

## THE FIRST RECORD OF *GALBA CUBENSIS* (L. PFEIFFER, 1839) (MOLLUSCA: GASTROPODA: LYMNAEIDAE) FROM OPEN FIELDS OF EUROPE

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**ABSTRACT:** A high density population of *Galba* sp. was found in rice fields in the Ebro Delta in Spain. Two specimens were subject to molecular identification. Their COI and ITS-2 sequences formed one cluster with the GenBank sequences of the neotropical *Galba cubensis* (L. Pfeiffer), *G. neotropica* Bargues, Artigas, Mera y Sierra, Pointier et Mas-Coma and *G. viatrix* (d'Orbigny), being clearly distinct from *G. truncatula* (O. F. Müller) or *G. schirazensis* (Küster). Since the COI sequences of the two specimens from Spain were identical with the two GenBank sequences of *G. cubensis* from Colombia, we identified the two Spanish specimens as *G. cubensis*. There is, however, some degree of uncertainty given the need for a large-scale revision of the neotropical *Galba*. This is the first record of *G. cubensis* in European natural habitats. Until now, the European records of the species were based on morphologically determined specimens from greenhouses of botanical gardens or horticultural companies. Morpho-anatomical studies of the shells and genitalia do not allow for a reliable discrimination from the native species *Galba truncatula* thus pointing to DNA markers as the best tools for identification.

**KEY WORDS:** *Galba cubensis*, Lymnaeidae, alien species, Europe, molecular genetics, morphology

### INTRODUCTION

Species of the freshwater snail genus *Galba* Schrank, 1803 act as intermediate hosts for medically and veterinarily important trematodes. Probably the most important vectors are *G. cubensis* (L. Pfeiffer, 1839) and *G. truncatula* (O. F. Müller, 1774). *G. cubensis* is one of the most important intermediate hosts of *Fasciola hepatica* Linnaeus, 1758 in the Caribbean area (POINTIER et al. 2009, ALBA et al. 2015, VÁZQUEZ et al. 2015), the southern United States (CRUZ-REYES & MALEK 1987), and a potential intermediate host of *Fascioloides magna* Bassi, 1875 (VIGNOLES et al. 2014). In the south-eastern United States *G. cubensis* plays an important role as an intermediate host of *Heterobilharzia americana*

Price, 1929 which infects several mammals, including horses (CORAPI et al. 2015). Fascioliasis is so important for veterinary medicine and human health that, recently, even a multiplex PCR for detection of *F. hepatica* in *G. cubensis* has been developed (ALBA et al. 2015). *G. viator* (d'Orbigny, 1835) is one of the fascioliasis vectors in Argentina (STANDLEY et al. 2013) and Brazil (MEDEIROS et al. 2014). In 2007, *G. neotropica* was described by BARGUES et al. (2007). It was found in Peru, Argentina, Venezuela, and Uruguay (PUJADAS et al. 2015, BARGUES et al. 2017) and is also an intermediate host for *Fasciola hepatica* (PUJADAS et al. 2015, BARGUES et al. 2016, 2017). There is still

some uncertainty about the possible synonymy of *G. cubensis*, *G. viator* and *G. neotropica* (STANDLEY et al. 2013, LOUNNAS et al. 2017). *G. truncatula* is the main fascioliasis vector in Europe, Asia, and Africa (BARGUES et al. 2011). According to MAS-COMA et al. (2001) it was introduced from Europe to South America, but in the opinion of CORREA et al. (2010: p. 6) *G. truncatula* “represents a branch of an American clade that reached the Old World”.

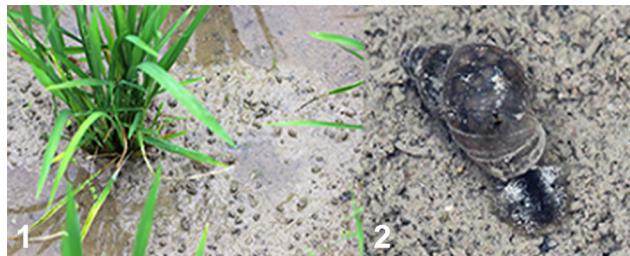
Of these species, so far only *G. truncatula* was recorded in Western Europe. However, another lymnaeid species which historically has distorted fascioliasis data in Europe and America is *G. schirazensis* (Küster, 1862), a cryptic taxon that was discovered in Egypt, Iran, Spain, the Dominican Republic, Mexico, Venezuela, Colombia, Peru (BARGUES et al. 2011) as well as in Ecuador (BARGUES et al. 2011, LOUNNAS et al. 2017) and the Reunion Island (LOUNNAS et al. 2017). In Spain this species has recently been recorded from anthropogenic habitats, such as canals and drainage ditches near rice fields in the Albufera de Valencia (BARGUES et al. 2011). While some authors suggest that the species is not a vector (BARGUES et al. 2011), others claim it is (DREYFUSS et al. 2015, CARON et al. 2017). The reproduction with high self-fertilisation rates recorded in some representatives of the genus *Galba* (e.g. TROUVE et al. 2004, BARGUES et al. 2011) explains the high potential for colonisation of new habitats (JARNE et al. 1993, TROUVE et al. 2004) or re-colonisation after a bottleneck event (e.g. JARNE et al. 1993) of these species.

## MATERIAL AND METHODS

### SNAIL MATERIAL

All specimens used for morphological and molecular genetic studies are listed in Appendix 1 (only two of the three Spanish specimens were analysed with molecular methods). They are kept in the mollusc collection of the Senckenberg Natural History Collections Dresden, Museum of Zoology (SNSD).

The snails were immediately fixed in 70–80% ethanol or isopropyl alcohol without relaxing. Their shell morphology, mantle pigmentation and anatomy were documented from the specimens studied. The genital organs were dissected and measured using stereomicroscope (Nikon SMZ18). Photographs were taken with a digital camera system (Nikon DS-Fi2). Foot tissue samples were fixed in 100% ethanol. They were registered in the tissue collection of the SNSD by assigning a tissue voucher number and a corresponding collection number in the mollusc collection of SNSD, and are stored at –80°C.



Figs 1–2. *Galba* snails in Spain: 1 – plot of a rice field in the Ebro Delta (Spain, Tarragona province) with a high population density of *Galba* specimens; 2 – live specimen

In July 2015, some specimens of the genus *Galba* were collected for determination in rice fields in the Ebro Delta near Sant Carles de la Ràpita (Spain, Tarragona province) (Figs 1–2). Three of these were sent to the Senckenberg Natural History Collections Dresden, Museum of Zoology (SNSD) for molecular genetic analyses. As opposed to what could be expected from their shell morphology and origin, the comparison of sequences of the nuclear marker ITS-2 from two of the specimens with sequences of *G. truncatula* revealed that they were not conspecific. The aim of this study was to identify these individuals by the analysis of the nuclear marker ITS-2 as well as the mitochondrial marker COI, comparative morphology and molecular phylogeny. A comprehensive dataset was put together for sound phylogenetic analyses so as not to base the interpretations on possible phenetic similarity artefacts in simple species identification analyses, such as neighbour-joining.

For the taxonomy of the freshwater molluscs used in the molecular genetic analyses, we followed the current European checklists (FALKNER et al. 2001, BANK 2011) and VINARSKI (2012, 2013).

### MOLECULAR TECHNIQUES AND PHYLOGENETIC ANALYSES OF SEQUENCES

We used the ITS-2 spacer in addition to the barcoding gene COI to exclude inconsistent results concerning the identification of the Spanish *Galba* specimens. Earlier published and yet unpublished results show that COI is not suitable for barcoding within the genus *Stagnicola* (SCHNIEBS et al. 2016), as well as for some other Lymnaeidae, among others due to hybridisation.

For primers and protocols of DNA extraction, Polymerase Chain Reaction (PCR), purification of PCR products and DNA sequencing see VINARSKI et al. (2011). Only the primers used for COI as well as the temperature profile for the PCR of the two *Galba*



specimens from Spain were different: LCO1490-JJ and HCO2198-JJ (ASTRIN & STÜBEN 2008), 95°C 5 min (95°C 45 s, 45°C 45 s, 72°C 1 min) × 35, 72°C 10 min, 8°C hold.

For comparison in the molecular genetic analyses, we used newly generated sequences and GenBank sequences of the Palaearctic species *Aplexa hypnorum* Fitzinger, 1837 (freshwater Physidae), *Lymnaea stagnalis* (Linnaeus, 1758), *L. taurica* (Clessin, 1880), *Omphiscola glabra* (O. F. Müller, 1774), *Stagnicola palustris* (O. F. Müller, 1774), *S. corvus* (Gmelin, 1791), *Ladislavella terebra* (Westerlund, 1885), *Radix auricularia* (Linnaeus, 1758), *R. balthica* (Linnaeus, 1758), *R. lagotis* (Schrank, 1803) and *R. labiata* (Rossmässler, 1835) (Lymnaeidae). We included sequences of *G. truncatula* as well as those of *G. cousinii* (Jousseaume, 1887), *G. viator*, *G. schirazensis*, and *G. cubensis* available from GenBank in the ingroup to facilitate inter- and intraspecific comparisons. We used such an extensive data set to avoid artefacts caused by incomplete taxon sampling. Moreover, the ITS-2

alignment is more accurate in our experience when more genera and species are included.

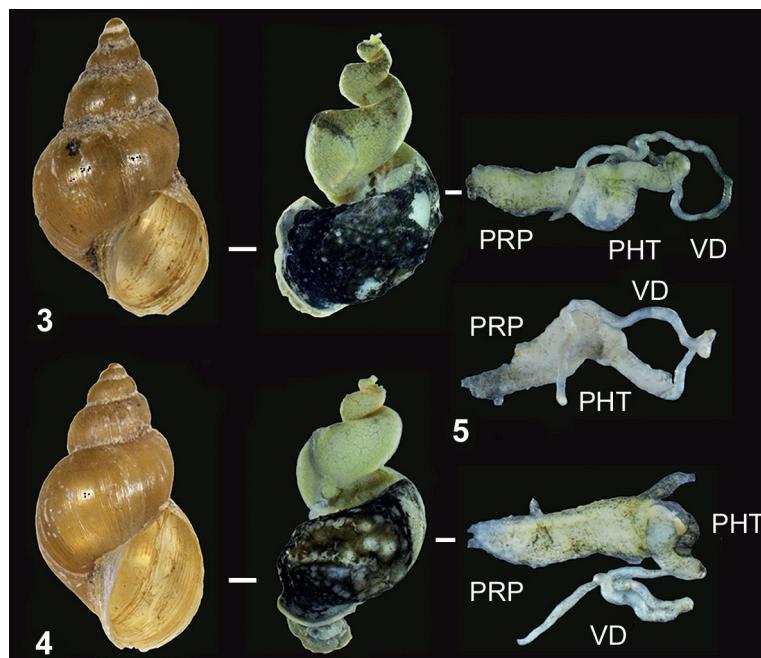
The alignments followed SCHNIEBS et al. (2017). For phylogenetic analyses we chose the maximum parsimony (MP) criterion in order to be able to include the gap code information following SCHNIEBS et al. (2011, 2013, 2017). The maximum number of trees with the setting maxtree = 100 did not have to be increased for COI, since the number of best trees remained below 100; for ITS-2 spacer we used the setting maxtree = 200; number of bootstrap replicates = 10,000 for COI and 5,000 for ITS-2. For presentation of the MP results of each ITS-2 and COI, one of the best trees was chosen to illustrate branch lengths (one showing the same overall topology as the majority rule consensus tree was chosen). In addition, the influence of outgroup choice on the topology of the whole tree was tested using *Planorbarius corneus* (Linnaeus, 1758) (469 bp) of the family Planorbidae as an alternative outgroup to *Aplexa hypnorum*.

## RESULTS

### MORPHOLOGY

The height of the brownish shells of the two Spanish *Galba* specimens (collection numbers Moll S7956 & Moll S7957) analysed by molecular genetics is 8.1 and 7.7 mm and the width is 4.7 and 4.5 mm

(Figs 3, 4). The height of the aperture is 3.4 and 3.8 mm. The 5 and 4.5 whorls are less stepped and separated by a shallower suture than in *G. truncatula*. The mantle pigmentation of the two specimens is very different. One specimen has a bluish-black mantle with numerous roundish small more or less diffuse



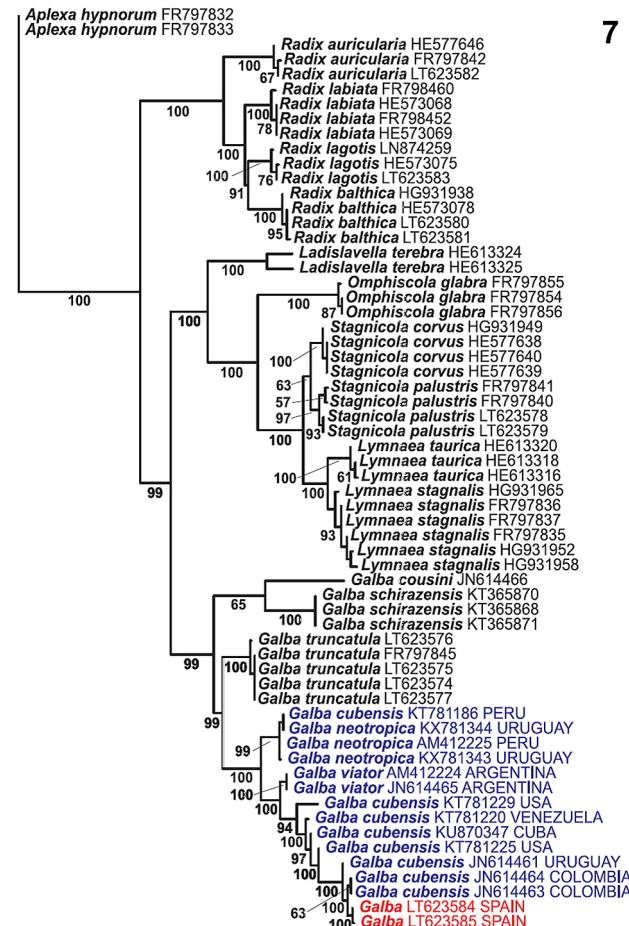
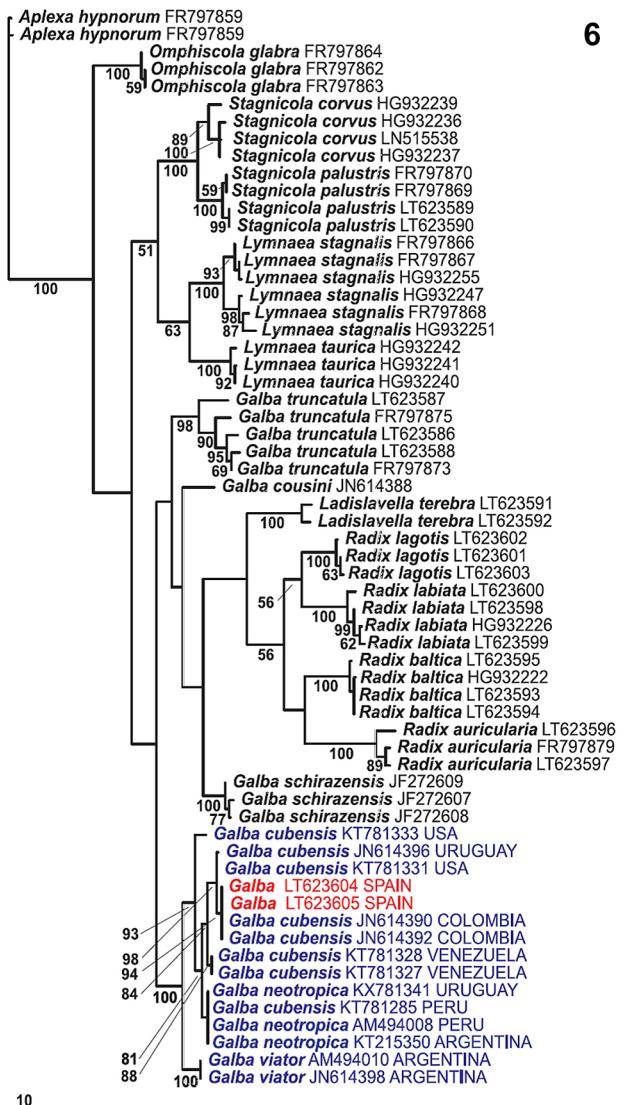
Figs 3–5. Shells, mantle pigmentation and anatomy of the two *Galba* specimens from the Ebro Delta (Spain, Tarragona province) and morphology of male genitalia of *G. truncatula* from Germany: 3 – *G. cubensis* SNSD Moll S7956 (*Galba* Spain 1), shell height 8.12 mm; 4 – *G. cubensis* SNSD Moll S7957 (*Galba* Spain 2), shell height 7.68 mm; 5 – male genitalia of a *G. truncatula* from Saxony (Germany) SNSD Moll S8234 (*G. truncatula* 6). Abbreviations: PHT – phallotheca, PRP – praeputium, VD – vas deferens

spots of yellowish grey (Fig. 3). The other one has a blue-black and partially dark greenish-grey mantle with a few diffuse greenish-grey patches in the dark greenish-grey mantle zone and numerous small, more or less diffuse, spots of bluish-grey (Fig. 4). In both specimens the praeputium is nearly twice as long as the phallotheca (1.05 : 0.48 mm and 0.92 : 0.45 mm). The anterior and posterior width of the phallotheca is 0.19 mm in specimen S7956 and 0.13 mm in specimen S7957. The bursa copulatrix is roundish-ovoid. Its duct is narrow and relatively long. Therefore the bursa is situated near the heart. For comparison, a specimen of *G. truncatula* from Saxony was dissected (Fig. 5).

Live specimens show a conspicuous white pigmentation on their heads and tentacles (Fig. 2). A similar pigmentation is visible on a photograph of a live specimen of *G. cubensis* from the Caribbean island Guadeloupe (POINTIER 2008: p. 34, fig. c). The pigmentation is no longer visible in alcohol-preserved specimens.

## MOLECULAR GENETICS

Twenty new sequences of COI (LT623586-LT623605) and twelve of ITS-2 (LT623574-LT623585) were deposited in the European Nucleotide Archive (ENA, <http://www.ebi.ac.uk/ena/>), available also



Figs 6–7. Hypothesis of species affiliation of the two *Galba* specimens from the Ebro Delta (Spain, Tarragona province): 6 – based on one of the best 24 maximum-parsimony trees of the mitochondrial marker COI (tree length = 949, CI = 0.4247, RI = 0.8414); 7 – based on one of the best 2,970 maximum-parsimony trees of the nuclear marker ITS-2 (tree length = 2255, CI = 0.7574, RI = 0.9537). The overall topology of both trees corresponds to that of the strict consensus. Branch lengths are proportional to the number of substitutions. Bootstrap support values above 50% are given below nodes. Specimen labels of *G. cubensis*, *G. neotropica* and *G. viator* (sequences from GenBank) indicated in blue, the two specimens from Spain labelled in red



from GenBank (for details see [Appendix 1](#)). They were compared with several GenBank sequences ([Appendix 1](#)).

The hypothesis of phylogenetic relationship of the two Spanish individuals (collection numbers Moll S7956 and Moll S7957, labelled in red as *Galba* LT623604 Spain and *Galba* LT623605 Spain for clarity in [Fig. 6](#)) based on the mitochondrial marker COI shows a 100% identity with the two individuals of *G. cubensis* from Colombia ([CORREA et al. 2011](#)). The genus *Galba* appears to be polyphyletic and the only three *Galba* species grouping together are *G. cubensis* (together with the two Spanish individuals), *G. neotropica* and *G. viator* (full support). The other *Galba* species used in this study for comparison (*G. truncatula*, *G. cousinsi*, *G. schirazensis*) do not group together.

In the MP tree of the nuclear marker ITS-2 the two analysed individuals from the Ebro Delta (marked

red LT623585 & LT623585 in [Fig. 7](#)) group together with some other *G. cubensis* specimens from GenBank (chosen for comparison) from Colombia and Uruguay in a subclade with a 100% support. Most of *G. cubensis* specimens constitute a sister group to the specimens of *G. viator* (with 100%, [Fig. 7](#)). One specimen of *G. cubensis* from Peru (KT781186) shows a 100% identity with a specimen of *G. neotropica* from Uruguay (KX781344). The specimens of *G. cubensis*, *G. neotropica* and *G. viator* from GenBank group together with the two *Galba* specimens from Spain in a cluster with full support. All species of the genus *Galba* group together in one cluster with a 99% bootstrap support.

We found no difference in the topology of the phylogenetic trees of the ITS-2 marker aligned with *A. hypnorum* or *P. corneus* as outgroup.

## DISCUSSION

### MORPHOLOGY

The shell height of our two *Galba* specimens from Spain falls within the range of European *G. truncatula* of 5–9 mm ([GLÖER 2002](#)) or, in some cases, up to 15 mm ([GITTINGERBERG et al. 1998](#)). Although [BAKER \(1911\)](#) found that *G. cubensis* had a shorter spire, wider and more rotund whorls, and a rounder aperture than *G. truncatula*, we could not detect any reliable morphological or anatomical differences between *G. truncatula* and the two *Galba* specimens from Spain. Conchometric studies of [SAMADI et al. \(2000\)](#) revealed that the parameters they examined did not allow for a distinction between *G. cubensis* and *G. truncatula*. The same authors found that anatomical characters (prostate circumference and surface, length ratios between praeputium and penis and width ratios of the anterior and posterior penis parts) were relevant for the differentiation between *G. cubensis* from Bolivia and the Caribbeans and *G. truncatula* from France, Portugal, Morocco, and Bolivia. We are not at all convinced that the phallotheca width can be used to distinguish between *G. cubensis* and *G. truncatula*. [SAMADI et al. \(2000\)](#) show a thicker phallotheca in a *G. cubensis* than in *G. truncatula* from Bolivia and France in the same publication. We found that the width of phallotheca in a *G. truncatula* specimen from Germany (*Galba truncatula* 6), which was randomly chosen for dissection in our study ([Fig. 7](#)), was the same as that in one of the two *G. cubensis* specimens from Spain ([Fig. 6](#)). Recent studies on the Lymnaeidae ([SCHNIEBS et al. 2011, 2013, 2016, VINARSKI 2011](#)) showed that the relative length of penis sheath and praeputium in the analysed individuals varied widely. Ontogenetic, sea-

sonal, geographic, parasitic, and some other factors (e.g. degree of relaxation) could influence the length of phallotheca and praeputium ([VINARSKI 2011](#)), and they could certainly influence the width of phallotheca and penis. We are convinced that especially ontogeny and seasonal aspects may influence the size and surface of prostate.

We agree with the results of [CORREA et al. \(2011\)](#) concerning the insufficiency of morpho-anatomical characters for a reliable discrimination between *G. truncatula* and *G. cubensis*.

We are not sure at present if the white speckles on the head and tentacles of live *G. cubensis* are a good distinguishing character from *G. truncatula*. Until now white pigmentation on the head and tentacles of live *G. truncatula* was not observed or mentioned in the literature, nor seen in any other Spanish specimen from different populations. The character requires examining more live specimens of both species.

### MOLECULAR GENETICS

The molecular genetic analyses of the mitochondrial marker COI and the nuclear ITS-2 spacer ([Fig. 7](#)) show that the two *Galba* specimens from the Ebro are genetically identical, or very similar, to three specimens of *G. cubensis* from Colombia and Uruguay (JN614390, JN614392, JN614396, JN614461, JN614463, JN614464; [CORREA et al. 2011](#)). We thus conclude that the two specimens represent *G. cubensis*. Contrary to what could be expected based on morphology and zoogeography, the Spanish snails analysed are not *G. truncatula*.

The correctness of assignment of the two Spanish specimens to *G. cubensis* is, to some extent, ques-

tioned by the relative closeness of *G. viator* and *G. neotropica* GenBank sequences to *G. cubensis* sequences in our trees. Our trees confirm the results of CORREA et al. (2010, 2011) and STANDLEY et al. (2013), in which *G. cubensis* together with *G. neotropica* form a sister group to *G. viator*, and the topology of the monophyletic *Galba* clade in our ITS-2 tree is basically the same as in the combined 18S/ITS-1/ITS-2 tree in BARGUES et al. (2011). On the other hand, the sequences of all three species in our trees are mixed within a single cluster which can be interpreted as a species cluster. Although based on only a few GenBank sequences, these results support the hypothesis of LOUNNAS et al. (2017) that *G. cubensis* and *G. neotropica* are conspecific or synonymous, as well as the results of STANDLEY et al. (2013) on the questionable validity of *G. neotropica* and *G. viator* as separate taxonomic entities. We are convinced that a large-scale revision of *G. cubensis*, *G. neotropica* and *G. viator* is urgently needed. This, consequently, questions the correctness of the classification of GenBank sequences of these species. Nevertheless, the clear distinctiveness from *G. truncatula* or *G. schirazensis* shows that another neotropical *Galba* species has been introduced in the Ebro delta.

## DISTRIBUTION

Initially described from Cuba (PFEIFFER 1839), *G. cubensis* was mentioned by BAKER (1911) with a West Indian, Central American, and Lower Mississippian distribution, with an occurrence in southern Lower California. HUBENDICK (1951: p. 128) described the species as “circum-Caribbean”. According to BURCH (1989), *G. cubensis* was distributed in the southern United States from Florida to southern Texas. Besides Cuba, the species was reported from other Antilles Islands: the Dominican Republic (GOMEZ et al. 1986), Puerto-Rico (HARRY & HUBENDICK 1964), Guadeloupe (POINTIER 1974, 2008), Martinique (GUYARD & POINTIER 1979), and St. Lucia (MCKILLOP & HARRISON 1980). *G. cubensis* occurs in muddy soils of ponds, grassy marshes, watercress beds, or in marshy habitats behind mangrove swamps (POINTIER 2008).

The occurrence in Spain is the first European record in the wild, and also the first one in Europe verified by molecular genetics. *G. cubensis* was previously recorded from Europe, based, however, solely on morphology: from Germany in greenhouses of the horticultural company Hermann Rothe in Berlin-Zehlendorf (BOETTGER 1929) and of the Botanical Garden in Berlin-Dahlem (PLATE & FRÖMMING 1953), from a greenhouse in Flensburg (WIESE 1991), from a horticultural company in Rostock (ZETTLER et al. 2006) as well as from greenhouses in Austria (LEISS & REISCHÜTZ 1996).

Before the specimens were collected for molecular genetic analyses and determined as *G. cubensis*, live *Galba* specimens with white spots on tentacles and head were found in the rice fields in the same site during three consecutive years, and in relatively high densities. Providing these were *G. cubensis*, it would mean that the species is established here despite the regular use of pesticides, the yearly drainage and flooding of the rice fields as well as other human interventions (LÓPEZ SORIANO & QUIÑONERO SALGADO, personal observation). As observed in the rice fields in Suriname the use of pesticides may not affect the entire population of freshwater gastropods because of their ability to avoid molluscicides in the water by active behaviour (VAN DINOTHER 1973). No recent observations of big populations of *G. truncatula* were reported from the rice fields or even the whole Ebro delta, where this species seems to be rather rare (QUIÑONERO SALGADO & LÓPEZ SORIANO 2014); this suggests that the huge numbers of white-speckled *Galba* specimens recently found (of which two were randomly chosen for molecular analyses) should also represent *G. cubensis*, although additional molecular confirmation based on a larger number of specimens is needed. The species seems to have an amphibious behaviour, and normally is present at the margin of the fields out of the water.

*G. cubensis* is yet another human-mediated introduction of an aquatic alien species in Spain, most of them apparently originating from releases from aquaculture facilities, including the rice pest *Pomacea maculata* Perry, 1810 (LÓPEZ-SORIANO et al. 2009), *Austropeplea viridis* (Quoy & Gaimard, 1833) (SCHNIEBS et al. 2017), and different fish species (FRANCH et al. 2008, QUIÑONERO SALGADO & LÓPEZ SORIANO 2013). *A. viridis* and *G. cubensis* come from two opposite geographical areas (Australasia and Caribbean/South America, respectively), thus giving additional support to the possible human-mediated transport with plants or other commercial activities. While we cannot be certain of the origin of the two *G. cubensis* specimens, we consider an escape from a horticultural facility as the most probable. The area holds many horticultural facilities which, in our experience, can well host non-native molluscs (LÓPEZ SORIANO & QUIÑONERO SALGADO, personal observation). YANAI et al. (2017) have shown that in Israel aquarium and aquatic ornamental plant nurseries are pathways of introduction of non-indigenous freshwater molluscs. Other putative sources, such as import with rice cultivation or animal-mediated transport, appear highly unlikely given the strict control of any rice culture-related activities and lack of any *G. cubensis* populations nearby.

Our report on *G. cubensis* is one of the many on the presence and naturalisation of alien mollusc species in the Ebro Delta, such as *Hawaiia minuscula* (Binney, 1851), *Zonitoides arboreus* (Say, 1810),



*Succinea (Calcisuccinea)* sp., *Physa acuta* (Draparnaud, 1805), *Gyraulus chinensis* (Dunker, 1848), *Ferrissia fragilis* (Tryon, 1863), *Pomacea maculata* Perry, 1810 (QUIÑONERO SALGADO & LÓPEZ SORIANO 2013, 2014 & 2015), or *Austropeplea viridis* (SCHNIEBS et al. 2017). A number of marine mollusc allochthonous

species have also been recorded from this area, all of them associated with marine aquaculture facilities (oyster and mussel culture; LÓPEZ SORIANO & QUIÑONERO SALGADO 2015). Altogether, these findings make the Ebro Delta a hotspot of biological – and mollusc – invasions.

## CONCLUSIONS

This is the first record of *G. cubensis* in the wild in Europe. Since its reliable morphology-based differentiation from *G. truncatula* is still uncertain, monitoring the spread of the species will be very difficult without molecular genetic methods. However, the white speckles seen in the *G. cubensis* specimens analysed and in all the individuals from their population should be further studied as a potential diagnostic character.

The discovery of *G. cubensis* in rice fields in Europe should be of high interest for parasitologists. The Spanish health authorities have already been warned of this fact and of the occurrence of *A. viridis* since December 2016.

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## APPENDIX 1.

Material used in molecular genetic studies

Code	Collection No. SNSD	Locality	GenBank ##		References
			COI	ITS2	
<b><i>Aplexa hypnorum</i> (Linnaeus, 1758)</b>					
<i>Aplexa hypnorum</i> 1	Moll S348	Germany, Mecklenburg-Vorpommern, Lake Nebel, 53°15'32"N, 12°42'02"E	FR797859	FR797832	<a href="#">VINARSKI et al. 2011</a>
<i>Aplexa hypnorum</i> 2	Moll S350	Germany, Mecklenburg-Vorpommern, Lake Nebel, 53°15'32"N, 12°42'02"E	FR797860	FR797833	<a href="#">VINARSKI et al. 2011</a>
<b><i>Galba cousinsi</i> (Jousseaume, 1887)</b>					
<i>Galba cousinsi</i> 1		Venezuela, Mucubají	JN614388	JN614466	<a href="#">CORREA et al. 2011</a>
<b><i>Galba cubensis</i> (L. Pfeiffer, 1839)</b>					
<i>Galba cubensis</i> 1		Colombia, Barbosa	JN614390	JN614464	<a href="#">CORREA et al. 2011</a>
<i>Galba cubensis</i> 2		Colombia, Barbosa	JN614392	JN614463	<a href="#">CORREA et al. 2011</a>
<i>Galba cubensis</i> 3		Uruguay, Paysandú	JN614396	JN614461	<a href="#">CORREA et al. 2011</a>
<i>Galba cubensis</i> 4		Peru	KT781285	–	<a href="#">ALDA et al. 2015</a>
<i>Galba cubensis</i> 5		Venezuela	KT781327	–	<a href="#">ALDA et al. 2015</a>
<i>Galba cubensis</i> 6		Venezuela	KT781328	–	<a href="#">ALDA et al. 2015</a>
<i>Galba cubensis</i> 7		USA, Florida	KT781331	–	<a href="#">ALDA et al. 2015</a>
<i>Galba cubensis</i> 8		USA, Florida	KT781333	–	<a href="#">ALDA et al. 2015</a>
<i>Galba cubensis</i> 9		Peru	–	KT781186	<a href="#">ALDA et al. 2015</a>
<i>Galba cubensis</i> 10		Venezuela	–	KT781220	<a href="#">ALDA et al. 2015</a>
<i>Galba cubensis</i> 11		USA, Florida	–	KT781225	<a href="#">ALDA et al. 2015</a>
<i>Galba cubensis</i> 12		USA, Florida	–	KT781229	<a href="#">ALDA et al. 2015</a>
<i>Galba cubensis</i> 13		Cuba, Contramaestre	–	KU870347	<a href="#">LOUNNAS et al. 2016</a>
<i>Galba</i> 1	Moll S7956	Spain: Ebro Delta, Tarragona, Sant Carles de la Rápita, 40°37'42.1"N, 00°36'04.0"E	LT623604	LT623584	This paper
<i>Galba</i> 2	Moll S7957	Spain: Ebro Delta, Tarragona, Sant Carles de la Rápita, 40°37'42.1"N, 00°36'04.0"E	LT623605	LT623585	This paper
<b><i>Galba neotropica</i> (Bargues, Artigas, Mera y Sierra, Pointier &amp; Mas-Coma, 2007)</b>					
<i>Galba neotropica</i> 1		Peru: Lima	AM494008	–	<a href="#">BARGUES et al. 2007</a>
<i>Galba neotropica</i> 2		Peru: Lima	–	AM412225	<a href="#">BARGUES et al. 2007</a>
<i>Galba neotropica</i> 3		Uruguay: Paysandú	–	KX781343	<a href="#">ARMUA-FERNANDEZ et al. 2016</a>
<i>Galba neotropica</i> 4		Uruguay: Tacuarembo	–	KX781344	<a href="#">ARMUA-FERNANDEZ et al. 2016</a>
<i>Galba neotropica</i> 5		Uruguay: Paysandú	KX781341	–	<a href="#">ARMUA-FERNANDEZ et al. 2016</a>
<i>Galba neotropica</i> 6		Argentina: Tanton & Ipizca	KT215350	–	<a href="#">BARGUES et al. 2016</a>



Code	Collection No. SNSD	Locality	GenBank ##		References
			COI	ITS2	
<i>Galba schirazensis</i> (Küster, 1862)					
<i>Galba schirazensis</i> 1			JF272607	–	BARGUES et al. 2011
<i>Galba schirazensis</i> 2		Mexico, Xalpatlaco in Atlixco, Puebla	JF272608	–	BARGUES et al. 2011
<i>Galba schirazensis</i> 3		Ecuador, La Buena Esperanza	JF272609	–	BARGUES et al. 2011
<i>Galba schirazensis</i> 4		Iran	–	KT365868	NASIBI et al. 2015 (unpublished)
<i>Galba schirazensis</i> 5		Iran	–	KT365870	NASIBI et al. 2015 (unpublished)
<i>Galba schirazensis</i> 6		Iran	–	KT365871	NASIBI et al. 2015 (unpublished)
<i>Galba truncatula</i> (O. F. Müller, 1774)					
<i>Galba truncatula</i> 1	Moll 52544	Germany, Saxony, Oelsnitz/Erzgebirge, former pond, 50°43'02"N, 12°42'04"E	FR797875	LT623574	VINARSKI et al. 2011 This paper
<i>Galba truncatula</i> 2	Moll 52443	Italy: Val d'Inzino	LT623586	LT623575	This paper
<i>Galba truncatula</i> 3	Moll S1130	Bulgaria, Osogovo Mountains, Smolichane Village, karst spring, 42°07'58.1"N, 22°48'25.2"E	FR797873	FR797845	VINARSKI et al. 2011
<i>Galba truncatula</i> 4	Moll S2381	Sweden: Rådetorp/Bjurtjärn near Storfors, field margins, residual water puddles, 59°25'42"N, 14°20'09"E	LT623587	LT623576	This paper
<i>Galba truncatula</i> 5	Moll S4047	Italy: Veneto, tributary of Lago di Alleghe, 46°24'49.4"N, 12°00'52.3"E	LT623588	LT623577	This paper
<i>Galba viator</i> (d'Orbigny, 1835)					
<i>Galba viator</i> 1		Argentina	AM494010	AM412224	BARGUES et al. 2007
<i>Galba viator</i> 2		Argentina, Rio Negro	JN614398	JN614465	CORREA et al. 2011
<i>Ladislavella terebra</i> (Westerlund, 1885)					
<i>Ladislavella terebra</i> 1	Moll 52850	Russia, Tjumen Region, vicinity of Labytnangi, branch of Vylposl Challel, 66°38'54.1"N, 66°25'25.3"E	LT623591	HE613324	This paper VINARSKI et al. 2012
<i>Ladislavella terebra</i> 2	Moll S1169	Russia, Altai Republic, Lake Teletzkoje, 51°47'29.5"N, 87°16'56.1"E	LT623592	HE613325	This paper VINARSKI et al. 2012
<i>Lymnaea stagnalis</i> (Linnaeus, 1758)					
<i>Lymnaea stagnalis</i> 1	Moll 53093	Germany, Baden-Württemberg, Lake Constance, peninsula Mettnau, north side, 47°43'52"N, 09°00'04"E	FR797867	FR797836	VINARSKI et al. 2011
<i>Lymnaea stagnalis</i> 2	Moll 53094	Germany, Baden-Württemberg, Lake Constance, peninsula Mettnau, north side, 47°43'52"N, 09°00'04"E	FR797868	FR797837	VINARSKI et al. 2011
<i>Lymnaea stagnalis</i> 3	Moll 53109	Germany, Baden-Württemberg, Konstanz-Egg, ditch Hockgraben, 47°40'57.3"N, 09°11'34.2"E	FR797866	FR797835	VINARSKI et al. 2011
<i>Lymnaea stagnalis</i> 4	Moll 53485	France: vicinity of Toulouse	HG932251	HG931958	VINARSKI et al. 2014 (unpublished)
<i>Lymnaea stagnalis</i> 5	Moll S339	Germany: Mecklenburg-Western Pomerania, Krümmel, Lake Tralowsee	HG932247	HG931952	VINARSKI et al. 2014 (unpublished)
<i>Lymnaea stagnalis</i> 6	Moll S2311	Bulgaria: Plovdiv, floodplain of Mariza River, 42°09'13.5"N, 24°43'34.8"E	HG932255	HG931965	VINARSKI et al. 2014 (unpublished)

Code	Collection No. SNSD	Locality	GenBank ##		References
			COI	ITS2	
<i>Lymnaea taurica</i> (Clessin, 1880)					
<i>Lymnaea taurica</i> 1	Moll S1678	Russia, Omsk Region, Isilkul District, small nonpermanent steppe pool, 54°54'43.7"N, 71°10'49.5"E	HG932240 HE613316		VINARSKI et al. 2014 (unpublished) VINARSKI et al. 2012
<i>Lymnaea taurica</i> 2	Moll S2922	Russia, Altai Region, pond in floodplain of Kulunda River near Shimolino, 52°59'30.4"N, 80°59'30.4"E	HG932241 HE613318		VINARSKI et al. 2014 (unpublished) VINARSKI et al. 2012
<i>Lymnaea taurica</i> 3	Moll S3245	Ukraine, Odessa Region, floodplain of Kogilnik River near Tatarbunar, 45°49'32.3"N, 29°37'43.8"E	HG932242 HE613320		VINARSKI et al. 2014 (unpublished) VINARSKI et al. 2012
<i>Omphiscola glabra</i> (O. F. Müller, 1774)					
<i>Omphiscola glabra</i> 1	Moll S304	Germany, Hamburg, Kollau, Mühlenau, 53°36'34"N, 09°55'33"E	FR797862	FR797854	VINARSKI et al. 2011
<i>Omphiscola glabra</i> 2	Moll S305	Germany, Hamburg, Kollau, Mühlenau, 53°36'34"N, 09°55'33"E	FR797863	FR797855	VINARSKI et al. 2011
<i>Omphiscola glabra</i> 3	Moll S306	Germany, Hamburg, Kollau, Mühlenau, 53°36'34"N, 09°55'33"E	FR797864	FR797856	VINARSKI et al. 2011
<i>Radix auricularia</i> (Linnaeus, 1758)					
<i>Radix auricularia</i> 1	Moll 52859	Russia, Novosibirsk Region, Novosibirsk, Reservoir near Kirza River, 54°14'14.6"N, 81°39'21.7"E	LT623596 HE577646		This paper SCHNIEBS et al. 2012
<i>Radix auricularia</i> 2	Moll 53070	Germany, Bavaria, Weichering near Ingolstadt, pond in riverside forest of Danube, 48°43'34.1"N, 11°19'23.6"E	FR797879	FR797842	VINARSKI et al. 2011
<i>Radix auricularia</i> 3	Moll 53086	Switzerland, Lake Constance near Guettingen	LT623597	LT623582	This paper
<i>Radix balthica</i> (Linnaeus, 1758)					
<i>Radix balthica</i> 1	Moll 51139	Russia, Tomsk Region, Lake Motshishtshe, 56°13'10.2"N, 84°54'48.0"E	HG932222	HG931938	VINARSKI et al. 2014 (unpublished)
<i>Radix balthica</i> 2	Moll 53111	Germany, Baden-Württemberg, Konstanz-Egg, pond near University, 47°41'09"N, 09°11'29"E	LT623593 HE573078		This paper SCHNIEBS et al. 2016
<i>Radix balthica</i> 3	Moll S1342	Germany, Mecklenburg-Western Pomerania, Lake Großer Pätschsee	LT623594	LT623580	This paper
<i>Radix balthica</i> 4	Moll S2829	Sweden: Gotland, coast of the Baltic Sea, northeast of Lickershamn	LT623595	LT623581	This paper
<i>Radix labiata</i> (Rossmässler 1835)					
<i>Radix labiata</i> 1	Moll 51275	Germany, Saxony, pond near Langenberg, 50°33'09"N, 12°51'21"E	HG932226 HE573068		VINARSKI et al. 2014 (unpublished) SCHNIEBS et al. 2011
<i>Radix labiata</i> 2	Moll 51276	Germany, Saxony, pond near Langenberg, 50°33'09"N, 12°51'21"E	LT623598 HE573069		This paper SCHNIEBS et al. 2011
<i>Radix labiata</i> 3	Moll 51277	Germany, Saxony, pond near Langenberg, 50°33'09"N, 12°51'21"E	LT623599 HE798452		This paper SCHNIEBS et al. 2013
<i>Radix labiata</i> 4	Moll 52431	Italy, Lombardy, Province of Brescia, Ponte di Legno, Bleis Lake, 46°16'43"N, 10°33'29"E	LT623600 HE798460		This paper SCHNIEBS et al. 2013



Code	Collection No. SNSD	Locality	GenBank ##		References
			COI	ITS2	
<b><i>Radix lagotis</i> (Schrank 1803)</b>					
<i>Radix lagotis</i> 1	Moll 52815	Germany, Saxony, Grethen, pond Kleiner Kirchenteich, 51°14'27.46"N, 12°39'24.91"E	LT623601	LT623583	This paper
<i>Radix lagotis</i> 2	Moll 53239	Saxony, dam Doellnitzsee near Mutzschen, 51°15'45"N, 12°55'18"E	LT623602	HE573075	This paper <a href="#">SCHNIEBS et al. 2011</a>
<i>Radix lagotis</i> 3	Moll S3770	Bulgaria, Dragoman marshland 40 km west of Sofia, 42°56'11.7"N, 22°57'09.3"E	LT623603	LN874259	This paper <a href="#">SCHNIEBS et al. 2015</a>
<b><i>Stagnicola corvus</i> (Gmelin, 1791)</b>					
<i>Stagnicola corvus</i> 1	Moll 49821	Germany, Saxony, Niederspree, pond Großer Tiefzug, 51°24'20"N, 14°53'38"E	HG932236		<a href="#">VINARSKI et al. 2014</a> (unpublished)
<i>Stagnicola corvus</i> 2	Moll 49872	Germany, Saxony, pond Vierteich near Freitelsdorf, 51°15'43"N, 13°41'57"E	LN515538	HE577638	<a href="#">SCHNIEBS et al. 2012</a>
<i>Stagnicola corvus</i> 3	Moll 52830	Germany, Saxony, Grethen, ditch on the west side of pond Kleiner Kirchenteich, 51°14'29"N, 12°39'22"E	HG932237	HE577640	<a href="#">VINARSKI et al. 2014</a> (unpublished) <a href="#">SCHNIEBS et al. 2012</a>
<i>Stagnicola corvus</i> 4	Moll S1750	Germany, Mecklenburg-Western Pomerania, small pond near Neperstorf	HG932239	HG931949	<a href="#">VINARSKI et al. 2014</a> (unpublished)
<b><i>Stagnicola palustris</i> (O. F. Müller, 1774)</b>					
<i>Stagnicola palustris</i> 1	Moll 48715	Germany, Saxony, wetland west of Burghausen, 51°21'33"N, 12°14'44"E	FR797869	FR797840	<a href="#">VINARSKI et al. 2011</a>
<i>Stagnicola palustris</i> 2	Moll 48716	Germany, Saxony, wetland west of Burghausen, 51°21'33"N, 12°14'44"E	FR797870	FR797841	<a href="#">VINARSKI et al. 2011</a>
<i>Stagnicola palustris</i> 3	Moll S5798	Germany, Brandenburg, Lake Schulzensee near Chorin, 52°54'19.38"N, 13°52'14.71"E	LT623589	LT623578	This paper
<i>Stagnicola palustris</i> 4	Moll S5799	Germany, Brandenburg, Lake Schulzensee near Chorin, 52°54'19.38"N, 13°52'14.71"E	LT623590	LT623579	This paper