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Helix straminea Briganti, 1825 in Italy (Gastropoda: Pulmonata): taxonomic history, morphology, biology, distribution and phylogeny

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Abstract
The land snail taxon *Helix straminea* Briganti, 1825 has been reintroduced as a valid species in 2014. We provide here a comprehensive account of its taxonomy, distribution, anatomy, phylogeny and karyology in Italy. An overview of the historical views on the validity of the species is presented and faunistic data are reviewed and implemented with new records from Campania and Basilicata. A lectotype is fixed for *H. straminea* from the syntypes stored in the Muséum d'Histoire Naturelle of Genève, as well as for three other taxa (*Helix straminiformis* Bourguignat, 1876, *Helix yleobia* Bourguignat, 1883 and *Helix straminea* ssp. *elongata* Bourguignat, 1860). Genital system, radula and karyotype are described for the first time. Molecular analysis of two mitochondrial genes combining GenBank data and the new sequences presented in this paper showed no differentiation between the northern and southern Italian populations. The conservation status of the species and its possible threats are discussed.

Keywords: Helix straminea, *anatomy, distribution, karyotype, phylogeny, taxonomy*

Introduction

Vincenzo [or Vincenzio] Briganti (Salvitelle (Salerno), 7 June 1766 – Naples, 5 April 1836), naturalist and physician, assistant professor of botany and then professor of "materia medica" [= pharmacology] at the University of Naples (Anonymous 1840; Del Giudice 1866) (Figure 1), described a new species of pulmonate land snail that he named *Helix straminea* V. Briganti, 1825. The validity of this taxon has been debated for years, and it was mistaken for other species: initially for *Helix pomatia* Linnaeus, 1758 and *Helix cincta* O. F. Müller, 1774, subsequently and for a long time, for *Helix lucorum* Linnaeus, 1758. Korábek et al. (2014) definitively confirmed the species status of *H. straminea* on molecular phylogenetic and conchological basis and identified the Balkan *H. vladika* Kobelt, 1898 as its closest relative. The species is present on Italian and Balkan peninsulas; however, its distribution has not been fully clarified by the authors.

The aim of this paper is to provide an up-to-date overview of the species, its taxonomy, distribution and biology based on a critical review of literature and museological data, and on a conchological, anatomical and molecular phylogenetic analysis of new specimens. This paper also describes the genital system, the radula and the karyotype of *H. straminea* for the first time. Finally, this paper also aims to document the current state of *H. straminea* populations and the threats they are facing.

Original description

Briganti (1825) described this *Helix* species, which he found to occur uncommonly in the territory of

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Figure 1. Vincenzo Briganti. Vincenzo [or Vincenzio] Briganti (1766–1836). Unedited photo by Iconoteca dei Botanici, Library of Orto Botanico, Università degli Studi di Padova. Arrangement: IB.NN.26.

the Kingdom of the Two Sicilies (1815–1860), by distinguishing it from *Helix pomatia* Linnaeus, 1758 and calling it "*straminea*" [feminine of the Latin *stramineus*: straw, straw-colored] for the background color of its shell. The type locality was indicated as "*in Principatu Citeriori, Aprutio, aliisque nostri Regni locis*" (from Latin: "in the Principato Citeriore [which today corresponds to the Province of Salerno, in the Campania Region, and to some municipalities in the Province of Potenza in the Basilicata Region], in Abruzzo, and in other places of our Kingdom"). Briganti (1825) further reported that he found this species for the first time in the dense woods of the village of Muro in the Principato Citeriore [today Muro is located in the Province of Potenza, since by 1863 the town is named Muro Lucano] and in the neighboring villages where it was called by the natives "marrucone". He noted that it was used and sold as food, even underlining that the taste of its meat had been highly appreciated by local inhabitants and that it had also been raised in nurseries.

The original diagnosis only concerns the shell: "*Testa solida, haud umbilicata, subglobosa, oblique striata, fasciis transversis rubro-fuscis, apertura cordata*. Table II, Figures 1, 2" (Figure 2) (Latin: "robust shell, without umbilicus, more or less globose, obliquely striated, with brown-reddish transversal bands, rope-shaped aperture).

In the description, he emphasized the absence of the umbilicus again, the color (stramineous or white opaque, ivory at the apex), four transversal reddish-brown bands, the two central wider, the upper-most thinner than the others. The aperture lip was thin and the margins dark red from inside. The color of the live animal was grey-cinereous, with a network of almost black wrinkles. In his observations he stated that this is the largest snail species of the Kingdom, and that it reached the width of about two [Neapolitan] inches (54–55 mm), the length of one inch and five lines (38 mm) and the weight of more than ¼ [Neapolitan] pound (about 80 g). He

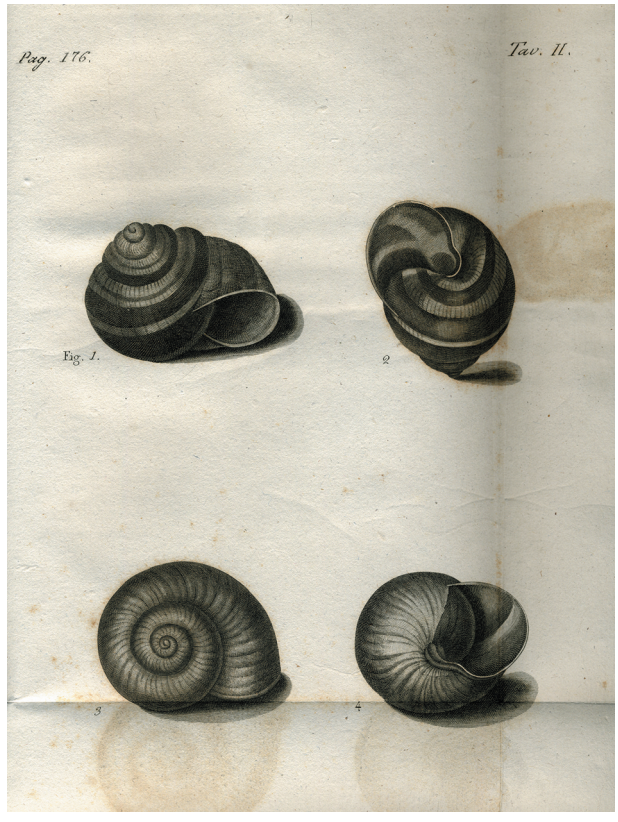

Figure 2. Original plate of Briganti. The original Table II by Briganti (1825) showing the shell of two examples of "Elice straminea" (Figures 1–2) and of the variety γ "shell with almost nil stramineous coloured bands (Figures 3–4) (Photo by G. Di Dato).

confirmed the difference with *H. pomatia* due to the different shape, size and colour. He stated that *H. straminea* resembles figure B in Table I by Gualtieri (1742: *Cochlea terrestris vulgaris, rufescens, fasciata*) (Figure 3(a)) except for the thickness of the margin of the aperture. Ironically, it is fig. C of the same plate (Figure 3(b)) that may be more similar to *H. straminea* (no major breaks in banding, thin aperture margins, large apex, distinct bands) while fig. B resembles more *H. lucorum*, but none of the depicted shells is extant in the Gaultieri's collection in the Museo di Storia Naturale dell'Università di Pisa (M. Dellacasa pers. comm.). Finally, Briganti (1825) mentioned the winter behavior of *H. straminea*, when the animal dug in the ground and formed a "solid, plaster white, externally convex" lid, that the animal shed once the hibernation phase is completed.

In addition, to the typical form, he mentions five other varieties or forms: var. α "with convex shell whorls of the shell"; var. β "with oval shell whorls of the shell"; var. γ "shell almost without stramineous bands, "plate II, fig. 3, 4" (Figure 2); var. δ "shell with 2, 3 stramineous bands"; var. ε "off-white shell, with attenuated red belly and oblique brown rows (striations) intersecting with uniformly colored transversal strips".

Briganti donated several individuals of *H. straminea* to the Anatomical Museum of Antonio Nanula for preparations of comparative anatomy (Maio et al. 1995; Maio 2009). In fact, in the "Elenco di oggetti di Anatomia umana e comparativa" of Nanula (1834) there are listed two preparations in alcohol (n. 336: "*Helix straminea*, Briganti, breathing organs with pulmonary vein injected with mercury", and n. 337: "other snail showing the major ganglion (brain) with nerves"). These specimens then passed to the Museum of Comparative Anatomy of the University founded by Paolo Panceri (1868) where they were registered under nos. 973 and 1127; then the trace is lost because of war events and the various vicissitudes linked to it (Maio et al. 1995; Maio 2009).

Table I. Specimens of *H. straminea* of the J. R. Bourguignat Collection of the MHNG.

Inventory number	Original labels	Early type status according to Korábek et al. (2014)	New type status as revised here
MHNG-MOLL-118064	(Figure 15).	Syntype *straminiformis*	Lectotype
MHNG-MOLL-118129	"*Helix lucorum*" containing the paper label "*H. straminea*? Brig., Muro, dans la Basilicate" inside. (Figure 8).	Not type	
MHNG-MOLL-118135	"*Helix straminea* Brig. Type. Roccamuria, Abruzzes". (Figure 6).	Syntype *straminea*	Lectotype
MHNG-MOLL-118136	"*Helix straminea* Brgt. Roccamuria, Abruzzes". (Figure 7).	Syntype *straminea*	Paralectotype
MHNG-MOLL-118139	"*Hel. yleobia* Bourg. Tricarico" [Basilicata]. (Figure 15(a–e)).	Syntype *yleobia*	Lectotype
MHNG-MOLL-118145-1	Outside label: "*Helix grisea* Linn, Italie". (Figure 17(a)). Label inside the mouth: "*grisea* Roccamuria (Abruzzes)".	Syntype *straminea*?	Not type
MHNG-MOLL-118145-2(4)	Label inside the mouth: "*grisea* Roccamuria *elongata* 4". Fig. 4, Table 20 of Bourguignat (1860a, 1860b). (Figure 17(d, e)).	Syntype *elongata*	Lectotype
MHNG-MOLL-118145-3	Label inside the mouth: "*grisea* Roccamuria (Abruzzes)"	Syntype of *straminea*?	Not type
MHNG-MOLL-118145-4	"*grisea*, Monte Gargano" [near San Marco la Catola? see Tiberi 1869]	Not type	
MHNG-MOLL-118145-5	"*grisea*, Monte Gargano" "4"	Not type	
MHNG-MOLL-118145-6	"*grisea*, Foligno" [Umbria]	Not type	

Historical overview

Oronzio [Oronzo] Gabriele Costa [1830] 1829 initially indicated *H. straminea* as *Helix lucorum* L. (= *H. mutata* Lamarck, or Hélice changée) very similar to *Helix pomatia* L.G. (Hélice vigneronne Lam.), and reported it living in South Italy "in wooded places of the high mountains". In his "Fauna del Regno di Napoli" Costa [1832]1839 considered the species barely different from *Helix cincta* and *Helix*

Table II. Comparison of anatomical and morphological data (in mm) in *H. mileti* (4 specimens: Posta, Rieti, Latium, 715 m a.s.l.; Gallo Matese, Caserta, Campania, 830 m a.s.l.; Mount Miletto, San Massimo, Campobasso, 1600 m a.s.l. and Oratino, Campobasso, 360 m a.s.l., Molise), *H. straminea* (4 specimens: Laviano, 600 m a.s.l.; Pietracamela, Teramo, 1050 m a.s.l. and Padula, Cortino, Teramo, 950 m a.s.l., Abruzzo; Oratino, Campobasso, Molise, 360 m a.s.l.) and *H. lucorum* (12 specimens, 3 per station: Govone, Cuneo, 237 m a.s.l.; Cà Conti, Granze, Padova, 6 m a.s.l.; Mandriole, Ravenna, 4 m a.s.l.; Jesolo, Venezia, 1 m a.s.l.).

Characters	*Helix mileti*	Mean	*H. straminea*	Mean	*H. lucorum*	Mean
Vas deferens	Range: 11.5–26.0	20.4	Range: 31.0–38.7	33.8	Range: 18.8–34.0	17.5
Epiphallus	Range: 4.5–7.9	5.6	Range: 4.7–5.5	5.0	Range: 6.0–10.0	8.1
Flagellum	Range: 11.0–29.5	24.0	Range: 45.0–66.0	57.0	Range: 63.0–83.6	71.8
Epiphallus/Vas deferens ratio	Range: 0.15–0.69	0.33	Range: 0.13–0.16	0.15	Range: 0.27–0.53	0.33
Epiphallus/Flagellum ratio	Range: 0.15–0.72	0.30	Range: 0.08–0.11	0.09	Range: 0.09–0.14	0.11
Diverticulum of the duct of the bursa copulatrix	Absent		Absent		Present	
Mantle border; Left side lobe	Elongated sickle-shaped		Elongated, globose		Elongated, more expanded on the backside	
Mantle border; Right side lobe	Trapezoidal, elongated posterior basal part		Trapezoidal, oval		Triangular	
Basal section of the digital glands	Short1, 1.8–4.0	2.50	Long, 5.0–7.0 mm	6.0	3.7–10.5	6.7
Dart	Short		Long		Long	
Protoconch development (Figure 13)	Narrower		Broader		Narrower	
Columellar edge	Whitish		Chestnut-brown		Dark-brown	
Labial profile last whorl	Simple		Slightly everted		Slightly everted	

ligata O.F. Müller, 1774 except for its bigger size (cf. Tiberi 1869).

Bourguignat (1860a, 1860b) described *H. straminea* Briganti in his "*Aménités malacologiques*", partially differing from the original description and amending it with new observations such as on shell size. The work first appeared in the "*Revue et Magasin de Zoologie pure et appliquée*", and later with different pagination and plate numbering as a separate print; we refer with the plate number to the latter edition throughout. Interestingly, the author asserted that a large specimen (62 mm × 68 mm) was present in the collection of Oronzio Costa. Bourguignat depicted two shells: one labeled as a "type" (Bourguignat 1860b, plate 20 figure 3; intended as "typical form" and not as "type" in the modern taxonomic sense, at the times of Bourguignat, this technical modus operandi did not exist) and the other named as var. *elongata* (plate 20, figure 4) (Figure 4). He reported the species, both the typical form and the var. *elongata*, from Abruzzo in the text, but in figure caption to figure 4 of plate 20 he indicated as origin in Calabria, a region where, to date, this species has never been reported (Adami 1873; Paulucci 1879; Cesari 1978; Korábek et al. 2014).

Tiberi (1869) confirmed the presence of *H. straminea* in Abruzzo, near "Monte Majella" (in the Comune of Gessopalena, Chieti Province), but claimed that the figure by Bourguignat (1860a, 1860b) does not depict a specimen from Abruzzo but one coming from "Monte Gargano in Capitanata [the old name of the Province of Foggia]" in the Apulia Region (near San Marco la Catola, Foggia Province), a specimen that Tiberi donated him during Bourguignat's visit to Portici (Naples), Tiberi's residence until 1879. Pfeiffer (1868: 234) just repeated the description of Bourguignat and the distribution in Abruzzo. Eight years later, Bourguignat (1876: 53) described a new species: *Helix straminiformis* Bourguignat, 1876, based on specimen from Monte Amaro (Chieti Province, Abruzzo Region), which was later considered a synonym of *H. straminea* (Westerlund 1889; Korábek et al. 2014).

Kobelt (1876: 18) listed *H. straminea* Briganti as a synonym of *H. lucorum* Linné, stating that this is a "mountain" form present in southern Italy, in Basilicata and on Monte Gargano (Apulia), where the specimens eaten in Naples come from. In figure 1027, he illustrated a shell from Bari (currently *H. lucorum*; SMF 9878) as *H. straminea* var. *elongata* of Bourguignat.

Paulucci (1878: 35) considered *H. straminea* just a larger and more wrinkled variety of *H. lucorum*. She stated that she possessed in her collection a specimen from Sammezzano (a castle in Regello, Florence

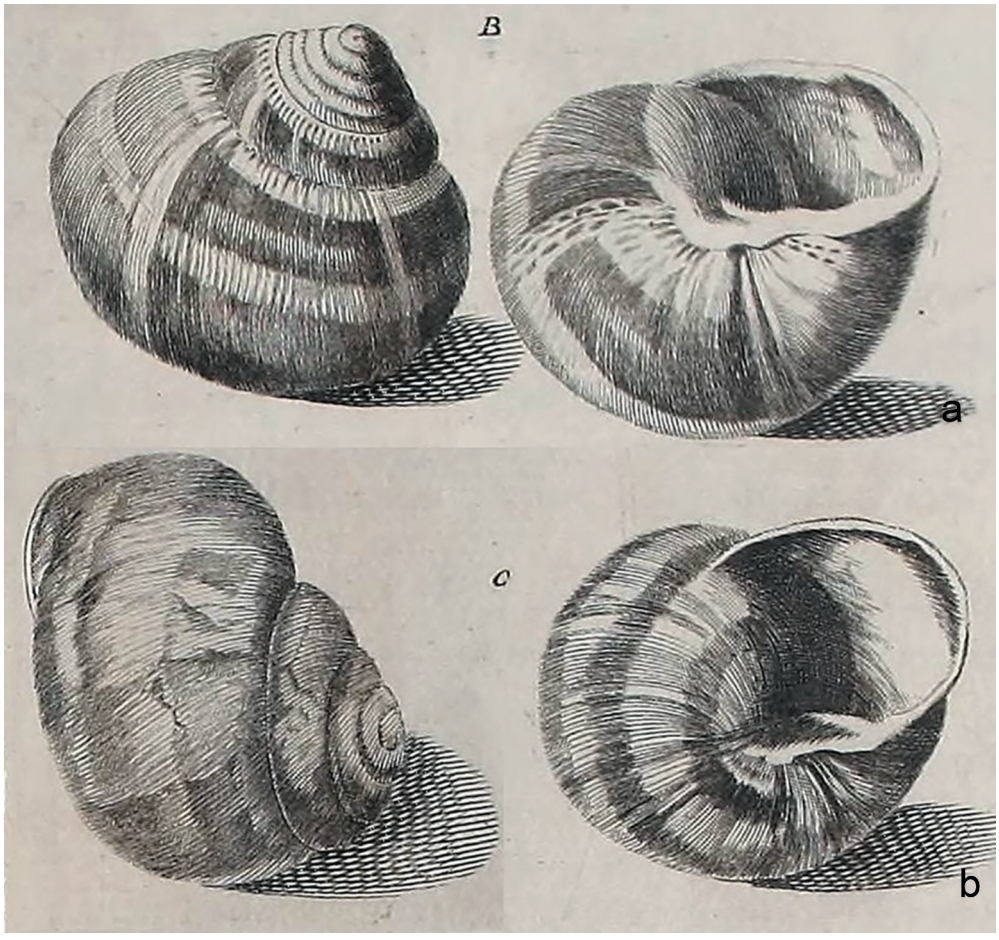

Figure 3. Original plate of Gualtieri. (a) *Cochlea terrestris vulgaris, rufescens, fasciata* (figure B, plate 1) of Gualtieri (1742) cited by Briganti (1825). (b) *Cochlea terrestris vulgaris, cinerea, aliquando pulla, fasciis quatuor fulvis distincta* (figure C, plate 1) of Gualtieri (1742)

Province) and cited Bourguignat's specimens from Murlo (sic!) and Tricarico (Basilicata), an evidence that Paulucci had been in correspondence with the French malacologist. In her subsequent work, Paulucci (1881) reiterated the synonymy with *H. lucorum* and said *H. straminea* was also present in San Marino and Avellana (perhaps Fonte Avellana in the Marche Region on the border with Umbria Region) as well as in Abruzzo (Caramanico, Pescara Province).

Bourguignat (1883) revised his description of 1860 and answered to Tiberi's controversy regarding the localities. In particular he stated that *H. straminea* was a gift by Briganti's son in 1858, labeled with a locality "Roccamuria" and that his figure 3 in "*Aménites*" represents the typical form of this species. The var. *elongata* (Figure 4) was based on shells donated by Tiberi and Costa (probably from Apulia). In the same work he described a new taxon, *Helix yleobia* Bourguignat, 1883, based on specimens of *H. straminea* from Tricarico (a Comune in the Province of Matera, Basilicata).

Westerlund (1889: 471) treated *H. straminea* as distinct from *H. lucorum* and referred to a large specimen from central Italy mentioned by Bourguignat (1860a, 1860b) as forma *maxima* (non *Helix aspersa maxima* Taylor, 1883). He incorrectly considered *Helix yleobia* described by Bourguignat in 1883 a variety of *H. lucorum*, but placed the Kobelt's "var. *elongata*" (Kobelt 1876: figure 1027) from Bari in *Helix lucorum* as a newly described var. *anaphora* Westerlund, 1889 (Figure 5). In the same book, Westerlund listed *Helix straminiformis* Bourguignat, 1876 from Amaro Mount, Majella Massif, Abruzzo as a variety of *H. straminea*. The taxon is represented by a single shell (MHNG-MOLL-118064; Figure 15), which lacks diagnostic features of *H. straminea* and has a partially open umbilicus. The placement was followed by Korábek et al. (2014) only due to the indicated geographic origin of the specimen.

Mascarini (1892) considered *H. straminea* a variety of *H. lucorum*, and described two new varieties coming from new localities of the Abruzzo and Marche Region,

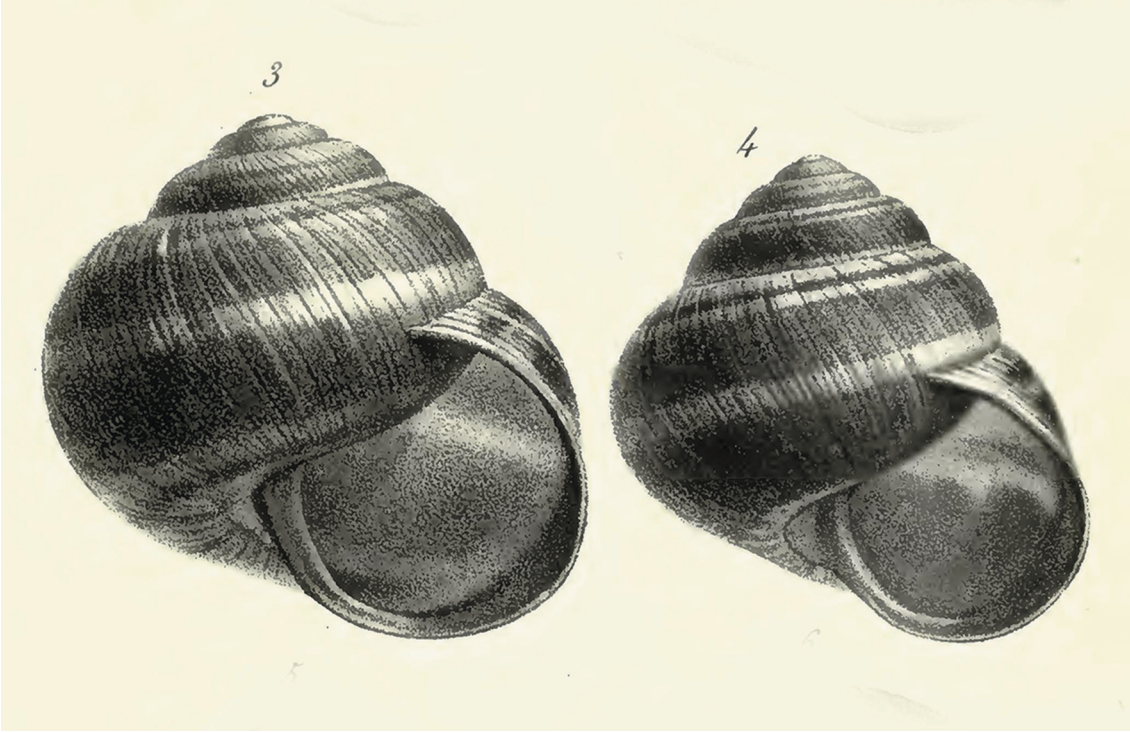

Figure 4. Original plate of Bourguignat. The Table 20, Figures 3–4 by Bourguignat (1860b)

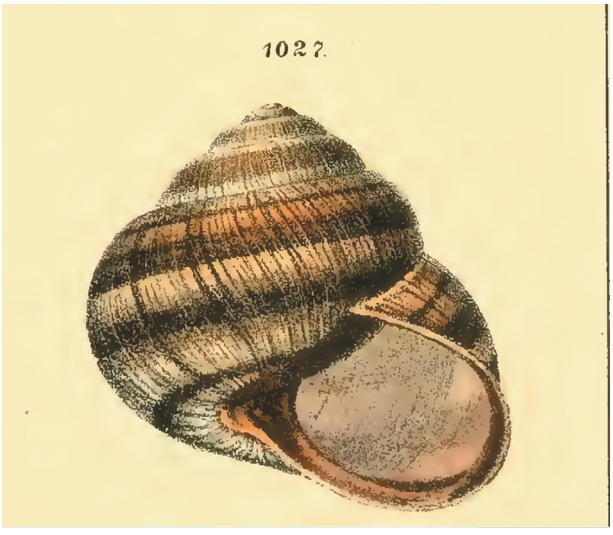

Figure 5. Original plate of Kobelt. Figure 1027, var. *anaphora* coming from Bari (Kobelt 1876).

namely *H. lucorum* var. *candida* Mascarini, 1892 (type locality: Settecerri, Teramo Province, Abruzzo) and *H. lucorum* var. *annosa* Mascarini, 1892 (type locality: Rocca Fluvione, a district of Agelli, Ascoli Piceno Province, Marche).

Kobelt (1903: figure 1913, 1906: page 210) considered *H. straminea* a valid species within the "Formenkreis" of *H. lucorum* and cited a specimen in the Neapolitan Museum (see MZUN) coming from the "wood of Castelnuovo in Capitanata, southwest of Sansevero" (now Castelnuovo della Daunia, Foggia Province, Apulia).

Giusti (1971) considered *Helix straminea* of Briganti, Bouguignat and Tiberi a synonym of *Helix lucorum*. Alzona (1971) and Cesari (1978) also considered *H. straminea* Briganti, 1825 and *yleobia* Bourguignat, 1883 synonyms of *H. lucorum*, along with *straminiformis* Bourguignat, 1883 and the var. *anaphora* Westerlund, 1889. Finally, Korábek et al. (2014, 2015) re-described *H. straminea* as a valid species, distinct from and unrelated to *H. lucorum*.

Materials and methods

Historical, field investigation and sampling

The data were collected following three different research methodologies: bibliographical, museum and field ones. At first, a bibliographic investigation was undertaken in order to draw a historical picture of the studies dealing with *H. straminea*. The libraries of the following Neapolitan institutions were consulted: Accademia delle Scienze Matematiche e Fisiche, Accademia Pontaniana, Biblioteca Nazionale Vittorio Emanuele III,

Biblioteca Universitaria, Orto Botanico Library of University of Padua.

Thereafter, the malacological collections of various museums (see below) and private collections (G. Martucci, Foggia; G. Fasulo, Napoli; Coll. N. Pirozzi, Napoli; coll. M. Cuomo, Napoli) were inspected. Further distribution data were obtained from unambiguously identifiable photographs published online. The Museum's and private collection acronyms are:

MHNG Muséum d'Histoire Naturelle, Genève;

NMBE Naturhistorisches Museum der Burgergemeinde, Bern;

MZUF Museo Zoologico "La Specola", sezione del Museo di Storia Naturale dell'Università di Firenze;

MZUN Museo Zoologico dell'Università di Napoli Federico II;

ZMB Museum für Naturkunde der Humboldt Universität, Berlin;

SMF Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main;

MANN Museo Archeologico Nazionale di Napoli.

In the Bourguignat collection in MHNG we examined two samples claimed by Bourguignat to be original material from Briganti (MHNG-MOLL -118135 and MHNG-MOLL-118136, Figures 6(a), 7) have been found by Korábek et al. (2014) in the Bourguignat collection of the Muséum d'Histoire Naturelle of Genève (MHNG) and identified as probable syntypes (see also Cailliez 1995).

MHNG-MOLL-118135 (Figure 6(a)), which measures 48.0 × 47.5 mm, bears a label that says: "*Helix straminea* Brig., type. Localité Roccamuria, Abruzzes" (Figure 6(b)) and is accompanied by a hand-written note by Bourguignat explaining the origin of the shell (Figure 6(e)).

Figure 7. Paralectotype of *Helix straminea* Briganti, 1825. Original sample of V. Briganti catalogued n. MHNG-MOLL -118136, collection of J.R. Bourguignat, Muséum d'Histoire Naturelle of Genève, here designated as paralectotype of *Helix straminea* V. Briganti 1825. (Photos by E. Tardy).

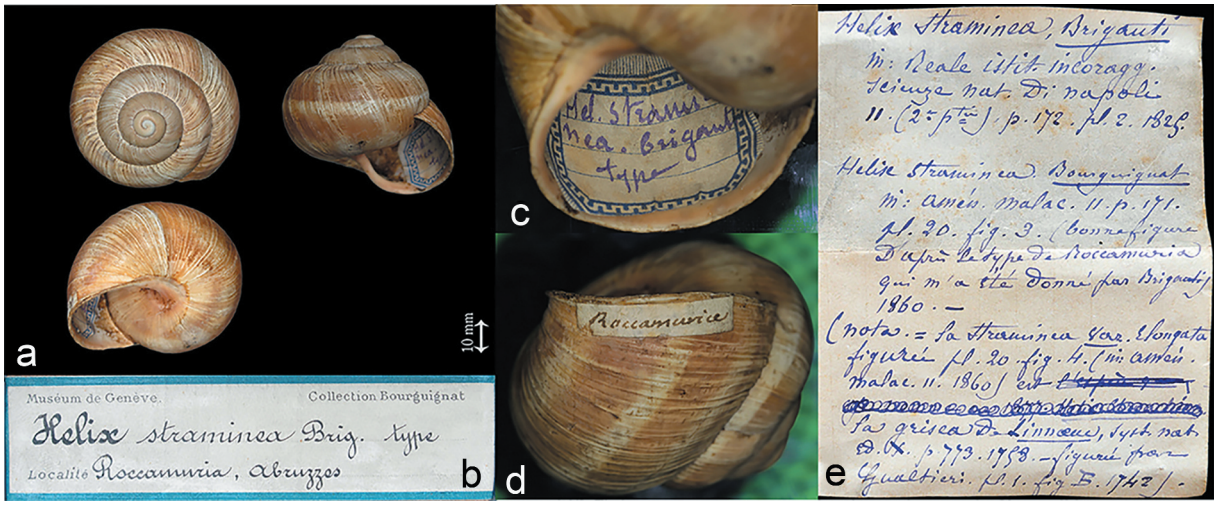

Figure 6. Lectotype of *Helix straminea* Briganti, 1825. (a) Original sample of V. Briganti catalogued n. MHNG-MOLL-118135, collection of J.R. Bourguignat, Muséum d'Histoire Naturelle of Genève, herein designated as lectotype of *Helix straminea* V. Briganti 1825. (b) Original anonymous label not autograph. (c,d) Details with other labels. (e) Associated autograph label of Bourguignat (Photos by E. Tardy).

MHNG-MOLL-118136 (Figure 7) (48.0 × 48.4 mm) has a label stating: "*Helix straminea* Brgt. [=Bourguignat]. Locality Roccamuria, Abruzzes". This specimen clearly matches the drawing of Table 20, Fig. 3 (Bourguignat 1860a) and could possibly match also with the shell depicted in figures 1 and 2 of Table II of Briganti (Korábek et al. 2014).

In the Bourguignat collection there are seven more specimens: six under no. MHNG-MOLL -118145 and the last under no. MHNG-MOLL -118129 (Table I). The first lot includes three specimens bearing a label: "*grisea* Roccamuria (Abruzzes)" (Figure 3). Inside one shell (Figure 17 (e)) is written: "[number] 4, *grisea elongata* Roccamuria"; the specimen corresponds to plate 20 figure 4 of Bourguignat (1860b) and has been considered syntype by Korábek et al. (2014) (Figures 8, 9(a,b)).

Two other specimens of the lot carry a label: "*grisea*, Monte Gargano" (probably referring to the locality of San Marco la Catola, Foggia Province, indicated by Tiberi (1869) as "near Monte Gargano". The sixth specimen has a label: "*grisea*, Foligno" (Perugia Province). The specimen MHNG-MOLL-118129

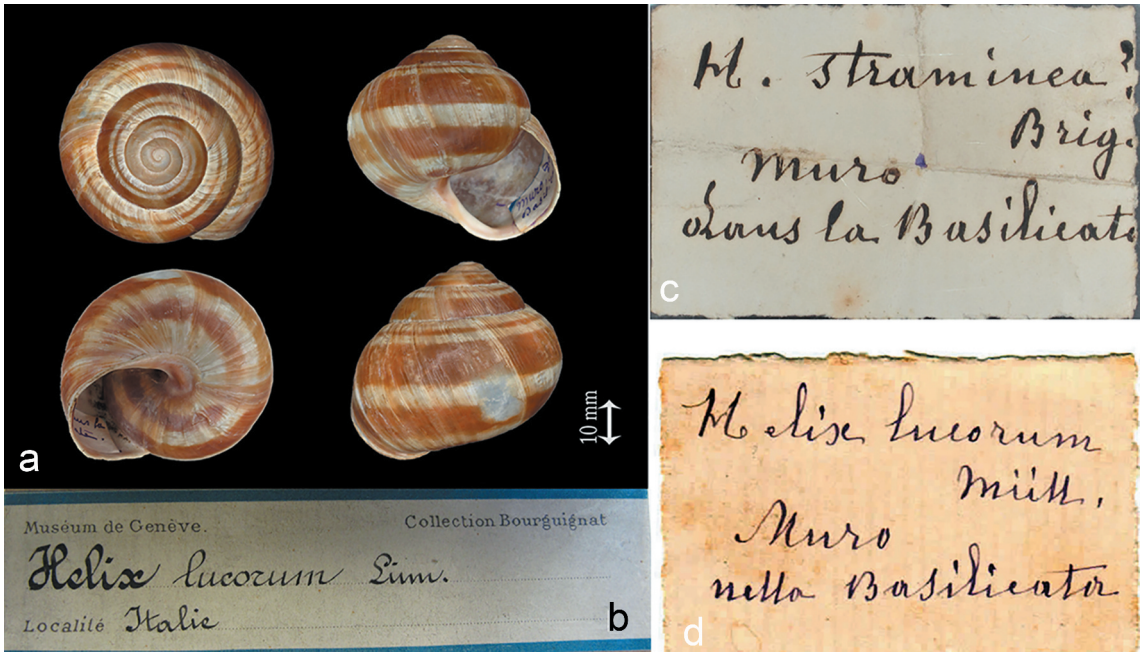

Figure 8. Specimen of Bourguignat collection. (a) Specimen no. MHNG-MOLL-118129 of Bourguignat collection. (b) Outside original Label. (c) Inside original label with a different calligraphy from that of Bourguignat (Photos by E. Tardy). (d) Original label of a specimen of *H. straminea* from Paulucci collection n. MZUF-1698, collected by I. Blanc in 1877 (Photos by S. Cianfanelli). The calligraphy of the two labels clearly corresponds.

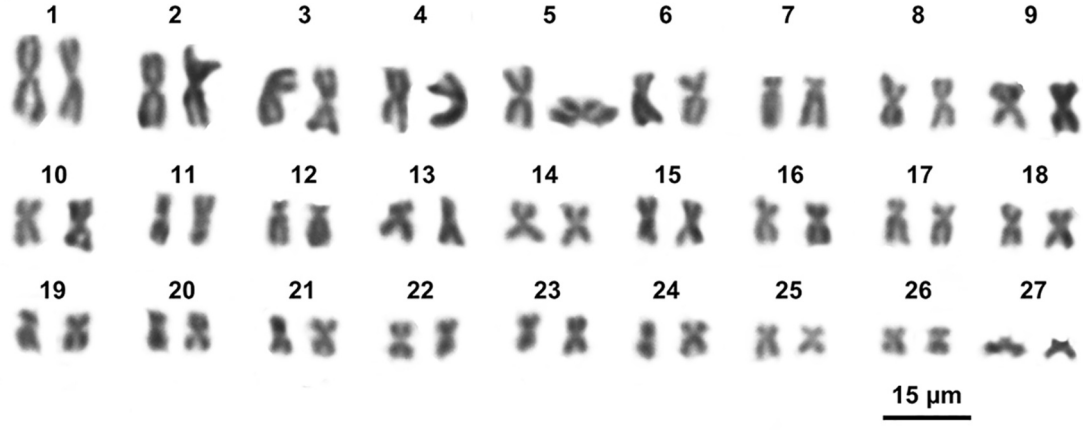

Figure 9. Giemsa stained karyotype of the sample from Ruvo del Monte (Potenza, Italy) (Photo by G. Odierna).

has an internal label: "*H. straminea*? Brig., Muro, dans la Basilicata" in different handwriting and an external card with the words: "*Helix lucorum* Linn. Localité Italie" (Figure 8(a–d)). The specimen MHNG-MOLL-118064 (Figure 15), has an outer label: "Mont Amaro dans les Abruzzes" and an inner label with: "*straminiformis*". The specimen MHNG-MOLL -118139, has an inner and outer label: "*Hel. yleobia* Bourg. [=Bourguignat] Tricarico" (Figure 15).

Several field trips were carried out in various localities of Campania, Basilicata and Apulia considered suitable in order to the presence of this species. In particular we surveyed the following localities: Capri, Ischia, Procida, Phlegraean Fields, Vesuvius and Monte Somma, Sorrento (Naples Province); Roccamonfina Regional Park, Matese Massif (Caserta Province); Matese Massif (Benvento Province); Partenio Regional Park, Terminio Mount (Avellino Province); Alburni Massif, Paestum archaeological Park, Pollica, Ascea, Romagnano, San Gregorio Magno, Ricigliano, Laviano (Salerno Province) in Campania Region; Muro Lucano, Balvano, Pescopagano, Ruvo del Monte (Potenza Province) in Basilicata Region; Monte Faeto, Gargano Regional Park (Foggia Province) in Apulia Region. An updated (see Neubert 2017) extent of occurrence (EOO; I.U.C.N 2012) was estimated in ESRI environment with the software: ESRI ArcGIS 9.2 and QGIS 2.18. The data were georeferenced with ESRI Satellite (free edition, 2018).

DNA extraction, PCR amplification and sequencing

All the previously sequenced individuals originated from the northern half of the Italian range of *H. straminea* (Manganelli et al. 2005; Korábek et al. 2014; Fiorentino et al. 2016). We sequenced here two more from the southern half (Laviano, 40° 46'46"N, 15°18'26"E; Ruvo del Monte, 40°50'09"N, 15°31'37"E). DNA was extracted from a piece of foot according to Solokov (2000). The muscle was minced in Carnoy's fixative (methanol, acetic acid, 3:1), washed in PBS 1× and transferred overnight in lysis buffer (Tris HCl 50 mM, EDTA 10 mM, NaCl 100 mM) containing proteinase K (100 µg/100 µl) and 1% SDS. Thereafter, to the cell suspension was added 1/10 of volume of saturated KCl solution. After incubation on ice for 10 min. and centrifugation at 13000 rpm for 15 min., the supernatant was extracted with an equal volume of chlorophorm-isoamylic alcohol 24:1. After centrifugation at 7500 rpm for 15 min, DNA was precipitated with two volumes of 100% ethanol, washed with 70% ethanol, dried and rehydrated with Tris-EDTA buffer 1×.

A partial sequence of the mitochondrial 16S rRNA was amplified using the primer pair 16sar (CGCCTGTTTACCAAAAACAT) and 16sbr (CC GGTCTGAACTCAGATCACGT) (Palumbi et al. 1991) and a fragment of COI with the primer pair HCO2198M (TCWACAAATCATAAAGATATTG G) and LCO1490 (TAAACTTCAGGGTGACCAA AAAATCA) (Folmer et al. 1994; with slight modification to the forward). PCR settings were as follows: an initial denaturation at 94°C for 5 min, 36 cycles at 94°C for 30s, 50°C for 45s and 72°C for 45s followed by a final step at 72°C for 7 min for both genes. Amplicons were sequenced in both directions on an automated sequencer ABI 377 (Applied Biosystems, Foster City, CA, USA) using BigDye Terminator kit 3.1 (ABI). To verify the sequences we manually checked all the chromatograms using Chromas Lite 2.1.1 and Bioedit 7.0.5.3 programs (Hall 1999) and blasted them against the NCBI nucleotide database. All sequences were deposited in GenBank (Accession Numbers: MW364279 MW364280 MW376263 MW376264).

For the phylogenetic analysis the obtained sequences were compared with homologous sequences of both loci available in GenBank from other populations of *H. straminea* (Korábek et al. 2014; Fiorentino et al. 2016). Sequences of two individuals of *H. vladika* (Korábek et al. 2014) and two of *H. schlaeflii* (Korábek et al. 2014) were used as outgroup.

The best fit nucleotide substitution models were selected with Jmodeltest 2.1.3 (Darriba et al. 2012) under the Akaike information criterion (AIC) (HKY +G for both 16S and COI). Phylogenetic analysis with Bayesian inference (BI) was performed in MrBayes 3.2.1 (Ronquist et al. 2012) using default number of chains of 10.000.000 of generations each sampled every 1000 generations. First 25% of the sampled trees were discarded as burn-in.

Chromosome analysis

A specimen from Ruvo del Monte (Potenza, Italy) was injected with 0.1 ml/10 g body weight of colchicine solution (1 mg/ml). After 2 hours the intestine and gonads were dissected and immersed for 30 min in hypotonic solution (0.5% sodium citrate-0.56% KCl, 1:1). Thereafter, the organs were transferred in Carnoy's fixative and immediately scraped on a sieve 100 mesh/inch. The cellular suspensions were centrifuged for 10 min at 1000 rpm. Immediately after, the fixative was renewed, and the cellular suspensions were newly centrifuged twice; 25 µl of the

cellular suspension was dropped on a cleaned slide. The chromosomes were stained with a 5% Giemsa solution at pH 7 in order to identify the metaphase plates.

Thereafter, sequential C-banding + Giemsa + Chromomicine A_3 (CMA) + DAPI were performed on metaphase plates (Petraccioli et al. 2010, 2015; Mezzasalma et al. 2013, 2017). Karyotypes were chosen from five Giemsa and five sequential C-banding stained well-spreaded metaphase plates. Chromosome morphometric parameters (relative length, RL, and centromeric index, CI) have been measured according to Levan et al. (1964).

Anatomical studies

Preserved animals were dissected using tweezers, scalpels and scissors under LEICA EZ4 with integated digital camera. The genital organs of the specimens were removed from the body, the genital situs morphology and further morphological details were investigated. The drawings of the genitalia were sketched by means of a camera lucida mounted on a Leica stereomicroscope, with both incident and transmitted light. Radulae from two individuals were mounted on aluminum stubs, coated with a thin gold film using an Agar Auto Sputter Coater model 108A, and observed with a FEI (Hillsboro, OR, USA) Quanta 200 ESEM in high vacuum mode at 30 kV.

Results

Designation of the lectotypes

Briganti (1825) studied specimens from Campania, Basilicata and Abruzzo without specifying their number and did not mention or select a holotype, therefore the type series is formed by syntypes. Korábek et al. (2014) discovered and catalogued what may be part of the type series of *Helix straminea* in the collection of Jules René Bourguignat (1829–1892) in the MHNG, which they considered as "presumed syntypes". They had some doubt whether the Bourguignat's account of their origin was accurate given his personal profile, as he engaged in questionable practices including deceiving on multiple occasions (Connolly 1934; Audibert & Breure 2017; Holyoak & Holyoak 2017). Indeed there is no independent evidence that the specimens were part of the original type series, and Bourguignat's claim that they originated from Briganti was not publicly made in the original publication, but 23 years later. However, in the Bourguignat collection there is also a specimen labeled as "type" of *Helix setulosa* Briganti, 1825, the other species described in Briganti (1825), but without any claim about its origin from Briganti's collection. Moreover, Bourguignat (1860a, 1860b) interpreted the name in the sense of Briganti (1825) and was the first to provide good illustration, and his account was the basis for later interpretation of *H. straminea*. Therefore, assuming that the two specimens, as well as others from "Roccamuria", represent part of the type series and their nomenclature is safe. At the same time, we are not aware of any other extant possible syntypes. The individuals donate by Briganti to Nanula are lost (see above). Therefore, we accept that these specimens are the original type specimens.

We designate here one of the two "probable syntypes" of Korábek et al. (2014) as the lectotype so that there is a single name-bearing specimen for the nominal taxon *Helix straminea* Briganti, 1825. We selected the specimen no. MHNG-MOLL-118135 (Bourguignat 1860a, 1860b plate 20 figure 3) because only this specimen contains the writing: "type" (Figure 6(c)), and an outer label glued to the shell with a different handwriting: "Roccamorice" (Figure 6(d)). For this reason, we believe that it represents the "type de cette espéce" of Roccamuria (sic!) given to Bourguignat by Briganti son (Bourguignat 1883). The specimen no. MHNG-MOLL-118136 is designated as paralectotype (art. 74.1.3). All designations included a red label, on which was written "Lectotype" or "Paralectotype", the taxon name and "Petraccioli A., Niero I., Carandente F., Crovato P., De Vico G., Odierna G., Picariello O.L.A., Tardy E., Viglietti S., Maio N., *det.* 2021". All the specimens comply well with the original description and are well preserved.

Familia Helicidae Rafinesque, 1815
Genus *Helix* Linnaeus, 1758
Helix straminea Vincenzo Briganti 1825: pages 172–175. Plate II, figures 1-2

Type specimens. straminea, no. MHNG-MOLL -118135 (Figure 6(a–d), herein selected and designated as lectotype [hic!]. No. MHNG-MOLL -118136 (Figure 7), here designated as paralectotype.

Additional specimens. (see Table I). MHNG-MOLL -118129. The specimens MHNG-MOLL-118145-1 and MHNG-MOLL-118145-3, both labeled inside the mouth "*grisea* Roccamuria (Abruzzes)" were identified as doubtfull syntypes by Korábek et al. (2014) and shall not be taken into account for the typical series.

Diagnosis. Medium to large *Helix* species possessing a broadly conical shell with broad first whorls, no umbilicus, and rounded aperture that is small in relation to the shell size. The shell usually has four longitudinal bands and its aperture is usually brown colored (by Korábek et al. 2014).

Description. Shell, foot and mantle: see Korábek et al. (2014: 80); reproductive system and radula: see following paragraphs of current paper.

Locus typicus. The label of the lectotype selected here reports the place of origin as "Roccamuria, Abruzzes". In compliance with The Code (I.C.Z.N. 2012, art. 76.2: "the place of origin of the lectotype becomes the type locality of the nominal species-group taxon, despite any previously published statement of the type locality"), the type locality should be "Roccamuria". We propose that "Roccamuria" is a misspelling of Roccamorice (compare with Figure 6(d)), a comune in the Province of Pescara, Abruzzo (42.21°N, 14.03° E), where the species is still present today (Cianfanelli pers. comm).

Remarks. The lot no. 118135 is associated with a second autograph label of Bourguignat in which the work by Briganti (1825) was mentioned with the respective plate II (Figure 6(e)) and where Bourguignat affirmed that the figure 3 of the Table 4, respective plate 20 in Bourguignat (1860a, 1860b) (Figure 4) well represents the "type of Roccamuria given to him by Briganti [son]". This specimen was suggested by Korábek et al. (2014) to possibly correspond to the variety γ represented in Table II, figure 3, 4 in Briganti (1825). The figure 3 on plate 4 (or 20) of Bourguignat (1860a, 1860b) depicts the lot no. 118136 (Figure 18(d,e)).

The specimens in the MHNG are ascribable to Briganti (1825), but it is impossible to establish an indisputable correspondence between the specimens in question and the figures in that work, unlike those of Bourguignat (1860a, 1860b). For this reason, the Article 72.5.6, which considers the specimen illustrated or described with certainty by the author as the type bearing specimen, cannot be invoked.

The specimen MHNG-MOLL-118129 kept in the MHNG has an internal label "*H. straminea*? Brig., Muro, dans la Basilicata" but with different calligraphy from that of Bourguignat, probably ascribable to Ippolito Blanc of Nice, a friend of Tiberi. That is confirmed by comparison between the handwriting of the label of a specimen from Blanc in Paulucci collection (MZUF-1698), which corresponds to that of the label of MHNG-MOLL -118129 (see Table II, Figure 8(c,d)). For this reason, it cannot be considered part of the type series, as it originated from Tiberi, not from Briganti.

Karyotype and molecular phylogeny

The studied specimen of *H. straminea* shows a karyotype of 2 n = 54 chromosomes decreasing in length, all metacentric except for the pairs 7 and 12, which are submeta/subtelocentric, and those of last pair, which are telocentric (Figure 9). Tiny, chromomicine and DAPI positive heterochromatic C-bands are showed on the centromeric regions of almost all chromosomes (Figure 10).

We amplified 400 bp of 16S and 529/590 bp of COI from specimens from Laviano (Salerno, Campania) and Ruvo del Monte (Potenza, Basilicata). Combined with GenBank data, all *H. straminea* samples form a strongly supported clade consisting of two well supported lineages, Italian (6 sites) and Balkan (2 sites) (Figure 11). The Italian contains a clade uniting the samples from Ripe di Civitella del Tronto (Teramo), Laviano (Salerno), Micigliano (Rieti) and Montalbuccio (Siena) to the exclusion of those from Ruvo del Monte (Potenza) and Torrente Bozzone (Siena).

Anatomy

One of the first authors to describe the anatomy of genus *Helix* was Marco Aurelio Severino (Tarsia, 2 November 1580 - Naples, 12 July 1656), considered by many to be the "father of modern comparative anatomy". Severino (1645) roughly describes in his *Zootomia Democritea* a study made in 1620 on the anatomy of a species of Helicidae. According to Delle Chiaie (1825 [1826]) the species studied by Severino corresponded to *Helix pomatia* a species not present in the Kingdom of Naples and probably confused with *H. straminea*, at the time, however, very common in the territory. Severino's work was criticized by Redi (1684) who made the first accurate description of several specimens of the genus *Helix* collected on Monte Morello (Florence Province). "Some specimens very large" cited in Redi (1684) probably were *Helix straminea* and not *H. lucorum*. Indeed, genitalia depicted in "Tavola Decima Terza, Fig. 3" (see Benocci & Manganelli 2012) matched those of this species, especially the absence of the diverticulum of the bursa copulatrix, even if there's a reported inconsistency: the vas deferens is detached from the oviduct and does not specify the flagellum and the penial retractor

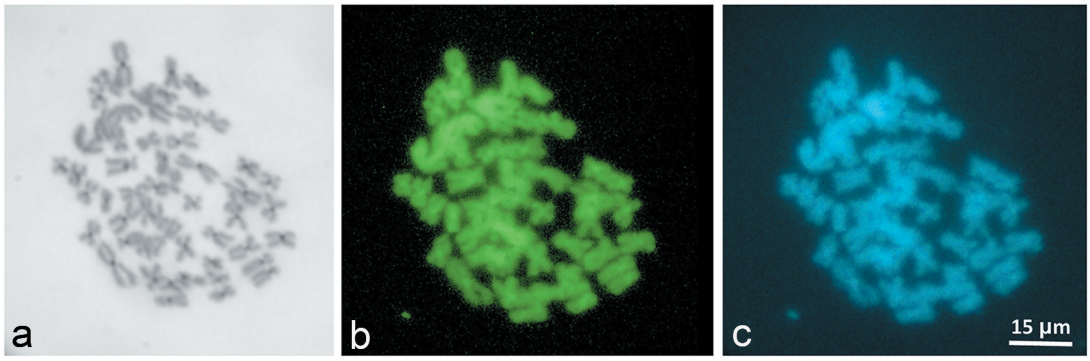

Figure 10. Metaphase plate of *H. straminea* from Ruvo del Monte sequentially stained with C-banding+Giemsa (a), CMA (b) and Dapi (c) (Photo by G. Odierna).

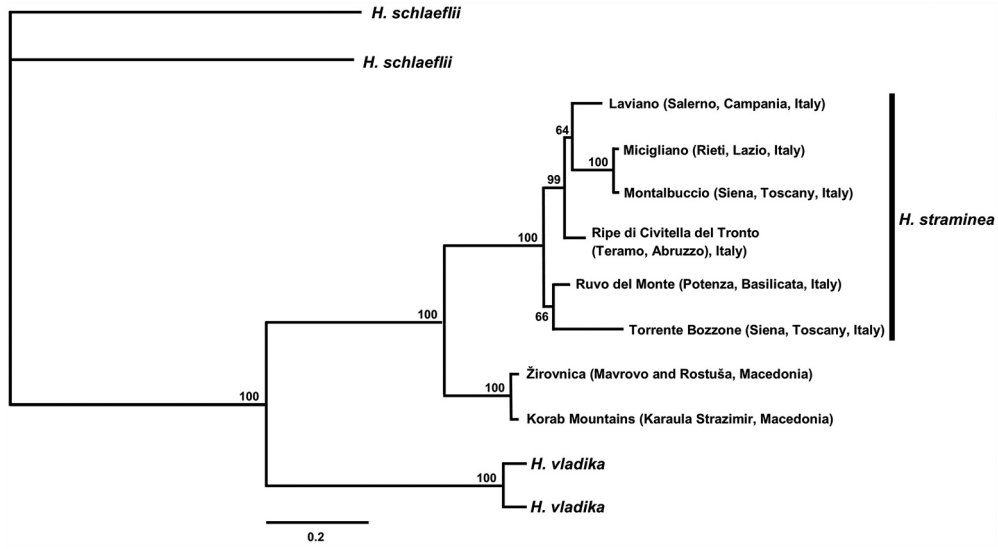

Figure 11. MRBAYES consensus 16S+COI tree of *Helix straminea*. Numbers at nodes are posterior probabilities.

muscle. Hesse (1920; tav. 657 pag. 197) probably for the first time described the anatomy of *Helix lucorum* on specimens from Northern Italy. After Redi the genitalia of *H. straminea* have not been redescribed, but as it generally holds within *Helix* (Neubert 2014), they do not offer unique traits for reliable identification of the species (Giusti & Lepri 1981).

The reproductive system of *H. straminea* is similar to that of the other species of the subgenus *Helix* Linnaeus, 1758 (Hesse 1920, 1931; Giusti 1971; Cesari 1978; Neubert 2014): short penis with very short epiphallus; a dart sac and digital glands are present; a diverticulum of the duct of the bursa copulatrix is absent in *H. straminea* and *H. mileti*, but present in *H. lucorum*. (Figure 12(a–f)). Details of the morphology are presented in Table II.

Radula is not elongated, but wider then long (up to 4.7 mm wide and 3.5 mm long). Dentition is formed by a large number of small teeth (up to over 7000), arranged in about 55 transverse straight rows, perpendicular to mid-line (the central column) (Figure 14(a–p)). There are up to 130–140 teeth per row, with only one "central" tooth; all the others are "laterals" and "marginals". The central (rachidian) tooth is tricuspid, with a primary median larger triangular denticle (mesocone) and lateral smaller cusps (ectocones) very weak at about half of height on either side, somewhat smaller

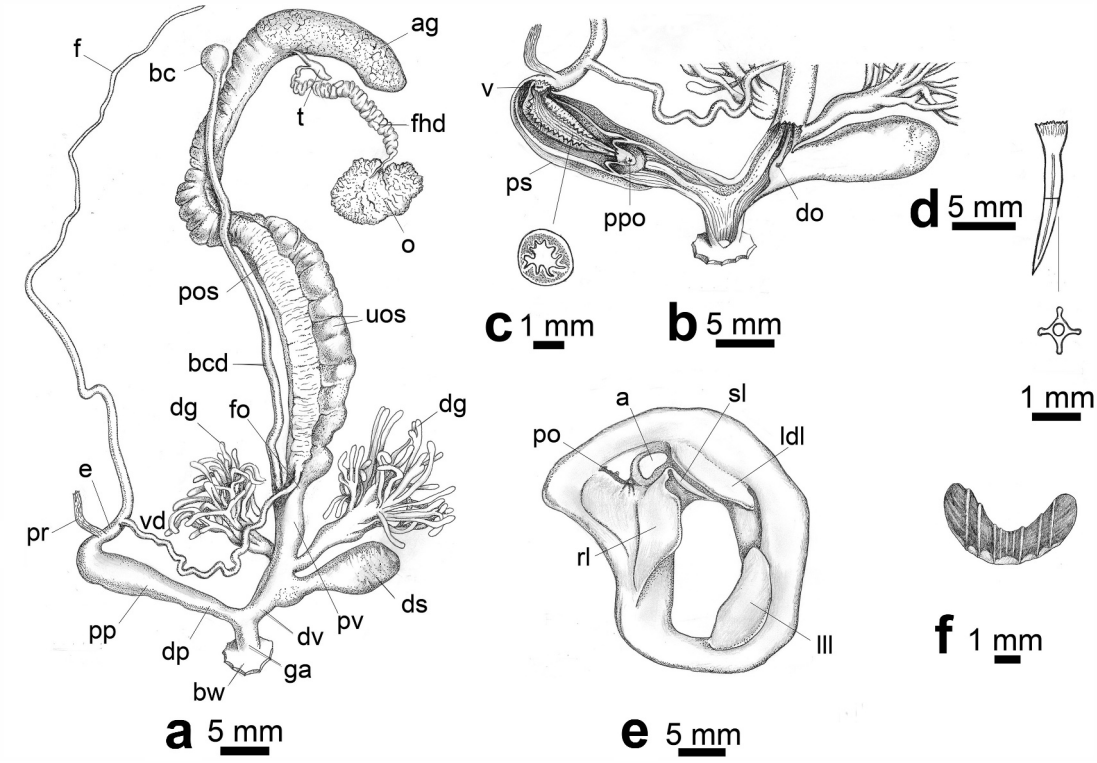

Figure 12. Reproductive system of *Helix straminea* from Laviano (Salerno). (a) Complete genitalia. (b) Distal genitalia and internal structure of penis and vagina. (c) Proximal penis section. (d) Dart and dart section. (e) Mantle edge and body lobes. (f) Jaw. a = anus, ag = albumen gland, bc = bursa copulatrix, bcd = duct of the bursa copulatrix, bw = body wall, dg = digital gland, do = dart opening, dp = distal penis, ds = dart sac, dv = distal vagina, e = epiphallus, f = flagellum, fhd = first hermaphrodite duct, fo = free oviduct, ga = genital atrium, ldl = left dorsal lobe, lll = left lateral lobe, o = ovotestis, po = pneumostomal opening, pp = proximal penis, ppo = proximal penis opening, pos = prostatic ovispermiduct, pr = penis retractor muscle, ps = penial sheat, pv = proximal vagina, rl = right lobe, sl = subpneumostomal lobe, t = talon, uos = uterine ovispermiduct, v = verge, vd = vas deferens. (Draft by I. Niero).

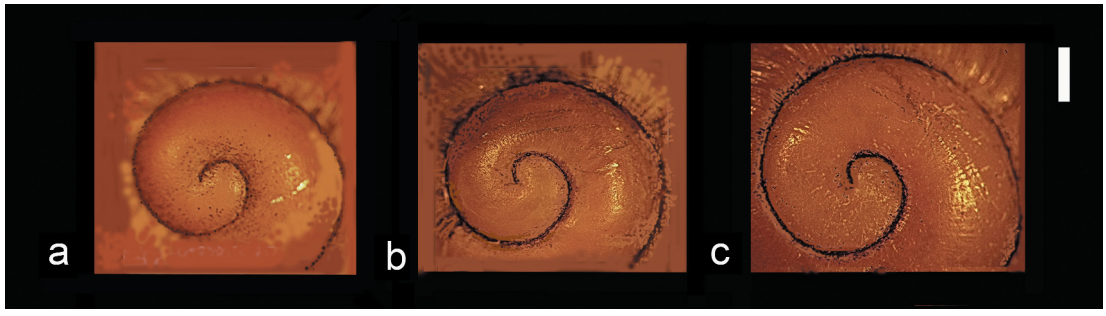

Figure 13. Protoconch development. (a) *Helix lucorum*. (b) *Helix mileti*. (c) *Helix straminea*. (Scale bar on the right 1 mm).

than those of the innermost lateral teeth. The basal plate is present and quite trapezoidal (Figure 14(a–d)). +Lateral teeth are larger than central ones, bicuspid, with inner cusp (endocone) well developed, subtriangular, while the outer cusps (ectocones) are smaller and weak. The basal portion of these teeth is subrectangular and wide (Figure 14(e–h)). The first 30–35 teeth on each side are "laterals", the remaining are "marginals" (30–35), but it is rather difficult to decide which teeth are the last laterals and the first marginals, because the transition from laterals to marginals is gradual (Figure 14(i–j)). The outermost lateral teeth are longer and sharper than the central ones: the shape of primary cusp is somewhat variable with a slight groove and frequently is not symmetrical along its mid-line (Figure 14(h)). Anterior radula teeth are clearly worn (Figure 14(l–n)). Approximate radular

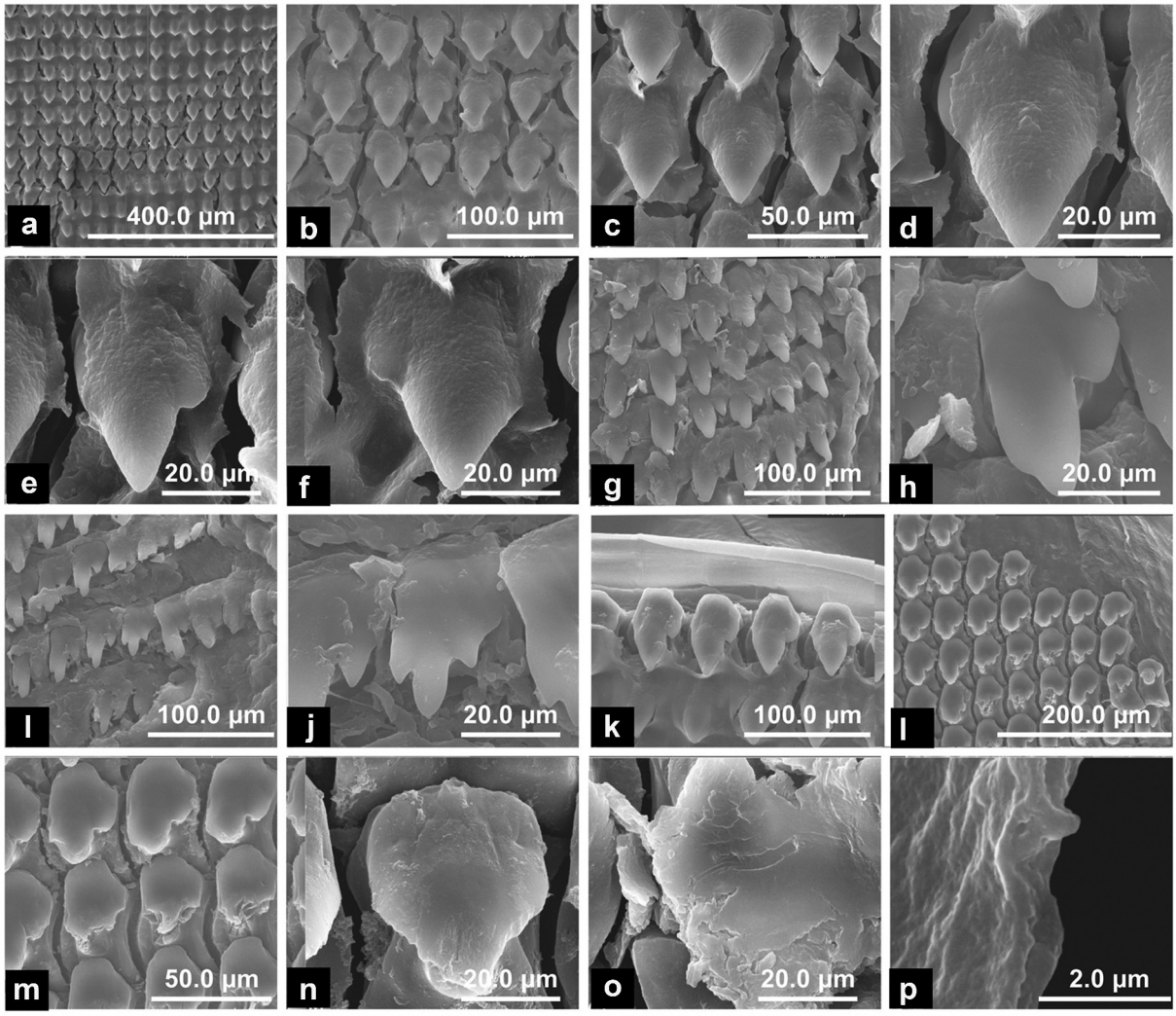

Figure 14. SEM images of radula of *H. straminea*. (a) Anterior-posterior (from top to bottom) middle section of the radula. (b) Detail. (C) Central and first two lateral teeth. (d) Central tooth. (e) First right lateral tooth. (f) First left lateral tooth. (g) Outermost lateral teeth. (h) Detail of the outermost lateral tooth. (i) Marginal teeth. (j) Detail of a marginal tooth. (k) Lateral teeth seen from the outer edge. (l) Lateral teeth of the anterior part of the radula clearly worn. (m) Detail. (n) An anterior lateral teeth clearly worn. (o) A thin film of dehydrated mucous that covers a tooth. (p) Detail of the film. (Photos by S. Sorbo).

formula: R1 + L(30–35)+M (±35). A thin film of mucous covers all the radula in between the teeth, probably for protective function (Figure 14(k,o,p)). In the anterior part of the radula the film is very deteriorated, probably due to wear.

Synonymized taxa

Helix straminiformis Bourguignat, 1876: pag. 53

Bourguignat (1883) described a new species discriminating against it by *H. straminea* and did not state the number of sampled specimens examined for his description of species and the original description neither implies that there were syntypes. A single specimen is kept in his collection of MHNG which we designate here as lectotype. The following lectotype designation is necessary to fix the status of junior synonym of this taxon name clearly.

Type specimen. straminiformis, MHNG-MOLL -118064 (Figure 15), herein designated as lectotype [hic!].

Locus typicus. "Mont Amaro dans les Abruzzes" [Majella Massif, Chieti Province, Abruzzo, Italy].

Remarks. The specimen was distinguished as syntype by Korábek et al. (2014) and the taxon was considered synonym of *H. straminea* following the geographic origin of the specimen and Westerlund

Figure 15. Lectotype of *Helix straminiformis* Bourguignat, 1876. Samples no. MHNG-MOLL-118064 from "Mont Amaro, Abruzzes" of Bourguignat collection, here designated as lectotype of *Helix straminiformis* Bourguignat, 1876. (Photo by E. Neubert & E. Bochud).

(1889); the shell lacks diagnostic features of *H. straminea* and has a partially open umbilicus.

Helix yleobia Bourguignat, 1883: pag. 265

When describing this taxon as a new species distinct from *H. straminea*, Bourguignat (1883) did not state the number of specimens on which he based his description, neither that there were syntypes. The specimen preserved in his collection in MHNG is designated here as the lectotype of *H. yleobia*. This designation is necessary to clearly fix the taxonomic status of this nominal taxon.

Type specimen. yleobia, MHNG-MOLL-118139 (Figure 16), herein designated as lectotype [hic!].

Locus typicus. Tricarico, Matera Province, Basilicata, Italy.

Remarks. The specimen was indicated as syntype of *H. yleobia* by Korábek et al. (2014). The original label is written with a calligraphy ascribable to Ippolito Blanc of Nice (Figure 16(b)).

Helix straminea ssp. *elongata* Bourguignat, 1860: pag. 172, plate 4, figure 4 (non *Helix elongata* Röding, 1789 = *Drymaeus elongatus*)

The name *Helix straminea* var. *elongata* was published by Bourguignat (1860a, 1860b) without a description except for mentioning the conicity, but with an illustration (plate 20 figure 4). The illustrated specimen is extant in the Bourguignat coll. of MHNG (Figure 17(a,b)).

Type specimen. MHNG-MOLL-118145-2 (4) shell labeled inside the mouth "*grisea* Roccamuria" with '4' written in the aperture, probably with reference to fig. 4 of the plate (Figure 17(e)). A large label for the whole lot MHNG-MOLL-118145 lists *Helix grisea* Linnaeus, 1758 and *H. straminea* var *elongata* as names, and was written by the same person who wrote "*grisea*" inside the mouth (Figure 17(c)). Same ink, same calligraphy: from Bourguignat himself who probably revised his view on his subspecies and re-identified it as "*grisea*". The specimen was designated as syntype by Korábek et al. (2014). Herein it is designated as lectotype [hic!] of *Helix straminea* ssp. *elongata* Bourguignat, 1860.

Figure 16. Lectotype of *Helix yleobia* Bourguignat, 1883. (a) Specimen no. MHNG-MOLL-118139 from Tricarico of Bourguignat collection, here designated as lectotype of *Helix yleobia* Bourguignat, 1883. (b) Original label with a calligraphy ascribable to Ippolito Blanc of Nice. (c) Inside label. (d,e) Other labels associated with the specimen. (Photo by E. Neubert & E. Bochud).

Additional specimens. The specimens MHNG-MOLL -118145-4 and MHNG-MOLL-118145-5 both labeled "*grisea*, Monte Gargano" and MHNG-MOLL -118145-6, labeled inside the mouth: "*grisea*, Foligno" [Umbria]" are regarded here as additional specimens.

Locus typicus. The specimen of the lot MHNG-MOLL -118145 indicated as *elongata* comes from locality: "Roccamuria" [Abruzzo]. Doubts remain, however, about the true type locality: at first Bourguignat (1860a) stated in the caption of the figure (pag. 201) that the var. *elongata* comes from "Calabre" while mentioning Abruzzo in the text, then (1883) he stated that it was described based on shells donated by Tiberi and Costa (thus from Apulia) as already stated by Tiberi (1869; he mentions San Marco la Catola) and Kobelt (1876). Two other specimens of the lot bear a label with the wording: "Monte Gargano". Perhaps the correct type locality is San Marco la Catola, Foggia Province, indicated by Tiberi (1869) as "near Monte Gargano".

Remarks. The name *elongata* takes on a subspecific rank because it was published before 1961 and its author expressly used the term "variété" without indication for an infrasubspecific entity (art. 45.6.4). So the name can be made available.

Habitat, new faunistic data and distribution overview

The altitudinal distribution in Italy ranges from 140 m a.s.l. (Mazzocco Torrent, loc. Agenzia, between Pietramaura and Villanova, San Leo, Rimini Province, Emilia Romagna, MZUF, 19/04/ 1998, Cianfanelli S., Calcagno M. & Ficarra A. *leg*.) to 1190 m a.s.l. (Civitella del Tronto Ripe, near Comune of S. Giacomo a Ripe, Teramo Province, Abruzzo, MZUF, 04/06/1995, Cianfanelli, Calcagno, *leg*.). However, *H. straminea* is more common between 500 and 800 m a.s.l. This species seems to prefer highlands and mountainous areas where it inhabits woodlands and shrubs, with beech and/or chestnut as dominant species, in mature broadleaved forests, deciduous woods and in their margins, under shrubs, but can also be found on meadows and in orchards (Korábek et al. 2014).

The oldest known record of *H. straminea* from the Campania Region perhaps was preceding the work by Briganti. Férussac (1821) reported "species no. 31 *Helix pomatia* Linné (= var. β *Helix scalaris* Müller, fig. 9) Habit.; Naples. Édule", which was considered by von Martens (1870) as probable *H. straminea* of Bourguiguat.

The first record certainly referring to *H. straminea* is provided by two specimens from the Robert James Shuttleworth collection, preserved in the NMBE (NMBE 512977/4 and NMBE 512978/6, Figure 19(a,b)) collected by C. Sury (the father-in-law of Shuttleworth, Earl of Sury, in Soulere or Canton of Solothurn, Switzerland) between 1835 and 1850 (E. Neubert pers. comm.) respectively from Capri and Naples, initially cataloged as *Helix lucorum* Müll.

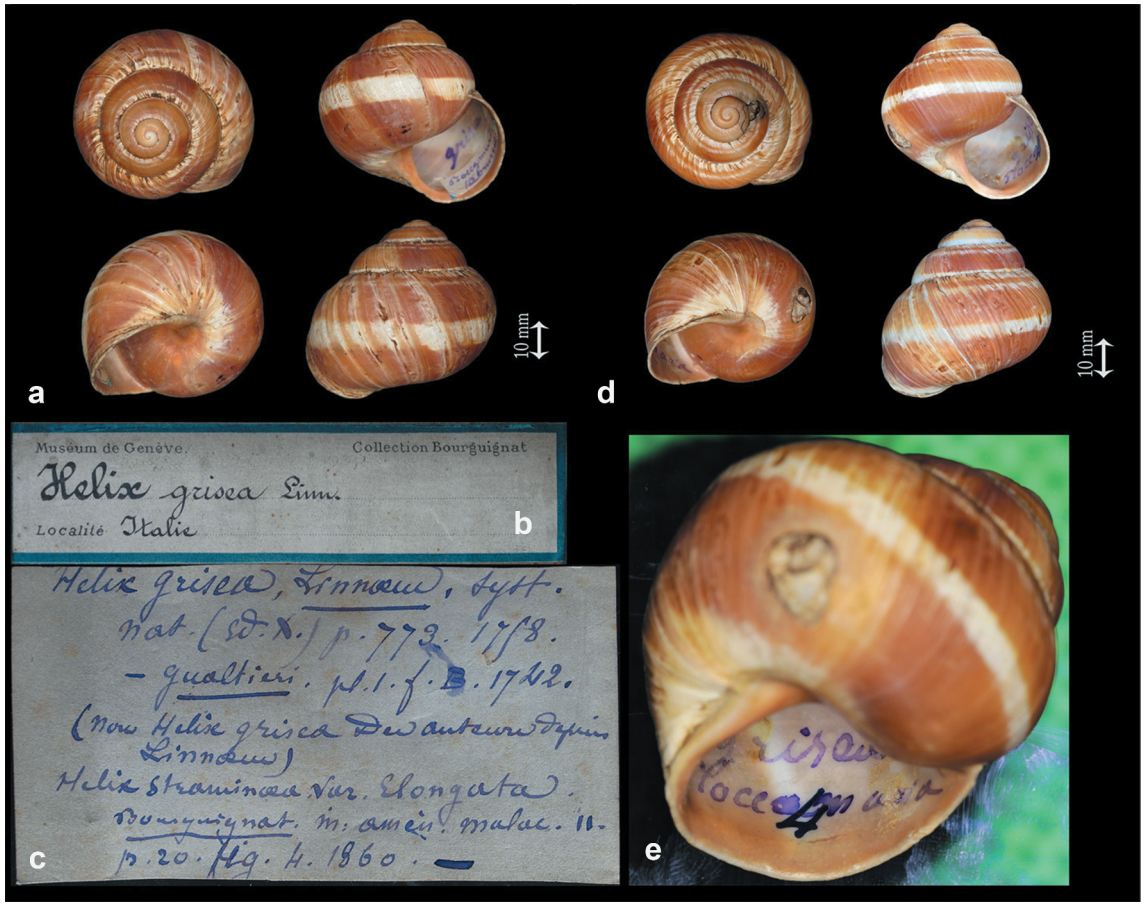

Figure 17. Lectotype of *Helix straminea elongata* Bourguignat, 1860. (a) Specimen catalogued under No. MHNG-MOLL-118145-1, Bourguignat collection, not taken into account for the typical series. (b) Original label of lot. (c) Associated autograph label of Bourguignat. (d) Specimens catalogued under No. MHNG-MOLL-118145-2 (4), here designated as lectotype of *Helix straminea* ssp. *elongata* Bourguignat, 1860. (e) Detail. (Photos by E. Tardy).

(Korábek et al. 2014). However, the species has been never found on Capri later (Petraccioli et al. 2005, 2006, 2007), so it is likely that these individuals were imported and perhaps purchased on a market.

von Martens (1870) received in 1855 in Naples a specimen "very similar to the figure of Briganti between the real *H. pomatia* and *H. lucorum*, or rather more similar to the second". He also believed that samples of "*H. pomatiae affinis*" found among food remnants in the archaeological ruin of Pompeii and preserved in the Bourbon Museum (now the MANN), are *H. straminea* Briganti (Monterosato 1872, 1879). Tiberi (1879a, 1879b) affirmed that the same shells preserved in the mentioned Museum probably came from Salerno Province, imported by Romans for food purposes. In the Book IX of his *Naturalis Historia*, Pliny the Elder described snail nurseries fattened with "sapa" (cooked wine must) and spelt. Still today, 18 specimens are preserved in the MANN (Figure 20), but they are not *H. straminea*. Instead, they represent *Helix asemnis* Bourguignat, 1860 (O. Korábek det.), an endemic species of southern Turkey (Triantis 2011; Neubert 2014): this represents a nice evidence of imports from Anatolia.

A specimen of *H. straminea* is stored in the natural history museum of the Salesian Institute "Sacro Cuore di Maria" of Caserta, bearing a label with the wording "Monte di Procida [Naples Province], 14.12.1998" and initially cataloged as "*Helix ligata*".

Some living specimens (Figure 15(a–f)) were found by us in Laviano (Salerno Province near Muro Lucano) in a mixed wood-chestnut grove at about 600 m a.s.l. in September - November 2015. A shell of a juvenile specimen was found by us (S. V. *leg.*) in Morcone (Benevento Province), near the Tammaro River, at 375 m a.s.l., in September 2017

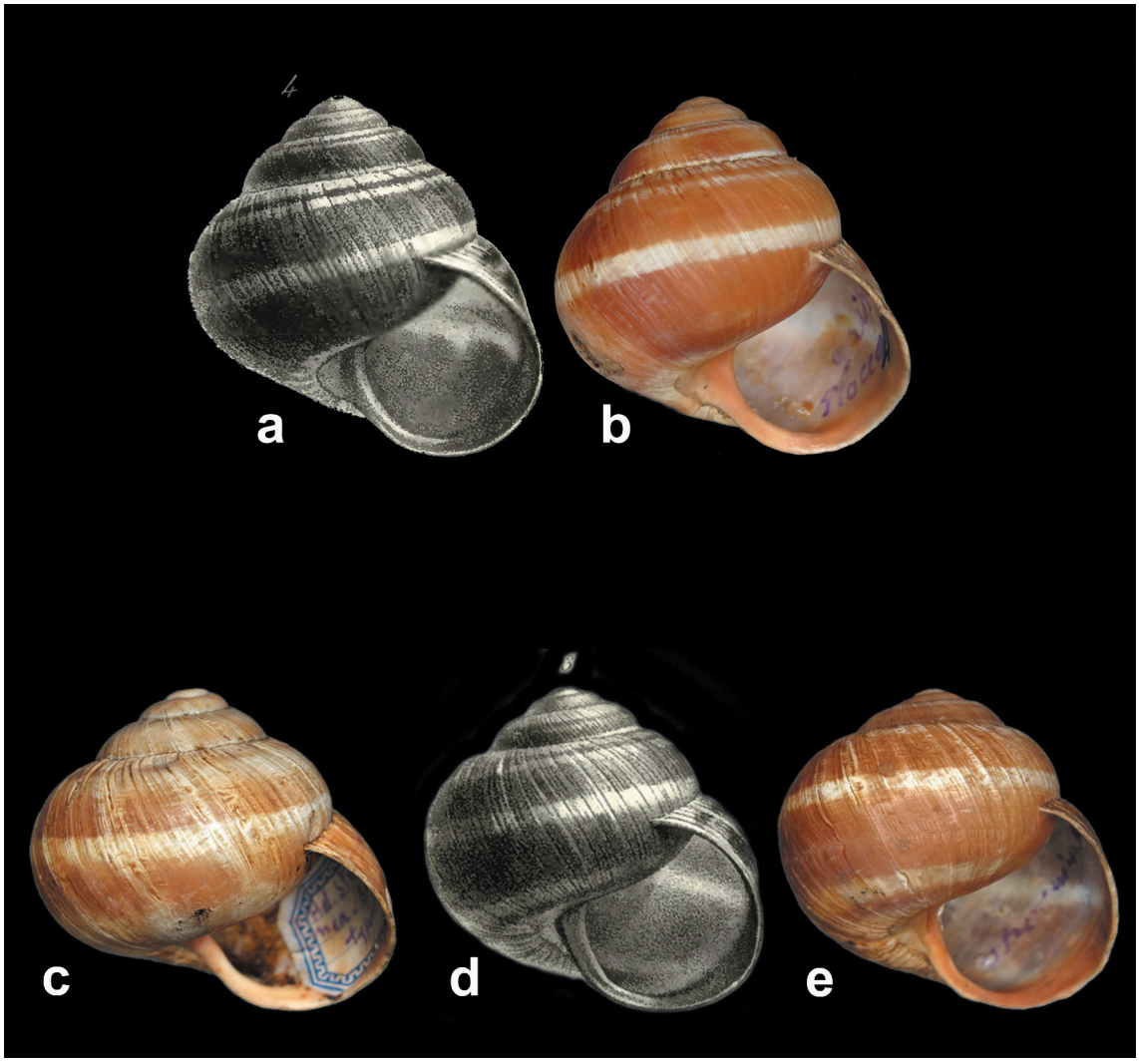

Figure 18. Comparison between Bourguignat figures and specimens present in MHNG. (a) Original figure of *H. straminea* var. *elongata*, fig. 4, pl. 20 (Bourguignat 1860b). (b) Specimen no. MHNG-MOLL-118145-2 (4). (c) Specimen no. MHNG-MOLL-118135. (d) Original figure of Bourguignat (1860b) fig. 3, pl. 20 of *H. straminea*. (e) Specimen no. MHNG-MOLL-118136.

in a willow-oak forest. A large specimen (W52.7 x H51.3 mm) was found by G.D.V. on the Matese Massif (Caserta Province) in September 2017.

Tiberi (1869) for the first time reported the species for the Province of Foggia. However, the specimens of Oronzio G. Costa cited by Bourguignat (1883) and maybe even the ones preserved in the MZUN (Table III) were collected in Apulia already between 1845 and 1873, when the Costa's collection has been donated to the museum (Maio et al. 1995; Maio 2009; De Stasio & Maio 2010). The *H. straminea* lots in various museums with the label "Monte Gargano" do not refer to Gargano Promontory, because the species is absent in this area (Holdhaus 1912; Korábek et al. 2014). Probably the locality corresponds to the Monte Gargano (Province of Foggia) of Tiberi (1869). Successively Kobelt (1903, 1906) collected a specimen from the "Bosco di Castelnuovo in Capitanata".

In addition to the work by Briganti, who designated Muro [Lucano] as one of the type localities, Philippi (1844) also mentioned a variety of *Helix lucorum* Müller, present in Lucania (today Basilicata), which he considered very similar to *H. pomatia* and *H. cincta* Müll. According to von Martens (1870) this form corresponds to *H. straminea*.

Two specimens of *H. straminea* were found by us (O.P. *leg.*) in November 2008 in the forest of Pescopagano (Potenza Province), a beech-oak forest at 600–800 m a.s.l., less than 20 km from Muro

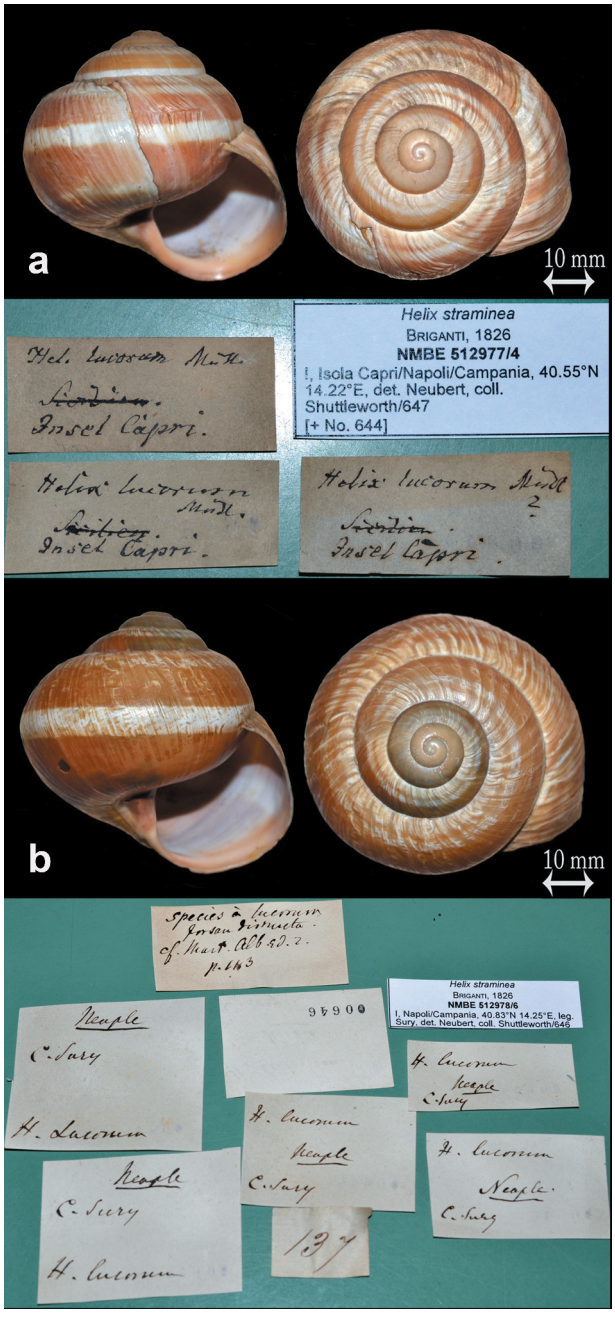

Figure 19. Samples of Shuttleworth collection in the NMBE. Samples collected by C. Sury from the Robert James Shuttleworth collection, preserved in the NMBE, respectively from Capri and Naples. (Photos by O. Korábek). (a) NMBE512977/4. (b) NMBE512978/6.

Lucano. It has recently also been found near Melfi (PZ), 600 m a.s.l. in 2009 (http://www.naturamediterraneo.com). The specimen described by Bourguignat (1883) as *Helix yleobia* was collected at Tricarico, Matera Province. It is probable that the specimen used by Bourguignat was donated to him by Paulucci (1878) several years before the publication of *H. yleobia*, and collected by "I. [Ippolito] Blanc" in 1877, as confirmed by a specimen kept in her collection (n. 1698, MZUF, Table III). Tricarico is the southernmost locality known for the species.

A living specimen (Figure 21(g–i)) has been found on Monte Prato, district of Ruvo del Monte (Potenza Province, near Muro Lucano) in a mixed wood grove at about 750 m a.s.l. in May 2018 (G. Aprea *leg.*).

Few reports are known from Latium: the oldest is represented by two specimens of the Paulucci collection (MZUF) from Soratte Mountain (Rome Province), collected in 1869 by Giovanni Rigacci (1816–1871); these shells were initially cataloged as *H. mutata* Lamarck and *H. lucorum* (Cianfanelli pers. comm.). Two further samples, one from comune of Acque Albule (Tivoli Municipality, Rome Province) (Zimmermann collection: ZMB 74122) and another from Rome (ZMB n.c.), both undated, are stored in the ZMB. The specimens found by Giusti (1971) on the Reatini Mountains (Rieti Province, Latium Region) and described as *Helix lucorum lucorum* Linnaeus should be referred to *H. straminea* (Korábek et al. 2014; Fiorentino et al. 2016). Two other recent reports are from Lago del Salto (Rieti Province) in 2007 and Sutri (Viterbo Province) in 2014 (Bilancioni 2007, http://www.naturamediterraneo.com).

The species is abundant and well distributed in Abruzzo, Molise, Marche and Umbria (Paulucci 1878, 1881; Valentini 1879; Korábek et al. 2014; museum data). Most recently, we found it in Civitanova del Sannio (Province of Isernia, Molise) in a mixed wood grove at about 627 m asl. on August 2018 (O. Picariello *leg.*).

The northern limit of the species is represented by the Province of Florence in Tuscany (Tosi, Regello and Vallombrosa), of Rimini in Emilia Romagna (Comune of San Leo) and San Marino (Paulucci 1881; museum data; Cianfanelli pers. comm). Some specimens were recently found in Monteveglio, Valsamoggia municipality, Bologna Province, Emilia Romagna (Bassi & Palmieri 2017), but these data need to be confirmed. The shell recovered during the archaeological excavation of Funo di Argelato (Bologna Province) could provide the oldest evidence (IX–XI century AD) of the northern limit of the species range in Italy (Bassi & Palmieri 2017) (Figure 22). Snails with appearance resembling *H. straminea* can be found even more northerly (e.g. Ravarano by Calestano or broader vicinity of Bardi, both Parma Province), but most individuals in the region correspond conchologically to *H. pomatia* and all those

Figure 20. Some of 18 specimens of *Helix asemnis* Bourguignat, 1860 preserved in the collections of MANN (Photos by N. Maio).

analyzed yielded *H. pomatia* haplotypes (Korábek et al. unpubl.). It remains to be established whether and where the ranges of the two species meet and whether *H. pomatia* and *H. straminea* hybridize.

Two specimens stored in the MZUF (cat. No. GC/16032) coming from Palermo (Sicily) dated in 1968 with the catalog annotation: "purchased by Mr. Reina" at first inventoried as *Helix lucorum* Müller, are probably stranded or imported specimens (Cianfanelli pers. comm; Table III).

Another specimen stored in the MZUF (cat. No. 20952) coming from Sassari (Sardinia, dated 27 June 1986), at first determined as *Helix lucorum*, is probably an allochthonous acclimatized specimen coming from heliciculture breedings (Cianfanelli pers. comm; Table III).

The distribution in Balkans was described by Korábek et al. (2014), but the locality at Stenje, Macedonia (by lake Ohrid) was erroneous (in fact it refers to *H. lucorum*) and the records from south of Albania was not verified by us. In the north the range extends to the northern part of the Prokletije Mountains. There is a record 20 km N of the Moraca Monastir in Montenegro and some others from the Albania-Kosovo-Montenegro border area. These ones are the northernmost ones (according to the material in the Hungarian Natural History Museum of Budapest, Zoltán Fehér pers. comm.). The populations geographically closest to Italy are known from South-east of Tiranë (valley of Erzen River by Ibë), where some individuals are very similar to Italian ones.

Discussion

Helix straminea in Italy is basically an Apennine species living from the Basilicata and Apulia regions, through Campania, Molise, Abruzzo and Latium to Marche, Umbria and Tuscany (Figure 22). The areal limit in the northern Apennines is probably the South side of the Po Valley (Emilia Romagna

Table III. Specimens of *H. straminea* of the museum collections with interesting faunistic data.

Locality	Collector, date of collection	Collection, inventory n.	Original determination, notes
Insel Capri [Campania]	coll. Shuttleworth, *leg*. C. Sury, n. 347 [1835–1850]	NMBE 512977/4	*H. lucorum* Müll. (H = 46.7 mm, D = 45.2 mm) (Neubert pers. comm.; Korábek et al. 2014)
Neaple [Campania]	coll. Shuttleworth, *leg*. C. Sury, n. 137 [1835–1850]	NMBE 512978/6	H = 51.1, D = 51.3 mm; Napoli (Korábek et al. 2014)
Italia meridionale	Costa coll.? [1845–1872]	MZUN Cat. RMZ 12006	*H.* (*pomatia*) *lucorum* Müll. 2 shells.
S. Marco la Catola [Foggia Prov., Apulia]	Costa coll.?, 1873.	MZUN Cat. RMZ 15864	*H. (pomatia) straminea* Briganti (= *lucorum* var.) 1 shell.
Gargano [Foggia]		MZUN, n.c.	*H. (pomatia) cincta*. 2 shells.
Italia meridionale		MZUN, n.c.	*H. (pomatia) lucorum* var. *straminea*. 3 shells.
M. Italien, Monte Gargano [Foggia]	coll. C. R. Boettger, 1909	SMF 74554/1	(Korábek et al. 2014)
Capitanata Appennino [Foggia]	Coll. Paulucci M., 1877. Tiberi N. *leg.*	MZUF 1689	*H. lucorum* Linneo forma (a) *quadrifasciata* (Cianfanelli pers. comm.)
Capitanata Appennino[Foggia]	Coll. Paulucci M., 1877. Tiberi N. *leg.*	MZUF 1716	*H. lucorum*, Müller v. *straminea* Brig. *Helix lucorum* Linneo forma (g) *elongata* (Cianfanelli pers. comm.)
Capitanata [Foggia]	Coll. Caramagna G., 1897, n. 1373D	MZUF 17565 vari es.	*H. lucorum*, Müller v. *straminea*
Tricarico nella Basilicata	Coll. Paulucci M., 1877. Blanc I. *leg.*	MZUF 1697	*H. lucorum* Linneo forma (g.) *elongata*
Muro in Basilicata	Coll. Paulucci M., 1877. Blanc I. *leg.*	MZUF 1698	*H. lucorum* Linneo forma (g.) *elongata*
Basilicata	Coll. Paulucci M., 1898	MZUF 1738 vari es.	*H. lucorum*
Roccamorice (Pescara Prov., Abruzzo)	Cianfanelli S. Calcagno M. *leg.*, 10/05/1993	MZUF n.c. ID 21862	*H. lucorum*
Palermo (Sicily)	1968. Reina don.	MZUF GC/16032	*H. lucorum*
Sassari, Via Baldedda (Sardinia)	27/06/1986	MZUF GC/20952	*H. lucorum*

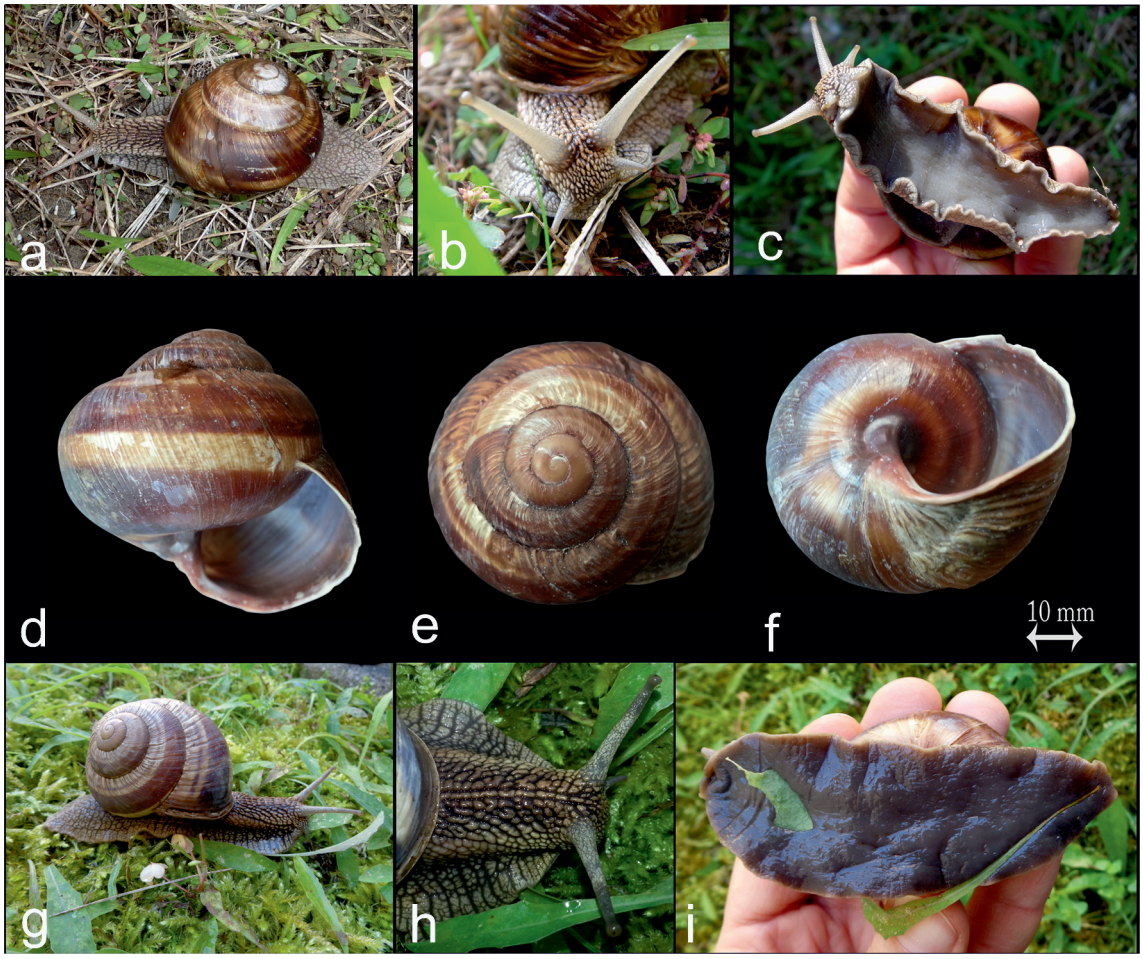

Figure 21. Living specimen of *H. straminea* from South of Italy. (a–c) A living specimen found in Laviano (Province of Salerno) in 2015. (d–f) Shell of a specimen from Laviano. (g–i) A living specimen found in Ruvo del Monte (Province of Potenza) in 2018. (Photos by N. Maio).

and Northern Tuscany). The species is absent from Calabria (Cesari 1978) and Gargano Promontory (Holdhaus 1912; Korábek et al. 2014). In Sardinia the species has recently acclimatized due to its introduction for food source (Sosso & Cianfanelli pers. comm.). Some references, which may include relevant faunistic records cannot be taken into account because the two species cannot be clearly discriminated against (Issel 1866; Gentiluomo 1868; Bonelli 1873; Pantanelli 1877 [1876]; De Stefani 1883; Lepri 1909; Bisacchi 1933; Paget 1962; Forcart 1965, 1968; Giusti & Mazzini 1970; Manganelli et al. 1995).

The known extent of occurrence (EOO) was estimated at 48,000 km^2 (Neubert 2017), with our new data it covers 55,000 km^2. The above-mentioned 73 localities of presence in Italy include also 33 historical records; among all these localities, 8 are doubtful, 2 accidental or due to introduction of the species. Some records are based only on pictures or shells from collections, for this reason it is not known whether the populations are abundant or not in these areas.

In conclusion, we confirm the synonymy of *Helix yleobia* Bourguignat, 1883, and *H. straminea elongata* Bourguignat, 1860 with *H. straminea* Briganti, 1825. It remains questionable if *H. straminiformis* Bourguignat, 1876 is to be included as it is based on misshaped and probably parasited specimen, whose place of origin and species identity cannot be sure.

So far, chromosomal studies have been carried out for a limited number of helicid species, namely 19 (Vitturi et al. 1982, 2005; Thiriot-Quiévreux 2003; Bakhtadze et al. 2016). The diploid (2 *n*) chromosome number of *H. straminea* (2 *n* = 54) is the same as reported for *H. pomatia, H. lucorum* and *H. buchii* Dubois de Montpéreux, 1839 (Perrot 1937; Bakhtadze et al. 2016). For the related genus *Caucasotachea* (Boettger 1909) the numbers are 2 *n* = 50 and 52 (Gill & Cain 1986; Bakhtadze et al.

Figure 22. Distribution of *Helix straminea* in Italy. The updated extent of occurrence (EOO) (bluish area). Historical data before 1980 (white triangles); recent published and new unpublished records (azure triangles). Type localities of the species and synonyms are indicated by yellow triangles. Localities where the species is introduced or accidental (red triangles). ?Doubtful data. (Picture by ESRI satellite, free edition, 2018, modified by S. Viglietti).

2016). *Cepaea* and the related genera *Iberus* and *Pseudotachea* have 2 *n* = 44 (Aparicio & Ramos 1988), while the number varies among the relatives of *Cornu* (*Theba* 60, *Cornu* and *Cantareus* 54, *Otala* and *Massylaea* 52, *Macularia* 50; Vitturi et al. 1982, 2005; Aparicio & Ramos 1988; Thiriot-Quiévreux 2003). Mostly (but see *Cepaea*; Gill & Cain 1986) the chromosomes are biarmed and gradually decreasing in length. This limits the utility of karyotype studies for the systematics of Helicidae, and *H. straminea* in particular. For example, the *H. straminea* distantly related *Cornu* (Neiber & Hausdorf 2015) also has 2 *n* = 54 chromosomes with a very similar karyotype (with meta-, submeta- and telocentric chromosomes) and pattern of C-band positive heterochromatin, which is few and distributed on the centromeric regions of almost all chromosomes (Vitturi et al. 2005).

Even after more sequence data become available since the publication of Korábek et al. (2014), the Italian and Balkan *H. straminea* appear monophyletic sister clades, although more sampling would be desirable especially in Albania. In the Italian clade, the similarity between haplotypes does not coincide with geographic proximity between sampling sites. The sample from Laviano (Campania) groups with populations of Montalbuccio by Siena (Toscana), Rieti (Latium) and Teramo (Abruzzo) and not with the geographically close sample of Ruvo del Monte (Basilicata), distant from Laviano about 12 Km. On the other hand, the sample from Torrente Bozzone of Siena appears closer to Ruvo del Monte than to the geographically close sample of Montalbuccio (Siena, Tuscany). These relationships suggest low geographic structure and a relatively recent (Late Pleistocene?) colonization of the present Italian range, not yet followed by formation of geographic structuring of the mitochondrial diversity. After this, environmental and climatic changes probably led to the present punctuated distribution.

The genitalia of *H. straminea* have not been described from Redi (1684) onwards, but as it generally holds within *Helix* (Neubert 2014), they do not offer unique traits for reliable identification of the species.

H. straminea has been given a Least Concern (LC) status in the Red List of the International Union for the Conservation of Nature (I.U.C.N., version 2020–3). Neubert (2017) assumed that there are enough populations to compensate any losses, due to the large potential distribution area in Italy and because the species occurs also on the opposite side of the Adriatic Sea. Nevertheless, massive exploitation of specimens from the wild may seriously influence the population dynamic. This species is historically used as food source by the local populations (since IX–XI century AD, Bassi & Palmieri 2017), because it is large sized and tastes good (Briganti 1825). Moreover, this species was customarily sold on the markets in Naples in the 19th century (Kobelt 1906) and continues to be consumed. These facts cause a real threat for the species. In addition, the strong anthropization of mountain areas and the relative reduction of woodlands and forest habitats, the livestock grazing in shrublands and wooded areas in the mountainous areas can also cause damage to the habitat of the species. In consideration of the fact that there is no information on the population size of this species, we believe that *H. straminea* is potentially near threatened, so we believe that this species is worthy of protection at European, national and regional level (Maio et al. 2017).

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