

A NEW ALIEN SPECIES IN EUROPE: FIRST RECORD OF *AUSTROPEPLEA VIRIDIS* (QUOY & GAIMARD, 1833) (MOLLUSCA, GASTROPODA, LYMNAEIDAE) IN SPAIN

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Abstract Freshwater snails of the genus *Austropeplea* were found on rice fields in Spain. It is the first record of this genus in Europe, very distant from all previously known localities of this taxon. By comparison of sequence data of the nuclear marker ITS-2 two specimens analysed fell into one cluster with GenBank sequences from *Austropeplea viridis* (Quoy & Gaimard, 1833) from Thailand and Australia, a *Radix* sp. sequence (Genbank) from Turkey and own sequences from *Orientogalba* specimens from China and Mongolia. Morphologically, the newly found snails correspond to specimens of *A. viridis* from Central Asia as well as to the syntypes of *Lymnaea viridis* collected in Guam. This finding confirms the high potential of aquatic pulmonate snails as successful transcontinental invaders.

Key words *Austropeplea viridis*, *Lymnaeidae*, alien Species, Europe, molecular genetics

INTRODUCTION

In July 2015 some lymnaeid specimens were collected for determination on rice fields in Deltebre (Spain, Tarragona province) (Fig. 1). Shell morphology and anatomy suggested them to belong to the genus *Radix*, species of which occur frequently in the region. Two of them were sent to the Senckenberg Natural History Collections Dresden, Museum of Zoology (SNSD) for molecular genetic analyses by KS. The comparison of the two mitochondrial cyt-b sequences (fragment of 329 bp) obtained with KS's database of about 230, partly yet unpublished, sequences of Palaearctic species of the genus *Radix* Montfort, 1810 and other Lymnaeidae related to *Radix* showed that the two specimens do not belong to the genus *Radix* as expected by morphology and origin, but cluster with some yet undetermined specimens from China and Mongolia of the genus *Orientogalba* Kruglov et Starobogatov, 1985 from the SNSD mollusc collection. The taxonomy of this group of lymnaeids is under debate (see Discussion).

By comparison of additional GenBank sequences and morphological characters of snails

collected in Australasia, the aim of this study was to determine the species and generic affiliation of the two Spanish individuals analysed.

MATERIAL AND METHODS

Snails were fixed in 70–80% ethanol or isopropyl alcohol. Shell morphology, mantle pigmentation and anatomy were documented from the specimens studied. Genital organs were dissected and measured using stereo microscope (Nikon SMZ18). Photographs were taken with a digital camera system (Nikon DS-Fi2).

All specimens used for morphological and molecular genetic studies are listed in Table 1. They are stored in the mollusc collection of the Senckenberg Natural History Collections Dresden, Museum of Zoology (SNSD).

We also examined morphological characters of the type series of *Lymnaea viridis* Quoy et Gaimard, 1833 (containing three syntype shells) housed in the National Museum of Natural History, Paris, as well as numerous individuals of this species collected by M.V. in Western Mongolia (July 2012) and kept in the Museum of Siberian Aquatic Molluscs, Omsk State Pedagogical University, Russia.

For the taxonomy of the freshwater molluscs used for outgroup comparison in the molecular genetic analyses, we followed the current European checklists (Falkner *et al.*, 2001; Bank 2011).

Tissue samples taken from the foot were fixed in 100% ethanol. They were registered in the tissue collection of the SNSD with a tissue voucher number and the corresponding collection number in the mollusc collection of SNSD and stored at -80°C.

MOLECULAR TECHNIQUES AND PHYLOGENETIC ANALYSES OF SEQUENCES

For the molecular genetic analyses we obtained the sequences of the nuclear ITS-2 spacer of the two specimens. For primers and protocols of DNA extraction, Polymerase Chain Reaction (PCR), purification of PCR products and DNA sequencing see Schniebs *et al.* (2011).

For outgroup comparison in the molecular genetic analyses, we used sequences of the Palaearctic species *Aplexa hypnorum* (Linnaeus, 1758) from the freshwater gastropod family Physidae Fitzinger, 1837 as well as *Lymnaea stagnalis* (Linnaeus, 1758) and *Stagnicola corvus* (Gmelin, 1791), another lymnaeid. We included sequences of *Radix auricularia* (Linnaeus, 1758), *R. balthica* (Linnaeus, 1758), *R. ampla* (Hartmann, 1821), *R. lagotis* (Schrank, 1803) and *R. labiata* (Rossmässler, 1835) in the ingroup to facilitate inter- and intraspecific comparisons. In addition we included ITS-2 sequences of *Austropeplea viridis*, *A. tomentosa* (Pfeiffer, 1855) and an undetermined *Radix* specimen from Turkey available from GenBank. The length of the ITS-2 spacer is 280 bp in *A. hypnorum* and up to 495 bp in *L. stagnalis*.

All DNA-sequences have been placed in the European Nucleotide Archive (ENA, see <http://www.ebi.ac.uk/ena/>).

The ITS-2 sequences were combined to a file using the sequence alignment editor BioEdit (Hall, 1999), aligned using the Clustal algorithm of MEGA version 4 (Tamura *et al.*, 2007) and the alignment was subsequently improved by eye. The post-alignment editing is necessary because MEGA is only able to recognize and order very similar gene fragments, whereas our dataset includes deeply diverged sequences that are additionally of very different lengths. The program is unable to align outgroup sequences from other families (Physidae) to the Lymnaeidae alignment. To avoid circular arguments, sequences were only edited without the taxon labels. We created the most parsimonious alignment by first grouping sequences according to similarity of hypervariable segments. We then separated grouped segments of sites by inserting gaps of the same length as the segment, rather than making a subjective alignment hypothesis. The alignment obtained is about two times longer (1009 sites) than its longest sequence (495 bp). This approach is less subjective than aligning stretches of nucleotides that show no similarity at all and thereby creating hypotheses, which are based only on noise.

Consequently, we chose an analytical approach under the maximum parsimony (MP) criterion to be able to include the gap code information. Losing this information by analysing under distance or maximum likelihood criteria would mean losing the greatest part of the phylogenetic signal. The phylogenetic analysis for the ITS-2 spacer was carried out using PAUP (version 4.0b10; Swofford, 2002; settings: gap-mode=NewState, addseq=closest, the maximal



Figure 1 A) The rice field habitat (Spain, Tarragona province, Deltebre) of the specimens to be identified by this study; B) the high population density observed; C) a living specimen.

Table 1 Material used for the molecular genetic and morphological analyses. ENA=European Nucleotide Archive.

| Code | Collection No. SNSD | Locality | ENA No. ITS-2 |
|---|---------------------|--|---------------|
| <i>Aplexa hypnorum</i> (Linnaeus, 1758) | Moll S348 | Germany, Mecklenburg-Western Pomerania, lake Nebel, 12°42'02"E 53°15'32"N | FR797832 |
| <i>Aplexa hypnorum</i> 1 | | | |
| <i>Aplexa hypnorum</i> 2 | Moll S350 | Germany, Mecklenburg-Western Pomerania, lake Nebel, 12°42'02"E 53°15'32"N | FR797833 |
| <i>Lymnaea stagnalis</i> (Linnaeus, 1758) | | | |
| <i>Lymnaea stagnalis</i> 1 | Moll 49239 | Germany, Saxony, Dresden, old branch of River Elbe, N 50°59'50.80" E 13°52'29.39" | HE573064 |
| <i>Lymnaea stagnalis</i> 2 | Moll 49835 | Germany, Saxony, Niederspree, small pond, N 51°24'28" E 14°54'03" | HE573065 |
| <i>Lymnaea stagnalis</i> 3 | Moll 53108 | Germany, Baden-Württemberg, Konstanz- Egg, ditch Hockgraben, 9°11'34.2"E 47°40'57.3"N | FR797834 |
| <i>Lymnaea stagnalis</i> 4 | Moll 53109 | Germany, Baden-Württemberg, Konstanz- Egg, ditch Hockgraben, 9°11'34.2"E 47°40'57.3"N | FR797835 |
| <i>Lymnaea stagnalis</i> 5 | Moll S1760 | Ukraine: province Zaporozhye, Zarechnoe village, Yushanly River | HG931962 |
| <i>Lymnaea stagnalis</i> 6 | Moll S2311 | Bulgaria: Plovdiv, floodplain of the Mariza River, 24°43' 34.8"E 42°09' 13.5"N | HG931965 |
| <i>Stagnicola corvus</i> (Gmelin, 1791) | | | |
| <i>Stagnicola corvus</i> 1 | Moll 49821 | Germany, Saxony, Niederspree, pond Großer Tiefzug, 14°53'38"E 51°24'20"N | HE577638 |
| <i>Stagnicola corvus</i> 2 | Moll 49872 | Germany, Saxony, pond Vierteich near Freitelsdorf, 13°41'57"E 51°15'43"N | HE577639 |
| <i>Stagnicola corvus</i> 3 | Moll 52830 | Germany, Saxony, Grethen, ditch on the west side of the pond Kleiner Kirchenteich, 12°39'22"E 51°14'29"N | HE577640 |
| <i>Stagnicola corvus</i> 4 | Moll 52831 | Germany, Saxony, Grethen, ditch on the west side of the pond Kleiner Kirchenteich, 12°39'22"E 51°14'29"N | HE577641 |
| <i>Radix auricularia</i> (Linnaeus, 1748) | | | |
| <i>Radix auricularia</i> 1 | Moll 50005 | Germany, Saxony, Niederspree, pond Neuwiesenteich, 14°52'57"E 51°24'19" | HE573066 |
| <i>Radix auricularia</i> 2 | Moll 50079 | Germany, Saxony, pond Vierteich near Freitelsdorf, 13°41'57"E 51°15'43" | HE573067 |
| <i>Radix auricularia</i> 3 | Moll 52857 | Russia, Novosibirsk Region, Novosibirsk, Reservoir near Kirza River, N 54°14.224' E 81°39.63114' | HE557647 |
| <i>Radix auricularia</i> 4 | Moll 52859 | Russia, Novosibirsk Region, Novosibirsk, Reservoir near Kirza River, N 54°14.224' E 81°39.63114' | HE557648 |
| <i>Radix auricularia</i> 5 | Moll 53070 | Germany, Bavaria, Weichering, pond in riverside forest, 11°19'23.6"E 48°43'34.1"N | FR797842 |

| Code | Collection No. SNSD | Locality | ENA No. ITS-2 |
|--|---------------------|---|---------------|
| <i>Radix auricularia</i> 6 | Moll 53071 | Germany, Bavaria, Weichering, pond in riverside forest, 11°19'23.6"E 48°43'34.1"N | FR797843 |
| <i>Radix ampla</i> (Hartmann, 1821) | | | |
| <i>Radix ampla</i> 1 | Moll 51112 | Russia, Chelyabinsk Region, river Miass near Dynamo village, 60°02'E 45°57'N | HE798448 |
| <i>Radix ampla</i> 2 | Moll 51113 | Russia, Chelyabinsk Region, river Miass near Dynamo village, 60°02'E 45°57'N | HE798449 |
| <i>Radix ampla</i> 3 | Moll 53098 | Germany, Bavaria, lake Ammersee, Stegen, 11°08'07"E 48°04'32"N | HE573072 |
| <i>Radix ampla</i> 4 | Moll 53099 | Germany, Bavaria, lake Ammersee, Stegen, 11°08'07"E 48°04'32"N | HE573073 |
| <i>Radix balthica</i> (Linnaeus, 1758) | | | |
| <i>Radix balthica</i> 1 | Moll 51283 | Switzerland, canton Basel-Landschaft, Liestal, Orishof, 07°43'03"E 47°28'22"N | HE573082 |
| <i>Radix balthica</i> 2 | Moll 51292 | Switzerland, canton Basel City, Riehen, Wiesengriener, 07°38'32"E 47°35'21"N | HE573083 |
| <i>Radix balthica</i> 3 | Moll 51834 | Germany, Saxony, Dresden-Kleitzschachwitz, river Elbe, 13°52'21"E 51°00'03"N | HE573079 |
| <i>Radix balthica</i> 4 | Moll 51860 | Sweden, Øland, east shore near Lille Seby, 16.565°E 56.345°N | HE573090 |
| <i>Radix balthica</i> 5 | Moll 53111 | Germany, Baden-Württemberg, Konstanz-Egg, pond near University, 09°11'29"E 47°41'09" N | HE573078 |
| <i>Radix balthica</i> 6 | Moll 53112 | Germany, Baden-Württemberg, Konstanz-Egg, pond near University, 09°11'29"E 47°41'09" N | HE577649 |
| <i>Radix labiata</i> (Rossmässler, 1835) | | | |
| <i>Radix labiata</i> 1 | Moll 51276 | Germany, Saxony, pond near Langenberg, | HE573069 |
| <i>Radix labiata</i> 4 | Moll 51863 | Montenegro, Žabljak, Black Lake, sand pools, 19°05'42"E 43°08'50"N | HE798455 |
| <i>Radix labiata</i> 5 | Moll 51910 | Germany, Saxony, Dehnitz, renovated swimming baths, 12°44'24"E 51°20'56"N | HE798456 |
| <i>Radix labiata</i> 6 | Moll 51964 | Germany, Mecklenburg-Western Pomerania, Gressow, alder fenwood, 11°18.592'E 53°41.898'N | HE798454 |
| <i>Radix labiata</i> 7 | Moll 52415 | Austria, Carinthia, Hermagor, 13°22'E 46°37'N | HE798457 |
| <i>Radix labiata</i> 8 | Moll 52427 | Italy, Lombardy, Province of Brescia, Borno, lower Varicla Lake, 2°15'24"W 45°58'50"N (Monte Mario) | HE798459 |
| <i>Radix labiata</i> 9 | Moll 52580 | Germany, Saxony, Linz, five year old pond, 13°43'33"E 51°20'51"N | HE798463 |
| <i>Radix labiata</i> 10 | Moll S172 | France, Bourgogne, Département Cote-d'Or | HE798465 |
| <i>Radix labiata</i> 11 | Moll S2904 | Germany, Saxony, small brook north of Tharandt, 13°34'19"E 51°00'08"N | HE798469 |

| Code | Collection No. SNSD | Locality | ENA No. ITS-2 |
|--|---------------------|---|---------------|
| <i>Radix lagotis</i> (Schrank, 1803) | | | |
| <i>Radix lagotis</i> 1 | Moll 49868 | Germany, Saxony, pond Vierteich near Freitelsdorf, 13°41'59"E 51°15'39"N | HE573076 |
| <i>Radix lagotis</i> 2 | Moll 51858 | Germany, Brandenburg, Strodehne, 51.74555°N 12.22396°E | LN874255 |
| <i>Radix lagotis</i> 3 | Moll 52563 | Germany, Saxony, pond Goldgrubenteich near Linz, 13°43'09"E 51°19'46"N | HE573077 |
| <i>Radix lagotis</i> 4 | Moll S2190 | Germany, Mecklenburg-Western Pomerania, creek connecting the lakes Kirchstücker and Barnerstücker | LN874256 |
| <i>Radix lagotis</i> 5 | Moll S3765 | Bulgaria. Dragoman marshland 40km west of Sofia, N42°56'11.7" E22°57'9.3" | LN874257 |
| <i>Austropeplea viridis</i> (Quoy & Gaimard, 1833) | | | |
| <i>Orientogalba</i> sp. | Moll S5292 | Mongolia, Tov province, river not far from Bayan-Onjuul | LT220501 |
| <i>Orientogalba</i> sp. | Moll S5294 | Mongolia, Tov province, river not far from Bayan-Onjuul | LT220502 |
| <i>Orientogalba</i> sp. | Moll S5410 | China: Gansu Province, Chuanzhen, Weiyuan, N 34.94385°E 104.04995° | LT220503 |
| <i>Orientogalba</i> sp. | Moll S5418 | China: Gansu Province, Wenxian, N 32.93013° E 104.72156° | LT220499 |
| <i>Orientogalba</i> sp. | Moll S6832 | Western Mongolia, small pond in the floodplain of the river Khovd, N 48°13.718' E 91°55.408' | LT220500 |
| Spain | Moll S7916 | Spain: Tarragona province, Deltebre, rice field, N 40.770885°, E 0.703721° | LT220497 |
| Spain | Moll S7917 | Spain: Tarragona province, Deltebre, rice field N 40.770885°, E 0.703721° | LT220498 |
| <i>Austropeplea viridis</i> | | Thailand, Chiang Mai (Kaset <i>et al.</i> 2010) | GU167912 |
| <i>Austropeplea viridis</i> | | Australia, Western Australia, Perth, S 31°56.000' E 115°50.000' (Puslednik <i>et al.</i> 2009) | EU556313 |
| <i>Radix</i> sp. | | Turkey, Soeke, Aydin Sira Daglari (Bargues <i>et al.</i> , 2001) | AJ319641 |
| <i>Austropeplea tomentosa</i> (Pfeiffer, 1855) | | New Zealand, North Island, North of Napier, S 39°13.040' E 176°53.380' E (Puslednik <i>et al.</i> 2009) | EU 556280 |
| <i>Austropeplea tomentosa</i> | | Australia, South Australia, Millicent S 37°09.688' E 140°06.075' (Puslednik <i>et al.</i> , 2009) | EU 556287 |

number of trees with the setting of maxtree=2500 did not have to be increased, since the number of best trees remained below 2500; number of bootstrap replicates=10000). For presentation of the MP results for ITS-2 one of the 2139 best trees was chosen to be able to illustrate branch lengths (one showing the same overall topology as the majority rule consensus tree was chosen). Please note that the phylogenetic hypothesis presented by the topology is not the focus of this study. A very different taxon sample would be necessary to clarify the phylogenetic position of *Austropeplea*.

RESULTS

Molecular genetics A comparison of the ITS-2 sequences of the two lymnaeid specimens from rice fields in the Spanish province Tarragona with sequences in GenBank using the Basic Local Alignment Search Tool showed a 97% identity to sequences of *Austropeplea viridis* and a 96% identity to a sequence of an undetermined *Radix* specimen from Turkey (*Radix* sp.). The hypothesis of their phylogenetic relationships based on the nuclear marker ITS-2 is illustrated as one of the 2139 best maximum parsimony (MP) trees in Fig. 2. It shows full or nearly full support for most basal branches, for an example the monophyly of Lymnaeidae, the clade *Lymnaea* plus *Stagnicola*, as well as *Radix* plus *Orientogalba*. Lower bootstrap support is observed for the clade consisting of *R. ampla*, *R. lagotis*, *R. balthica* and *R. labiata* (73%), the subclade of *R. ampla*, *R. lagotis* and *R. balthica* (71%) and the only clade of interest for the focus of this study: only 53% bootstrap support for two specimens of *A. tomentosa* (sequences from GenBank) grouping sister with two specimens of *A. viridis* (sequences from GenBank), one *Radix* sp. specimen from Turkey (GenBank), the two specimens from the rice fields in Spain and our *Orientogalba* specimens from the SNSD collection. The species-clades received full support including the cluster of *A. viridis*, the *Radix* from Turkey and our *Orientogalba* specimens and the two specimens from the rice fields in Spain.

MORPHOLOGY

The brownish shells of the two Spanish lymnaeid specimens have 4.0 and 4.5 whorls. They are conical egg-shaped with slightly truncated spire

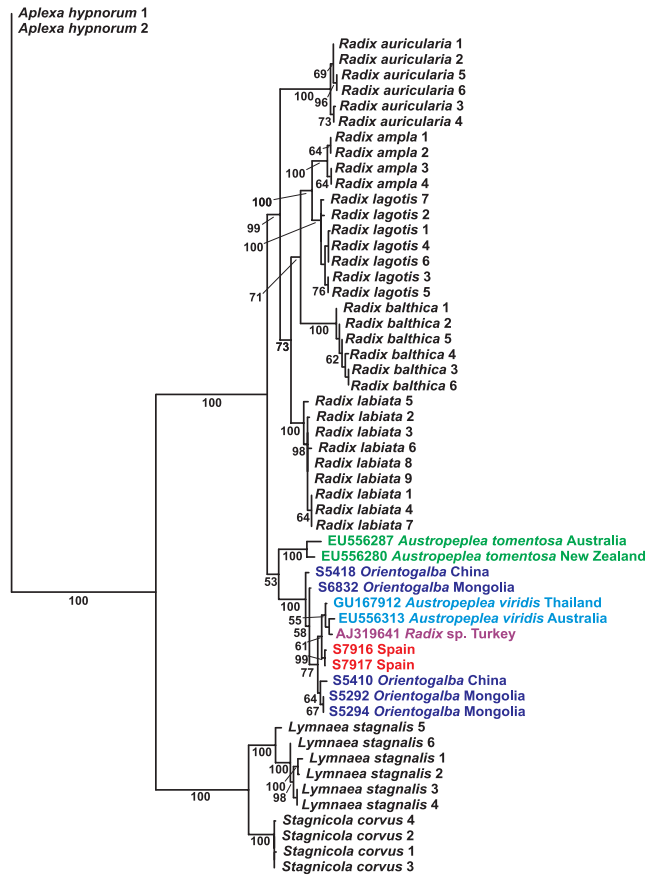


Figure 2 Hypothesis of the species affiliation of the two lymnaeid specimens from rice fields in the Spanish province Tarragona, based on one of the best 2139 maximum-parsimony trees of the nuclear marker ITS-2 (tree length=1249, CI=0.8205, RI=0.9651). The overall topology corresponds to that of the strict consensus tree and does not contain interpretable phylogenetic information due to the restricted taxonomic representation of lymnaeids. Branch lengths are proportional to the number of substitutions. Bootstrap support values above 50% are reported below nodes. Specimen labels of *Austropeplea tomentosa* (sequences from GenBank) are marked green, those of the *A. viridis* (sequences from GenBank) used for comparison light blue, the label of the undetermined *Radix* specimen from Turkey (sequence from GenBank) purple and those of the specimens formerly assigned to the probably synonymous *Orientogalba* from the mollusc collection of SNSD are marked dark blue. The labels of the two specimens from Spain are marked red.

whorls (Fig. 3, A, B). The shell heights are 12.8 and 11.9mm. They show the same shell shape as an *Orientogalba* specimen examined from the Gansu province in China (Fig. 3, F, shell height 11.2mm, 4.15 whorls) and another one from Western Mongolia (SNSD Moll S6832, shell height 8.3mm,

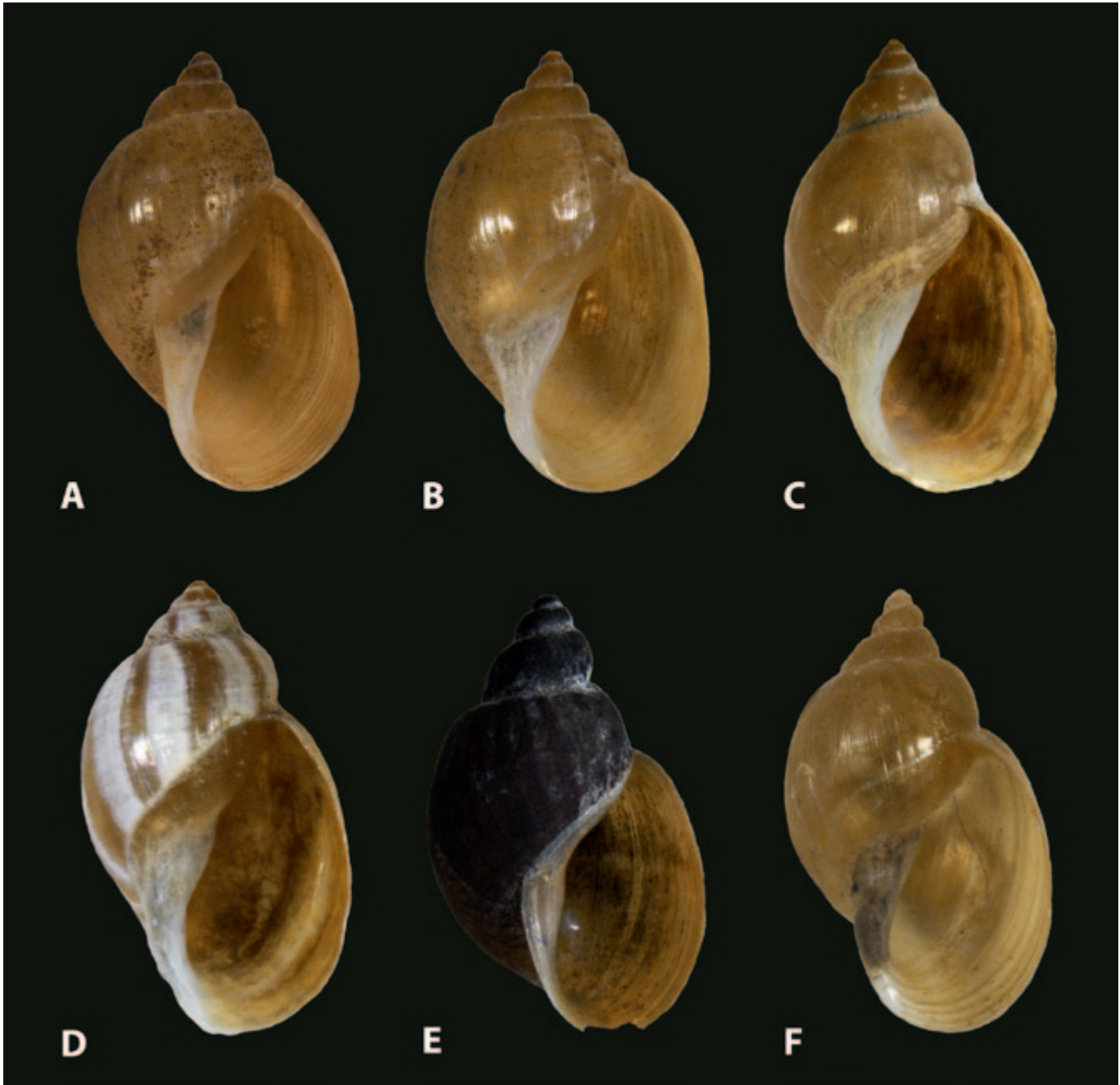


Figure 3 Shell variation of the *Austropeplea* specimens analysed. The specimens are from A) Spain: Tarragona Province, Deltebre, rice field (SNSD Moll S7916, shell height 12.8mm), B) Spain: Tarragona Province, Deltebre, rice field (SNSD Moll S7917, shell height 11.9mm), C) Mongolia, Tov Province, river not far from Bayan-Onjuul (formerly assigned to the probably synonymous *Orientogalba*, SNSD Moll S5292, shell height 14.5mm), D) Mongolia, Tov Province, river not far from Bayan-Onjuul (*Orientogalba* as above, SNSD Moll S5294, shell height 10.3mm), E) China: Gansu Province, Chuanzhen, Weiyuan (*Orientogalba* as above, SNSD Moll S5410, shell height 8.7mm), F) China: Gansu Province, Wenxian (*Orientogalba* as above, SNSD Moll S5418, shell height 11.2mm).

4 whorls). The shells of these Spanish and Asian specimens are very similar in size and shape to the shells of *L. viridis* syntypes from Guam Island housed in Paris (Fig. 4). But they differ in shape from the other three specimens analysed from Mongolia and China. In the latter the shells are

either more conical egg-shaped with rounded whorls (Fig. 3, C, shell height 14.5mm, 5 whorls) or more egg-shaped with rounded (Fig. 3, D, shell height 10.3mm, 4 whorls) or slightly elongated first whorls (Fig. 3, E, shell height 8.7mm, 4 whorls). One of the shells from Mongolia



Figure 4 Syntypes of *A. viridis* (originally described as *Lymnaea viridis*) from Guam Island, Pacific Ocean (National Museum of Natural History, Paris). Shell height of the left specimen 11.3mm, shell height of the right specimen 11.2mm.

(Fig. 3, D) is not uniformly brownish as all the other Spanish and Asian ones examined, but has a pattern of whitish and brownish radial stripes.

The mantle pigmentation of the two specimens from Spain is very similar. The mantle is grey-black with irregular light bluish-grey roundish blurred patches of different size and the mantle collar shows a dark bluish-grey colour with irregular patches of black (Fig. 5, B). This pigmentation is almost the same as in the two specimens analysed from China (Fig. 5, E, F). The mantle of one specimen from Mongolia (Fig. 5, A) is grey-black with more distinct white spots, most of them with nearly the same size, and a yellow-whitish mantle collar with irregular patches of black. The bluish-black mantle of the other two specimens from Mongolia analysed (Fig. 5, C, D) is very different, showing distinct whitish dots and spots of different size and a bluish-grey mantle collar with irregular patches of black. The length of the bursa duct could be analysed in five specimens. In one specimen from China (SNSD Moll S5418, Fig. 6, C) and one specimen from Spain (SNSD Moll S7917, Fig. 6, B) the duct of the bursa was nearly as long as the bursa. In two specimens from Mongolia the ratios of the length of the bursa duct to that of the bursa were 1:1.29 (SNSD Moll S5292 and S5294) and in the second specimen from Spain (SNSD Moll S7916, Fig. 5, A) this ratio was 1:2.29.

In all specimens examined from Asia, as well as in those from Spain, the bursa duct entered on the ventral side into the provagina above the female vent.

The ratio of the length of the praeputium to that of the penial sheath varies from 1:1.07 (Mongolia, SNSD Moll S5294) to 1:0.58 (Spain, SNSD Moll S7917, Fig. 6, B) in four specimens from which the male genitalia could be studied.

DISCUSSION

Molecular genetics The nuclear ITS-2 spacer was chosen for our molecular genetic analyses because ITS-2 sequences of *A. viridis* and *A. tomentosa* are available from GenBank for comparison. The results of our molecular genetic analyses of the nuclear ITS-2 spacer (Fig. 2) allow the conclusion that the two lymnaeid specimens from rice fields in the Spanish province Tarragona are genetically very close to two specimens of *A. viridis* from Thailand (GU167912, Kaset *et al.*, 2010) and Australia (EU556313, Puslednik *et al.*, 2009) as well as to the specimen *Radix* sp. AJ319641 from Turkey (Soeke, Aydin Sira Daglari) (Bargues *et al.*, 2001) and as the *Orientogalba* specimens from mollusc collection of SNSD from Mongolia and China. These seven ITS-2 sequences show a level of intra-clade variability in the same order of magnitude as that within e.g. *Lymnaea stagnalis*.

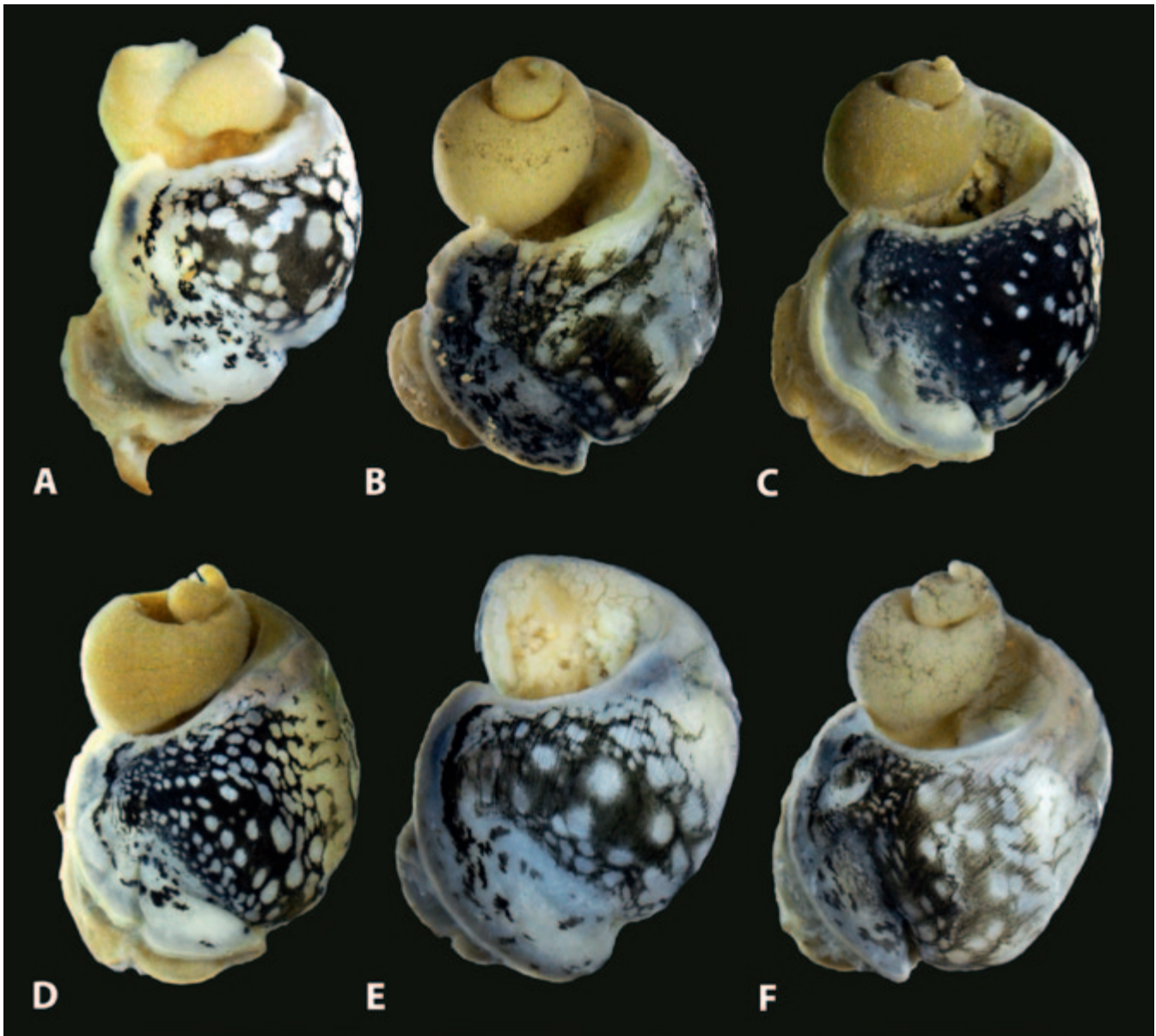


Figure 5 Variation of mantle pigmentation of the *Austropeplea* specimens analysed. A) Western Mongolia, small pond in the floodplain of the river Khovd (formerly assigned to the probably synonymous *Orientogalba*, SNSD Moll S6832), B) Spain: Tarragona Province, Deltebre, rice field (SNSD Moll S7917), C) Mongolia, Tov Province, river not far from Bayan-Onjuul (*Orientogalba* as above, SNSD Moll S5292), D) Mongolia, Tov Province, river not far from Bayan-Onjuul (*Orientogalba* as above, SNSD Moll S5294), E) China: Gansu Province, Chuanzhen, Weiyuan (*Orientogalba* as above, SNSD Moll S5410), F) China: Gansu Province, Wenxian (*Orientogalba* as above, SNSD Moll S5418).

We thus conclude that they come from specimens belonging to one species, in this case *A. viridis*. The Spanish snails do not belong to the genus *Radix* as originally expected by morphology and zoogeography.

MORPHOLOGY

The shell morphology of both the Spanish and Asian specimens analysed in this study show a

similar variability as Hubendick (1951) found within *Lymnaea viridis* (p. 163, Fig. 351), suggesting that the Spanish snails should be placed into this species. A comparison of these shells with those of the syntypes of *L. viridis* (see Fig. 4) also supports this hypothesis. But it is almost impossible to distinguish them with certainty from similar shells of representatives of the genus *Radix*, in Europe especially *R. labiata* (e.g. Schniebs *et al.*, 2013). The same applies for the mantle



Figure 6 Variation of the length of the bursa copulatrix, the length of the bursa duct and the male genitalia. The specimens are from A) Spain: Tarragona Province, Deltebre, rice field (SNSD Moll S7916), B) Spain: Tarragona Province, Deltebre, rice field (SNSD Moll S7917), C) China: Gansu Province, Wenxian (formerly assigned to the probably synonymous *Orientogalba*, SNSD Moll S5418).

pigmentation. Similar pigmentations could be found in *R. lagotis* (Schniebs *et al.*, 2015), *R. labiata* (Schniebs *et al.*, 2013), and *R. balthica* (Schniebs *et al.*, 2011). The ratio of the length of the bursa duct to that of the bursa shows more variability in the five specimens from Spain, China and Mongolia analysed, than mentioned in Kruglov (2005) for *Orientogalba viridis* (see taxonomy discussion below) with a bursa duct 1.6–1.7 times longer than the diameter of the bursa. In one specimen from Spain (SNSD Moll S7917, Fig. 6, B) and in one specimen from China (SNSD Moll S5418, Fig. 6, C) as well as in two specimens from Mongolia (SNSD Moll S5292 and S5294) analysed this character shows nearly the same values as in two specimens of *R. lagotis* from Germany: 1:1.1 and 1:1.20 (Schniebs *et al.*, 2015).

In three of four specimens analysed (the two specimens from Spain and one specimen from China SNSD Moll S5418) the phalloteca was shorter than the praeputium as described by Kruglov (2005). In one specimen (from Mongolia, SNSD Moll S5294) the phallotheca was nearly as long as the praeputium. This character is thus also not suitable for distinguishing *A. viridis* from similar *Radix* species (Schniebs *et al.*, 2011, 2013).

Further investigations of the morphology and anatomy with more individuals will probably show more variability.

TAXONOMY

Concerning the genus affiliation of *Lymnaea viridis* Quoy & Gaimard, 1833, two different views exist:

1. Inaba (1969) assigned the representatives of the genus *Lymnaea* with 16 pairs of chromosomes from the eastern part of Asia and Australia, thus including *L. viridis*, to the valid generic name *Austropeplea* Cotton, 1942. Hereafter, the name *A. viridis* was used for this species by a number of authors (e.g. Ponder & Waterhouse, 1997; Correa *et al.*, 2010; Kaset *et al.*, 2010; Dung *et al.*, 2013). Correa *et al.* (2010) showed in their phylogeny of Lymnaeidae that *A. viridis*, *A. hispida* (Ponder & Waterhouse, 1997) and *A. tomentosa* (L. Pfeiffer, 1855) (the latter is the type species of genus *Austropeplea*) form a cluster based on molecular markers that could be interpreted as a genus, confirming the taxonomic position of *L. viridis* within the genus *Austropeplea*.

The molecular genetic analysis of a specimen of *A. viridis* from Vietnam by Dung *et al.* (2013) together with two sequences of *A. viridis* from Australia and Thailand and three sequences of *A. tomentosa* from Australia, lead to the same result. Only Puslednik *et al.* (2009) concluded on the base of molecular analyses that the inclusion of *A. viridis* in the genus *Austropeplea* is questionable. The authors had included other species for comparison and a matrix of morphological characters, which are, however, determined by the environment and the age of the specimen according to our analyses. Morphological characters in Lymnaeidae, especially of shell and genital organs, are apparently of high variability (e.g. Schniebs *et al.* 2011; Vinarski 2009, 2011; Vinarski *et al.* 2016). Thus, we interpret these phylogenetic analyses as biased, due to the inclusion of hyper-variable, non-diagnostic and thus homoplastic characters.

2. In contrast, the name *Orientogalba* was introduced by Kruglov and Starobogatov (1985a) as a subgenus of the genus *Lymnaea* Lamarck, 1799 with *L. viridis* as type species of the section *Viridigalba* Kruglov & Starobogatov, 1985. In other publications, these authors also recognize the status of *Austropeplea* as subgenus of the genus *Lymnaea* (Kruglov & Starobogatov, 1985b; Kruglov, 2005). However, in their opinion *L. viridis* does not belong to *Austropeplea*, because snails of this species do not cover their shell with the mantle lobes. This character had been described as being characteristic for this genus by Cotton (1942). This character does, however, also occur in other genera, e.g. *Myxas* (G. B. Sowerby, 1822), probably by parallel evolution. Recently, both *Orientogalba* and *Austropeplea* were treated as separate genera (Vinarski, 2013). Ponder & Waterhouse (1997) treated *Orientogalba* as a subgenus of *Fossaria* Westerlund, 1885 (probably a synonym of *Galba* Schrank, 1803).

Our analyses of the nuclear ITS-2 spacer show that *Orientogalba* should probably be regarded as a synonym or, at most a separate subgenus of *Austropeplea*, and not of *Galba*. However, this needs further investigations as this contradicts the hypotheses for the taxonomic position of *A. viridis* by various authors.

We have to note that the type specimen of *A. tomentosa* is conchologically very different from *Orientogalba*. As already mentioned above, this species belongs to the phenotypic

(non-monophyletic) group of “mantlesnails”, or glutinous snails, with the European genus *Myxas* G. B. Sowerby I, 1822 as being its most typical representative (Hubendick, 1951; Kruglov, 2005). The shell similarity between *Myxas* and *Austropeplea* s. str. (sensu Vinarski, 2013) is explained by convergent evolution (Kruglov, 2005) but both genera are morphologically much closer to each other than to any species of *Orientogalba* (Kruglov & Starobogatov, 1985). Solving this taxonomic problem was not aim of this study. The entire group apparently needs careful revision by a comparative analysis of all taxa in question based on all characters that have been used, including those from molecular sequences.

DISTRIBUTION

Austropeplea viridis has an Asian-Australasian distribution from Japan (Hubendick, 1951; Ponder & Waterhouse, 1997; Köhler & Rintelen, 2011), the Russian Kuril Islands (Köhler & Rintelen, 2011), the Russian Maritime Province (Kruglov, 2005), Mongolia (Hubendick, 1951) and China in the north (Hubendick, 1951; Ponder & Waterhouse, 1997; Kruglov, 2005, Köhler & Rintelen, 2011) to Guam (locus typicus), and Papua New Guinea (Köhler & Rintelen, 2011). It was introduced to Australia (Boray 1978; Ponder & Waterhouse, 1997; Puslednik *et al.*, 2009; Köhler & Rintelen, 2011). Our analyses presented in this study show that it has been introduced to Europe (Spain) and possibly also occurs in Turkey.

INTRODUCED SPECIES IMPACT

The finding of an Asian-Australasian species of lymnaeids in Spain and, possibly Turkey, adds one more alien species of this family to the European malacofauna. To date, the only non-indigenous representative of Lymnaeidae in the European waterbodies was the Nearctic-Neotropical species *Pseudosuccinea columella* (Say, 1817) introduced via aquaria and greenhouses (Glöer, 2002) and now distributed in some Central European countries. Given the fact that phenotypically, *A. viridis* may be confused with individuals of *Radix* spp. (Hubendick, 1951; present study), it seems likely that this species may occur in other countries of Southern Europe in the area between Spain and Turkey. More molecular genetic studies are needed to confirm its cryptic presence there.

This problem has a clear practical and strongly applied aspect, since *A. viridis* is thought to be involved in the fascioliasis epidemiology (Correa *et al.*, 2010; Kaset *et al.*, 2010; Dung *et al.*, 2013). The discovery of another potential intermediate host of the parasitic trematode *Fasciola hepatica* in Europe should be of high interest to medicine and should be taken into account in rice field agriculture in the province of Tarragona. Indigenous (European) intermediate hosts are the Lymnaeid species *Galba truncatula* (O. F. Müller, 1774) (e. g. Berghen, 1964; Bargues *et al.*, 2001; Rondelaud *et al.*, 2001; Medeiros *et al.*, 2014), *Lymnaea stagnalis* (Linnaeus, 1758) (Berghen, 1964), *Stagnicola palustris* (O. F. Müller, 1774) (Berghen, 1964; Bargues *et al.*, 2001), *Omphiscola glabra* (O. F. Müller, 1774) (Bargues *et al.*, 2001; Rondelaud *et al.*, 2001) or *Radix labiata* (Caron *et al.*, 2007). Obviously, our data cannot comment on the frequency of introduction of *A. viridis* to Europe. It cannot be ruled out though, that this is an ongoing process with infected individuals from areas with higher densities of outdoor livestock coming into Europe regularly.

POTENTIAL INVASION MECHANISMS

The invasion mechanism of *A. viridis* from Asia and Australasia to Europe is unclear and only speculations are possible. The first hypothesis is a slow and gradual westward dispersion of this species from Central Asia to Europe. In this case, its finding in Turkey may represent a “stepping stone” of this route. This hypothesis could be confirmed if *A. viridis* is found in other regions of the Middle East, the Caucasus and South-Eastern Europe.

The alternative possibility is a direct migratory bird- or human-mediated transfer of *A. viridis* from Asia and Australasia to Spain. In the last years, a number of aquatic alien species were found to have been introduced to this area by humans, most of them apparently originating from releases from an aquaculture facility, including the rice pest *Pomacea maculata* Perry, 1810, and different fishes (Quiñonero Salgado & López Soriano, 2013; Franch *et al.*, 2008).

CONCLUSION

In conclusion, our results report the first finding of an Asian-Australasian lymnaeid species

in Europe. They confirm the high potential of Lymnaeidae and other taxa of aquatic pulmonates as very successful invaders able to sustain transcontinental voyages. High densities of individuals were observed in the rice field habitats sampled (Fig. 1). There is no reason to assume that the entire population should not be homogeneous with respect to their mitochondrial species assignment to *A. viridis*, although this needs to be confirmed by sequencing further individuals. If this dense population would indeed entirely consist of *A. viridis*, this would indicate that the species is able to form stable occurrences in areas very remote from their native ranges. Perhaps, the most spectacular example of a successful global colonization is *Physella acuta* (Draparnaud, 1805), a physid snail of presumably Nearctic origin, now widely distributed throughout the World including most tropical countries, Europe and even southern Siberia. Dillon *et al.* (2002: 233) nominate it as “the world’s most cosmopolitan freshwater gastropod”.

Unfortunately, the exact determination of lymnaeid snails on the basis of phenotypic traits only, may be very misleading due to high intraspecific variation preventing the existence of diagnostic interspecific characters (see also Schniebs *et al.*, 2011, 2013; Vinarski *et al.*, 2016). This makes the molecular techniques the only reliable tool for identification of *A. viridis* in Europe and thus for further monitoring of its spread beyond the native range. This is very important, since it is a common intermediate host of human and animal fascioliasis (e.g. Correa *et al.*, 2010; Kaset *et al.*, 2010; Dung *et al.*, 2013).

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