Research Article

Five new introduced terrestrial slugs in Hungary

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Abstract

Five terrestrial slug species are reported from Hungary, either for the first time or confirming earlier records that had been considered unreliable: Limacus maculatus, Deroceras invadens, Ambigolimax valentianus, Ambigolimax parvipenis, and Milax nigricans. In all cases identification was supported by anatomical examination and barcoding sequences of the COI mitochondrial gene. For M. nigricans we also sequenced individuals of this species from Italy and France so as to establish differences to sequences of M. gagates. Most records came from garden centres in Budapest and from two botanical gardens; these habitats may therefore be crucial in the spreading of non-indigenous species. A Facebook appeal was used to involve citizen scientists in searching for further sites for L. maculatus, but the Limacus populations reported proved all to be L. flavus. Studies over the last four years have added altogether seven slug species to the 26 hitherto recognised in the Hungarian fauna. This demonstrates the importance of continuing to check for additional introduced species.

Key words: non-indigenous species, citizen science, distribution, greenhouse fauna, invasive species, Mollusca

Introduction

Invasive species both cause economic damage (Pimentel et al. 2005; Gall and Tooker 2017) and threaten native species (Didham et al. 2007; Pejchar and Mooney 2009; Butchart et al. 2010). Today they are spreading at an increasing rate (Seebens et al. 2017; Gladstone et al. 2020), likely because of global climate change (Hellmann et al. 2008; Mainka and Howard 2010) and increased international transportation, especially of agricultural and horticultural products (Meyerson and Mooney 2007; Cowie et al. 2008; Hulme 2009; for reviews, see Peltanová et al. 2012 and Pyšek et al. 2020). Transportation as a means of species introduction is especially important for terrestrial molluscs (Bergey et al. 2014), which otherwise have low vagility.

Invasive molluscs are understudied compared to vertebrates, insects and plants (Cowie et al. 2009). Amongst terrestrial species, certain slugs are successful invaders, and particularly important ones because they can cause...
serious economic damage in agriculture and horticulture (Buschmann et al. 2005; Douglas and Tooker 2012; Kozłowski 2012). However, slugs are often neglected in general surveys of terrestrial molluscs, because their preservation, in alcohol, is less convenient than for dry snail shells and because identification to species often requires dissection of adult specimens or genetic sequencing. The latest monograph specifically on Hungarian slugs is Wiktor and Szigethy (1983), which was based on Wiktor’s reidentification of extensive collections in Hungarian museums. Subsequently, Fehér and Gubányi (2001) and Pintér and Suara (2004) compiled distributional data on Hungarian molluscs in general, but their data collection ended in the mid 1990s. In the two decades since, a number of alien mollusc species have been introduced to Hungary (Gerber 1994; Varga et al. 2010; Csányi and Varga 2017; Páll-Gergely et al. 2020, 2021). Two years ago, Turóci et al. (2020a) reported the introduced slug species Krynickillus melanocephalus Kaleniczenko, 1851 and Tandonia kusceri (H. Wagner, 1931), which were already widespread in Hungary. Here we report five additional introduced slugs: Limacus maculatus (Kaleniczenko, 1851), Deroceras invadens Reise, Hutchinson, Schunack & Schlitt, 2011, Ambigolimax valentianus (A. Férussac, 1821), Ambigolimax parvipenis Hutchinson, Reise & Schlitt, 2022 and Milax nigricans (Philippi, 1836).

**Materials and methods**

**Sample collection**

In the four years since 2018, two of us (ÁT and BP-G) have been collecting slugs from all over Hungary as preparation for a slug identification book. Whenever we encountered a species new for Hungary, we targeted our searching to find it at additional sites. For instance, the first specimens of D. invadens and A. valentianus were found in the Füvészkkert (botanical garden of Eötvös Loránd University, Budapest), and therefore we aimed to survey garden centres, which might provide a similarly sheltered environment. For his M.Sc. thesis (Rapala 2021), one of us collected in 22 garden centres in Budapest from 10.v.2021 to 23.ix.2021. This represents about half of all garden centres in Budapest. The garden centres were open to the general public, selling primarily plants. They had outside yards and some of the plants on sale had been grown elsewhere (mainly in Hungary but also abroad, although we did not record the plants’ origins systematically). Searching covered the whole publicly accessible territory of each garden centre; it took one to two hours, depending on how much needed to be inspected. The aim was to collect as many snails and slugs as possible by lifting up flower pots, rain barrels, and bricks, looking around polytunnels and into plastic containers (among roots of trees and in the soil), and searching under ground-cover sheeting or in places where dead plant material was stored (Rapala 2021). Temperatures ranged over 15–36 °C. We did not differentiate within each garden centre where we collected each species.
Following our discovery of *L. maculatus* in one district of Budapest, we utilised citizen science to try to obtain additional records (Páll-Gergely et al. 2019). An appeal on BP-G’s private Facebook profile consisted of a short description accompanied by photographs. The post was released on 7.vi.2020. As the external morphology and colouration of slugs do not reliably identify the species (Balashov and Markova 2021), we collected and dissected specimens from all populations that citizen scientists located.

**Storing, handling and imaging of specimens**

Living specimens were kept in plastic containers punctured with holes and lined with a damp paper towel to prevent desiccation. The slugs were stored in a refrigerator until processed. Living individuals and preserved specimens were photographed from three standard views: dorsal, right side (in both extended and contracted states) and ventral (sole). We killed the individuals in 20% ethanol to prevent extreme contraction and preserved them in 75% ethanol. Ethanol (75%) was changed daily for three days or until it remained clear.

We photographed the slugs using a Canon EOS 2000d camera with Tamron SP AF 90 mm F/2.8 Di MACRO 1:1 macro objective and one camera-mounted flash, with two studio flash units (BlitzBirne Mikrosat) set up to the left and right of the subject, reflecting off white umbrellas.

Dissection was performed under a Zeiss Stemi 305 stereomicroscope. The genital organs were photographed with the same camera and lens as above, or with a Canon MP-E 65 mm F/2.8 1–5x macro objective (for *A. parvipenis*) or using a Keyence VHX5000 digital microscope (for *D. invadens*).

**Identification**

All slug species were identified on the basis of genital characters as well as external morphological traits, mainly using the works of Wiktor (Wiktor 1983, 1987; Wiktor et al. 2000) and Rowson et al. (2014b), supplemented by Reise et al. (2011) for *D. invadens* and Hutchinson et al. (2022) for *A. parvipenis*.

*Limacus maculatus* might easily have been mistaken for *Limacus flavus* (Linnaeus, 1758) previously. Therefore, we checked anatomically the previous identifications of *L. flavus* preserved in the Hungarian Natural History Museum (HNHM) in Budapest and in the Petró-collection. Ede Petró (1942–2012) was a Hungarian malacologist, whose collection is stored in the Mátra Museum of the Hungarian Natural History Museum, in Gyöngyös, Hungary.

In the Hungarian fauna, only *Deroceras sturanyi* (Simroth, 1894) and *Deroceras laeve* (O. F. Müller, 1774) externally resemble the newly recorded *D. invadens*. Since the collection of the HNHM was checked by A. Wiktor in 1982 (Wiktor and Szigethy 1983) and G. Majoros in 1989 (*unpublished*), and these species possess very different genital structures to those of *D. invadens*, rechecking the identities of these *Deroceras* species was considered not necessary.
Introduced slugs in Hungary


Table 1. Number of individuals of four slug species in garden centres and in Füvészker (botanical garden of Eötvös Loránd University, Budapest). Inventory numbers of the HNHM are indicated for each species, with the number of individuals in brackets. (Each inventory number applies to the set of individuals collected at the same location at the same time.) *Limacus maculatus* occurred neither in the garden centres nor in Füvészker.

<table>
<thead>
<tr>
<th>Location</th>
<th>Latitude (°N)</th>
<th>Longitude (°E)</th>
<th>D. invadens</th>
<th>A. valentianus</th>
<th>A. parvipenis</th>
<th>N. nigricans</th>
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Number of locations for the species

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<th>15</th>
<th>11</th>
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<td>Number of locations for the species</td>
<td>15</td>
<td>11</td>
<td>5</td>
<td>2</td>
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</table>

After 1990, c. 30–40 lots of undetermined material were collected and stored in the HNHM (Budapest). We (ÁT) checked these items, but none of the newly discovered species occurred among them.

In the case of *Ambigolimax* species, anatomical examination is necessary for distinguishing *A. valentianus* and *A. parvipenis*. In the collection of the HNHM, there were only three lots of questionable *Lehmannia* or *Ambigolimax*, which we checked.

In the family Milacidae, two species are native to Hungary—*Tandonia budapestensis* (Hazay, 1880) and *Tandonia rustica* (Millet, 1843)—and *T. kusceri* also occurs; their size and other external traits differentiate them from our new discovery, *M. nigricans*. But dissection was essential to distinguish *M. nigricans* from *M. gagates*.

Ethanol-preserved voucher specimens are deposited in the Hungarian Natural History Museum, Budapest (HNHM) and in the Plant Protection Institute, Centre for Agricultural Research, Budapest (inventory numbers are listed in Table 1).
DNA analysis

For at least one specimen of each species, the morphology-based identification was confirmed by DNA barcoding, using the usual partial sequence of the mitochondrial cytochrome oxidase subunit I (COI).

A sample of tail tissue was taken from selected animals of each species. Methods of DNA extraction and sequencing follow those described in Hutchinson et al. (2020), also using the same primer sets. They resulted in a sequence of up to 655 base pairs.

As there were no partial-COI sequences of *Milax nigricans* in GenBank, we sequenced six animals, from Dunkirk (France; two animals), Corsica (France), near Nice (France), Sicily (Italy) and Sardinia (Italy). We compared them with four haplotypes in GenBank from the British Isles identified as *M. gagates* by Rowson et al. (2014a) and with our four new sequences of *M. gagates* from the Algarve (Portugal), Madeira (Portugal), the Netherlands, and Belgium. Localities are listed in Table 2.

Results

For each of the newly recorded species, we present a brief account of its taxonomy and known distribution before detailing our own findings.

*Limacus maculatus* (Figure 1)

The names *Limax grossui* Lupu, 1970 and *Limax pseudo flavus* Evans, 1978 have been synonymised with this species (Wiktor and Norris 1982). *Limax ecarinatus* Boettger, 1881 is the correct name if *Limacus* is treated as a subgenus of *Limax* (Welter-Schultes 2013: 65). The native range is believed to be around the Black Sea, where it occurs in deciduous forest (Wiktor and Norris 1982; Wiktor 1983). *Limax maculatus* has been known in Great Britain since 1884, but it was in Ireland that it became widespread already in the twentieth century (Kerney 1999). Over the last 20 years it has been spreading rapidly through Great Britain, particularly but not exclusively in synanthropic habitats. In the same period apparently new populations have been reported from the Netherlands, Germany, the Czech Republic, Ukraine, and Belarus (Balashov and Markova 2021; Čejka et al. 2020; Langeraert et al. 2021; Jueg et al. 2022). The species is distinguishable from its congener *Limacus flavus* by the attachment point of the bursa duct, but conflict between COI sequences and genital anatomy have indicated hybridisation (Rowson et al. 2014a).

We found *L. maculatus* at two sites in Budapest about 100 m apart. In 2019 (8.iv–10.v), five specimens were found at the Kass János lépcső, II. district, Budapest. This is a narrow paved path with c. 2 m wide strips of grass on either side. Beyond those are fences and hedges of private gardens. We found the specimens under these hedges. Three additional specimens were found in 2020 crawling on the pavement at Herman Ottó út 9, II. district, Budapest.
Table 2. Details of slugs for which a partial-COI sequence was obtained. Only one example of each haplotype was submitted to GenBank: entries without a GenBank number share the haplotype of that preceding it (but the sequence may be shorter). An additional short sequence from another *A. valentianus* was compatible with both haplotypes found in other individuals of this species. Our analysis also utilisedKF894243.1, KF894314.1, KF894340.1, and KF894342.1 GenBank sequences of *M. gagates* from Britain and Ireland. Collection numbers of the Senckenberg Museum of Natural History Görlitz start with a p; the others are from the Hungarian Museum of Natural History.

<table>
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<th>Species</th>
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<th>Longitude (°E)</th>
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The Facebook post about *L. maculatus* had been shared 346 times up to 25.iii.2022, predominantly during the first three weeks (starting 7.vi.2020). This number underestimates how many people will have viewed the post, probably considerably. Amongst some misidentifications (e.g. photographs showing *Limax maximus* Linnaeus, 1758), 14 populations of *Limacus* were reported, all with photographs. As a consequence we were able to study 59 specimens altogether, from 9 sites in Budapest (all private gardens) and from 3 towns elsewhere (5 specimens from Eger, 13 specimens from Debrecen).
and 2 specimens from Kecskemét). Dissection proved that all these populations were *L. flavus*.

The museum specimens of *Limacus* also proved to be *L. flavus*. We dissected altogether nine specimens, from localities widely spread over Hungary: Böhönye, Budatétény, Debrecen, Etyek, Komárom, Martonvásár, Sükösd, Székesfehérvár, and Verpelét.

We observed some differences in external appearance between Hungarian populations of *L. maculatus* and *L. flavus*. In *L. maculatus* the head was more similar in colour to that of the body: the neck and the base of the tentacles were greenish-yellowish and the grey colour occurred only towards the eyes (Figure 1A). In *L. flavus* the neck was also yellowish, but the whole tentacles appeared grey from their bases, contrasting more strongly with the colour of the mantle (Figure 1B). The sole was more uniformly...
coloured in *L. maculatus* than in *L. flavus*, in which an intense yellow frame bordered the edges of the sole (due to the more yellowish mucus). Contrary to descriptions in the literature (e.g. Wiktor 1983), the mucus of *L. maculatus* was often transparent rather than yellow.

One external character found to distinguish the two species in the British Isles was the presence of a pale line along the back of *L. flavus*, which was absent in *L. maculatus* (Rowson et al. 2014b). In Hungarian *L. flavus* usually this line was more or less continuous only along the posterior third of the back; more anteriorly it was broken into streaks (Figure 1B). This is not so different from Rowson et al.’s (2014b) illustration of an immature British *L. flavus* (“M19”), although in other British specimens it was less broken. In contrast, Balashov and Markova (2021) reported that introduced populations of *L. flavus* in Kyiv lacked a line. The line was absent in our specimens of *L. maculatus*, but they were too few to judge whether this is consistent in the Hungarian population.

All eight specimens of *L. maculatus* were distinguishable from *L. flavus* anatomically (i.e. by the attachment of the bursa duct to the penis and the shorter penis: Rowson et al. 2014b; Figure 1C, D).

We sequenced four individuals of Hungarian *L. maculatus* (including animals from both sites) and seven individuals of Hungarian *L. flavus* (from three sites in Budapest and from four other towns). This yielded a single partial-COI haplotype of *L. maculatus* and three haplotypes of *L. flavus* (Table 2). The *L. maculatus* haplotype matches that recorded from the Czech Republic (MT947694.1) and from the most common haplotype of *L. maculatus* found in Great Britain and Ireland (e.g. KF894225.1). It also matches two Genbank sequences from Britain that were reported as *L. flavus* (AM259713.1, AM259714.1), but we consider these liable to have been erroneously identified. A *L. maculatus* haplotype from Niedersachsen in Germany (Jueg et al. 2022; ON756107.1) is just 1 bp different. It thus seems plausible that the Hungarian population of *L. maculatus* derives directly or indirectly from the long-established populations in the British Isles rather than arriving independently from the native area (believed to include the neighbouring countries of Romania and Ukraine: Wiktor and Norris 1982).

The *L. flavus* sequences are identical to, or only 1 bp different from, haplotypes found in the Czech Republic, Slovakia and Germany.

*D. invadens* (Figure 2)

Until 2011 (Reise et al. 2011), this species was not distinguished from *D. panormitanum* (Lessona & Pollonera, 1882), formerly also known as *D. caruanae* (Pollonera, 1891) and *D. pollonerae* (Simroth, 1889). Earlier records of *D. panormitanum* mostly refer to *D. invadens* since the latter has spread much more widely. The origin of *D. invadens* is southern Italy (Hutchinson et al. 2020), but it has spread globally in temperate regions,
including North and South America, Africa and various oceanic islands, although apparently almost not at all in Asia (Hutchinson et al. 2014). Although abundant in much of Western Europe, its expansion eastwards appears limited by cold winter temperatures. Concerning nearby countries, it is widespread in Austria, but records are still sparse in Poland, the Czech Republic and Slovakia (Hutchinson and Reise 2015; Čejka et al. 2020, 2021), and it has not been reported from further neighbouring countries. Pintér (1974) reported “D. caruanae” from the botanical garden in Vácrátót and from Budapest. But later, Pintér (1984: 89) included the following note against the list for Agriolimacidae, “Likharev et Wiktor (1980). D. caruanai temporarily should be deleted.” The deletion might have been because Likharev and Wiktor (1980) did not list this species from Hungary or perhaps following Wiktor’s re-examination of Hungarian museum material (Wiktor

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**Figure 2.** *Deroceras invadens* Reise, Hutchinson, Schunack & Schlitt, 2011. Living individual (A) (HNHM 105369), preserved specimen (B) (HNHM 105379) and reproductive anatomy (C) (HNHM 105377). Abbreviations: a = atrium, c = (penial) caecum, l = (penial) lobe, ovi = oviductus, p = penis, pg = penial glands, pr = penis retractor, vd = vas deferens. Photographs by Ágnes Turóci.
and Szegedy 1983). Subsequently, Pintér and Suara (2004) also omitted the species from their revised list of Hungarian species, here without any note of explanation.

We found *D. invadens* in the Füvészkert (botanical garden of the Eötvös Loránd University in Budapest) on 17.ix.2019. We identified 47 adults from this site (all of them from outdoors), with perhaps more among the juveniles. This species also occurred in 15 out of 22 garden centres in Budapest but did not turn up outside of Budapest. Budapest lies slightly further east than any outdoors population known from mainland Europe (Hutchinson et al. 2014). However, minimum temperatures in Budapest from 2001–2020 never dropped below −15 °C, with a median yearly minimum of −10 °C (Országos Meteorológiai Szolgálat 2022), which is considerably warmer than some sites from which *D. invadens* has been recorded (Hutchinson et al. 2014).

The slugs varied from light to dark brown (Figure 2A, B). The external features and internal anatomy matched the original species description (Reise et al. 2011). The penis usually has two pockets, the lobe and the caecum (Figure 2C). In our populations both were always present, but the lobe was shorter than the caecum.

We sequenced the usual barcoding segment of the COI gene in three animals from the Füvészkert (Table 2). One had the commonest haplotype worldwide and two had the second commonest (the red and blue haplotypes in Hutchinson et al. 2020: JQ743070.1 and KX977426.1).

### Ambigolimax valentianus (Figure 3)

This species appears in the literature also as *Limax valentianus* A. Férussac, 1821, *Lehmannia valentiana* (A. Férussac, 1821) and *Limax poirieri* Mabille, 1883. Its most distinctive anatomical character is the large penial appendage with a rounded end (Figure 3C), but this sometimes can be inverted into the penis, which must then be cut open to reveal it. Hutchinson et al. (2022) further suggested that the length of the rectal caecum was an informative character. *Ambigolimax valentianus* is often said to originate from the Iberian Peninsula, but the only evidence for this is its early discovery there. Already Waldén (1961) documented a worldwide distribution (besides Europe, also North and South America, South Africa, Australia and several oceanic islands), and Stojnić et al. (2011) summarised subsequent records. Nearby countries from which it has been reported are Austria (Reischütz 1986), the Czech Republic (Horsák et al. 2004), Poland (Wiktor 2004), Slovakia (Čejka et al. 2021), Serbia (Stojnić et al. 2011) and Romania (Grossu 1983). It is widespread in greenhouses and often abundant enough to be a pest (e.g. Horsák et al. 2004 in the Czech Republic), and increasingly it is found outdoors (e.g. near Bratislava: Čejka et al. 2021), although that need not necessarily imply that it can survive winters there. In Hungary it was recorded in 1964 from the Füvészker (botanical garden of the Eötvös Loránd University, Budapest) (Flasarová and Flasar 1965) and included in
Pintér’s (1974) checklist of Hungarian species, but later lists omitted it. A possible reason is that Hungarian specimens preserved in museums were all identified as this species only tentatively.

These museum specimens are 38–42 years old and in poor condition. The earliest lot is from the greenhouse of Vácrátót botanical garden (leg. János Podani, 11.ix.1979, cat. no. 105329). The vial contains two specimens, very faded but with two darker bands on the mantle, indicative of *Ambigolimax*. The genitalia had been cut out and only one genital organ was in the vial; it is unknown to which specimen it belongs. The penis is roughly as long as the bursa copulatrix and its duct combined. This rules out *A. parvipenis*, whose penis is half as long as the bursa and its duct. The penial appendage is not visible at the tip of the penis, but further dissection revealed it to be inverted inside the penis. The rectal caecum was not identifiable owing to
the poor condition. Given the mantle markings and the penis anatomy, we confirm the identification as *A. valentianus*.

The second lot is from FFI Kertészete (garden centre), Tengerszem utca 59, XIV. district, Budapest (leg. Éva Kiss, 21.i.1984, cat. no. 105330). The single specimen has an entirely homogeneous, pale brownish skin on the body, faded patterns on the mantle, but without any bands visible (which might be an artefact of preservation). The genital tract is dissected out but complete, and the penis consists of two sacs with what might be interpreted as a huge penial appendage at the tip, which once coiled spirally. Taking into account the external morphology and the structure of the penis, a possible identification is a euphallic *Deroceras laeve*.

The third lot is from a greenhouse in the botanical garden at Vácrátót (leg. Fürjes, Kiss, Pintér, 16.v.1984, cat. no. 105331). The single specimen is pale, with two bands on the mantle continuing posteriorly along the back. That confirms that it is *Ambigolimax*, but the specimen was too juvenile to reveal the genital anatomy. We checked the rectal caecum, which continues to the tip of the visceral sac, suggesting *A. valentianus* (Hutchinson et al. 2022), but we observe below that this distinguishing character is not fully diagnostic.

We have now also confirmed the modern-day presence of *A. valentianus* in the Füvészkert (outdoors, leg. Alexandra Juhász, 07.ix.2020 and 17.ix.2021), which means that it either has persisted there for 58 years, or it was re-introduced. It also occurred in 10 of 22 garden centres in Budapest (Table 1).

The colouration of our fresh specimens was very diverse, but most common was a creamy-brown background colour with a pair of bands running along the dorsal side of the animal, and usually an additional patch on the centre of the mantle (Figure 3A, B). The pair of bands were also well visible in preserved specimens (Figure 3B). Very pale individuals also occurred, in which bands were visible only on the mantle, not on the back. Some specimens were translucent.

Four specimens of *A. valentianus* were sequenced, each from a different garden centre (Table 2). They yielded two partial-COI haplotypes, both widespread elsewhere in the species’ introduced range.

**Ambigolimax parvipenis** (Figure 4)

Until very recently (Hutchinson et al. 2022), this was one of several species confused under the name *Lehmannia nyctelia* (Bourguignat, 1861) or *Ambigolimax nyctelius*. However, Rowson et al. (2014b) and Vendetti et al. (2019) had already described the distinctive anatomy of a small penis without an appendage. Externally it is not distinguishable from *A. valentianus* with certainty. It is spreading rapidly in the British Isles (Rowson et al. 2014b) and is also well established in California (Vendetti et al. 2019) and the Iberian Peninsula (Hutchinson et al. 2022). No doubt *A. parvipenis* is still under-
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Figure 4. *Ambigolimax parvipenis* Hutchinson, Reise & Schlitt, 2022. Living individual (A) (HNHM 105371), the same specimen preserved (B), and reproductive anatomy (C) (HNHM 105378). Abbreviations: bc = bursa copulatrix, p = penis, so = spermoviductus, vd = vas deferens. Photographs by Ágnes Turóci.

recorded, but the nearest known occurrences to Hungary are Athens and northern France (Hutchinson et al. 2022).

*Ambigolimax parvipenis* was recorded from five of the 22 garden centres in Budapest. We initially overlooked this species as juvenile *A. valentianus*. Compared to that species, *A. parvipenis* was often smaller. Its colouration was often darker and characterised by more bands, which were often discontinuous, giving the animal a distinctive mottled appearance (Figure 4A, B). The penis is half the length of the bursa and its duct combined and lacks a penial appendage (Figure 4C). Hutchinson et al. (2022) suggested the shorter rectal caecum as a useful character to distinguish *A. parvipenis* from *A. valentianus*, but this proved not to be consistent among our specimens. The rectal caecum of one juvenile ended at the tip of the visceral sac, a character state that Hutchinson et al. (2022) associated with *A. valentianus*. We have also recently
Milax nigricans (Philippi, 1836). Living individual (A), preserved specimen (B), reproductive anatomy (C), atrium stimulator (D) with some of the papillae marked with red arrows (HNHM 105380). Abbreviations: a = atrium, ag = atrium glandula, at = atrium tubuli, bc = bursa copulatrix, dh = ductus hermaphroditicus, epi = epiphallus, ga = glandula albuminalis, gh = glandula hermaphroditica, ovi = oviductus, p = penis, pr = penis retractor, sti = (atrial) stimulator, vd = vas deferens. Photographs by Ágnes Turóci.

encountered other such specimens from Scotland (JMCH unpublished). One specimen was sequenced (Table 2). It yielded a partial-COI sequence otherwise known only from the Iberian Peninsula (e.g. MF982922.1).

**Milax nigricans** (Figure 5)

This species has often been confused with *Milax gagates* and doubts have sometimes been expressed whether they are separate species (Rowson et al. 2014b). Although *M. nigricans* is often darkly coloured this is not a consistent character. Reliable identification requires examining the surface structure of the atrial stimulator (Hutchinson and Reise 2013). The species is widespread around the Mediterranean, with the nearest such population to Hungary the single reported occurrence in Croatia (Wiktor 1996). The species has established...
itself at several sites in north-east France and the Netherlands, and at one time there was a population in Munich (Hutchinson and Reise 2013).


Because many of the specimens found were pale, we initially suspected a mixed population with *M. gagates*, especially as our individuals had light foot soles (Figure 5A, B); Wiktor (1987) stated that *M. nigricans* usually has a dark sole. The rim of the pneumostome was the same shade as the surrounding skin, whereas in *M. gagates* the rim may stand out as darker (Rowson 2014b). However, dissection indicated that all were *M. nigricans* (Figure 5C). In agreement with Wiktor (1987), the massive atrial stimulator has lots of papillae, all situated on its basal two-thirds (Figure 5D).

Our eight sequenced specimens of *M. nigricans* from diverse localities yielded three distinct haplotypes compared with five haplotypes from our sample of *M. gagates*. Maximum variation within *M. nigricans* was 1.4% (9 bp) and within *M. gagates* was 2.5%, compared with the minimum between-species difference of 8.5%. Hence, the pattern of variation in the mitochondrial COI gene is supportive of these being distinct species. This conclusion is unchanged if we incorporate the 51 “*Milax* sp.” sequences in GenBank provided by Gómez-Rodríguez et al. (2019) from the Iberian Peninsula, all of which group with our *M. gagates* haplotypes. Our conclusions concur with the genetic study of Zając et al. (2022). Of the two sequenced *M. nigricans* from Budapest (from different garden centres), one had the same haplotype as a specimen from Corsica (France), and the other matched specimens from Sardinia (Italy) and near Nice (France) (Table 2).

**Discussion**

Pintér and Suara (2004) listed 26 slug species for Hungary. In the last four years we have discovered seven additional introduced species, two of which had earlier claims of occurrence (*D. invadens*: Pintér 1974; *A. valentianus*: Flasarová and Flasar 1965). Two species have spread countrywide (*K. melanocephalus* and *T. kusceri*: Turóci et al. 2020a), but three species were found only in garden centres in Budapest (*A. parvipenis, A. valentianus* and *M. nigricans*). They might be restricted to such sites because the greenhouses provide protection from winter cold, but garden centres might be richer in species also because they import plants from abroad. Some garden centres provide an environment intermediate between greenhouse and garden, which could facilitate adaptation through both phenotypic plasticity and evolution. In any case, the garden centres will be playing a major role in spreading slugs into gardens. Slugs can easily hide in plant pots and tolerate long-distance journeys in soil.

In contrast, the role of climate change in furthering the spread of alien species must be tested by examining whether the slug species survive and
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reproduce outdoors. Winters might be too cold for them or summers too dry. As winter weather becomes less extreme because of global climate change, the situation may alter. One suggestive example is the slug *T. kusceri*, which originated in the Balkans (where the winter climate is warmer than in Hungary) but was able to spread through Hungary in synanthropic habitats (Turóci et al. 2020a).

The spread of introduced species is a great challenge globally. In Hungary *Arion vulgaris* Moquin-Tandon, 1855 provides an example of a slug that is causing appreciable economic damage, having spread rapidly since its first reported occurrence in 1985 (Turóci et al. 2020b). We do not have information about what damage the new introduced species are currently causing, and it is difficult to predict which of them, if any, will be similarly economically important. Monitoring the populations of all non-native terrestrial molluscs is thus worthwhile, although it is a big step from that to stopping their further spread.

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**Authors’ contribution**

BP-G and ÁT were responsible for the research conceptualization, sample design and methodology. BP-G, MR and ÁT collected the specimens (MR in the garden centres and ÁT in Füveszkert, Budapest); ÁT took the photographs and made the anatomical determinations with advice from HR and BP-G. Data management was ÁT’s task. BS generated the DNA sequences, which JMCH analysed. BP-G and ÁT organised funding (see Funding declaration). ÁT wrote the original draft of the manuscript to which JMCH, BP-G, MR and HR further contributed.

**References**


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Web sites and online databases