


FIRST RECORD OF *ARION ATER* S.L. (GASTROPODA: ARIONIDAE) IN UKRAINE

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ABSTRACT: Slugs in the *Arion ater* complex have been reliably recorded in Ukraine for the first time. In recent years, large specimens with an intense black colouration and very prominent skin tubercles have been observed in two neighbouring villages of the Bohorodchany district of the Ivano-Frankivsk region, Stara Huta and Huta. Even externally, they were very different from *Arion vulgaris*, which has already become common in western Ukraine. The structure of the distal genitalia of such slugs collected in Stara Huta in early July 2024 is similar to the older descriptions and images of *Arion rufus*. However, DNA barcoding classifies them as *A. ater* s.s. We assume that *A. ater* s.l. could have been accidentally introduced into the studied area from abroad together with ornamental plants. The initial source of the slug dispersal here could have been the administrative territory of the Syniohora National Nature Park or the presidential residence of the same name located nearby. For comparison, a map of known records of *A. vulgaris* in Ukraine is shown, only a few of which have been anatomically verified so far.

KEY WORDS: *Arion ater*; *Arion rufus*; introduction; land molluscs; slugs; Ukraine

INTRODUCTION

Arion vulgaris Moquin-Tandon, 1855 was first recorded in Ukraine in 2007 (GURAL-SVERLOVA & GURAL 2011a) and then quickly spread in the western and central parts of the country (BALASHOV et al. 2018). Before this, not a single taxon of the subgenus *Arion* s.s. was reliably known here (LIKHAREV & WIKTOR 1980, SVERLOVA & GURAL 2005). Mentions of *Arion empiricorum* A. Férussac, 1819 in some older publications most likely referred to large specimens of *Arion subfuscus* s.l. (GURAL-SVERLOVA & GURAL 2011a, BALASHOV & GURAL-SVERLOVA 2012) – a complex represented in the plain areas of Ukraine only by *Arion fuscus* (O. F. Müller, 1774) (GARBAR et al. 2014, GURAL-SVERLOVA & GURAL 2015). Adults of *A. vulgaris* were clearly distinguished in appearance from all other *Arion* species previously known

in Ukraine. Therefore, numerous amateur naturalists, posting their photographs with dates and places of observations on the social network Facebook and in citizen science databases (INATURALIST 2024, UKRBIN 2024), played a major role in collecting information about the spread of *A. vulgaris* in Ukraine. On the contrary, anatomical studies of *A. vulgaris* in Ukraine were carried out relatively rarely and related to a few localities (GURAL-SVERLOVA & GURAL 2011a, 2011b, KADLUBOVSKA & GARBAR 2013, BALASHOV et al. 2018, MOSKALYK et al. 2020, LEONOV 2021). The most detailed of them, which included the structure of the ligula and spermatophores, was made in the Lviv region (GURAL-SVERLOVA & GURAL 2011b). Thus, there was always a risk of not noticing another large arionid slugs, externally similar to *A. vulgaris*,



which could have been introduced to Ukraine. This applies primarily to *Arion ater* s.l., a complex of very similar species or subspecies (REISE et al. 2020).

In recent years, photographs of large black arionid slugs, taken in the village of Stara Huta, Ivano-Frankivsk region, mainly in the administrative territory of the Syniohora National Nature Park, began to appear in the database INATURALIST (2024). We

found similar images from the neighbouring village of Huta in a thematic Facebook group dedicated to the animal world of Ukraine. A trip to this area confirmed the presence there of both *A. vulgaris*, now widespread in western Ukraine, and *A. ater* s.l. This paper is devoted to the analysis of the collected material.

MATERIAL AND METHODS

STUDY AREA

The villages of Stara Huta and Huta are located in the mountainous part of the Bohorodchany dis-

trict of the Ivano-Frankivsk region, in the Ukrainian Carpathians (part of the Eastern Carpathians). The average altitude above sea level is about 600 m. According to the census of 2001, these two villages



Figs 1–3. Habitats of *A. ater* s.l.: 1, 2 – the administrative territory of the Syniohora National Nature Park in Stara Huta, 3 – alder thickets on the bank of the Bystrytsia Solotvynska River in Huta

had a population of 326 and 738 people, respectively. Most of the studied material was collected on the administrative territory of the Syniohora National Nature Park (Figs 1–2) or in its immediate vicinity. In addition to the park administration, there is a hotel and cottages for tourists, a variety of tree and shrub plantings, including hedges of ornamental bushes (Fig. 1). Nearby is the presidential residence with the same name Syniohora, organised on the basis of an old departmental sanatorium in 2001. In 2009, part of the territory was transferred to the newly formed nature park.

COLLECTED MATERIAL

The main sample of *A. ater* s.l. was collected in early July 2024 on the administrative territory of the Syniohora National Nature Park (Figs 1–2) in Stara Huta, 48°37'29.5"N, 24°12'49.0"E. One small slug of the same species was found in the thickets of *Alnus incana* on the bank of the Bystrytsia Solotvynska River (Fig. 3) in the neighbouring village of Huta, 48°39'18.5"N, 24°13'09.5"E. This area is located on the outskirts of the village and, judging by the fireplaces, is used for recreation.

The specimens of *A. vulgaris* used for comparison were collected near the main collection site of *A. ater* s.l., on the side of Syniohirska street overgrown with tall grass, next to household plots, 48°37'31.1"N, 24°12'39.3"E. In addition, between the two collection sites of *A. ater* s.l. (see above) we several times found living or recently dead individuals of *A. vulgaris* along the highway.

The collected slugs were photographed and immediately fixed with 75% ethanol, so-called hard fixation according to LIKHAREV & WIKTOR (1980). Material for DNA barcoding was preserved in undiluted ethanol. Some specimens of *A. ater* s.l. from Stara Huta were transferred to the collection of land molluscs of the Schmalhausen Institute of Zoology in Kyiv, Ukraine, inventory number SIZK (IZAN) GT7223.

RESULTS

APPEARANCE OF SLUGS

All specimens of *A. ater* s.l. in the studied area, collected by authors personally or observed in previous years by others (see Acknowledgments), had a uniform black colouration (Figs 4–6). The exception was the light central part of the sole, which contrasts with the dark edges (Fig. 7). Because of this, the colouration of the sole of living slugs resembled that of adult *Limax cinereoniger* Wolf 1803. As a consequence,

LABORATORY STUDY

Slug dissections were carried out according to LIKHAREV & WIKTOR (1980). DNA was extracted from tissue samples of one ethanol preserved specimen of *A. ater* s.l. from the first locality using Blood and Tissue kit (Qiagen, Germany) according to the manufacturer's protocol. PCR reaction was prepared using standard HCO1490 and LCO2198 primers and reaction conditions (FOLMER et al. 1994). PCR products were checked in electrophoresis, and a fragment of cytochrome oxidase subunit I gene was later purified and sequenced by commercial sequencing service Explogen LLC (Lviv, Ukraine). A trimmed sequence was uploaded to the NCBI GenBank under accession number PQ252644. The sequence similarity to other sequences in GenBank was tested using NCBI BLAST tool (ALTSCHUL et al. 1990).

MAPPING

To map the records of large arionid slugs in Ukraine, we used the results of our own long-term observations in the west of the country, literature sources (BALASHOV et al. 2018, MOSKALYK et al. 2020, LEONOV 2021) as well as numerous photographs in citizen science databases (iNATURALIST 2024, UKRBIN 2024) and in specialised groups on Facebook. In addition to our own and literature data, we also considered some photos demonstrating the slug copulation as anatomically confirmed finds of *A. vulgaris*. The latter concerned only those cases where the images clearly showed the expanded distal parts of the oviducts, characteristic of *A. vulgaris* and absent in *A. ater* s.l. (DREIJERS et al. 2013).

Observations made in the same settlement or its immediate vicinity were shown on the map as a single dot. In areas with a large concentration of observations, in some cases the same approach was applied to neighbouring communities. Most often this concerned small settlements adjacent to the administrative borders of Lviv and Kyiv.

first observers often mistook them to be *L. cinereoniger* (iNATURALIST 2024).

Due to their intense black colouration, the individuals of *A. ater* s.l. collected by us were clearly different from *A. vulgaris*. In the studied area, the colour of the upper part of the body in *A. vulgaris* varied from pale orange to brown (Fig. 8), and the colour of the sole from whitish to dark grey (Fig. 9), but without such a contrast as in *A. ater* s.l. In all cases, *A. vulgaris* was easily distinguished from *A. ater* s.l.



(Figs 10–11). After fixation with ethanol, differences in colouration between *A. vulgaris* and *A. ater* s.l. remained, although they became less pronounced (Fig. 12). The sole in fixed specimens of *A. ater* s.l. also lost such contrast as seen in living slugs.

A. vulgaris and *A. ater* s.l. also differed in the mucus colour on the upper part of the body: from yel-

lowish to bright orange in *A. vulgaris*, colourless or milky white when irritated in *A. ater* s.l. The collected specimens of *A. ater* s.l. also had larger body sizes and more pronounced skin tubercles on the sides and back. The length of *A. ater* s.l. in motion was not measured. After fixation, the length of the largest specimen was about 6 cm.



Figs 4–9. Slugs of the subgenus *Arion* s.s. from Huta (4, photo by SVITLANA SHMIDT made in 2023) and Stara Huta (5–9): 4–7 – *A. ater* s.l., 8, 9 – *A. vulgaris*

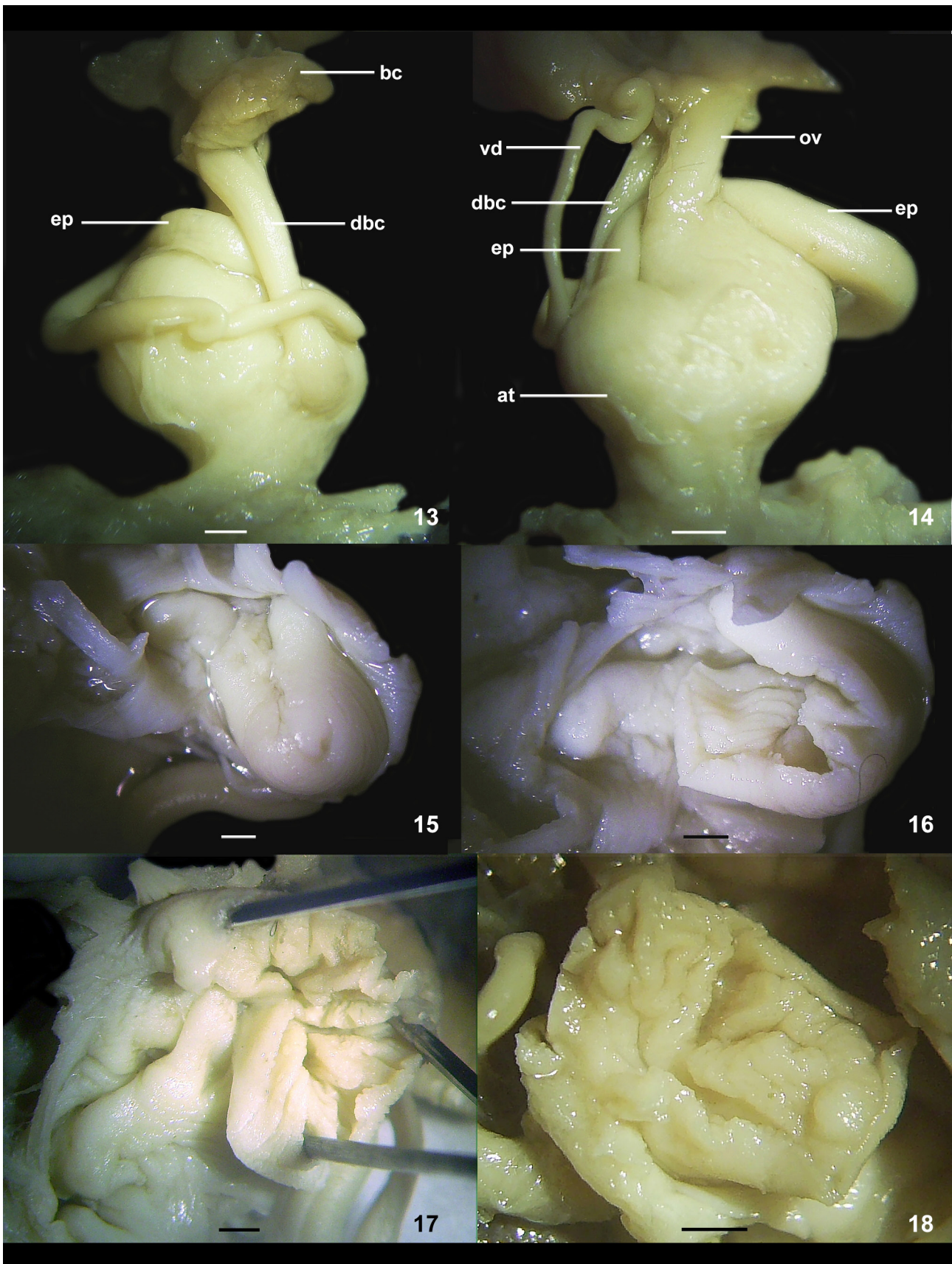


Figs 10–12. Comparison of the appearance of *A. ater* s.l. and *A. vulgaris* from Stara Huta: 10, 11 – live slugs, 12 – after fixation. Asterisks indicate *A. vulgaris* specimens

DISTAL GENITALIA

In both dissected large specimens of *A. ater* s.l., the structure of the distal genitalia was similar. The atrium was large, with a relatively short and narrow

lower (distal) part and a massive upper (proximal) one (Figs 13–14). The upper part of the atrium had a well-expressed lateral swelling, which gave the atrium an asymmetrical shape (Fig. 13). The epiphallus and the duct of the bursa copulatrix entered the atri-



Figs 13–18. *A. ater* s.l. from Stara Huta: 13, 14 – distal genitalia, 15–17 – ligula in the opened atrium at different stages of dissection, 18 – ligula of another specimen. Legend: at – atrium, bc – bursa copulatrix (spermatheca), dbc – duct of the bursa copulatrix, ep – epiphallus, ov – oviduct, vd – vas deferens. Scale bars 1 mm

um far lower than the vagina (Fig. 14). Inside the expanded upper part of the atrium there was a large ligula (Figs 15–18), which at a certain stage of dissection had a distinct C-shape (Fig. 16). The right part of the ligula was somewhat larger than the left (Figs 17–18), forming a kind of tongue at the end (Figs 16–17). A clearly visible ridge ran from the ligula base to the lower part of the atrium (Fig. 17). Its upper part was covered by the right edge of the ligula (Fig. 16).

DISCUSSION

TAXONOMIC AFFILIATION OF THE COLLECTED MATERIAL

The views on the systematics of slugs of the *A. ater* complex are not stable (in more detail described in REISE et al. 2020): from recognising the species independence of *Arion rufus* (Linnaeus, 1758) and *A. ater* (Linnaeus, 1758) (LIKHAREV & WIKTOR 1980, KERNEY et al. 1983, CAMERON et al. 1983, WELTER-SCHULTES 2012, CASTILLEJO et al. 2019, etc.) to reducing them to subspecies (QUICK 1960, EVANS 1986, NOBLE 1992) or even synonyms (e.g. in PILSBRY 1948: 668). ROWSON et al. (2014) distinguished *A. rufus* not only from *A. ater* but also from another taxon within the *A. ater* s.l. complex, which they named provisionally *A. empiricum*. This was based on 16S differences. REISE et al. (2020) confirmed these differences with COI (verified with 16S), but interpreted the distinct groups as subspecies. This was evidenced by a geographical pattern, anatomical differences, and clear indications of free interbreeding. The existence of three subspecies of *A. ater*, distinguished on the basis of genital anatomy and mitochondrial DNA (REISE et al. 2020), was later confirmed using microsatellite markers (HUTCHINSON et al. 2021). Instead of *empiricum*, it was recommended to use the older name *ruber* (REISE et al. 2020). Thus, the form from continental Europe, previously designated *A. rufus* or *A. empiricum*, should be called *A. ater ruber* (Garsault, 1764). The name *Arion ater rufus* was proposed to be retained for the morphotype commonly found in England.

The shape and proportions of the atrium in slugs collected in Stara Huta are similar to descriptions and images of *A. rufus* in some identification guides (LIKHAREV & WIKTOR 1980, KERNEY et al. 1983). However, according to REISE et al. (2020: 16), the external shape of the atrium in *A. ater ater* is highly variable and may therefore be indistinguishable from the other two subspecies. In both *A. ater rufus* and *A. ater ruber*, the epiphallus and the duct of the bursa copulatrix usually entered the atrium quite far from

DNA BARCODING

A 565 base-pair long fragment of mitochondrial cytochrome oxidase subunit I gene showed the highest (99.82%) identity with MN958008 sequence from Germany. This sequence belongs to the widespread haplotype 5 of the *A. ater* haplotype group *ar* from Germany, British Isles and Scandinavia (REISE et al. 2020). The haplotype group *ar* corresponds to *A. ater* s.s.

the oviduct, in contrast to *A. ater* s.s., but these differences are also not constant.

The ligula shape in the slugs from Stara Huta seems to us to be very different from that of *A. ater ater* depicted in REISE et al. (2020: fig. 39). According to the same authors, a strong ridge inside the atrium extending from the ligula base is characteristic of *A. ater rufus* and *A. ater ater*, but not of *A. ater ruber*. Based on a combination of anatomical features, we have preliminarily identified the studied slugs as *A. ater rufus*. However, this contradicts the barcoding results, identifying them as *A. ater* s.s., see above. As noted by REISE et al. (2020: 10), any correspondence between morphology and genetics in the *A. ater* complex is liable to be disrupted by hybridisation and introgression.

PRESENT DISTRIBUTION OF SLUGS OF THE SUBGENUS ARION S.S. IN UKRAINE

Recently, the number of reports recording large arionid slugs in Ukraine has increased significantly. This is clearly demonstrated by a comparison of the map we compiled (Fig. 19) with a similar map published not so long ago (BALASHOV et al. 2018: fig. 2). However, most of the finds remain concentrated in western Ukraine and around Kyiv in its central part. At present, large arionids appear to avoid the steppe zone of Ukraine, except single reliable records in the Odesa, Mykolaiv, Dnipropetrovsk and Donetsk regions. In Crimea, where *A. vulgaris* has been known since 2013 (LEONOV 2021), a few records have been made in the foothills in the south of the peninsula, but not in its steppe part. Perhaps this is due to the climatic conditions of the steppe zone or the specifics of the landscaping of populated areas.

Before the record of a further taxon of the subgenus *Arion* s.s. in Ukraine, all finds of large arionid slugs were automatically attributed to *A. vulgaris* (see Introduction). Taking into account different sources of information (see Material and Methods), the records of *A. vulgaris* in the following administrative re-



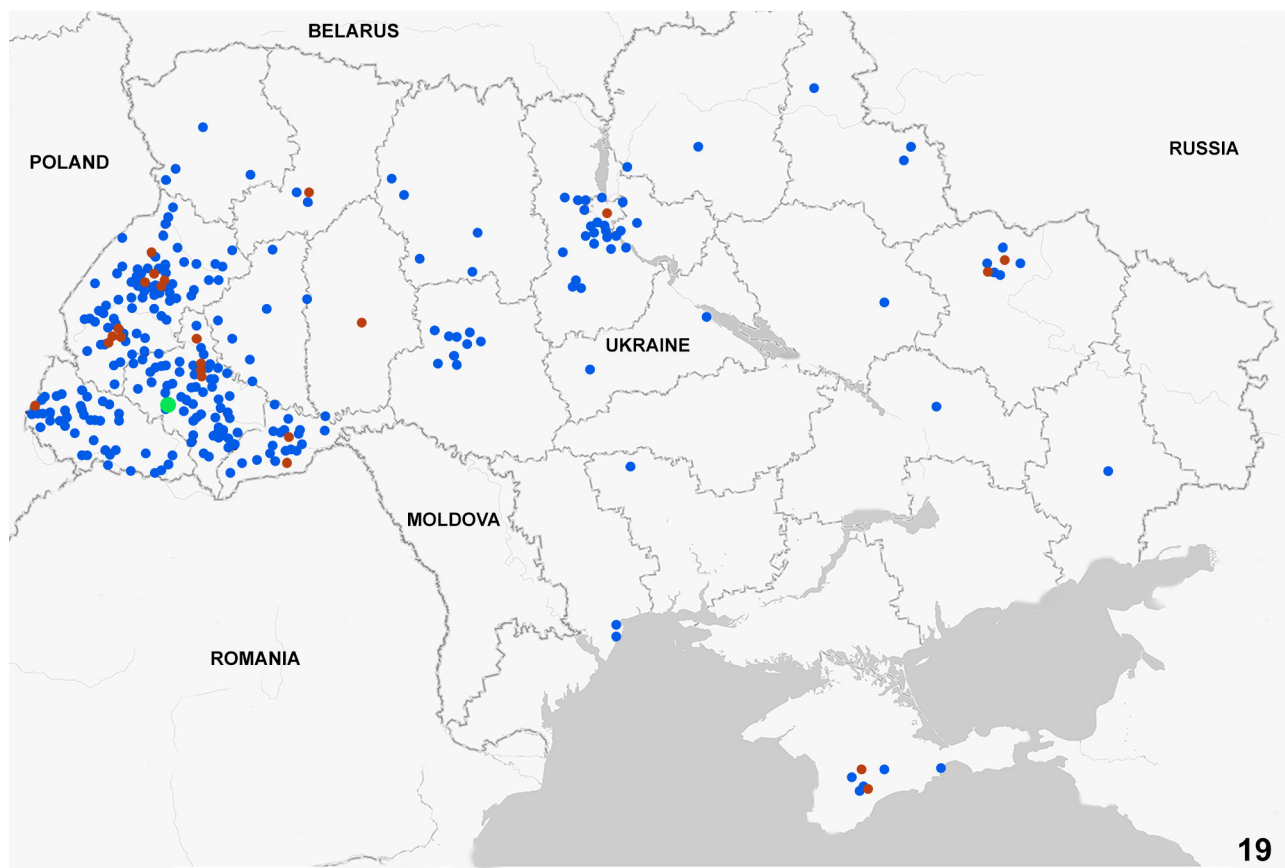
gions of Ukraine can be considered anatomically confirmed (marked in red in Fig. 19): Lviv (Lviv, Boryslav, Davydiv, Drohobych, Obroshyne, Skhidnytsia, Truskavets, Vynnyky, Zhovkva), Ivano-Frankivsk (Ivano-Frankivsk and its environs, Burshtyn, Tiaziv), Chernivtsi (Chernivtsi, Hlyboka), Transcarpathian (Uzhhorod), Rivne (Rivne) and Khmelnytskyi (Khmelnytskyi) in the west of the country, as well as in Kyiv (Kyiv), Kharkiv (Kharkiv, Budy) and Crimea (Perevalne and Donske in the Simferopol district).

A. vulgaris and *A. ater* s.l. cannot always be reliably distinguished by appearance alone (CAMERON et al. 1983, WIKTOR 2004, REISE et al. 2018, etc.), although adult specimens of the latter are somewhat larger in size and have more embossed skin tubercles. In the two villages we studied (Stara Huta and Huta), these two taxa are very different externally. There, only the black form of *A. ater* s.l. occurs, which is clearly distinguished from any colouration form of *A. vulgaris* adults (Figs 20–24). In general, the body colouration of *A. ater* s.l. is very variable. Besides black ones, brown and orange (red) specimens are also often found in this taxon, similar to the usual colouration variants of *A. vulgaris*. In addition to the common orange and brown slugs (Fig. 21), grey individuals (Fig. 23) are occasionally present in western Ukrainian populations of *A. vulgaris*. Sometimes

such slugs are so dark that they appear almost black (Fig. 20, right). These dark grey and sometimes also brown specimens of *A. vulgaris* are often mistaken for *A. ater* by non-professional observers.

The variability of the body colouration of *A. vulgaris* shown in Figures 20–24 was recorded in the first populations of this species discovered in Lviv (2010) and then examined in detail anatomically (GURAL-SVERLOVA & GURAL 2011b). Therefore, it cannot be caused by possible hybridisation of *A. vulgaris* with *A. ater* s.l. The latter has not yet been recorded in Lviv, despite 30 years of targeted study of the city's malacofauna and its changes. Also, not a single large *Arion* from Lviv or other localities in the west of Ukraine, anatomically examined by us personally, could be classified as a hybrid of *A. vulgaris* and *A. ater* s.l. (REISE et al. 2020).

Over many years of observation in western Ukraine, we have never found a specimen of *A. vulgaris* with such an intensely black body colouration as in *A. ater* s.l. from Stara Huta and Huta. Having looked through many photographs of large arionids from other populated areas in different administrative regions of Ukraine, we also did not find a single similar individual. This also applies to other settlements of the Bohorodchany district of the Ivano-Frankivsk region. Therefore, it can be preliminary concluded that



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Fig. 19. Known records of large arionid slugs in Ukraine: green – *A. ater* s.l. and *A. vulgaris* together, red – anatomically confirmed *A. vulgaris*, blue – observations most likely related to *A. vulgaris*

A. ater s.l. is sporadically distributed in two adjacent villages and has not yet been observed in neighbouring areas.

The remaining known records of large arionids in Ukraine (blue in Fig. 19), or at least the vast majority of them, are very likely to belong to *A. vulgaris*.

Before *A. vulgaris* began to spread rapidly in Ukraine, similar processes were observed in Central European countries, in particular in Germany (WIESE 2014) and neighbouring Poland (KOZŁOWSKI 2007). The present range of *A. ater* s.l. is also significantly expanded due to anthropochory (REISE et al. 2018).



Figs 20–24. Colouration variability of *A. vulgaris* in Lviv, western Ukraine



However, the dispersal of the forms of *A. ater* s.l. does not occur as rapidly as that of *A. vulgaris* (VON PROSCHWITZ 1997, KNOP et al. 2013), which can be explained by the biological peculiarities of the latter (ZAJĄC et al. 2017), in particular a higher locomotor activity (KNOP et al. 2013).

A. vulgaris can hybridise with *A. ater* s.l. (REISE et al. 2020, HUTCHINSON et al. 2021). However, in the west (see above) or in other parts of Ukraine, hybrid individuals have not yet been found. Even in Stara Huta, where both taxa are introduced, we have not yet observed them living together, which could lead to hybridisation.

POSSIBLE WAYS OF *A. ATER* S.L. PENETRATION INTO THE STUDIED AREA

The import of ornamental plants from neighbouring Poland was considered to be one of the possible ways of primary penetration of *A. vulgaris* into the Lviv region (GURAL-SVERLOVA & GURAL 2011a). In Poland, the first known records of *A. vulgaris* (since 1993, see in KOZŁOWSKI & KORNOBIS 1995) were made in the south-east of the country (WIKTOR 2004: map 56) bordering the Lviv region of Ukraine. In the first years after the discovery of *A. vulgaris* in Lviv, its populations were often found near private houses with recently planted ornamental plants, especially the now fashionable conifers (thuja, juniper, etc.). Another introduced land mollusc species, *Cepaea nemoralis* (Linnaeus, 1758), which is spreading from garden centres, is currently showing a similar trend (GURAL-SVERLOVA et al. 2021, GURAL-SVERLOVA et al. 2024). Among the first records of *A. vulgaris* in Crimea, two were made on the territories of garden centres (LEONOV 2021).

It is likely that *A. ater* s.l. was also introduced into the study area together with ornamental plants. In particular, in the administrative territory of the Syniohora National Nature Park, this species is found together with the above-mentioned *C. nemoralis*, first discovered in the Ivano-Frankivsk region in 2018 (GURAL-SVERLOVA et al. 2020). One adult specimen of *C. nemoralis* was also found by us on the fence of a household plot in Huta, not far from the second collection site of *A. ater* s.l. Thus, the joint introduc-

tion of these two species into the studied area cannot be ruled out.

However, if the introduction of *A. ater* s.l. into Ukraine occurred through any garden centre operating in the Ivano-Frankivsk region, this species could appear almost simultaneously in different settlements of the region, and not just in two neighbouring villages. Moreover, introduced species of land molluscs that spread through garden centres are usually first recorded in the centres of administrative regions or districts, their immediate environs or other large settlements, i.e. where there are more of their potential clients. Therefore, it is possible to assume direct import of ornamental plants from abroad, without the mediation of a garden centre. We believe that the most likely source of dispersal of *A. ater* s.l. in the studied area is the administrative territory of the national park or the nearby presidential residence. Judging by the photos and videos available on the Internet, many ornamental shrubs, especially conifers, are planted also in the residence.

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