Heleobia charruana (Gastropoda, Truncatelloidea, Cochliopidae), a South American brackish water snail in northwest European estuaries

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van Haaren, T., Worsfold, T.M., Stelbrink, B., Collado, G.A., Gonçalves, I.C.B., Serra, W.S., Scarabino, F., Gittenberger, A., & Gittenberger, E., 2021. *Heleobia charruana* (Gastropoda, Truncatelloidea, Cochliopidae), a South American brackish water snail in northwest European estuaries. — Basteria 85 (1): 82–91. Leiden. *Published 7 May 2021.*

ABSTRACT

The South American brackish water snail *Heleobia charruana* (d'Orbigny, 1841), abundant in Uruguay, is newly recorded for three western European countries: the United Kingdom (2003), the Netherlands (2014) and Belgium (2017). Its identity was confirmed using morphological and molecular methods. The method successfully used to isolate DNA and to further amplify and sequence the full mitochondrial COI barcoding fragment from an old dry shell without damage is described in detail. A short synonymy with references to the main literature is presented. The species' range and ecological data are reviewed for both South America and Europe and the implications of the finds are discussed.

Key words: *Heleobia*, non-native, distribution, Neotropical Region, Europe

INTRODUCTION

During the initial two decades of this century, a hydrobioid snail has been found in brackish waters, independently in three European countries. It was first noticed in samples from Barking Creek (tidal Thames, England, U.K.) and later found in samples collected in 2003 (Battersea) and subsequent years from other parts of the tidal Thames and associated dock basins. The conspicuous structure of the penis of the animals showed that it belonged to the family Cochliopidae, probably being part of *Heleobia*, but that it was clearly not the indigenous *Eupaludestrina stagnorum* (Gmelin, 1791), which is not considered congeneric with *Heleobia* by Kroll et al. (2012: 1524) or Willing & Rowson (2020). The first European material to be sequenced, by Thomas Wilke (Justus Liebig University Giessen), was collected in 2005 from Battersea (tidal Thames). Material sent to Robert Hershler (Smithsonian Museum), from Greenwich (tidal Thames), was confirmed as Cochliopidae but could not be determined at species level, as morphology alone is problematic for determination of Cochliopidae (De Francesco, 2007) and DNA analysis did not result in a clear match with any of the cochliopid species that were represented in GenBank at the time. The first published record for Europe, from Antwerp, Belgium in May 2017 (Gittenberger et al., 2018: 30), was originally named as *Heleobia* cf. *australis* (d'Orbigny, 1835), based on a comparison of shell shapes only.

From a comparison of shell shapes only, and taking into account species for which DNA data were available in GenBank, the first author hypothesized that the non-native *Heleobia* could be *H. charruana* (d'Orbigny, 1841), a species known from eastern South America. Eventually, the identification was confirmed through comparison of original DNA sequences of specimens from the three European countries and South America. Here, we present some taxonomic, ecological, and biogeographical data for *H. charruana*. Our main goal is to record the introduction of this non-native brackish water hydrobioid snail for the European malacofauna.

As De Francesco (2007: 632) and Scarabino et al. (2011:19) noted that diagnostic morphological differences are lacking between *H. charruana* and *H. conexa* (Gaillard, 1974), we have considered the distributions of both in the context of the European records.

MATERIAL AND METHODS

Material. — For shells, see the systematic part.

Abbreviations for collection numbers: LMSM = Laboratorio de Malacología y Sistemática Molecular, Universidad del Bío-Bío; RMNH = National Biodiversity Center, Leiden; UGSB = University of Giessen Systematics and Biodiversity collection; USNM = National Museum of Natural History, Washington D.C.

For DNA sequencing 10 specimens were used, eight of which were from Europe and two from Uruguay, as follows.

2: England, London, West India Dock (Blackwall Basin), 0.011°W, 51.5033°N, Jamie Dyson & Victoria Mallott leg. 09.x.2005; UGSB 25910-25911, GenBank acc. nos MW717665–MW717666.

2: Belgium, Antwerp harbour, 4.37°E, 51.25°N, Adriaan Gittenberger leg. v.2017; UGSB 21800-21801, GenBank acc. nos MW717667–MW717668.

2: The Netherlands, Amsterdam, North Sea Canal, near entrance channel H, 4.8577°E, 52.4172°N, Ton van Haaren leg. 30.xii.2018; UGSB 23076-23077, GenBank acc. nos MW717669–MW717670.

2: The Netherlands, Amsterdam, NW corner Noorder IJ-Plas, 4.8607°E, 52.4199°N, Ton van Haaren leg. 30.xii. 2018; UGSB 23079–23080, GenBank acc. nos MW717671–MW717672

1: Uruguay, Montevideo, Punta Trouville; 34.92111°S, 56.14774°W, LMSM HTrouv8FG; GenBank acc. no. MW 717673.

1: Uruguay, Maldonado, Isla de Lobos (8 km SE of Punta del Este), 54.87417°W, 35.05028°S, Daniel Korkos leg. ix.1998; Jan Delsing colln, GenBank acc. no. MW717674.

Collecting. — British material was collected from several routine monitoring and impact assessment surveys throughout the tidal Thames region, using standard methods: 0.01 m² core samples intertidally, kick samples in the infralittoral, 0.1 m² Day grab samples and qualitative dredges subtidally. Samples were sieved and preserved on collection and fauna extracted using a stereo microscope. In Antwerp harbour, Belgium, a hand dredge was used together with a Petit Ponar sediment grab. The underground water systems that are connected with the harbour's waters to be used for fire extinguishing purposes were investigated with a PacBag® system from Corexeed B.V. (i.e., a mesh bag designed to be attached to a hydranth to sample organisms that live inside a water system). The turbidity, pH, and acidity were measured with equipment from Hanna Instruments. Live snails and shells were collected by searching by eye, following sieving of the substrata. Dutch material was collected by either 0.1 m² van Veen grab sampling or with a pond-net (mesh size 500 µm). Core samples were sieved on board, preserved and sorted in the lab and pond-net material was sorted alive on site. The material from Punta Trouville was collected with a hand sieve. The collection method on the one from Maldonado is unknown.

DNA isolation, PCR amplification and DNA sequencing. — For the majority of European samples, DNA was isolated using a standard CTAB protocol for molluscs (Winnepenninckx et al. 1993). To obtain a gene fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI, 658 bp length), we used the standard metazoan primers LCO1490 and HCO2198 (Folmer et al., 1994) and the following PCR conditions: initial denaturation at 95 °C for 1 min, 35

cycles (denaturation: 95 °C for 30 s, annealing: 45 °C for 30 s, elongation: 72 °C for 30 s) and a final elongation at 72 °C for 3 min. DNA sequencing was performed on an ABI 3730XL sequencer using the Big Dye Terminator Kit (both Life Technologies). The same CTAB procedure and primers were used for *H. charruana* from Punta Trouville, Montevideo.

For a single shell of *H. charruana* that was collected in September 1998 from Isla de Lobos, Uruguay, and kept dry at variable room temperatures, a special 3-day CTAB DNA extraction method was used. First, the shell was placed in a 2 mL tube with a mixture of 500 µL CTAB lysis buffer (BioChemica/VWR A4150.0500), 3 µL proteinase K (20mg/mL dissolved in TE pH 8.0) and 1 µL 2-mercaptoethanol (99% pure, Acros Organics). The last whorl of the shell, where dried tissue was found, was washed with this buffer using a pipette. The tube was left in a heat block at 60 °C for ~15 hours. Then 500 µL chloroform/isoamyl alcohol (24:1 VWR) were added. After gentle hand shaking, the tube was centrifuged for 10 min at 8,000 rpm. The supernatant was carefully pipetted into a new tube and 280 µL isopropanol (propan-2-ol VWR/Electan® Molecular biology grade) were added. The tube was hand-shaken and placed at 4 °C overnight (for ~15 hours) before centrifuging for 10 min at 10,000 rpm. The supernatant was discarded and 700 µL ethanol (70%) were added to wash the pellet. After gently hand shaking the tube, it was again centrifuged for 2 min at 10,000 rpm. This step was repeated, the ethanol was discarded and the pellet was dried by leaving the tube open for several hours. The DNA pellet was then dissolved in 20 µL TE buffer (10 mM Tris, 1 mM EDTA; pH 8.0, VWR).

To increase the chance of a successful PCR, the universal COI primers of Geller et al. (2013) and Folmer et al. (1994) were used, in addition to 5 primer combinations that were specifically designed for molluscs at GiMaRIS for internal use (unpubl. data). For each of these primer combinations, a PCR was done with a reaction mix consisting of 1 µL DNA, 6 µL Qiagen RM mix, 4 µL PCR-water (Qiagen), 0.5 µL forward and 0.5 µL reverse primer. The PCR was done in a Qiagen Rotor-Gene Q apparatus with a HRM analysis at the end to check whether the PCR product formed melted at the temperature expected for a COI product. The only successful combination of primers that resulted in such a full 658 bp DNA barcoding fragment (with a HRM temperature of 80.6 °C), were the GiMaRIS_Mollo1-F and GiMaRIS_Mollo1-R (Table 1). The PCR program used comprised an initial denaturation step at 95 °C for 5 min, followed by 60 cycles at 95 °C for 1 min, 55 °C for 1 min and 70 °C for 1 min, followed by a hold step of 70 °C for 7 min and finally a HRM analysis ramping from 70 to 90 °C, with every 2 s a step of 0.1 °C, and a hold of 90 s of pre-melt conditioning on the first step. The PCR product was cleaned with a MEGAquick-spin Total Fragment DNA Purification Kit (iNtRON Biotechnology), following the manufacturer's instructions. Sequencing was done in both directions by Macrogen Europe.

Table 1. Primers.

GiMaRIS_Moll01-F GGTCAACAAATCATAAAGAYATYGG
GiMaRIS_Moll01-R TAAACTTCAGGGTGACCAAARAAYCA

Molecular analysis. — We used TCS 1.2.1 (Clement et al., 2000) with a 95% connection limit to construct a statistical parsimony haplotype network for all individuals sequenced.

RESULTS

Systematic part

Superfamily Truncatelloidea Gray, 1840

Family Cochliopidae Tryon, 1866

Subfamily Semisalsinae Giusti & Pezzoli, 1980

Genus *Heleobia* Stimpson, 1865

Type species, by original designation: *Paludina* (*Paludestrina*) *culminea* d'Orbigny, 1838. The species was figured in 1838 on plate 47 in Livraison 32 of d'Orbigny's Voyage dans l'Amérique méridionale (1834–1847), with legends that validate the name. See Coan & Kabat (2020: 29) for bibliographical data.

Heleobia charruana (d'Orbigny, 1841)

Figs 1–13

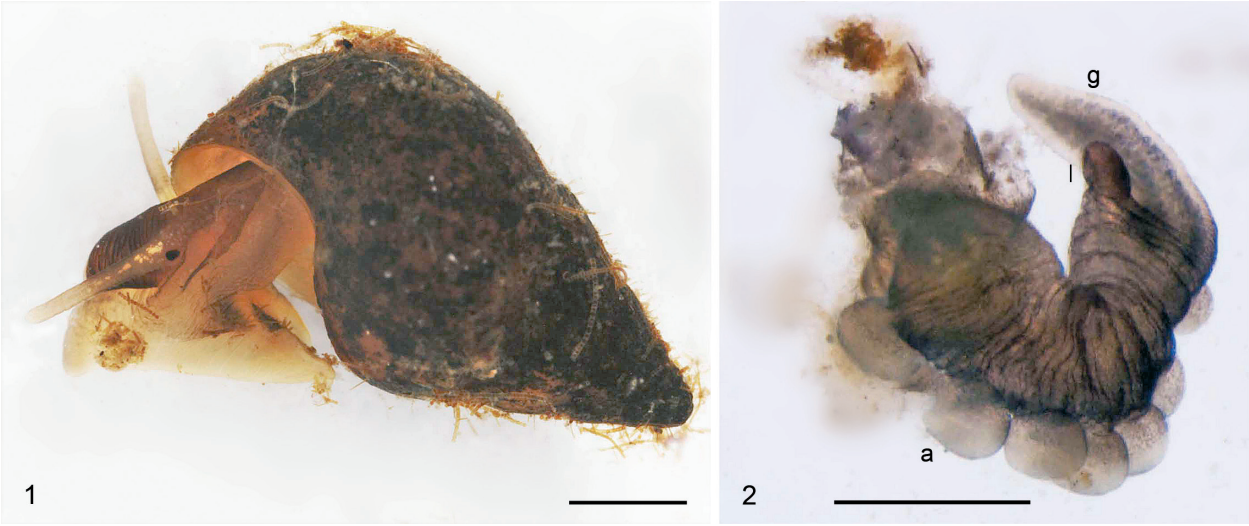
Paludina (*Paludestrina*) *charruana* d'Orbigny, 1841: 384, pl. 75 figs 1–2 ("au nord de [north of] Montevideo", Uruguay). See Coan & Kabat (2020: 30) for bibliographical data. d'Orbigny (1841: 381) confusingly mentions that *Paludestrina* should be considered a subgenus of *Paludina*.

Heleobia charruana; Pons da Silva & Davis, 1983: 129, 132 figs 5 (lectotype), 6–8 (paralectotypes), 139. Hershler & Thompson, 1992: 52. Cenzano & Würdig, 2006: 156. De Francesco, 2007: 632. Cazzaniga, 2011a: 16. Cazzaniga, 2011b: 36. Ortega et al., 2018: 146. De Oliveira Saad et al., 2019: 4.

Littoridina charruana; Pilsbry, 1911: 558, pl. 41C figs 1–2. Marcus & Marcus, 1963: 46. Marcus & Marcus, 1965: 71–72. Cazzaniga, 1982: 11.

Heleobia cf. *australis*; Gittenberger et al., 2018: 30. Not d'Orbigny, 1835.

Material. — For this study, shells were studied from (1) United Kingdom, England, tidal Thames (several sites, APEM Colln), deposited material from Greenwich (USNM 1456648), (2) Belgium, Antwerp harbour (RMNH.MOL.346846/33), (3)



Figs 1–2. *Heleobia charruana* (d'Orbigny, 1841). The Netherlands, Noorder IJ-Plas, leg. D. Drukker & T. van Haaren 16.vi.2018 (RMNH.MOL.346840). **1**. Live snail. **2**. Penis, with apocrine glands (a), lobe (l), and glans penis (g). Scale bars: 1 mm (**1**) and 0.5 mm (**2**).

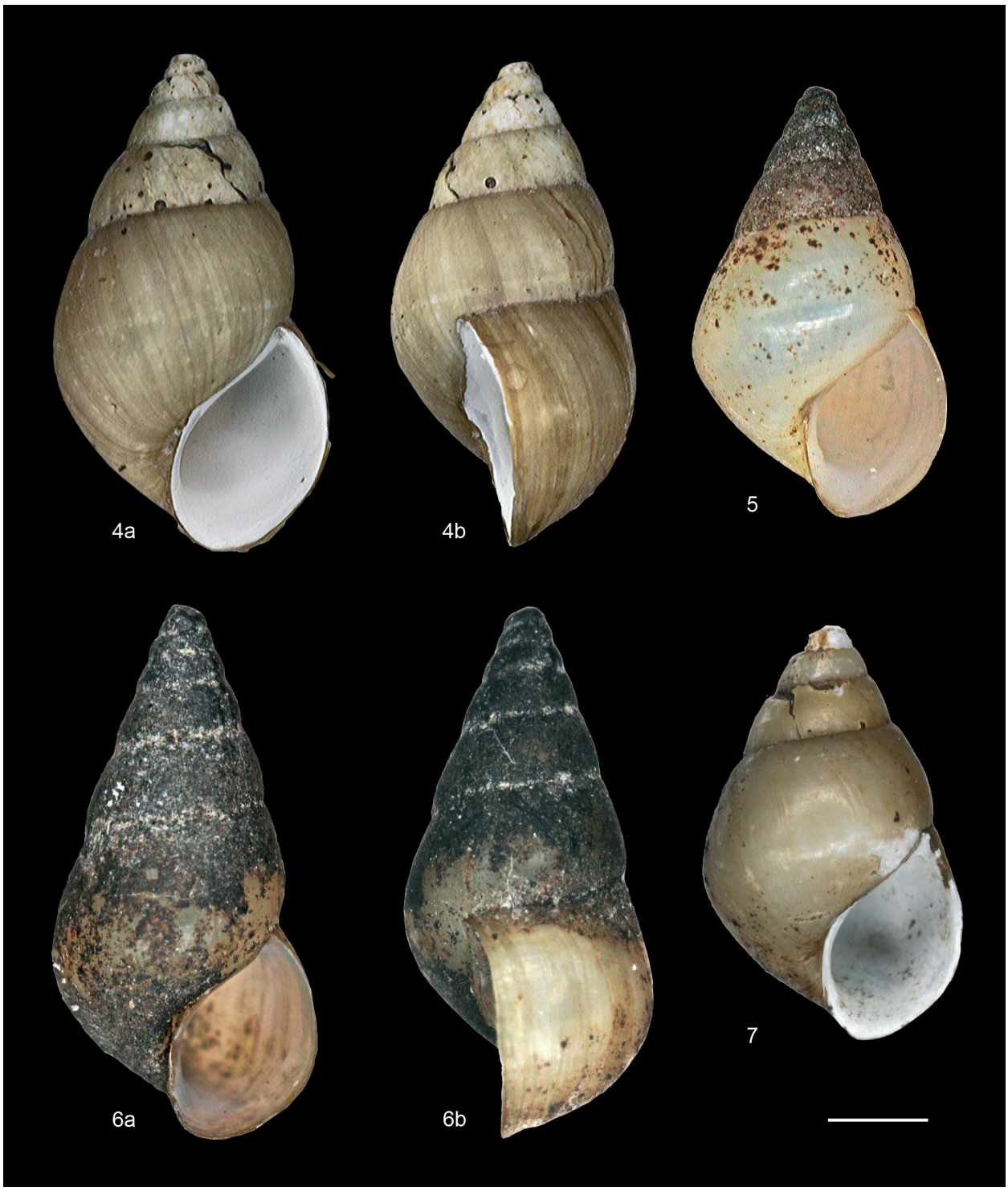
Fig 3. Live snail *Heleobia charruana* (d'Orbigny, 1841) in situ. The Netherlands, North Sea Canal near IJmuiden, photo R. Offermans, April 2014.

the Netherlands, Noord Holland, North Sea Canal and surroundings (RMNH.MOL.346839/7, 366840/24, 346845/25), and (4) Uruguay, Montevideo, Punta Trouville (LMSM HTrouv8 FG) and Maldonado, Isla de Lobos (Jan Delsing Colln).

Shell (Figs 4–13). — The very strong shell varies from almost white, through dull yellowish grey to brownish. It has a sculpture of inconspicuous growth lines, some of which may be more prominent near the aperture. Shells that are not encrusted have a vague silky gloss. There is a slender, strictly conical spire, with 6–6¾ flattened whorls that are separated by a barely incised suture. The outline of the periphery varies from regularly convex to slightly angular. The aperture border is vaguely S-shaped (lateral view) and opisthocline in rare, fully grown shells. These may have a narrowly thickened peristome inside, recognizable at the outside of the shell (Figs 12–13). They look quite different from the more commonly found shells. The aperture is pyriform, with a slightly protruding columellar border and a characteristically flaring basal side, which is also seen in juveniles. The umbilicus varies from narrowly open to closed.

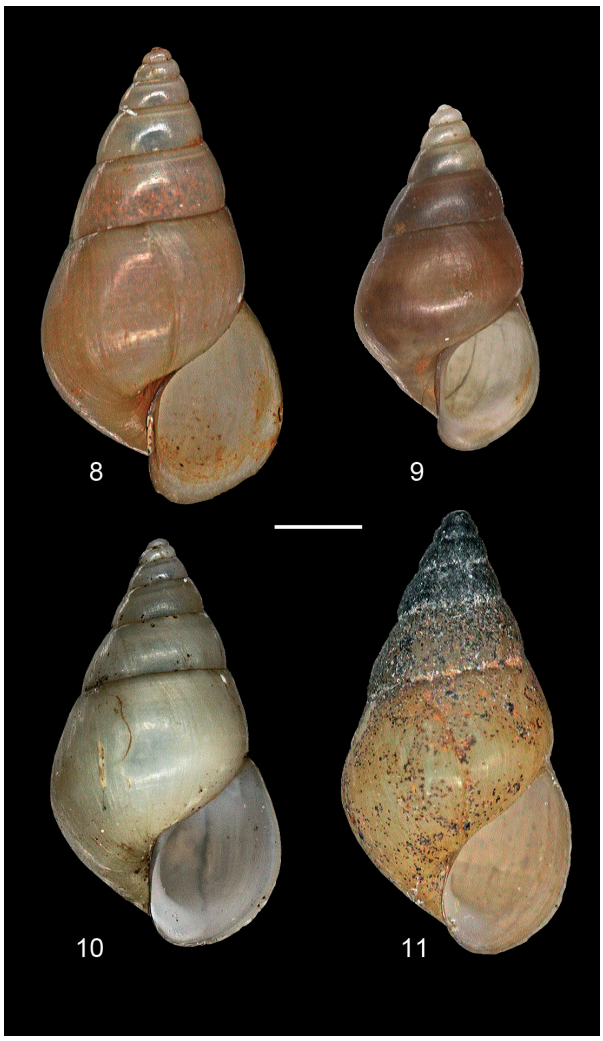
The shells are quite variable in size. For the sample from Belgium, Antwerp harbour (n = 33) the measurements are: height 3.9–6.4 mm, width 2.1–3.0 mm, height/width 1.7–2.1. No larger shells are known from the United Kingdom or the Netherlands. Since it is impossible to determine with certainty whether a shell is fully grown, the minimum measures are less reliable than the maximum. The shells are superficially similar to those of other hydrobioid species including *Potamopyrgus antipodarum* (Gray, 1843) and *Peringia ulvae* (Pennant, 1777), both of which may be found in the same samples as *H. charruana*, at either end of its salinity range. *Potamopyrgus antipodarum* has more tumid whorls than *H. charruana* and the body is generally darker, showing through the shell, which is more translucent. The shell of *P. ulvae* typically has a more yellowish or brownish tinge, compared to the grey or white most commonly seen in *H. charruana*. Shells of *H. charruana* usually differ additionally from these species by the obliquely narrowed lower half of the aperture, together with a more or less conspicuously expanding basal part of the peristome. The penis structure immediately distinguishes *H. charruana* from both species. *Eupaludestrina stagnorum* (syn. *Semisalsa stagnorum*), which is reminiscent of *Heleobia* in anatomy, differs by a much less solid shell with more convex whorls and a basally more regularly curved peristome.

Anatomy. — In living Dutch material (Figs 1, 3), the entire head was dark, in contrast to the light tentacles. Underneath

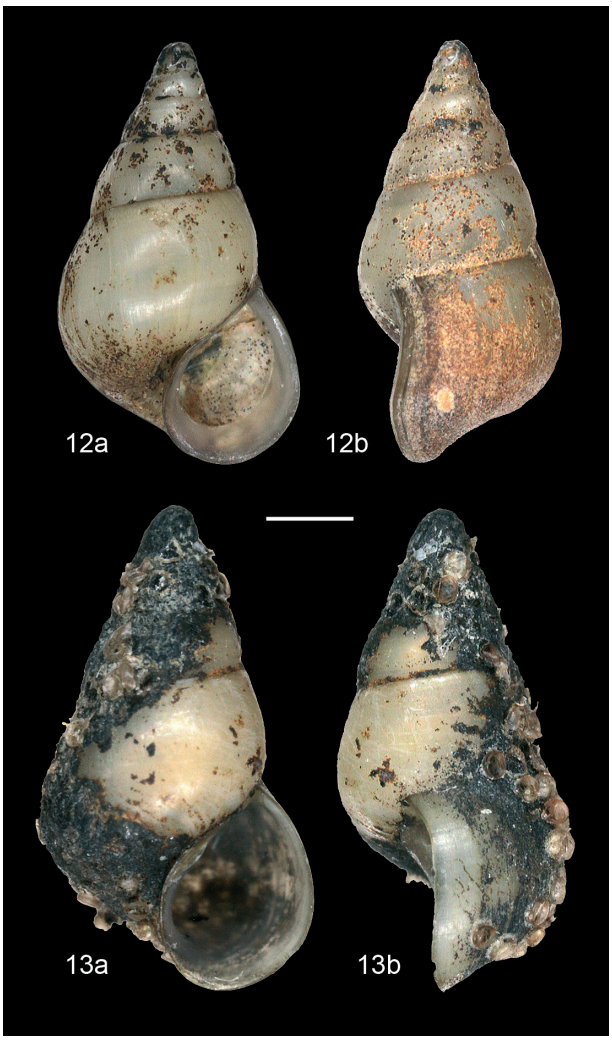


Figs 4–7. *Heleobia charruana* (d'Orbigny, 1841). **4.** Uruguay, Isla de Lobos, 8 km SE of Punta del Este, 35.05028°S 54.87417°W, Jan Delsing colln, Daniel Korkos leg. ix.1998. **5–7.** The Netherlands, southern Noorder IJ-Plas, E. Gittenberger & T. van Haaren leg. 06.iii.2019 (RMNH.MOL.346845). **4.** H = 5.2 mm, **5.** H = 4.3 mm, **6.** H = 5.6 mm, **7.** H = 4.45 mm. Scale bar: 1 mm. Photos by E.G.

the blackish eyes there is a unpigmented longitudinal stripe. An animal from the tidal Thames had grey and white mottling over the dorsal surface of the head and tentacles, with a white foot and underside of the head. Black eyes are present at the base of the tentacles. The tentacles of *H. charruana* do not have a blackish pigment band at the tip as in *P. ulvae*, which separates both species easily. A single studied Dutch male specimen had a relatively broad, dark, wrinkled main part to the penis, with 8 round apocrine glands on its convex side and a small, oval stalked lobe, inserting where the short,



Figs 8–11. *Heleobia charruana* (d'Orbigny, 1841). Belgium, Antwerp harbour; A. Gittenberger leg. v.2017 (RMNH.MOL.346846). **8.** H = 5.3 mm, **9.** H = 4.0 mm, **10.** H = 4.8 mm, **11.** H = 5.2 mm. Scale bar: 1 mm. Photos by E.G.



Figs 12–13. *Heleobia charruana* (d'Orbigny, 1841) shells with a strongly thickened peristome. Belgium, Antwerp harbour; A. Gittenberger leg. v.2017 (RMNH.MOL.346846). **12.** H = 4.85 mm, **13.** H = 5.65 mm. Scale bar: 1 mm. Photos by E.G.

tapering, smooth glans penis with a light border zone begins (Fig. 2). In the Thames specimen, the penis structure was similar but paler.

According to Marcus & Marcus (1963: 46) *H. charruana* has up to 16 penial "warts". Taking all other characters into account, we consider this intraspecific variation.

COI data — Three haplotypes were identified by the statistical parsimony network (Fig. 14). The majority of the European samples (including those from London, Antwerp, North Sea Canal and Noorder IJ-Plas) and the specimen from Isla de Lobos, Uruguay belonged to haplotype "H1", which is assumed to be the ancestral haplotype by the analysis. Haplotype "H2" was represented by the individual from Punta Trouville, Montevideo, Uruguay, which differs by a single substitution. Haplotype "H3" included a second individual sampled from the North Sea Canal, which differs from "H1" by three substitutions.

Ecology. — Within its native range, the species is known from a variety of saline habitats on more or less muddy sediments in stagnant to slowly moving water. Cenzano & Würdig (2006) provide detailed data for the Itapeva lagoon, where *H. charruana* is common. At Isla de Lobos, the snails were collected from intertidal rocks at low tide; they have been found on similar substrata in England (see below).

The species may occur at a variety of salinities and the animals can survive considerable changes in salinity during their lifespan. Marcus & Marcus (1963: 46) mentioned variations in salinity from 34.5 to 4.5‰ or even 1.5‰ within 10 hours at a single site, related to tides and winds. These authors observed hundreds of living *H. charruana* among the roots of floating aquatic macrophytes (*Eichhornia* and *Salvinia*) that were washed ashore after transport of over 70 km, and a change of oligohaline to polyhaline brackish surface water (Marcus & Marcus, 1963: 46). It remains unproven

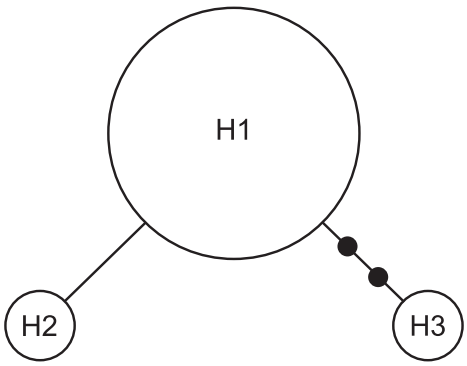


Fig. 14. Statistical parsimony network of all *Heleobia charruana* individuals sequenced by applying a 95% connection limit. See text for details on the different haplotypes.

that the snails originated from the same area as the plants, but it is not unlikely taking the local situation into account (Collado, pers. comm.). The morphologically identical *Heleobia conexa* (Gaillard, 1974) is reported from hypersaline backwaters in the Peninsula Valdés (Cazzaniga, 1982: 11) and at salinities between 1 and 40‰ in the Mar Chiquita (De Francesco & Isla, 2003: 795).

In the tidal Thames, England, it is abundant on the lower shore and in shallow subtidal mixed substrata, ranging from silty mud to stony gravel. Salinity records are not available for the actual samples but published means for the part of the Thames occupied by the species vary from about 0.5‰ (at the most upstream sites at low tide) to 30‰ (at the most downstream sites at high tide) (Attrill, 1998). In sheltered parts of Antwerp harbour, the species was widespread and common at depths between 5 and 12.5 m in water varying in salinity from 4.8 to 8.7‰, in acidity from pH 7.4 to 8.1, and turbidity between 2 ntu (clear water) to 308 ntu (very turbid). It was also recorded in the underground water systems of the harbour, where it occurs in complete darkness, with little to no current. In the Noorder IJ-Plas and secondary channels of the North Sea Canal, the species was very common in shallow (<1 m) sheltered areas while in the North Sea Canal it is found up to a depth of 19.2 m, with densities ranging from 10 to 3,573 individuals/m^2. Along the North Sea Canal it was also recorded from underground water systems similar to those of Antwerp harbour.

Distribution. — *Heleobia charruana* is known as a native species from the coastal region of eastern South America, from Brazil and Uruguay. Records for Brazil are: Province of Sao Paulo, Santos (Marcus & Marcus, 1963: 46), the northernmost locality, and Province of Rio Grande do Sul, Patos lagoon (Ortega et al., 2018: 142) and Itapeva lagoon (Cenzano & Würdig, 2006: 154). The type locality is north of Montevideo in Uruguay. Pilsbry (1911) misleadingly included *H. charruana* in reports of expeditions to Patagonia, without referring to any specific record south of Uruguay. *Heleobia conexa*, described from Albufera Mar Chiquita, Buenos Aires province, Argentina (Gaillard, 1974: 104) is reported from Buenos Aires province, Estuarios de Mar Chiquita, Quequén Grande and Quequén Salado (De Francesco, 2007), with its southernmost record at Peninsula Valdés, Chubut province (Cazzaniga, 1982). There are no sympatric records with *H. charruana*.

In Europe, *H. charruana* has been recorded in England, Belgium and the Netherlands. In England, it was first found in samples from the tidal Thames, London, the earliest of which were collected in 2003, and initially identified only as Cochliopidae. It is abundant between Battersea and Woolwich, including the East India Docks, and has been recorded in the grey literature as "Cochliopidae Species A" in most years since that time, with a family level published record from Deptford Creek in May 2016 (Worsfold & Kazubek, 2017: 67); there are fewer records from Barking Creek and downstream, as far as Mucking, such that live records of the species range over 40 km of the length of the Thames. In Belgium, it has been known from Antwerp harbour since May 2017 (Gittenberger et al., 2018). The first record for the Netherlands is from the North Sea Canal, April 2014 (Fig. 3); during subsequent years, the species was recorded frequently at various places in the North Sea Canal and waters connected with it like the Noorder IJ-Plas. They were found in The North Sea Canal over a length of 23 km from IJmuiden sluices (52.471°N 4.614°E) up to "het IJ" at Amsterdam (52.38044°N 4.92296°E).

DISCUSSION

This is the first report of the South American *H. charruana* outside its native range. That the snails have been found within the present study at several locations in northwestern Europe repeatedly for seventeen years, suggests that stable populations have become established. In the tidal Thames, for example, *H. charruana* is common and may be the dominant macrofaunal taxon at some sites. It was first sighted there in samples collected in 2003. First sightings during independent surveys followed in 2014 and 2017 in port areas of the Netherlands and Belgium, respectively. It seems likely that the European populations are derived from a single intercontinental introduction into the Thames area, followed by transport across the southern North Sea. Despite that the genetic analyses confirm that the European individuals represent *H. charruana*, the low number of specimens that were sequenced and the restricted diversity of haplotypes found in Europe and in Uruguay, do not allow further conclusions about their relationships or the colonization history. Due to its strong resemblance to the native species *Peringia ulvae*, it is possible that *Heleobia charruana* has been confused with *P. ulvae* in the past. It is therefore worth checking material from *P. ulvae* which may demonstrate that *H. charruana* may have been present in European estuaries for much longer.

The means of introduction of *H. charruana* to Europe are also unknown, but shipping would be the most obvious possibility. It is unlikely that the snails would have survived the journey from South America to Europe, across the Atlantic Ocean, attached to the outer hull. More likely, they were present somewhere inside a vessel in the sediment of a ballast water tank, a sea chest and/or another sheltered niche area. Such places are well known as a potential habitat for alien species (Carlton, 1985; Tsimplis, 2004; Frey et al. 2014). The animals may survive as adult snails in unnatural habitats, since *H. charruana* was found in the complete darkness of underground water systems in Antwerp harbour and near the North Sea Canal. Secondary spread throughout European waters could also be explained by ballast water, but other possible vectors may be taken into consideration. The New Zealand mudsnail *Potamopyrgus antipodarum* is able to survive the passage through the digestive tract of fishes and birds (Haynes et al. 1985; Vinson & Baker 2008). By feeding experiments it was found that *Peringia ulvae* could survive the passage in the intestinal tract of the mallard (*Anas platyrhynchos* L., 1758) but not *Potamopyrgus antipodarum* (van Leeuwen et al., 2012). The veligers of *H. charruana* with no true pelagic phase (Marcus & Marcus, 1963: 46–47) have been observed to remain amongst plants. Natural distribution can be considered whereby veligers and/or the adult snails on floating seaweeds may have drifted along with the residual currents in the southern North Sea. These residual currents run from England across the Channel to the European mainland, and from there northwards towards the Belgium and Dutch coasts (Sündermann & Pohlmann, 2011: 670, fig. 7). As both commercial vessels and pleasure crafts cross the North Sea, snails may also have been transported in fouling communities on hard surfaces. During the survey in the port of Antwerp, where *H. charruana* was repeatedly and commonly found in sediment samples across the area, however, no snails were recorded in any of the fouling community samples taken from the floating docks, suggesting that hull fouling and currents are less likely as transport vectors.

As *H. charruana* is often dominant where it occurs, impacts on native species would be expected. However, through much of its current introduced range, the fauna is already dominated by non-native species, particularly the gastropod *Potamopyrgus antipodarum*, which has similar colonizer traits (Alonso & Castro-Díez, 2008) and is very common in the Thames (Attrill, 1998), where it is often found in the same samples as *H. charruana*. In the USA and Chile, several native species may have become extinct with the introduction of *P. antipodarum* due to its high reproductive capability (Richards 2002; Collado et al., 2019). In the Thames, such impacts would already have happened since the *P. antipodarum* arrived in the 19th century. Several species considered to be of conservation value are restricted to brackish or tidal fresh waters (Chadd & Extence, 2004; Sanderson, 1996; Willing, 2020), including the small gastropods *Hydrobia acuta neglecta* Muus, 1963, which is not known from the tidal Thames, and *Mercuria anatina* (Poiret, 1801), which is found on the upper shore in tidal fresh water (Willing, 2020) near sites colonised by *H. charruana*, such as Barking Creek, but their tidal ranges do not overlap. In the more saline parts of Dutch estuarine sites, such as the North Sea canal, and in the lower Thames, *H. charruana* co-occurs in with the native *Peringia ulvae* but negative impacts from *H. charruana* would most likely be limited to areas with lower salinity and lower down the shore than the main population centres for *P. ulvae*. At present, there is no evidence for impacts on native European species. There would be cause for re-evaluation, should the new introductions spread to other estuaries, or to lagoonal systems.

The records presented here highlight the need for continued vigilance in the analysis of benthic samples. Some historical Thames samples were reviewed following discovery of the unknown snails but other samples may be available for review and the records show the importance of availability of archived material and data. The need for further research into the dispersal and identity of non-native species has also been highlighted, together with the value of more work into the taxonomy, ecology and biology of native species in all regions. A second species of non-native hydrobioid snail was found in the tidal Thames alongside *H. charruana* but material suitable for molecular studies are not yet available. Work continues on the taxonomy of South American Cochliopidae, including molecular studies into the relationship between *H. charruana* and *H. conexa*.

ACKNOWLEDGEMENTS

We are grateful to Thomas Wilke (Justus Liebig University Giessen) for the initial sequence and particularly to Robert Hershler (formerly NMNH) for review of early material. We thank Chad Walter (formerly NMNH), Kathryn Ahlfeld and Amanda Robinson (both NMNH) for assistance with retrieving data from that material and Mark Davison and Kevin O'Connell (Environment Agency, Thames Region), who provided sampling data and permission for much of the UK material. We also thank for assistance in various ways Ruud Bank (Hoogezand), Rykel de Bruyne (Halfweg), Joel Cuperus (Lelystad), Daan Drukker (Amsterdam), Claudio de Francesco (Mar del Plata, Argentina), and Ron Offermans (Amsterdam). We especially thank Jan Delsing (Echt), who provided the best-looking shell of *H. charruana* in his collection to be sacrificed for DNA sequencing. Björn Stelbrink was funded by two DFG grants (STE2460/3-1 and STE2460/4-1).

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