

FIRST RECORDS OF THE TERRESTRIAL SLUG *ARION ATER* S. L. (LINNAEUS, 1758) (PULMONATA: ARIONIDAE) FROM TURKEY

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ABSTRACT: A strong population of the terrestrial slug *Arion ater* s. l. is reported from the European and Asian parts of Istanbul, Turkey. This is the first confirmed report of this large, conspicuous taxon from Turkey and from Asia. Our samples from five synanthropic sites indicate that it is already well established. Partial sequences of the mitochondrial COI gene (cytochrome c oxidase subunit I) place the Turkish slugs in a small clade shared with a few specimens from western France, perhaps indicating the origin of the Istanbul population. The next closest haplotypes (9% difference) fall within the clade identified as *Arion ater* s. s. This fits with the genital morphology of the Turkish slugs, which is most similar to the *ater*-form of *A. ater* s. l. Our discovery also puts a new light on the recent report of the highly invasive pest slug *Arion lusitanicus* auct. non Mabille, 1868 (often called *Arion vulgaris* Moquin-Tandon, 1855) in Isparta, which was identified only on the basis of external morphology. As reliable morphological distinction of these two species requires examination of the genital anatomy, the specimen from Isparta should be reinvestigated.

KEY WORDS: invasive species, *Arion lusitanicus*, *Arion vulgaris*, *Arion rufus*, Istanbul

INTRODUCTION

Some synanthropic species of terrestrial molluscs are prone to spread: they hide under items such as bricks, plastic sheets and wood piled up in the open before being transported elsewhere, or they live on horticultural plants or vegetables. Young slugs or eggs may be transported in the soil of flower pots. An ability to self-fertilise may help single displaced specimens to establish a new population. But in any case, most terrestrial molluscs are hermaphrodites, so every adult that had mated prior to its dispersal may lay fertilised eggs, and every other mature conspecific encountered is a potential mate.

A particularly notorious case is the highly invasive *Arion lusitanicus* auct. non Mabille, 1868 (henceforth

called just *A. lusitanicus*), also often called *A. vulgaris* Moquin-Tandon, 1855 (nomenclature currently under consideration by the ICZN: [BALASHOV 2018](#)). This large slug has a considerable potential as an agricultural and horticultural pest. It has spread across Western and Central Europe and into Northern and Eastern Europe, and it has recently also turned up in North America ([HATTELAND et al. 2013](#), [PĂPUREANU et al. 2014](#) and references therein, [ZEMANOVA et al. 2016, 2018](#)). So, it was not too surprising that *A. lusitanicus* was recently reported from Turkey (as *A. vulgaris*, [YILDIRIM & GÜRLEK 2017](#)). The nearest earlier records are from Bulgaria, only 440–500 km away (see [PĂPUREANU et al. 2014](#)).





However, *A. lusitanicus* is not the only large *Arion* species inhabiting European synanthropic habitats. Externally extremely similar are forms of *Arion ater* s. l. (Linnaeus, 1758), a taxon which is widely distributed and common across Western and Central Europe (WIKTOR 1973, ROWSON 2017). It occurs in synanthropic habitats as well as natural ones such as woodland and open grassland (RÜETSCHI et al. 2012, WELTER-SCHULTES 2012, ROWSON et al. 2014b). As an inhabitant of synanthropic habitats, it has also been spread by human activities, partly even by deliberate introduction (WIKTOR 1973, VON PROSCHWITZ 1997, WIESE 2014). The eastern margin of its natural range is not clear, but it is assumed to lie somewhere in western Poland (WIKTOR 1973, WELTER-SCHULTES 2012, ROWSON 2017). There are also isolated populations in Eastern Europe such as in Latvia (RUDZĪTE et al. 2010) and Russia (LIKHAREV & WIKTOR 1980), indicating earlier introductions (ROWSON 2017). In contrast to *A. lusitanicus*, *A. ater* s. l. made the step across the Atlantic long ago, occurring in both Canada and the United States (PILSBRY 1948, FORSYTH 2004); more recently it has turned up in Australia (ZEMANOVA et al. 2018).

Here, we report the discovery of *A. ater* s. l. in Turkey and document that it is already quite widespread in the area of Istanbul.

The taxonomy of *A. ater* s. l. is somewhat unresolved. It has been disputed whether *A. ater* s. s. should be considered as conspecific with *A. rufus* (Linnaeus, 1758) (the former then representing a subspecies *A. ater ater* from the northern parts of the distribution range), but the two taxa tend to be treated as separate species by current taxonomists. Based on molecular data, ROWSON et al. (2014a) recently split *A. rufus* further into two species, *A. rufus* s. s. and *A. empiricorum*. But mitochondrial sequences alone are not sufficient evidence to make species distinctions within a group of taxa known to hybridise (contra PELÁEZ et al. 2018). REISE et al. (2017 and unpublished data) have observed a considerable anatomical variability of this group in Central Europe and recognised three morphological types including one corresponding to *A. ater* s. s. As taxonomy is not the task of this paper, and in order to avoid having to decide for one or the other name, we decided to use the name *A. ater* s. l., referring to *A. rufus* s. s., *A. empiricorum*, *A. ater* s. s. and potential further (western European) forms.

MATERIAL, METHODS AND RESULTS

COLLECTION

Our investigations were initiated by a garden owner in Istanbul (Turkey) inquiring about a heavy slug infestation. We collected a sample of slugs in this garden (site 3) and at four additional sites where we noticed further occurrences (Table 1, Fig. 1). Four of the sites were within the European area of Istanbul and had similarly high slug densities. Site 5 was on the Asian side of Istanbul and had a lower slug density.

All localities are not situated within the densely built-up urban centre of Istanbul at the southern end of the Bosphorus but at rural and village sites to the north and east, and always very close to forest (Fig. 1). However, we did not systematically check a diversity of localities and habitats.

All slugs (20 specimens from each of sites 1–4 and 10 specimens from site 5) were collected between September and November 2017. They were killed either in 75% ethanol or by drowning and were then

Table 1. Collection localities and the habitats of the immediate and surrounding areas. N_c – number of specimens collected; N_d – number dissected

Site	Locality, coordinates	N_c	N_d	Habitat	
				Collection habitat (0–10 m)	Surrounding habitat (10–200 m)
1	Bahçeköy 41°10'31"N 28°59'32"E	20	7	Concrete and stone wall, garden	Deciduous trees, shrubs, buildings, grass and weeds
2	Bahçeköy 41°10'42"N 28°59'44"E	20	7	Asphalt road, soil, weeds and grass, buildings, vegetable garden, deciduous trees	Deciduous trees (<i>Quercus</i> , <i>Fagus</i> , <i>Carpinus</i>), bushes, edge of forest, buildings, grass and weeds
3	Haciosman 41°08'14"N 29°01'22"E	20	8	Grass and weeds, garden	Bushes, building, coniferous forest (<i>Pinus</i>), edge of forest
4	Zekeriya köy 41°12'29"N 29°02'36"E	20	5	Grass, garden, ornamental plants, street	Fruit trees, vegetable garden, buildings, coniferous forest (<i>Pinus</i>) and deciduous trees, residential areas
5	Elmalı 41°04'29"N 29°07'07"E	10	5	Soil, grass and weeds	Buildings, bushes, deciduous trees, coniferous forest (<i>Pinus</i>), edge of forest

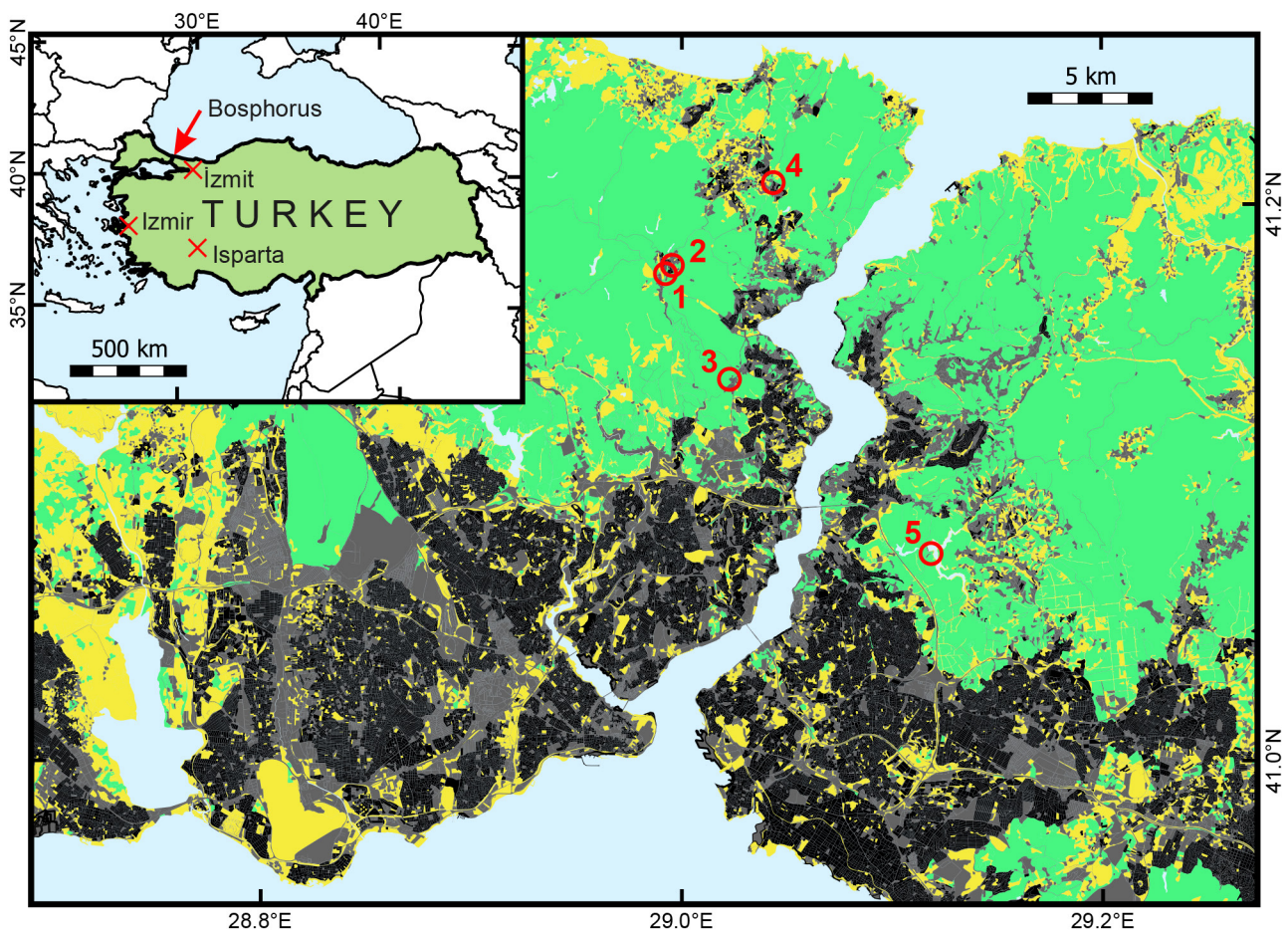


Fig. 1. The inset map of Turkey (green) shows the location of the cities of Isparta, Izmir and İzmit and of the Bosphorus strait between Europe and Asia. The main map shows the Bosphorus with the five numbered sites where we sampled *Arion ater* s. l. (numbers according to Table 1). The colour coding of the main map indicates land use: black and grey – built-up areas; green – forest and other more natural habitats; yellow – agricultural land and other disturbed vegetated habitats. Based on data from Natural Earth and the Copernicus Urban Atlas; drawn using QGIS v. 3.2 (QGIS DEVELOPMENT TEAM 2017)

preserved in 75% ethanol. Seven specimens from sites 1–3 were sent to the Senckenberg Museum of Natural History Görlitz (SMNG), Germany (with permission of the General Directorate of Nature Conservation and National Parks of Turkey).

Thirty-two of the collected specimens were dissected and determined anatomically: five from each of the five localities by ZA, and the seven sent to the SMNG by HR. The specimens in the SMNG are now stored in that collection (numbers p22687–p22689); 25 specimens (5 per site) are in the collection of the Department of Forest Entomology and Protection in the Istanbul University Cerrahpasa (Gastropoda 00001–00005).

MORPHOLOGY

Living specimens (Figs 2–5) were large, bulky slugs with coarse tubercles on the back and sides. The body colour ranged from orange or reddish to dark brown. One (smaller) specimen from locality 1

showed lateral bands (Fig. 4). The sole fringe was orange to red, the sole light cream. The body length when crawling ranged from 75 to 150 mm.

All dissected specimens were sexually mature or subadult, and the majority showed the typical characters of *A. ater* s. l., as presented in, for instance, ROWSON et al. (2014b) or WIKTOR (1973): the external appearance combined with the bulky, more or less spherical proximal atrium and the small, thin free oviduct (Fig. 6). All the specimens deposited in the SMNG (from localities 1, 2 and 3) are most similar to the *ater*-form identified by REISE et al. (2017). Relevant characters are the comparatively large lower atrium, the shape of the ligula and its insertion on the inner wall of the proximal atrium.

A few specimens from sites 1, 2, 3 and 5 had some genital characters untypical of *A. ater* s. l. (HR: unpublished data) but also of *A. lusitanicus*, requiring further investigation. Currently, we consider it possible that these specimens are hybrids between the two species.



Figs 2–5. External appearance of large *Arion* from Istanbul (from sites 3, 2, 1 and 3 respectively). Figure 4 shows one of the anatomically untypical specimens mentioned in the text and the only one with lateral bands

MOLECULAR STUDIES

For three specimens, one from each of sites 1, 2 and 3, we sequenced the standard barcoding region (655 bp) of the mitochondrial COI (cytochrome c oxidase subunit I) gene. Tissue samples were taken from the foot. DNA extraction followed WINNEPENNINCKX et al. (1993). For DNA amplification, we used Taq-polymerase, buffer from Peqlab (Erlangen) and the

standard barcoding primers LCO1490 and HCO2198 (FOLMER et al. 1994). PCR was carried out with total volumes of 10 μ l, $T_a = 40$ °C, 38 cycles. The DNA fragments were gel purified with the QiaQuick gel extraction kit (Qiagen, Hilden, Germany) and then sent to the Senckenberg BIK-F Laborzentrum (Frankfurt) for direct sequencing in both directions. The sequencing electropherograms were checked manually.

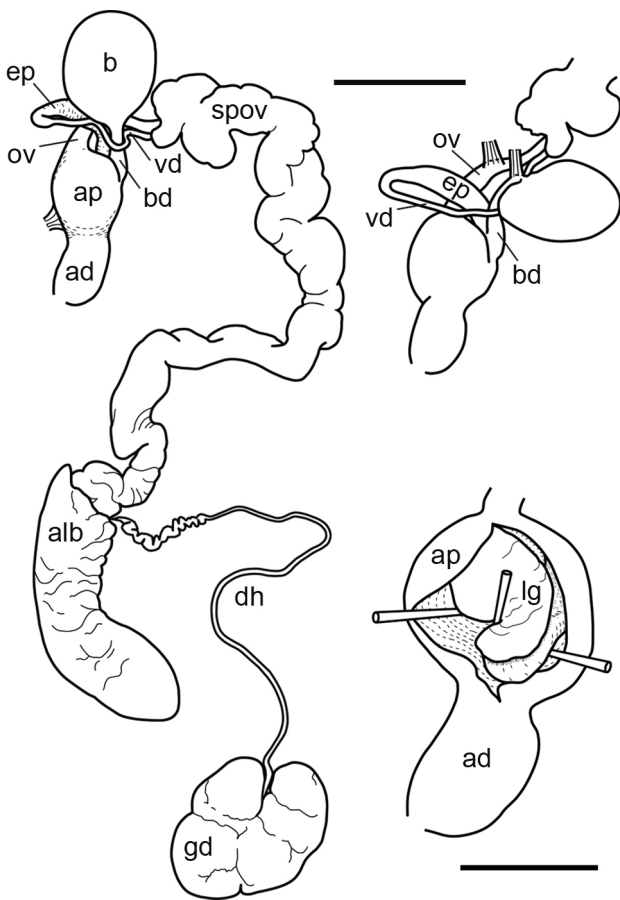


Fig. 6. Genital anatomy of a typical specimen from site 2.

In the top right-hand drawing, the genitalia have been slightly reorientated to reveal the insertions of epiphallus and bursa duct. The bottom-right drawing, at a higher magnification, shows the proximal atrium opened to reveal the ligula. Abbreviations: ad – distal atrium, ap – proximal atrium, alb – albumen gland, b – bursa, bd – bursa duct, dh – ductus hermaphroditicus, ep – epiphallus, gd – gonad, lg – ligula, ov – oviduct, spov – spermoviduct (upper scale bar 10 mm, lower scale bar 5 mm)

We compared the Istanbul partial-COI haplotype with the hundreds of sequences of large *Arion* available from Genbank (particularly from the studies of

PFENNINGER et al. 2014 and ZEMANOVA et al. 2016) supplemented by our own unpublished sequences. The three individuals from Istanbul were identical with each other (GenBank accession number MH830239), and most similar (differing by 3%, 18–20 bp) to a group of three haplotypes (KX834752, KJ842906, KJ842937) from western France, which differed by only 3–5 bp amongst themselves. The first of these is identified as *A. vulgaris* in GenBank and in Table S5 of the associated publication, ZEMANOVA et al. 2016 (although not in the phylogenetic tree in Fig. S2 of this same paper), but the sequence is very untypical of other *A. lusitanicus*. The other two were deposited as “*Arion* sp.” by PFENNINGER et al. (2014). The next most similar haplotypes to that from Istanbul are considerably more different (9%) and fall within the clade identified by ZEMANOVA et al. (2016) as *A. ater* s. s. (e.g. KJ842864, KJ842862, KJ842870). Differences from members of the clades that ZEMANOVA et al. (2016) identified as *A. rufus* and *A. vulgaris* are typically a little higher still (c. 10% and 11%).

DISCUSSION

Our records of *A. ater* s. l. constitute the first from Turkey, and locality 5 is the first from Asia. We know of no potential source populations in the close neighbourhood of Turkey. Records from Bulgaria are most likely referring to *A. lusitanicus* (OSHANOVA 1968, DAMJANOV & LIKHAREV 1975, IRIKOV & ERÖSS 2008), and old literature records from Romania, Albania and Bosnia need verification (WIKTOR 1996, PĂPUREANU et al. 2014 and references therein, FEHÉR & ERÖSS 2009). The southeastern margin of the distribution range of *A. ater* s. l. and its closest confirmed occurrence to Turkey is considered to be in western Hungary (PINTÉR & SUARA 2004, ROWSON 2017).

The genital anatomy places our specimens clearly into *A. ater* s. l. and excludes *A. lusitanicus*. However, it is more puzzling where within *A. ater* s. l. the Turkish specimens should be placed. The genetic results, based only on a mitochondrial sequence, indicate some distance from forms of *A. ater* s. l. commonly found in Central and Northern Europe, Britain and northern Spain, and a closer relationship

to populations from western France. The taxonomic identity of these haplotypes fitting best with our Istanbul haplotype is somewhat uncertain. They were generated by studies concentrating on genetics, but not on morphology. PFENNINGER et al. (2014) do not provide any information on the morphology of their two specimens, and labelled them as “*Arion* sp.”, probably because the sequences could not be assigned to a named species in GenBank. Subsequently a third similar sequence was labelled as *A. vulgaris* by ZEMANOVA et al. (2016), but it is very different from those of others from this species (= *A. lusitanicus*).

The taxonomy of large *Arion* is still somewhat unresolved and requires further research, particularly in Southwest Europe. CHEVALLIER (1972, 1974) distinguished four subspecies of *A. rufus* (and separated *A. ater* as its own species), of which two were limited to western parts of France. This was entirely based on morphological characters, but, nevertheless, it indicates the presence of genetically distinct populations in western France.



The most similar haplotypes besides these three uncertain specimens are from individuals named in Genbank as *A. ater* s. s., which fits with our morphological determination of the Turkish slugs as closest to the *ater*-form of *A. ater* s. l. (REISE et al. 2017). However, the Istanbul slugs' body colour, particularly those bright orange to reddish brown, is very untypical of *A. ater* s. s., which tends to be dark brown or black. The colouration of *A. ater* s. s. does vary over a wider range than this, from whitish yellow to black, but bright orange or red are not mentioned by, for example, ROWSON et al. (2014b) or WIESE (2014).

The genus *Arion* is not native to Turkey (WELTER-SCHULTES 2012), and only two species of *Arion* had been reported from there: *A. subfuscus* (subgenus *Mesarion*) from the town of Sapanca, northwestern Turkey, in 2003 (YILDIRIM & KEBAPÇI 2004) and a single individual of a large *Arion* (subgenus *Arion*) determined as *A. vulgaris* (YILDIRIM & GÜRLEK 2017). The latter was found in 2017 in a pot plant, which had been bought in the city of Isparta (c. 400 km SSE of Istanbul, Fig. 1), but, according to the florist who had sold the plant, it originated from the surroundings of Izmir on the west coast (Fig. 1). However, this determination as *A. vulgaris* (the same species that we term *A. lusitanicus* auct. non Mabilie) was based solely on external characters (YILDIRIM & GÜRLEK 2017, ÜMIT KEBAPÇI pers. comm.). So, in the light of our discovery of several strong populations of *A. ater* s. l. in Istanbul, it would be highly desirable to reinvestigate the specimen from Isparta. In addition to this record, already in 2015 a large *Arion* was photographed in the city of İzmit (c. 100 km E of our sites, Fig. 1, CARLISLE 2015); the external appearance is compatible with *A. ater* s. l. and perhaps *A. lusitanicus*, but information on genitalia or genetics would be required for a reliable species identification.

A. lusitanicus and *A. ater* s. l. both exhibit considerable colour polymorphism, and lighter or darker orange-brown individuals are very common in both taxa. Genital anatomy is thus essential for species determination, but rather straightforward unless hybrids occur: *A. ater* s. l. has a large, muscular and more or less spherical proximal genital atrium, and a thin, rather short oviduct (Fig. 6), whereas *A. lusitanicus* has a comparatively small atrium with a thick, tubular oviduct inserting onto it (see, e.g. ROWSON et al. 2014b).

We cannot exclude that *A. lusitanicus* is also present in Istanbul, but at least it seems not to be the predominant species. The fast spread of this conspicuous slug elsewhere has attracted much pub-

lic attention, because it can quickly establish populations with very high densities and cause much damage in gardens. It has been classified as one of the 100 worst pest species (RABITSCH 2006). New records of *A. ater* s. l. outside its native range are rarer and usually not correlated with reports of mass occurrences or considerable damage of garden crops. However, this species has also been assigned a potential as a horticultural pest (GODAN 1979, ROWSON et al. 2014b), and the gardener's complaint from Istanbul confirms this. Interestingly, species distribution modelling by ZEMANOVA et al. (2018) has not classified Turkey as providing suitable climatic conditions for invasion by *A. lusitanicus* or *A. ater* s. l., neither currently nor within the next 50 years. Possibly the form of *A. ater* s. l. from Istanbul (and potentially from western France) differs in its climatic tolerance.

The trend of range expansion of *A. ater* s. l. is countered by a decline in some Central European and Scandinavian countries caused by its invasive congener *A. lusitanicus*. Where *A. lusitanicus* has established strong populations, *A. ater* s. l. tends to be replaced in synanthropic habitats within a few years, and then remains restricted to natural habitats (e.g. VON PROSCHWITZ 1997, RÜETSCHI et al. 2012, HATTELAND et al. 2015). One reason for the displacement might be hybridisation between *A. ater* s. l. and *A. lusitanicus* (REISCHÜTZ & REISCHÜTZ 2007, RÜETSCHI et al. 2012, DREIJERS et al. 2013, HATTELAND et al. 2013, ZEMANOVA et al. 2017). Morphological intermediates have been observed at sites of co-occurrence (ZEMANOVA et al. 2017, REISE et al. unpublished). The few morphologically untypical individuals amongst the Istanbul specimens might indicate the presence of *A. lusitanicus*. Another indication is the body colouration of the mature or subadult slug depicted in Fig. 4 and belonging to those untypical individuals: the banded pattern resembles that of juvenile or subadult *A. lusitanicus* rather than of typical *A. ater* s. l., although the latter can also be banded (ROWSON et al. 2014b). It will be worthwhile to keep the population of *A. ater* s. l. in Istanbul under observation over the next few years.

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