1 mm. Photos: ANNA SULIKOWSKA-DROZD

Table 1. GenBank sequences used in phylogenetic analysis

Species	COI GenBank numbers	Reference
Agriolimacidae sp.	MF544601, MF544669	DeWaard et al. 2019
Deroceras agreste (Linnaeus, 1758)	KF894375	Rowson et al. 2014
Deroceras golcheri van Regteren Altena, 1962	JN248291, JN248293	Reise et al. 2011
Deroceras invadens Reise, Hutchinson, Schunack et Schlitt, 2011	JN248295, JN248302	Reise et al. 2011
	KF894259, KF894385	Rowson et al. 2014
	MH830204, MH830237	Hutchinson et al. 2020b
Deroceras laeve (O. F. Müller, 1774)	KM612199	Hebert et al. 2014
	MF544949, MF545090, MF545139	DeWaard et al. 2019
	MG421254	DeWaard 2017
Deroceras panormitanum (Lessona et Pollonera, 1882)	JN248308	Reise et al. 2011
	KM612148, KM612186	Hebert et al. 2014
Deroceras reticulatum (O. F. Müller, 1774)	KM612116	Hebert et al. 2014
	MF545107, MF545125, MF545161	DeWaard et al. 2019
	MG423285	DeWaard 2017
Krynickillus melanocephalus Kaleniczenko, 1851	MT827967	Hutchinson et al. 2020a
	OQ728907, OQ728908	This paper
Limax maximus Linnaeus, 1758	JN248294	Reise et al. 2011

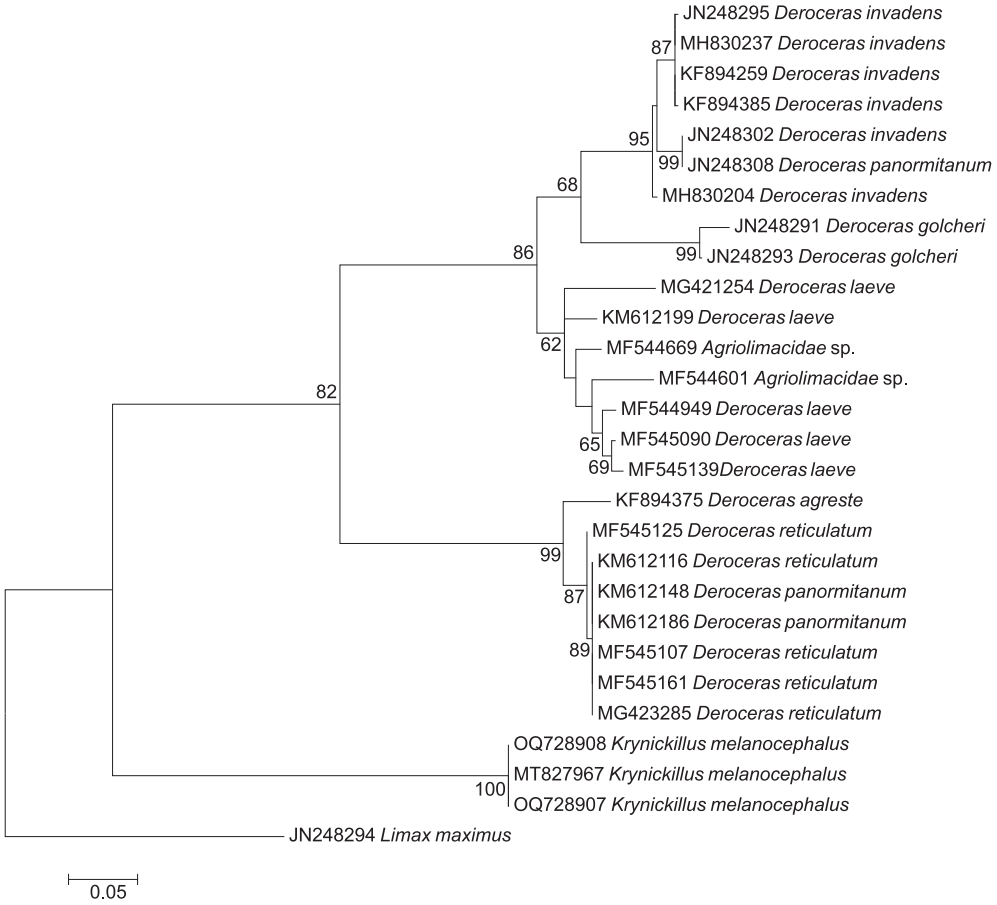


Fig. 8. Maximum Likelihood (ML) tree of COI sequences obtained from GenBank for Agriolimacidae species. The sequences were cut to 619 bp in length. Alignments were performed with ClustalW, implemented in BioEdit (Thompson et al. 1994). For alignments, HKY+G was specified as best nucleotide substitution model according to the Bayesian Information Criterion (Hasegawa et al. 1985, Kumar et al. 2016). The tree was built using MEGA7 (Kumar et al. 2016). Numbers next to the branches indicate bootstrap support above 50% calculated for 1000 replicates (Felsenstein 1985). The tree was rooted with *Limax maximus* COI sequence JN248294 deposited in GenBank by Reise et al. (2011)

BÖSSNECK 1999), further parts of Russia (SYSOEV & SCHILEYKO 2009) and Ukraine (BALASHOV 2016), Belarus (OSTROVSKY 2017), Lithuania and Latvia (DREIJERS et al. 2017), Estonia, Sweden (VON PROSCHWITZ 2020), Hungary (TURÓCI et al. 2020), and Slovakia (ČEJKA et al. 2021). In the iNaturalist database (https://www.inaturalist.org/observations?taxon_id=807716, accessed on 5 April 2023) there are also 15 records in Finland, the northernmost of the species' range. Despite the presence in the neighbouring countries, the species has not yet been reported in Poland[1].

K. melanocephalus is considered an invasive species (VON PROSCHWITZ 2020). In its introduced range, it is most commonly found in synanthropic habitats. Our record comes from a forest habitat within the administrative boundaries of the city of Warsaw. Despite the terrain being a legally protected area (MINISTERSTWO LEŚNISTWA I PRZEMYSŁU DRZEWNEGO 1980), Kabaty Woods are intensively used as a recreation area, being almost entirely surrounded by residential areas. As much as determining the source of the introduction is impossible, the surroundings of the forest may suggest an introduction by humans.

Because this alien species has been recorded within a legally protected area, we find it necessary to discuss guidelines on future responses to similar occurrences. There is no legal basis for mandatory eradication of the population. *K. melanocephalus* is absent from lists of invasive alien species of the European Union or Poland (RADA MINISTRÓW 2022, GDOŚ 2023). Nonetheless, as it is described as an invasive species (VON PROSCHWITZ 2020), actions should be taken to eliminate individuals from the protected area. We recommend manually picking up easily identifiable adult specimens and killing them humanely. We discourage the use of chemical approaches, as they do not seem to be effective or selective and pose high harm to the environment.

Learning from the cases of *K. melanocephalus* invasion in neighbouring countries, we suspect an incoming influx of species' sightings in Poland. In the iNaturalist database there are records of the slug's presence near the Polish border in three distinct regions: Kaliningrad Oblast (Russia), Brest and Grodno (Belarus) and Bardejov (Slovakia) (https://www.inaturalist.org/observations?taxon_id=807716, accessed on 5 April 2023). Information on new sightings should be reported to the Institute of Plant Protection – National Research Institute and the General Directorate for Environmental Protection. It is also recommended to inform the public about the presence and adverse effects of *K. melanocephalus*, as well as broader topics of species' invasions, through press reports and popular science literature.

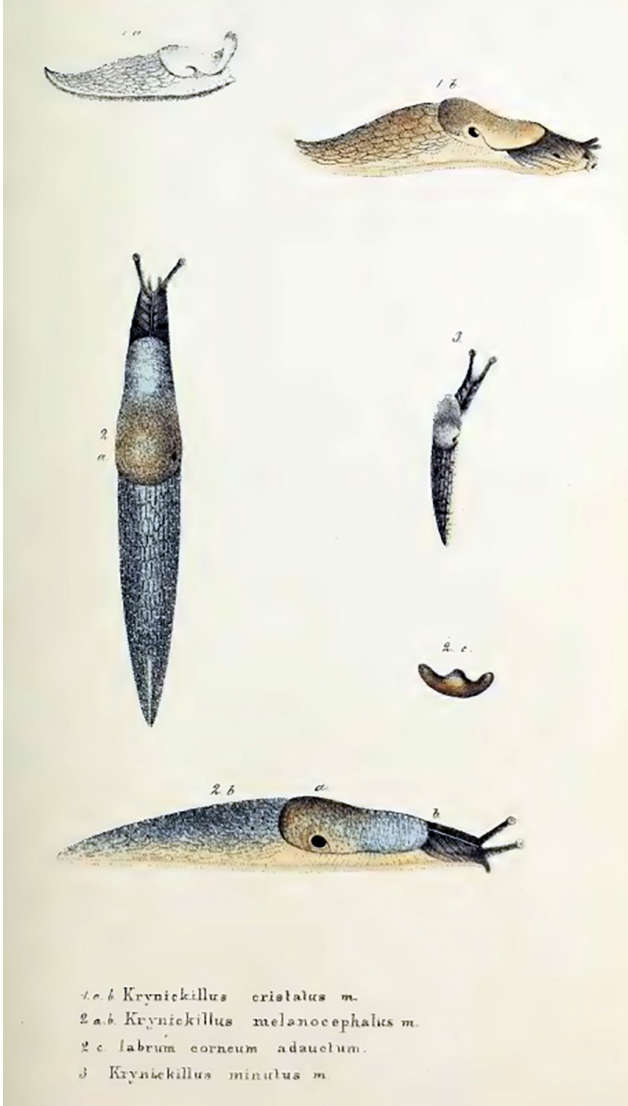


Fig. 9. *Krynickillus melanocephalus* as depicted by KALENICZENKO (1851)

In the case of the first record of *K. melanocephallus* it is important to acknowledge the vital role of citizen science networks, as exemplified by two other reports of the same species by BARBATO et al. (2021) and TURÓCI et al. (2020). In our case the iNaturalist network enabled to quickly identify and document the specimen. As citizen science networks enable connecting amateurs with the scientific community, they may greatly contribute to the malacology and biological natural sciences in general.

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[1] Note added to proofs: iNaturalist of 28 September 2023 reported *Krynickillus melanocephalus* from Raba near Bochnia (Poland) found on 7 October 2022.

the reproductive system and review of the report. Additionally, the authors would like to thank IGOR BALASHOV for preliminary identification and prof. ANDRZEJ LESICKI for sharing the literature. Second Autor would like to thank ZORZA RUCZAJ for the help with locating the specimens again and ZOFIA PARTYKA for the proofreading.

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