

MORPHO-ANATOMICAL CHARACTERIZATION OF THE UROGENITAL SCHISTOSOMIASIS VECTOR *BULINUS TRUNCATUS* (AUDOUIN, 1827) (HETEROBRANCHIA: BULINIDAE) FROM SOUTHWESTERN EUROPE

ALBERTO MARTÍNEZ-ORTÍ^{1,2}, SONIA ADAM¹, GIOVANNI GARIPPA³, JÉRÔME BOISSIER⁴, M. DOLORES BARGUES^{1,5} & SANTIAGO MAS-COMA^{1,5}

¹Unit of Sanitary Parasitology, Department of Parasitology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

²Museu Valencià d'Història Natural-i\Biotaxa, Alginet, Valencia, Spain

³Unità di Malattie Parassitarie, Dipartimento di Medicina Veterinaria, Università di Sassari, via Vienna, 2, 07100 Sassari, Italy

⁴IHPE, Univ. Montpellier, CNRS, IFREMER, Univ. Perpignan Via Domitia, Perpignan, France

⁵CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III, C/Monforte de Lemos 3-5, Pabellón 11. Planta 0, 28029 Madrid, Spain

Abstract Urogenital schistosomiasis has been present naturally in the South of Europe since the beginning of the 20th century and nowadays its presence is also known, at least imported by Sub-Saharan emigrants and tourists, in France, Italy, Portugal and Spain. One of the intermediate hosts of this trematode present in Europe is the bulinid mollusc *Bulinus truncatus*, non-native species that can be reached to Europe by humans and birds. In order to know this mollusc better, we carried out a morpho-anatomical study, of the shell, the reproductive system, radula, the respiratory organs and pseudobranch of several populations from Italy, France and Spain. Spanish conchological material studied comes from different populations, from material deposited in the "Museo Nacional de Ciencias Naturales" of Madrid and the "Museu de Ciències Naturals" of Barcelona, as well as from its own material deposited in the "Museu Valencià d'Història Natural" of Alginet (Valencia). The shell growth in captivity and the estimation of the population age of *B. truncatus* from El Ejido (Almería, Spain), has also been studied. Finally, the finding of aphyllid and euphyllid specimens in the different populations of southern Europe studied is presented and taxonomic and ecological data of the genus *Bulinus* are shown.

Key words *Bulinus truncatus*, *Bulinidae*, urogenital Schistosomiasis, morpho-anatomy, South Europe.

INTRODUCTION

It is evident that the world has been facing a new scenario of emerging infectious diseases in the last two decades (Jones *et al.*, 2008), including many threats to human health and potential repercussions on global stability (Morens & Fauci, 2013). Parasitic diseases are included within the so-called Neglected Tropical Diseases (NTDs) by the World Health Organization (WHO, 2010). One of them is human schistosomiasis caused by infection with the trematode *Schistosoma* Weinland, 1858. It is estimated that at least 240 million people worldwide are infected, 90% of them in Sub-Saharan Africa, and that it causes around 300,000 deaths annually (WHO, 2010, 2013). Two species, *Schistosoma haematobium* (Bilharz, 1852) and *Schistosoma mansoni* Sambon, 1907, are responsible for urogenital and hepatointestinal schistosomiasis respectively in humans (Malek & Cheng, 1974; Brown, 1994).

Urogenital schistosomiasis appears limited to Africa and a part of the Near East (Doumenge *et al.*, 1987). Unexpectedly, however, an outbreak of urogenital schistosomiasis has recently been detected on the French Mediterranean island of Corsica (Berry *et al.*, 2014; Holtfreter *et al.*, 2014). An overlap of climate change and global change factors appear to underlie this disease introduction (Boissier *et al.*, 2015; Kincaid-Smith *et al.*, 2017). Three schistosomes have been proved to be introduced into Corsica island, namely *S. haematobium*, *S. bovis* (Sonsino, 1876) and *S. haematobium*-*S. bovis* hybrids. Studies predict that these schistosome introductions to the South of Europe occur by Sub-Saharan emigrants and tourists in France, Italy, Portugal and Spain (Boissier *et al.* 2015; Salas-Coronas *et al.*, 2018, 2019; Martínez-Ortí *et al.*, 2019). The molecular analyses of these imported schistosomes indicated that the introduction most probably occurred from the northern part of Senegal (Boissier *et al.*, 2016). Recently, Salas-Coronas *et al.* (2021) indicate the autochthonous transmission of urogenital Schistosomiasis

Corresponding author: A. Martínez-Ortí. E-mail: amorti@uv.es

Code Orcid iD: <https://orcid.org/0000-0001-6816-1140>

in the province of Almería (Spain). Snail vectors are useful markers for the study of the impact of both climate change and global change on the transmission, epidemiology and also the spreading power of these trematodiasis, mainly by increasing the cercarial production (Mas-Coma *et al.*, 2008, 2009; Kincaid-Smith *et al.*, 2017). The freshwater snail vectors of schistosomiasis are of the genus *Bulinus* O.F. Müller, 1781, and *Bulinus truncatus* (Audouin, 1827) vectorise urogenital schistosomiasis in Europe (Berry *et al.*, 2014; Holtfreter *et al.*, 2014; Boissier *et al.*, 2015; Martínez-Ortí *et al.*, 2015, 2019). The underlying high and fast multiplication of these species is in great part due to their selfing capacity, mainly in asexual specimens, found regularly in *B. truncatus* (Brown, 1994; Dillon, 1994). Schistosomium–snail specificity is a complex phenomenon which involves the geographical location and macromolecular glycoproteins produced by the snail through its mucous secretions that provide a mechanism for host recognition by miracidia as in *Bulinus globosus* (Morelet, 1866) (Haberl *et al.*, 1995; Allan *et al.*, 2009). Consequently, the studies for the aforementioned purposes should not only focus on the bulinid vector but also involve an in-depth analysis of their environmental characteristics in the area or region where the gastropods are emerging or spreading. In fact, *B. truncatus* has a wide geographic range, extending along the coasts of the circum-Mediterranean region, a large part of Africa, the Middle East and the Turanic region. In Europe it has been cited from various countries such as Spain, mainland France and Corsica, mainland Greece and Crete, Italy (Sardinia and Sicily), Malta and Portugal (Brumpt, 1929; Germain, 1931; Larambergue, 1939; Coluzzi *et al.*, 1965; Deiana *et al.*, 1966a, 1966b; Berry *et al.*, 2014; Holtfreter *et al.*, 2014; Martínez-Ortí *et al.*, 2015, 2019).

The parasitological and veterinary interest of *B. truncatus* does not only concern its capacity to transmit *S. haematobium* and *S. haematobium*–*S. bovis* hybrid to humans and *S. bovis* to cattle, since it can transmit other species of trematodes such as *Paramphistomum cervi* (Schränk, 1790), *P. microbothrium* Fischöder, 1901 or *Echinostoma* spp. (Martínez-Ortí *et al.*, 2019). The susceptibility of the European *B. truncatus* to African schistosomes opens the query about the risk of introduction of the African *S. haematobium* into southern countries of Europe where this bulinid

species is also known to be present and countries where African immigration is a continuous movement (Martínez-Ortí *et al.*, 2015, 2019; Boissier *et al.*, 2016).

Following with the risk map of urogenital schistosomiasis made by Martínez-Ortí *et al.* (2019) for the Iberian Peninsula and Balearic Islands, the present study has the aim to contribute to the knowledge of different aspects of *B. truncatus* populations from the Western Mediterranean frame: El Ejido in Almería province (Spain) and Villena in Alicante province (Spain) (Martínez-Ortí *et al.*, 2015), Porto-Vecchio in Corsica (France) and San Teodoro in Sardinia (Italy), including comparisons with samples deposited in several Spanish museums such as the “Museo Nacional de Ciencias Naturales” of Madrid (MNCN) and the “Museu de Ciències Naturals” of Barcelona (MCNB), as well as samples deposited in the “Museu Valencià d’Història Natural” of Alginet (MVHN).

Aspects studied in the current paper are among those of interest for snail vector specimen identification, geographical strain differentiation, population age estimation, epidemiological characterization and disease transmission follow-up studies, and include (i) the morphology and measurements of the shell, (ii) the shell growth followed experimentally in laboratory-borne and reared specimens, (iii) anatomical features comprising the respiratory system and digestive organs such as the pseudobranch, jaw and the radula, (iv) ecological characteristics of the environment of the populations and (v) to improve the capacity to identify and monitor populations of *B. truncatus* in South of Europe and the potential introduction and spreading of *S. haematobium* and *S. haematobium*–*S. bovis* hybrid.

MATERIALS AND METHODS

Material collected The specimens from Almería (Spain) were collected on December 5 2014 and July 8 2015 in a pond that occupies a large artificial excavation in the course of a ravine from the Sierra de Gádor (El Ejido, Almería). The specimens from Corsica were collected on July 15 2015 and those from Sardinia on July 11 2015. All the material was preserved in 70% ethanol for morpho-anatomical studies and 96% ethanol for molecular studies and are deposited in the MVHN of Alginet (Spain): El Ejido (Almería,

Spain) (UTM 30SXH8572) (MVHN-071214TP04), Cavu river in Porto-Vecchio (Corsica, France) (UTM 32TNM2717) (MVHN-240815AF01), and Stagno Brandinchi of San Teodoro (Sardinia, Italy) (UTM 32TNL5720) (MVHN-031215MA06). The details of their habitats appear in Martínez-Ortí *et al.* (2015, 2019). The water data was collected using Hanna HI98312 instrument for temperature and conductivity, the Hanna HI8424 for pH and the Hanna HI9147 for dissolved oxygen meter.

Material from museums Shells of several samples of *B. truncatus* deposited in MNCN of Madrid have also been studied and measured and the MCN of Barcelona also photographed. The four samples of the MNCN from the Ortiz de Zárate collection and Cobos collection are: “Barcelona, en una finca particular” (MNCN-15.05/40640) with five specimens recollected by Carlos Altimira determined as *Bulinus contortus* and “Barcelona, Huerto en la Bonanova” (MNCN-15.05/26320) with three specimens collected in February of 1957 by Carlos Altimira and determined as *Isidora contortus*; the sample MNCN-15.05/26314 from Menorca island with two small specimens from Cobos collection and the sample MNCN-15.05/40630 from “Palma de Mallorca” with 19 specimens. Of the samples from Barcelona (MNCN) only the four bigger specimens have been measured (Table 1). Another sample for conchological studies from MVHN corresponds to “Laguna de Villena” (Alicante, Spain) (MVHN-281214TY01) (Fig. 7). The pseudobranch studied of *B. truncatus* comes from El Ejido while the pseudobranch of *Helisoma duryi* (Wetherby, 1879) from the Ágora of Valencia (Spain).

Morpho-anatomical characters All obtained measurements of the shells come from literature and our measurements: length, (L), width, height of aperture (HA) and HA/L relation (Table 1). The ornamentation of the protoconch and the teleoconch is accurately shown, and the morphology of the radula, jaw and pseudo-branch, as well as the reproductive system of three Mediterranean European locations studied are described. Conchological measurements were obtained with the help of the Image Kappa 5.1 software coupled to the high resolution binocular loupe, forming part of an ICAS system (Computer Image Analysis System). Both the

study and the drawings of the reproductive system were made using the Leica M3Z stereomicroscope with clear coupled camera Wild 308700. For the study of both the ornamentation of the surface of the shells and the radula, the jaw and the male sexual pore photographs were made in the Scanning Electron Microscopy (SEM) Hitachi S-4100 with the digital program Sprit 1.8 (Bruker) and the Images application. All the material was coated with a layer of gold-palladium, for its exposure to the electron beam. The protoconch, teleoconch, radula and jaw studied with SEM correspond to specimens of the population of El Ejido (Almería). In the anatomical description of the different organs of the genitalia the use proximal denotes the part which is closer to the gonad, and distal the part which is closer to the gonopore.

Shell growth in captivity 30 specimens of *B. truncatus* were studied in captivity to determine their growth between March 2015 and June 2016. They were collected in December 2014 in El Ejido (Almería, Spain). The bulinids were transported under isothermal conditions to our laboratory. The possible natural infestation of schistosome of each of the collected specimens was checked individually, keeping them in Petri dishes with a small amount of mineral water. They were acclimated and maintained under controlled experimental conditions of 20°C, 50% relative humidity and a photoperiod of 12 h light / 12 h dark in climatic chambers (model Heraeus-Vötsch VB-0714). The snails were placed in completely transparent plastic boxes inside which a silicone tube was inserted that releases air to keep the water as oxygenated as possible. At the bottom of the culture boxes was a substrate layer of calcareous stones submerged in mineral water. Their diet was based on lettuce (*Lactuca sativa*) for adults and *Oscillatoria formosa* cyanophyceae algae for juveniles. From three specimens collected in El Ejido three generations were obtained in the laboratory. From each of these generations 30 specimens were selected. During the experiment, these specimens were kept individually in Petri dishes of 5cm in diameter with mineral water, fed with a small portion of lettuce, according to the size of the snail, and also with an aliquot of the *O. formosa* algae, in the case that they were recently hatched specimens. The length was monitored by biweekly scans of the 30 specimens

in ventral and apical positions, with the help of the Image Kappa 5.1 previously cited. The morphometric measurements were obtained through the software Image Pro Plus 5.1, from the images previously generated.

RESULTS

Taxonomic data *B. truncatus* was collected from Egypt (*locus typicus*) and figured for the first time by Savigni (1817: pl. 2, fig. 27) without specifying the collection location, to which it does not assign any specific name. Later, Audouin (1827: fig. 29) comments on the species represented by Savigni and indicates that it is very similar to the *Physa truncata* of Férussac indicating conchological characters, although being an unpublished name corresponding to a *nomen nudum*, in consequence, the authorship of this taxon must be attributed to Audouin (1827) (Haas, 1935). Bouchet & Danrigal (1982: fig. 29) show a specimen of the Savigny collection, deposited in the Musée National d'Histoire Naturelle of Paris, which must be considered a lectotype of the species, corresponding to the other specimen deposited in that collection as a paralectotype. The type species of the genus *Bulinus* is *B. senegalensis* O.F. Müller, 1781 and its *locus typicus* Podor (Senegal). To systematic level Germain (1931) designates the family Bulinidae P. Fischer & Crosse, 1880 to group the genus *Bulinus* O.F. Müller, 1781 and genus *Indoplanorbis* Annandale & Prashad, 1921. Some authors had considered this valid (Larambergue, 1939; Baker, 1945) based on two characteristic features of the group and separate it from any other known division of the Planorbidae Rafinesque, 1815 based on the fluted or lobular pseudobranch (Fig. 21) and the shape of the penial complex in the genitalia. However, the pseudobranch in Planorbidae (comprising SF. Bulininae, now F. Bulinidae) is smooth and shaped like a leaf or square (Baker, 1945) (Fig. 22). Recently, Bouchet *et al.* (2017) based mainly on Morgan *et al.* (2002) and Aldrecht *et al.* (2007), point out that Bulinidae and Planorbidae are different families belonging to Superfamily Lymnaeoidea Rafinesque, 1815 and included in the Superorder Hygrophila Férussac, 1822.

In Europe two subspecies of *B. truncatus* are known: *B. t. contortus* (Michaud, 1829) from southern France and *B. t. rivularis* (Phillipi, 1836) of Sicily (Italy). Falkner *et al.* (2002) consider *Physa*

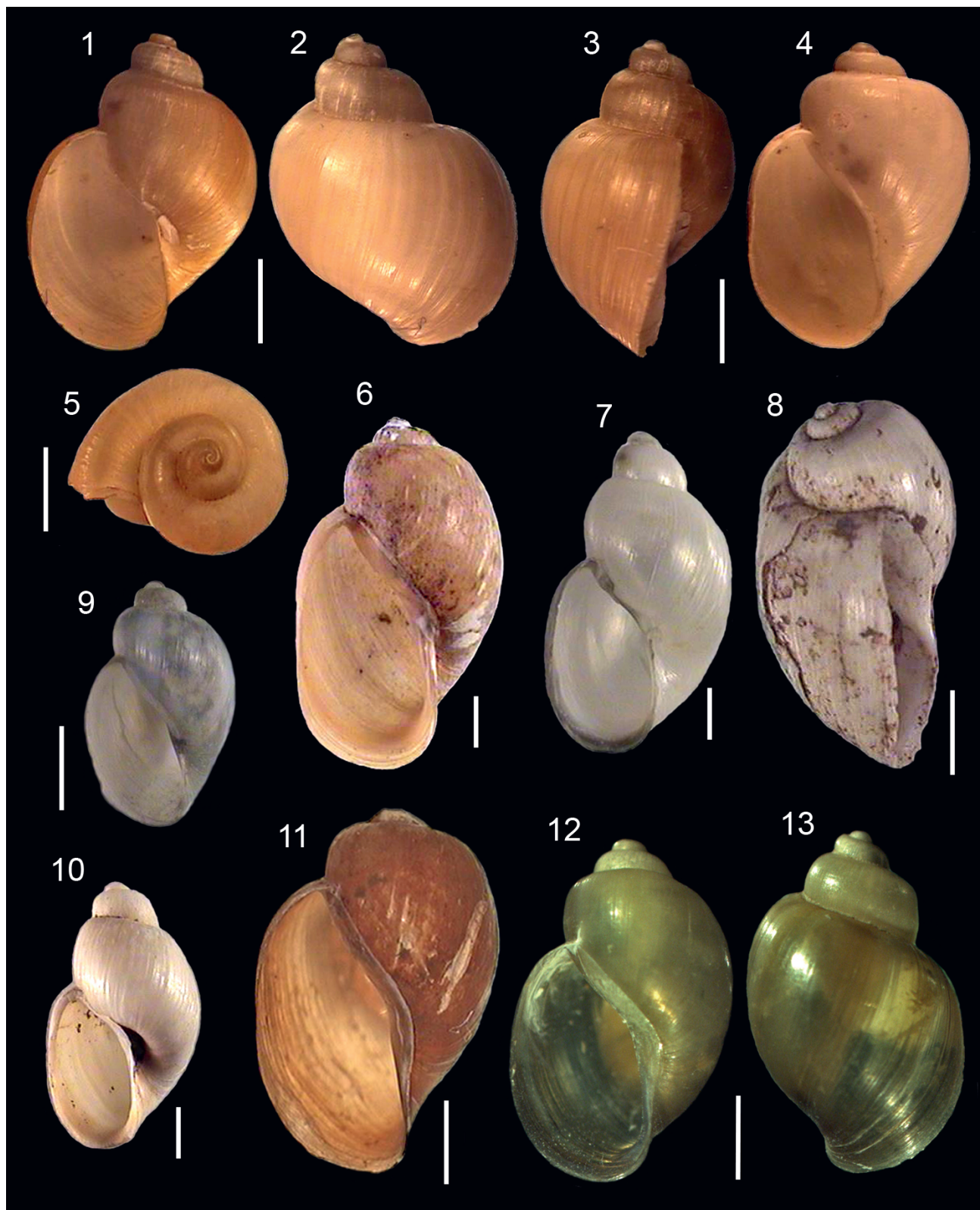
contorta a subspecies of *Bulinus truncatus* while Boyer & Audibert (2007) consider *B. contortus* a junior synonym of *B. truncatus*, based on the fact that the shells of both taxa are indistinguishable and because the dispersion by birds has played an important role in the ubiquitous distribution of *B. truncatus*. This character is also applicable for *B. truncatus rivularis*, which would justify its presence in Sicily. Until now, only the population of El Ejido (Spain) (Figs 1–3) has been molecularly studied by sequencing *cox1* (GenBank Acc. No. KR108924), confirming its specific assignment to *B. truncatus*, with 100% homology with other populations of this species available in GenBank. These first molecular data are the object of a more extensive study together with epidemiological studies (Martínez-Ortí *et al.*, 2015).

External and internal anatomical data A regular dark brown in colour and with thin and elongated tentacles, shorter than in *Physa acuta* (Draparnaud, 1805), which also has a much lighter colour all over. It has the reproductive system, respiratory system (pneumostoma and pseudobranch) and excretory organs on the left side of the body. The displacement on the substrate is slow, unlike *P. acuta*, with which it is often confused, which is much faster. Planorbids are characterized by having a basic haploid chromosome number of 18 and the bulinid *B. truncatus* is considered polyploid ($2n=36$) (Burch, 1960) and tetraploid ($2n=72$), the latter associated with aphyllity (Brown, 1994). This tetraploid form is common among species of *Bulinus* which are the intermediate hosts of *S. haematobium* and cause urogenital schistosomiasis in humans (Burch, 1960; Davis, 1978; Dillon, 2004).

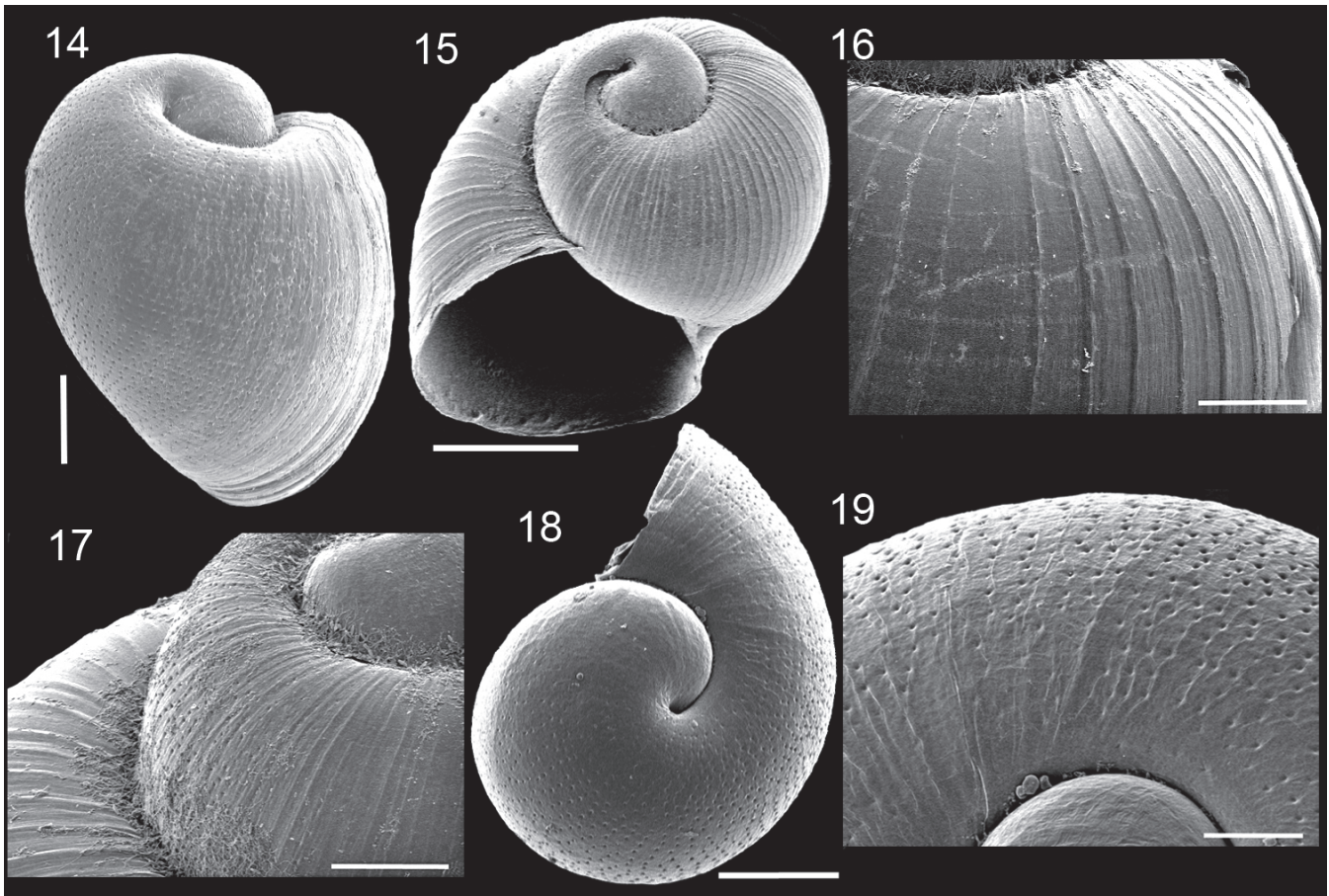
Conchological characters (Figs 1–19, Table 1) The genus *Bulinus* presents about thirty species described, characterized by having a sinistral shell with a spire that is very variable in size and shape, a relatively high opening, evenly curved turns, and sometimes angular and but rarely warped (Brown, 1994; Martínez-Ortí *et al.*, 2015, 2019). The shell of *B. truncatus* is sinistral and has a very fragile ovoid globose shape, of pale amber colour, short spire, composed of 3–4 domed convex rounds, with deep sutures, and the latter being a little higher than half the shell and sculptured with thin radial ribs, although in general not very marked, thin, uneven and somewhat

Table 1 Shell size of *Bulinus truncatus* populations in different countries of the western Mediterranean frame. Values in mm. (–) data not available.

Population	n	Shell Length (L)			Shell Width			Height of Aperture (HA)			HA/L		References
		range	mean±SD	extreme values	range	mean±SD	extreme values	range	mean±SD	extreme values	Relation	Relation	
El Ejido (Spain)	10	7.8–9.6	8.62±0.57	5.1–5.9	5.45±0.28	4.75–6.1	5.37±0.41	0.62	present study	0.62	present study	present study	
Villena (Spain)	6	5.2–7.1	6.43±0.85	3.5–4.4	4.16±0.50	3.7–5.1	4.66±0.55	0.72	present study	0.72	present study	present study	
Corsica (France)	7	5.9–8.5	7.0±0.9	4.1–5.0	4.52±0.5	4.45–5.6	4.8±0.45	0.68	present study	0.68	present study	present study	
Sardinia (Italy)	10	8.5–9.8	8.96±0.43	5.7–6.8	6.18±0.36	6.3–7.3	6.68±0.29	0.74	present study	0.74	present study	present study	
Barcelona (Spain); MNCN samples	4	15.5–16.9	16.12±0.65	10.6–11.6	11.07±0.55	–	–	–	present study	–	–	present study	
Palma de Mallorca (Spain); MNCN- 15.05/40630	1	14.0	–	9.2	–	–	–	–	present study	–	–	present study	
North and Center Portugal	–	13.0	–	8.0	–	–	–	–	Nobre (1941)	–	–	Nobre (1941)	
Algarve (Portugal)	–	9.0	–	5.5	–	5.2	–	0.57	Fraga de Azevedo <i>et al.</i> (1969)	0.57	Fraga de Azevedo <i>et al.</i> (1969)	Fraga de Azevedo <i>et al.</i> (1969)	
North Portugal	–	6.7	–	4.6	–	4.2	–	0.62	Fraga de Azevedo <i>et al.</i> (1969)	0.62	Fraga de Azevedo <i>et al.</i> (1969)	Fraga de Azevedo <i>et al.</i> (1969)	
Malta island	–	11.9–15.0	–	9.6–11.0	–	–	–	–	Giusti <i>et al.</i> (1995)	–	–	Giusti <i>et al.</i> (1995)	



Figures 1–13 Shells of *Bulinus truncatus* of Spain, Italy and France: **1–3** El Ejido (Almería, Spain) (MVHN-071214TP04) **4–5** Villena (Alicante, Spain) (MVHN-281214TY01) **6** Barranc de l’Algendar (Menorca, Balearic Islands, Spain) (MZB 80–3053) **7** Ampurias (Girona, Spain) (MZB 2009–0536) **8** San Antonio Abad, Ibiza (Balearic Islands, Spain) (MZB 84–1825) **9** Adra (Almería, Spain) (MZB 84–7124) **10** Font de Mestre Pere, Mallorca (Balearic Islands, Spain) (MZB 84–1822) **11** San Teodoro (Sardinia, Italy) (MVHN-031215MA06) **12–13** Porto-Vecchio, Corsica (France) (MVHN-240815AF01). Scales=2mm.



Figures 14–19 Microsculpture of the protoconch and the first whorls of *B. truncatus* (El Ejido, Almería, Spain). Scales: 14,17,18,=200µm; 15,=600µm; 16,=300µm; 19,=70µm.

flexible. The first whorls of the shell of *B. truncatus* are ribbed. Blunt apex, oblique oval opening and slightly angled on the upper part, with the thin, subcontinuous peristome not reflected, without thickening, with the columellar margin usually narrow and more or less coiled and with narrow navel (Brown, 1994; Giusti *et al.*, 1995; Martínez-Ortí *et al.*, 2015, 2019) (Figs 1–19).

The maximum dimensions known of this species reach 13.0mm in height and 8.0mm in diameter (Germain, 1931; Nobre, 1941) (Table 1), although some authors point out that they can reach 20–23mm (Malek & Cheng, 1974; Brown, 1994). The dimensions of the 10 specimens of the population of Villena (Alicante, Spain) (Figs 4–5) range between 5.2 and 7.1mm in height and between 3.5 and 4.4mm in diameter, while those in the population of El Ejido (Alicante, Spain) (Figs 1–3) range between 7.8 and 9.6mm in height and between 5.1 and 5.9 in diameter (Martínez-Ortí *et al.*, 2015, 2019). Of all the Spanish samples studied, the largest specimens

reach 16.9mm in height and 11.6mm in diameter in two MNCN samples (MNCN–15.05/40640 and MNCN–15.05/26320). Nobre (1941) notes for Portuguese specimens 13.0mm in height and 8.0mm in diameter. Fraga de Azevedo *et al.* (1969) curiously consider that the Algarve specimens are larger than those of the north and centre of Portugal, due to temperature, obtaining 9.0mm maximum height and 5.5mm maximum diameter. The seven measured specimens of Corsica (Figs 12–13) range between 5.9mm and 8.45mm in height and between 4.1 and 5.0mm in width. The largest specimen presented the dimensions of 8.45×5.0mm and the smallest of 5.9×4.0mm. Ten specimens from Sardinia (Italy) (Fig. 11) have maximum measurements between 8.5 and 9.8mm in height and between 5.7 and 6.8mm in width, presenting the larger one the following dimensions 9.8×5.9mm and the smaller 8.5×6.5mm. Giusti *et al.* (1995) indicate dimensions for this species in the Maltese islands between 11.9–15.0mm in height and between

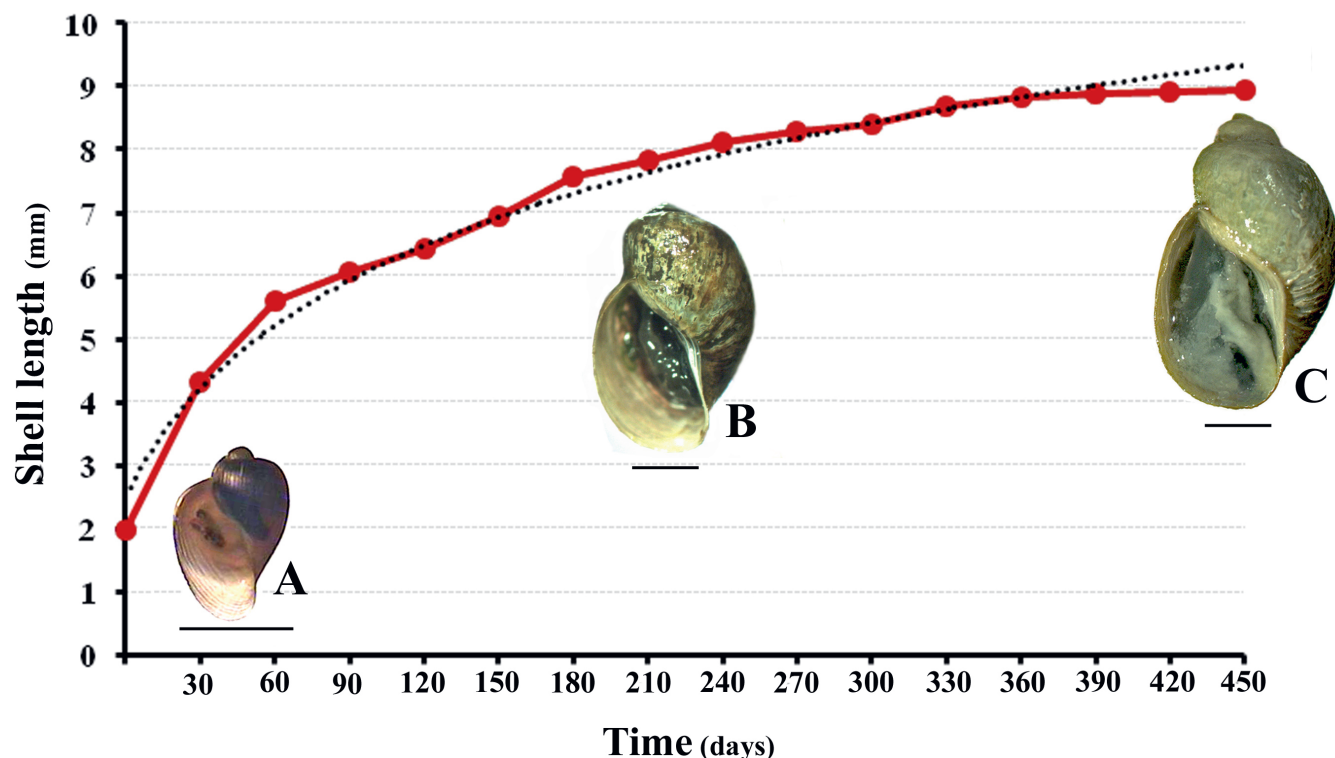


Figure 20 Variation of the length of the shell (in mm) of *Bulinus truncatus* with respect to the time in days, from El Ejido (Almería, Spain). Scales=2mm.

9.6–11.0mm in diameter. The maximum height can reach exceptionally 20mm (Giusti *et al.*, 1995; Malek & Cheng, 1974). The features of the habitat (e.g. marshy bottom or not, abundance of food or scarcity, limestone or not, etc.) can influence both the size and shape of the shell (Schwetz, 1954).

According to the data obtained in Table 1 of 37 specimens the shell length (L) varies between 8.62 ± 0.57 mm in specimens from Almería and 16.12 ± 0.65 mm from Barcelona (for the 4 bigger specimens), and the height of aperture (HA)/Length (L) relation of 33 specimens measured varies between 0.62 and 0.74. The height aperture (HA) of 33 specimens varies between 4.66 ± 0.55 of Almería and 6.68 ± 0.29 mm of Sardinia. The data obtained of Fraga de Azebedo *et al.* (1969) was 0.57, although we do not know the number of specimens measured. These data indicate conchological variation among the different populations of this species, meaning one is unable to differentiate any of them by the shell. Although the population of Almería corresponds to the one with the smallest shell and the largest one to that of Barcelona populations. The protoconch, as in the genus *Bulinus* and the majority of planorbids, presents a punctiform microsculpture, whose

spots appear arranged in spiral rows (Figs 14–15, 17–19) (Germain, 1931; Walter, 1962; Martínez-Ortí *et al.*, 2015).

Shell growth in captivity (Fig. 20) The growth in length of the shell has been followed in captive *B. truncatus* specimens from El Ejido (Almería, Spain). It has been shown that this growth is logistic type, since it increases rapidly from hatching and as the population approaches the carrying capacity of the environment, the growth rate becomes slower until it finally stabilizes (Molles, 2006). The shell size grows rapidly during the first ninety days, and during the following ninety days, the growth decreases and then from 150 to 180 days it increases scarcely. From September, the length of the shell continues to increase, although more slowly than at the beginning. After one year since hatching the growth is fully stabilized, giving rise to a linear growth during the following ninety days. Growth stabilization may be due, in part, to environmental resistance (Molles, 2006), which in our case includes abiotic factors such as temperature (20°C), light (photo-period 12 h light / 12 h dark), pH (close to 7), humidity (50%), properties of the mineral water

in which they are kept and the type of nutrients that are supplied. A way of recognizing at the conchological level from the juvenile phase to the adult, whose growth is stabilized, is observing the ridge that appears in the peristome along the parietal and columellar edge of the opening (Figs 20A–C). In the most juvenile phase, 30 days after hatching, no thickening is observed in these areas of the opening (Fig. 20A). During the growth of the shell, these two zones thicken and leave a mark in the parietal and columellar areas of the opening (Fig. 20B, 240 days), and reaches a very clear edge or callus after the year of growth (Fig. 20C, 450 days), since the shell will swell more and more but without growing.

Respiratory system (Figs 21–22) It is pulmonary and also consists of a pseudobranch on the left side of the body, that corresponds to an accessory gill that presents imbricate gill plates and is capable of capturing dissolved oxygen in the water while the animal is submerged but cannot obtain free oxygen. Traditionally, the pseudobranch is a taxonomic character having been used, together with the shape of the teeth of the radula and others, to differentiate the bulinids and planorbids (Germain, 1931; Baker, 1945). The rectum is placed above the pseudobranch and the anus opens between the pseudobranch and the pneumostoma, as in all planorbids and bulinids. Germain (1931) and Baker (1945) note that the shape of the pseudobranch is very characteristic in the bulinids, lobed and with folds, while in the planorbids it is compact, very prominent and not folded, smooth with variable shape, of leaf or square (Germain, 1931: p. 516; Baker, 1945: p. 50). In the two pseudobranchs observed in our specimens of *B. truncatus* from El Ejido, between 10 and 12 imbricate folds have been found (Fig. 21), being morphologically similar to those depicted by Hubendick (1978: fig. 88) for *Bulinus* sp. and by Stiglingh *et al.* (1962: fig. 19) and for *B. africanus* (Krauss, 1848). In *Helisoma duryi* (Wetherby, 1879) we have observed the two mentioned types. The only measured pseudobranch in the present work is of the square type (see Fig. 22), short and wide, and about 1.5mm long and 1.0mm high in size.

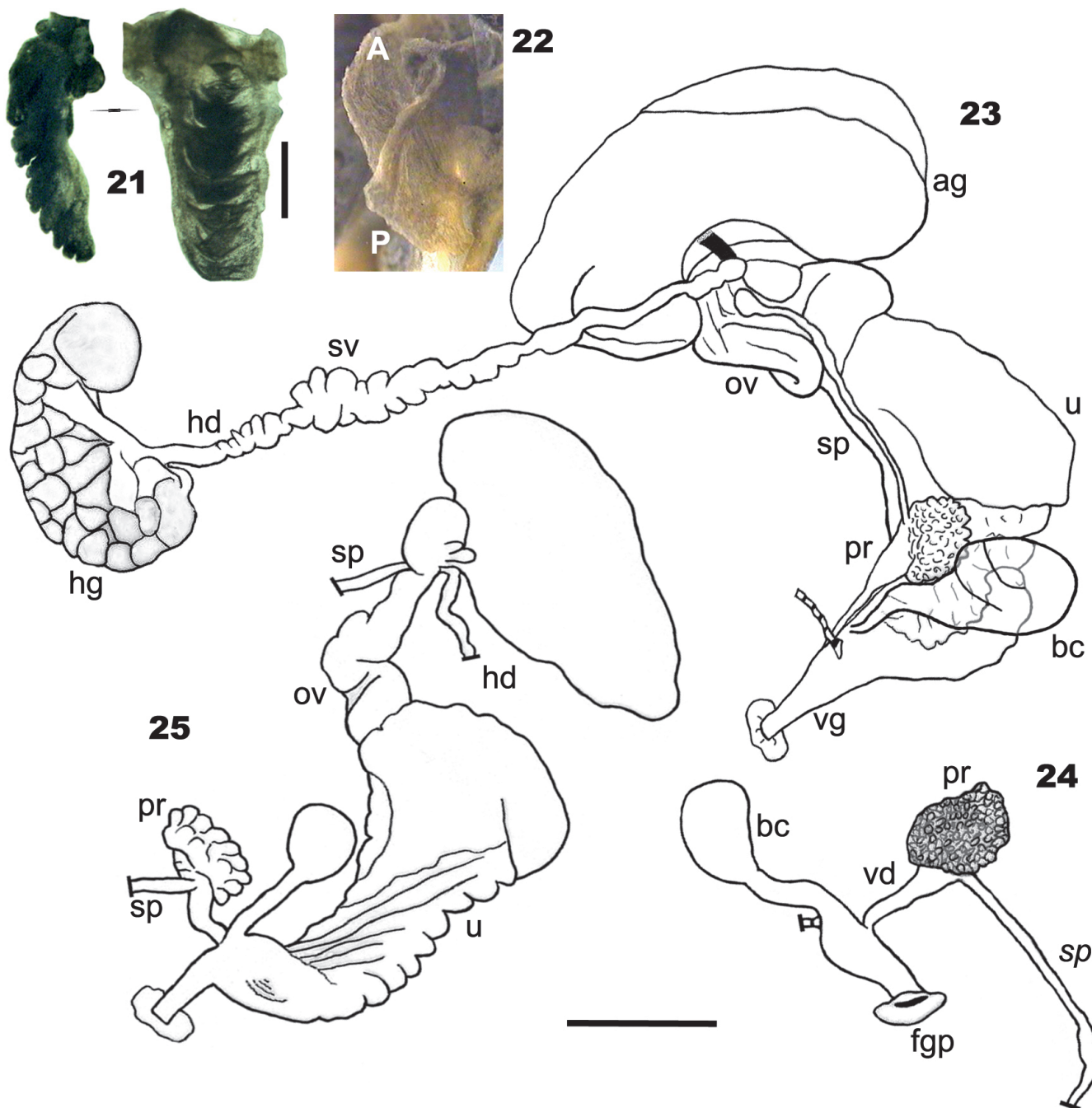
Reproductive system (Figs 23–27) Hygrophilan snails are hermaphrodites, with spermatogenesis prior to oogenesis for at least a few weeks, not

mating together at the same time. At least initially, in most cases, one snail must serve as the male and the other as the female. They have organs of both sexes, mostly separated, although other sexual organs are common (Baker, 1945). The reproductive system presents a general scheme of a diallic and ditremated reproductive system, with the presence of two genital pores, one male and the other female (Fig. 26). However, like all freshwater pulmonates, they seem to be able to reproduce successfully by self-fertilization (Dillon, 1994).

In *B. truncatus* it is quite frequent to find aphillic specimens, and therefore monotremated, showing a scar on the surface of the skin produced by the closure of the male genital pore (Larambsegue, 1939: fig. 20) (Fig. 28). The aphillic condition is also extremely common in the exanimate populations as well as the population from Sardinia (Italy), and the automixis could be the prevailing mode of reproduction (Wright, 1980; Lecis *et al.*, 1984). Lecis *et al.* (1984) indicating that in populations of San Teodoro, Río Vignola in Posada (Sardinia), only aphillic specimens were found in 360 examined specimens. Fraga de Azevedo *et al.* (1969) found only aphillic individuals in Portugal, both in northern and southern populations. Larambsegue (1939) studied two populations in Corsica (France), and although in Porto-Vecchio found both types in a euphallic/aphillic ratio 1:9.5 in Ajaccio they were discovered in 1:0.70. In Greece, this author only finds aphillic individuals in Heraklion, while in Kalamata both euphallics and aphillics individuals in ratio 1:5. The morphology of the aphillic specimens shows a reduction of the ducts and male organs, with no penial complex and with the vas deferens leading to the vagina from the prostatic gland (Larambsegue, 1939) (Figs 23–25). Depending on the degree of reduction, the prostatic gland can also become rudimentary and the canal that connects it to the hermaphroditic canal corresponds to the vestige of the spermoviduct. Externally the aphilly is translated in the absence of masculine pore, although a small whitish scar can be observed (Fig. 28).

The reproductive system of 20 specimens from El Ejido (Spain) and seven from Porto-Vecchio in Corsica (France) were examined and all found to be aphillics (Figs 23–25).

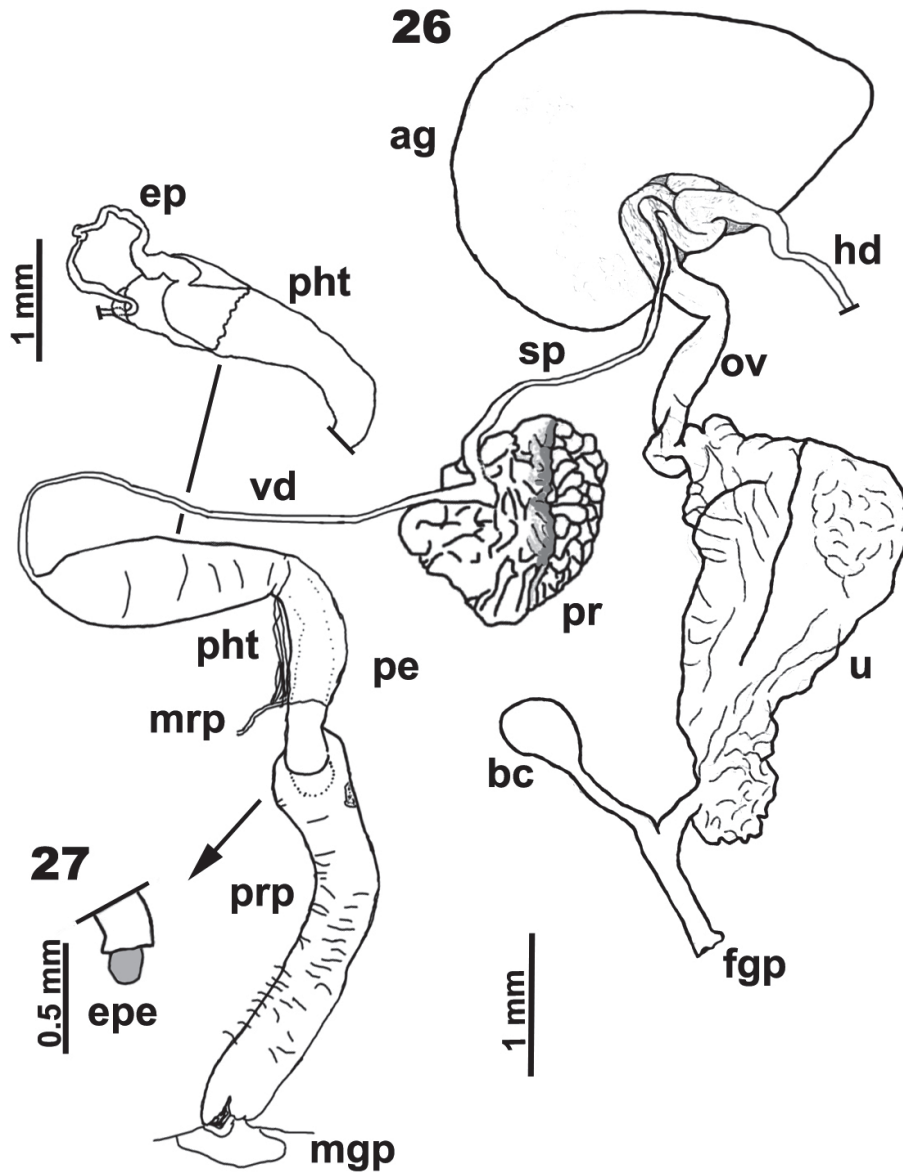
However, of 32 specimens of the Sardinia population examined, 7 euphallic and 25 aphillic



Figures 21–25 Pseudobranch and aphyallic reproductive system of *Bulinus truncatus* and the pseudobranch of *Helisoma duryi* **21** Pseudobranch of *B. truncatus*. El Ejido (Almería, Spain) **22** Pseudobranch of *H. duryi*. Ágora (Valencia, Spain) **23–24** Reproductive system of two specimens of *B. truncatus* of El Ejido (Almería, Spain) **25** Specimen from Porto-Vecchio. Corsica (France) (abbreviations: a=anus; ag=albumen gland; bc=bursa copulatrix; fgp=female genital pore; hd=hermaphroditic duct; hg=hermaphroditic gland; ov=oviduct; p=pseudobranch; pr=prostatic gland; sp=spermoviduct; sv=seminal vesicle; u=uterus; vd=vas deferens; vg=vagina). Scales=1mm.

specimens with a ratio 1:3.6 were found (Figs 26–27). Both reproductive morphologies, of euphallic and aphyallic specimens, have been scarcely illustrated up to now, with only two papers reporting where euphallic specimens of this species appear (Larambergue, 1939; Malek

& Cheng, 1974). The morphology of an euphallic specimen is characterized by the presence of the preputium, masculine organ more distal to the arrival of the phallotheca, which houses the retracted penis (Fig. 27), and goes outside through the proximal preputium that leads to



Figures 26–27 26 Reproductive system of a one euphallic specimen of San Teodoro (Sardinia, Italy) 27 Everted penis (abbreviations: ag=albumen gland; bc=bursa copulatrix; epe=everted penis; fgp=female genital pore; hd=hermaphroditic duct; hg=hermaphroditic gland; mcp=male genital pore; mrp=penis retractor muscle; ov=oviduct; pe=penis; pht=phallotheca; pr=prostatic gland; prm=penis retractor muscle; prp=preputium; sp=sperm- oviduct; u=uterus; vd=vas deferens). Scales: 26,=1mm; 27,=0.5mm.

the outside through the male genital pore. From the prostatic gland, placed on the uterus, the vas deferens arrives to the phallotheca (Fig. 26). All these male organs do not occur in an apha- llic individual, in which the vas deferens coming from the reduced prostatic gland empties directly into the vagina (Figs 23–25).

Jaw and radula (Figs 29–37) The jaws and the radula examined come from El Ejido (Almería, Spain). *B. truncatus* feeds on algae, ostracods, decaying organic matter and macrophytes,

which are found on the firm mud in the bed of the water body (Dillon, 2004). The jaw is of poly- placognata type, formed by diverse fused plates that confer striations in its outer face and a little bent down by the ends and equal for the species of the genus (Germain, 1931; Hubendick, 1978: fig. 102) (Figs 29–30). No big differences are observed with those of other planorbids of the subfamily Helisomatinae (Baker, 1945). They are covered with thick cuticle to facilitate the cut- ting of leaves, filaments and other large particles (Dillon, 2004).

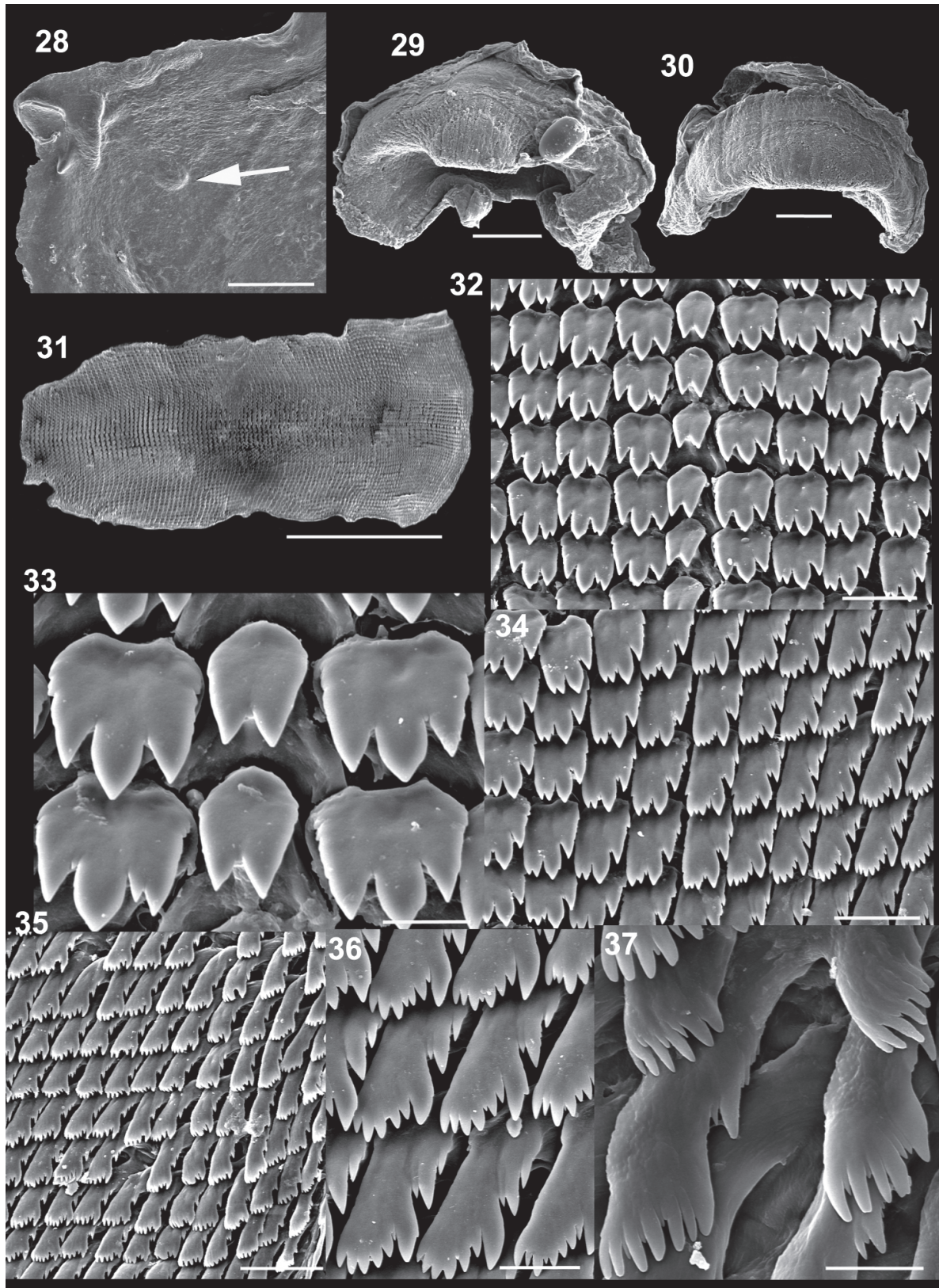
The radula has even been used to distinguish a group of species of the genus *Bulinus* (Schutte, 2009). The number of rows found in the three examined European radula of *B. truncatus* is variable, depending on the new rows that appear from the odontophore. Two of them have up to 117 rows and the other up to 120 rows (Fig. 31). Stiglingh *et al.* (1962) show that *B. tropicus* reaches 123 rows. The three radula of the Spanish population examined have the same radular formula: 29L+C+29L. Burch & Kye-Heon (1984) indicate for *B. truncatus* specimens from Sudan a radular formula of 31L+C+31L. The central tooth is bicuspid, with two cusps similar in size and with denticulate borders, arranged in the middle line of the radula and much shorter and narrower than the rest (Baker, 1945; Burch & Kye-Heon, 1984) (Figs 32–33). Burch & Kye-Heon (1984) indicate the presence in some occasions of a small central cusp to which they call “interstitial cusp”, located between the two largest cusps, although it has not been observed in the radula examined by us. The lateral teeth closest to the central tooth are basically tricuspid, with a central triangular mesocone with arrowhead shape much larger than the two lateral cusps (endoconus and ectoconus) (Figs 32–33). As we progress towards the edges of the radula, the endoconus lengthens, occupying the inner and central margins of the tooth, while both the mesocone and the ectocone shortens and divides forming small cusps and occupying the lateral, external margin of the tooth (Figs 32–33). This dental transition to the margin is gradual, not observing sudden changes in its general form (Figs 34–35) (Burch & Kye-Heon, 1984). The marginal teeth, that occupy the outermost margin, are much narrower, elongated and minutely denticulate (Figs 36–37).

Ecological data *B. truncatus* is an ubiquistic species with great capacity of self-fertilization that lives in coastal environments, whether they are lagoons, ponds, upwelling or river mouths where there is hardly any current or low flow-rate. The living Spanish population of *B. truncatus* studied comes from the pond of El Ejido (Almería). The snails have been found living on the surface of aquatic plants, with submerged or floating leaves of the phanerogam *Stuckenia pectinatus* (Linnaeus, 1753) and the green alga *Chara vulgaris* (Linnaeus, 1753) and on the mud of the bottom of the pond not far from the shore (Martínez-Ortí *et*

al., 2015). Thomas (1995) indicates that *B. truncatus* lives on *Stuckenia* spp., *Ceratophyllum* spp. and *Lemna* spp. Like other intermediate freshwater snails hosts of *Schistosoma* it is able to tolerate desiccation in shaded temporary water bodies that gradually dried up (Chu *et al.*, 1967; Brown, 1994; Van Aardt *et al.*, 2007). The Almería pond considerably reduces its extension due to the low rainfall and high evaporation that it undergoes throughout the year, especially in the summer season, in which time *B. truncatus* can survive being buried in the mud (Ghandour, 1987; Van Aardt *et al.*, 2007).

B. truncatus is a freshwater hermaphroditic snail with mean selfing rates exceeding 80% in natural populations. Therefore, when an individual of *B. truncatus* finds a new suitable ecosystem, the population grows rapidly and continuously, and allows it to expand its geographical area of occupation, through self-fertilization capacity, even becoming a pest, and its eradication is practically impossible. These isolated snails in the natural environment that grow more quickly can produce a much larger number of clusters, thus the population density increases and colonies originate, most likely as an effect of the absence of copulation (Bayomy & Joosse, 1987). The population of Almería was visited twice, once in 6th December 2014 when the population was abundant (approx. 50 specimens/m²), with visible clusters and a large amount of water while in 8th July 2015, the water body was quite small and the population much smaller (approx. 6 specimens/m²), with scarce clusters.

In nature, *B. truncatus* eggs require temperatures between 12.5°C and 35°C to hatch (McCrees & Booth, 2013). Under laboratory conditions the isolated individuals start to lay eggs when they are approximately 5mm long (e.g. 35 days of age at 25°C) (Doums *et al.*, 1998). In our specimens the average value reached 5.31mm (n=30). The physical-chemical parameters of water are important for the life of molluscs. A pH lower than 5.2 and high salinity inhibit the development of schistosomiasis vector snails (Okland, 1990; Thomas, 1995). In the south of Tunisia *B. truncatus* lives between 1220 and 2240 µS at 18°C (Brown, 1994) and *B. obtusispira* less than 600 µS. The parameters obtained from the water in El Ejido (Almería) in December 2014 were water temperatures of 18°C (11 h, with 16.0°C) pH of 7.1 and a conductivity of 1250 µS, while in July



Figures 28–37 Some morphological characters of *Bulinus truncatus* of El Ejido (Spain) 28 Closed male genital pore 29–30 Jaws 31 General view of radula 32–37 Radula details 32 Central and first lateral row 33 Central and first lateral teeth 34–35 Denticular transition of lateral teeth towards the margin of the radula 36–37 Detail of marginal teeth.

Scales: 28,=300 μ m; 29,30,=50 μ m; 31,=500 μ m; 32,34,=20 μ m; 33,=9 μ m; 35,=30 μ m; 36,=10 μ m; 37,=6 μ .

2015, with a large reduction of the pond extension, the data were as follows: dissolved oxygen: 2.5 mg/l (32%), temperature of water 34.6°C (13:45 h, with 29°C temperature) and a conductivity of 550 µS.

It is of interest to point out that the longevity of the planorbids in the wild is not well understood, and they can probably live between four and five years (Baker, 1945). Some species in captivity like *Helisoma trivolvis* (Say, 1917) lives up to 16 months, *H. duryi* one year and *Planorbarius corneus* (Linnaeus, 1758) up to two years. In our laboratory with water temperature at 20°C, *B. truncatus* from Almería (Spain), reach almost two years of life, and between a month and a month and a half reach sexual maturity.

During our visits to the El Ejido pond (Almería), between July 2015 and the end of 2019, we identified some bird species of interest: the Eurasian coot (*Fulica atra* Linnaeus, 1758), the little egret [*Egretta garzetta* (Linnaeus, 1766)], the grey heron (*Ardea cinerea* Linnaeus, 1758) and the black-winged stilt [*Himantopus himantopus* (Linnaeus, 1758)].

These bird species are of interest because they can also be implicated as passive transporters in the passage from Africa to Southern Europe, be they lay, juveniles and even adults of *B. truncatus*, on their wings, feathers or legs (Martínez-Ortí *et al.*, 2019).

Besides, the pond is often visited daily by a flock of about 300 sheep and some goats. The pond that we have recorded in these last two years at least has not never dried up. The water comes from the rains, from the surpluses of the greenhouses and from a broken drain pipe from the nearby hospital.

DISCUSSION

Because of the conchological characteristics of *B. truncatus*, specimens can be confused with those of the physid *Physa acuta*, an exotic species widely distributed throughout Europe, with which it can coexist. Walter (1962) besides showing the punctiform ornamentation of *B. truncatus* of Sardinia and Egypt also shows that of *B. forskalii* (Ehrenberg, 1831) and *B. globosus* (Morelet, 1866) and even that of the genus *Indoplanorbis*. Walter (1962) points out the great importance of this conchological character, of great help to differentiate the hyrophilic snails with high shells,

that transmit schistosomiasis from those that do not, as those species belonging to the family Physidae Fitzinger, 1833, whose protoconch is smooth (Martínez-Ortí *et al.*, 2015).

According to the reproductive system the aphyallic condition is predominant in *B. truncatus* and its acquisition of aphyally is still unclear (Leonard & Córdoba-Aguilar, 2010). The production of aphyallic specimens in *B. truncatus* involves both genetic factors, and other environmental factors such as higher temperatures, which increase the appearance of aphyallic specimens, as also happens in reptiles, or exposure to light in slugs (Nicklas & Hoffmann, 1981; Doums *et al.*, 1996; Dillon, 2004). An aphyallic specimen can only receive sperm and auto-fertilize, acting then as a female, when copulation occurs with a euphyallic specimen (Pokryszko, 1987). After the first laying, the aphyallic specimens of Posada (Sardinia) retain, in both the gonad and bursa copulatrix, the mature sperm (Lecis *et al.*, 1984). The euphyallic specimens seem to have mechanisms that favour fertilization by allosperm (donated by a partner) over autosperm. Autosperm, produced endogenously, is generally transported through the male duct into the vas deferens, along a prostatic gland, to the penis (Dillon, 2004). *Bulinus* can store sperm over seven weeks of starvation, eight weeks of low temperature, or four weeks of desiccation (Rudolph & Bailey, 1985). However, the euphyallic forms are also known in *B. truncatus* (Larambergue, 1939; Malek & Cheng, 1974; Jarne *et al.*, 1992). In Bulininae, the male reproductive system of euphyallic specimens is very characteristic, and the penial complex next to the prostatic gland has great taxonomic importance (Baker, 1945) (Fig. 26).

Infection of molluscs by digenean trematode parasites typically results in the repression of reproduction, so-called parasitic castration. The aphyally can be an adaptation to reduce the consequences of parasitism of flukes (Schrag & Rollinson, 1994). Parasitic castration results in the penis being greatly reduced, as is the whole reproductive system, and sometimes complete destruction of the gonads. This is known to occur by altering the expression of a range of host neuropeptide genes (Adiyodi & Adiyodi, 1994; Rice *et al.*, 2006). The size and prominence of the accessory sex organs (penis and vas deferens in male) become abnormal after the invasion of the gonads by parasites. It seems to indicate that

the size of these sex organs depends on gonadal activity (Reeves, 1936; Leonard & Córdoba-Aguilar, 2010). Rothschild (1938) found in *Hydrobia ulvae* (Pennant, 1777) parasitized specimens with abnormal penises, with sizes more reduced (hemiphally), and absence total of penis due to the total destruction of the testicle (aphally). However, the female organs and ducts are perfectly normal and allow the reproduction through self-fertilization, a unique way of reproduction for such specimens. The aphallic specimens are more fertile and lay more eggs than the euphallics, important for the colonization of new places. The aphally is transmitted preferably to its descendants, although they can also produce euphallic descendants (Larambergue, 1939; Jarne *et al.*, 1992; own dates). Although initially it was thought that these individuals can invest more energy in reproduction, avoiding it in the formation and maintenance of the male organs (Jarne *et al.*, 1992), later Ostrowski *et al.* (2003) suggest that the cost of the construction and maintenance of the phallus must be very low in this species and suggest investigating the cost associated with the use of the penis (e.g. search of a mate, courtship and copulatory behaviours, sperm production) to explain the maintenance of high frequencies of aphallic individuals in natural populations.

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REFERENCES

- ADANSON M 1757 Histoire naturelle du Sénégal. Coquillages, Paris.
- ADIYODI KG & ADIYODI RG (Eds.) 1994 Reproductive Biology of Invertebrates, vol 2, *Spermatogenesis and Sperm function*, Wiley, Chichester.
- ALDRECHT C, KUHN K & STREIT B 2007 A molecular phylogeny of Planorboidea (Gastropoda, Pulmonata): insights from enhanced taxon sampling. *Zoologica Scripta* 36(1): 27–39. <https://doi.org/10.1111/j.1463-6409.2006.00258.x>
- ALLAN F, ROLLINSON D, SMITH JE & DUNN AM 2009 Host choice and penetration by *Schistosoma haematobium* miracidia. *Journal of Helminthology* 83: 33–38. DOI: <https://doi.org/10.1017/S0022149X08073628>
- AUDOUIN V 1826 Explication sommaire des planches de Mollusques de l’Egypte et de la Syrie publiées par J. C. Savigny (1917). Description de l’Egypte ou recueil des observations et des recherches qui ont été faites en Egypte pendant l’expédition de l’armée française, publiée par les ordres de sa majesté l’empereur Napoléon le grand. Histoire Naturelle, Imprimerie impériale, Paris, Animaux invertébrés 1(4): 7–56.
- BAKER FC 1945 The molluscan family Planorbidae. The University of Illinois Press Urbana. Illinois, 1–530 pp. (Collation, Revision, and Additions by H.J. Van Cleave).
- BAYOMY MFF & JOOSSE J 1987 Effects of Isolation, Grouping and Population Density on Fecundity of *Bulinus truncatus*. *International Journal of Invertebrate Reproduction and Development* 12(3): 319–330.
- BERRY A, MONÉ H, IRIART X, MOUAHID G, ABBO O, BOISSIER J, FILLAUX J, CASSAING S, DEBUISSON C, VALENTIN A, MITTA G, THÉRON A & MAGNAVAL JF 2014 *Schistosomiasis haematobium*, Corsica, France. *Emerging Infectious Diseases* 20(9): 1595–1597. DOI: <http://dx.doi.org/10.3201/eid2009.140928>
- BOISSIER J, MONE H, MITTA G, BARGUES MD, MOLYNEUX D & MAS-COMA S 2015 Schistosomiasis reaches Europe. *Lancet infectious Diseases* 15: 757–8. DOI: [https://doi.org/10.1016/S1473-3099\(15\)00084-5](https://doi.org/10.1016/S1473-3099(15)00084-5)
- BOISSIER J, GRECH-ANGELINI S, WEBSTER BL, ALLIENNE J-F, HUYSE T, MAS-COMA S, TOULZA E, BARRE-CARDI

- H, ROLLINSON D, KINCAID-SMITH J, OLEAGA A, GALINIER R, FOATA J, ROGNON A, BERRY A, MOUAHID G, HENNERON R, MONE H, NOEL H & MITTA G 2016 Outbreak of urogenital schistosomiasis in Corsica (France): an epidemiological case study. *Lancet infectious Diseases* 16(8): 971–979. DOI: [https://doi.org/10.1016/S1473-3099\(16\)00175-4](https://doi.org/10.1016/S1473-3099(16)00175-4)
- BOYER F & AUDIBERT C 2007 Le matériel d'auteur conservé au Muséum de Lyon pour les taxa de Michaud, 1828 et 1829. *Cahiers scientifiques* 13: 149–159.
- BOUCHET P & DANRIGAL F 1982 Napoleon's Egyptian campaign (1798–1801) and the Savigny collection of shells. *Nautilus* 96(1): 9–24.
- BOUCHET P, ROCROI JP, HAUSDORF B, KAIM KANO Y, NÜTZE A, PARKHAEV P, SCHRÖDL M & STRONG EE 2017 Revised Classification, Nomenclator and Typification of Gastropod and Monoplacophoran Families. *Malacologia* 61(1–2): 1–526. DOI: <https://doi.org/10.4002/040.061.0201>
- BROWN DS 1994 *Freshwater snails of Africa and their Medical Importance*, Taylor & Francis Ltd., Second edition. London, 1–608.
- BRUMPT E 1929 Cycle évolutif du *Schistosoma bovis* (*Bilharzia crassa*). Infection spontanée du *Bulinus contortus* en Corse. *Comptes Rendus de l'Académie des Sciences, Paris* 189: 879.
- BURCH JB 1960 Cytological studies of Planorbidae (Gastropoda: Basommatophora). I. The african subgenus *Bulinus* s.s. *Malacologia* 1(3): 387–400.
- BURCH JB & KYE-Heon J 1984 The radular teeth of selected Planorbidae. *Malacological Review* 17: 67–84.
- CHRISTENSEN NO, FRANSEN F & ROUSHDY MZ 1980 The influence of environmental condition sand and parasite-intermediare host-related factors on the transmission of *Echinostoma*. *Zeitschrift für Parasitenkunde* 63: 47–63.
- CHU KY, ARFAA F & MASSOUD J 1967 The survival of *Bulinus truncatus* buried in mud under experimental outdoor conditions. *Annals of Tropical Medicine & Parasitology* 61: 6–10. DOI: <https://doi.org/10.1080/00034983.1967.11686449>
- COLUZZI A, NUVOLE A, ORECCHIA P & PAGGI L 1965 Indagini su *Bulinus truncatus* e *Schistosoma bovis* della Sardegna. *Parassitologia* 7(2–3): 173–229.
- DAVIS GM 1978 Experimental methods in molluscan systematics. *Pulmonates, Systematics, Evolution and Ecology*. Fretter & Peake eds. vol. 2A, chapter 3: 99–169.
- DEIANA S, ARRU E & NUVOLE A 1966a L'infestazione di *Bulinus contortus* da cercarie di *Schistosoma bovis* e di *Paramphistomum cervi* in Sardegna. *Bollettino della Società italiana di Biologia Sperimentale* 42(14): 902–903.
- DEIANA S, ARRU E & NUVOLE A 1966b Suscettibilità di *Bulinus contortus* del Rio Mascari all'infestazione di *Schistosoma bovis*. *Bollettino della Società italiana di Biologia Sperimentale* 42(14): 904–905.
- DILLON RT 2004 *The Ecology of Freshwater Molluscs*. Cambridge University Press, 1–509.
- DOUMS C, BRÉMOND P, DELAY B & JARNE P 1996 The genetical and environmental determination of phally polymorphism in the freshwater snail *Bulinus truncatus*. *Genetics* 142: 217–225. DOI: [10.1093/genetics/142.1.217](https://doi.org/10.1093/genetics/142.1.217)
- DOUMS C, PERDIEU MA & JARNE P 1998 Resource allocation and stressful conditions in the aphallic snail *Bulinus truncatus*. *Ecology* 79: 720–733. DOI: [10.2307/176965](https://doi.org/10.2307/176965)
- DOUMENGE JP, MOTT KE & CHEUNG C 1987 Atlas of the global distribution of schistosomiasis continued. Presses Universitaires de Bordeaux, 398 pp.
- FALKNER G, RIPKEN THEJ & FALKNER M 2002 Mollusques continentaux de France. Liste de Référence annoté et Bibliographie. *Patrimoines naturels* 52: 1–350.
- FRAGA DE AZEVEDO J, SAMPAIO XL, PEQUITO MM & SIMÕES M 1969 Contribution to morphological and biochemical identification of some strains of the *Bulinus truncatus* group. *Malacologia* 9(1): 25–34.
- GHANDOUR AM 1987 The resistance of snail intermediate hosts of Schistosomiasis in Saudi Arabia to desiccation. *Journal of Arid Environments* 13: 273–278.
- GERMAIN L 1931 *Mollusques terrestres et fluviatiles. Faune de France* 22 vol. 2: 479–897.
- GIUSTI F, MANGANELLI G & SCHEMBRI PJ 1995 *The non-marine molluscs of the Maltese Islands*. Monografie XV. Museo Regionale di Scienze Naturali 1–607.
- HAAS F 1935 Zur Systematik und geographischen Verbreitung der im pallärtischen Gebiet vorkommenden *Bulinus*-Arten. (Moll. Pulm.). Leipzig, Akademische Verlagsgesellschaft – Geschichte 230–244.
- HABERL B, KALBE M, FUCHS H, STRÖBEL M, SCHMALFY G & HAAS W 1995 *Schistosoma mansoni* and *S. haematobium*: Miracidial host-finding behaviour is stimulated by macromolecules. *International Journal for Parasitology* 25(5): 551–560. DOI: [10.1016/0020-7519\(94\)00158-K](https://doi.org/10.1016/0020-7519(94)00158-K)
- HOLTFRETER MC, MONÉ H, MÜLLER-STÖVER I, MOUAHID G & RICHTER J 2014 *Schistosoma haematobium* infections acquired in Corsica, France, August 2013. *Eurosurveillance* 19(22): 1–3. DOI: [10.2807/1560-7917.es2014.19.22.20821](https://doi.org/10.2807/1560-7917.es2014.19.22.20821)
- HUBENDICK B 1978 Systematics and comparative morphology of the Basommatophora. *Pulmonates, Systematics, Evolution and Ecology*, Fretter & Peake eds. vol. 2A, chapter 1, 1–47.
- JARNE P, FINOT L, BELLEC C & DELAY B 1992 Aphally Versus Eupally in Self-Fertile Hermaphrodite Snails from the Species *Bulinus truncatus* (Pulmonata: Planorbidae). *The American Naturalist* 139(2): 424–432.
- JONES KE, PATEL NG, LEVY MA, STOREYGARD A, BALK D, GITTLEMAN JL & DASZAK P 2008 Global trends in emerging infectious diseases. *Nature* 451: 990–993. DOI: <https://doi.org/10.1038/nature06536>
- KINCAID-SMITH J, REY O, TOULZA E, BERRY A & BOISSIER J 2017 Emerging Schistosomiasis in Europe: A need to Quantify the Risks. *Trends in Parasitology* 33: 600–609. DOI: <https://doi.org/10.1016/j.pt.2017.04.009>
- LARAMBERGUE M 1939 Étude de l'autofécondation chez les gasteropodes pulmonés recherches sur l'aphallie et la fécondation chez *Bulinus* (*Isidora*) *contortus* Michaud. *Bulletin Biologique de la France et de la Belgique* 73(1–2): 19–231.

- LECIS AR, RANDACCIO A & LAUDANI U 1984 Reproduction in the fresh water snail, *Isidora truncata* (Gastropoda: Planorbidae). *Bolletino di zoologia* **51**: 379–384.
- LEONARD J & CÓRDOBA-AGUILAR A (Eds.) 2010 The Evolution of Primary Sexual Characteristics in Animals. Oxford University Press, New York, 537 pp.
- MALEK EA & CHENG TC 1974 *Medical and economic malacology*, Academic press New York and London, 1–398.
- MARTÍNEZ-ORTÍ A, BARGUES MD & MAS-COMA S 2015 Dos nuevas localizaciones para España de *Bulinus truncatus* (Audouin, 1827) (Gastropoda: Planorbidae), hospedador intermediario de Schistosomiasis urinaria. *Arxius de Miscel·lània Zoològica* **13**: 25–31. DOI: <https://doi.org/10.32800/amz.2015.13.0025>
- MARTÍNEZ-ORTÍ A, VILAVELLA D, BARGUES MD & MAS-COMA S 2019 Risk map of transmission of urogenital schistosomiasis by *Bulinus truncatus* (Audouin, 1827) (Mollusca Gastropoda, Bulinidae) in Spain and Portugal. *Animal Biodiversity and Conservation* **42.2**: 257–266. DOI: <https://doi.org/10.32800/abc.2019.42.0257>
- MAS-COMA S, VALERO MA & BARGUES MD 2008 Effects of climate change on animal and zoonotic helminthiasis. *Revue scientifique et technique-office international des epizooties* **27**(2): 443–452.
- MAS-COMA S, VALERO MA & BARGUES MD 2009 Climate change effects on trematodiasis, with emphasis on zoonotic fascioliasis and schistosomiasis. *Veterinary Parasitology* **163**(4): 264–280. DOI: [10.1016/j.vetpar.2009.03.024](https://doi.org/10.1016/j.vetpar.2009.03.024)
- MCCREESH N & BOOTH MB 2013 Challenges in predicting the effects of climate change on *Schistosoma mansoni* and *Schistosoma haematobium* transmission potential. *Trends in Parasitology* **29**(11): 548–555. DOI: <https://doi.org/10.1016/j.pt.2013.08.007>
- MOLLES MC 2006 *Ecología. Conceptos y aplicaciones. Capítulo 11- Crecimiento de poblaciones*, Ed. Mc Graw-Hill-Interamericana, Tercera edición, University of Mexico, 297–300.
- MORENS DM & FAUCI AS 2013 Emerging Infectious Diseases: Threats to Human Health and Global Stability. *PLoS Pathog* **9**(7): e1003467. DOI: <https://doi.org/10.1371/journal.ppat.1003467>
- MORGAN JA, DE JONG RJ, JUNG Y, KHALLAAYOUNE K, KOCK S, MKOJI GM & LOKER S 2002 A phylogeny of planorbid snails, with implications for the evolution of *Schistosoma* parasites. *Molecular Phylogenetics and Evolution* **25**(3): 477–488. DOI: [10.1016/s1055-7903\(02\)00280-4](https://doi.org/10.1016/s1055-7903(02)00280-4)
- NICKLAS NL & HOFFMANN RJ 1981 Apomictic parthenogenesis in a hermaphroditic terrestrial slug, *Deroceras laeve* (Müller). *Biological Bulletin* **160**: 123–135.
- NOBRE A 1941 *Fauna malacológica de Portugal. II. Moluscos terrestres e fluviais*. Memórias e estudos do Museu Zoológico da Universidade de Coimbra, Porto.
- OKLAND J 1990 *Lakes and snails*, Universal Book Services, Dr. W. Backhuys, Oegstgeest, 1–156.
- OSTROWSKI MF, JARNE J & DAVID P 2003 A phallus for free? Quantitative genetics of sexual trade-offs in the snail *Bulinus truncatus*. *Journal of Evolutionary Biology* **16**: 7–16. DOI: [10.1046/j.1420-9101.2003.00508.x](https://doi.org/10.1046/j.1420-9101.2003.00508.x)
- POKRYSZKO BM 1987 On the aphyllity in the Vertiginidae (Gastropoda: Pulmonata: Orthurethra). *Journal of Conchology* **32**: 365–375.
- ROTCHSCHILD M 1938 Further observations on the effect of trematode parasites on *Peringia ulvae* (Pennant) 1777. *Novitates Zoologicae*, Tring, vol. XLI, pp. 84–102.
- RUDOLPH PH & BAILEY JB 1985 Copulation as females and use of allosperm in the freshwater snail genus *Bulinus* (Gastropoda: Planorbidae). *Journal of Molluscan Studies* **51**: 267–275.
- SALAS-CORONAS J, CABEZAS-FERNÁNDEZ MT, LOZANO-SERRANO AB, SORIANO-PÉREZ MJ, VÁZQUEZ-VILLEGAS J & CUENCA-GÓMEZ JA 2018 Newly Arrived African Migrants to Spain: Epidemiology and Burden Disease. *The American Journal of Tropical Medicine and Hygiene* **98**(1): 319–325. DOI: <https://doi.org/10.4269/ajtmh.17-0604>
- SALAS-CORONAS J, VÁZQUEZ-VILLEGAS J, LOZANO-SERRANO AB, SORIANO-PÉREZ MJ, CABEZA-BARRERA I, CABEZAS-FERNÁNDEZ MT, VILLAREJO-ORDÓÑEZ A, SÁNCHEZ-SÁNCHEZ JC, ABAD VIVAS-PÉREZ JI, VÁZQUEZ-BLANC S, PALANCA-GIMÉNEZ M & CUENCA-GÓMEZ JA 2019 Severe complications of imported schistosomiasis, Spain: A retrospective observational study. *Travel Medicine and Infectious Disease* 2020 May–Jun; **35**: 101508. DOI: <https://doi.org/10.1016/j.tmaid.2019.101508> Epub 2019 Nov 5. PMID: 31704484.
- SALAS-CORONAS J, BARGUES MD, LOZANO-SERRANO AB, ARTIGAS P, MARTÍNEZ-ORTÍ A, MAS-COMA S, MERINO-SALAS S & ABAD VIVAS-PÉREZ JI 2021 Evidence of autochthonous transmission of urinary schistosomiasis in Almeria (southeast Spain): an outbreak analysis. *Travel Medicine and Infectious Disease* **44**: 102165. DOI: [10.1016/j.tmaid.2021.102165](https://doi.org/10.1016/j.tmaid.2021.102165)
- SAVIGNY JC 1817 *Description de l’Égypte, ou recueil des observations et des recherches qui ont été faites en Égypte pendant l’Expédition de l’armée française, publiée par ordre du gouvernement, Histoire naturelle, Planches, vol. II*, Imprimerie royale, Paris.
- SCHRAG S & ROLLINSON D 1994 Effects of *Schistosoma haematobium* infection on reproductive success and male outcrossing ability in the simultaneous hermaphrodite, *Bulinus truncatus* (Gastropoda: Planorbidae). *Parasitology* **108**: 27–34. DOI: <https://doi.org/10.1017/S0031182000078483>
- SCHUTTE CHJ 2009 Notes on the radular mesocone as a criterion for distinguishing between the *Tropicus* and *Truncatus* groups of the genus *Bulinus* (Mollusca: Basommatophora). *Annals and Magazine of Natural History* **8**(91): 409–419. DOI: <https://doi.org/10.1080/00222936508651588>
- SCHWETZ J 1954 L’influence du milieu sur la taille et la forme du même Planorbe ou du même *Bulinus*. *Annales de la Société royale zoologique de Belgique* **85**(1): 23–34.

- SOUTHGATE VR & KNOWLES RJ 1977 On *Schistosoma margrebowiei* Le Roux, 1933: the morphology of the egg, miracidium and cercaria, the compatibility with species the *Bulinus*, etc. *Zeitschrift für Parasitenkunde* 54: 233–250.
- STIGLINGH I, VAN EEDEN JA & RYKE PAJ 1962 Contribution to the morphology of *Bulinus tropicus*. *Malacologia* 1: 73–114.
- THOMAS JD 1995 The Snail Hosts of Schistosomiasis: Some Evolutionary and Ecological Perspectives in Relation to Control. *Memórias do Instituto Oswaldo Cruz* 90(2): 195–204. DOI: <http://dx.doi.org/10.1590/S0074-02761995000200012>
- WALTER HJ 1962 Punctuation of the embryonic shell of Bulininae (Planorbidae) and some other Basommatophora and its possible taxonomic-phylogenetic implications. *Malacologia* 1(1): 115–137.
- VAN AARDT WJ & STEYTLER SSJ 2007 Shell permeability and desiccation physiology of the freshwater snail *Bulinus (Bulinus) tropicus* (Krauss). *Malacologia* 49(2): 339–349.
- WHO 2010 Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases. WHO Library Cataloguing-in-Publication Data, 172 pp.
- WHO 2013 Sustaining the drive to overcome the global impact of neglected tropical diseases. Second WHO report on neglected tropical diseases. WHO Library Cataloguing-in-Publication Data, 138 pp.
- WRIGHT CA 1980 Biology and systematics of the molluscan hosts of schistosomiasis in the Mediterranean basin in perspective. *Parassitologia* 22: 257–262.