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New records of *Plagyrona* Gittenberger, 1977
(Gastropoda: Eupulmonata: Valloniidae)
from Europe and problems about specific determination

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Keywords: Mediterranean area, *Plagyrona placida*, *Plagyrona angusta*, species distribution.

SUMMARY

The genus *Plagyrona* Gittenberger, 1977, includes only two small species of terrestrial snail: *Plagyrona angusta* Holyoak and Holyoak, 2012, and *Plagyrona placida* (Shuttleworth, 1852). While *P. angusta* is known only from Portugal, *P. placida* has a vast but fragmented distribution: it is known from some of the Macaronesian islands (Madeira and the Canary Archipelago) and some European countries: Portugal, France (in Corsica only), Italy (including Sardinia and the Tuscan Archipelago), Albania, Greece (in the Ionian Islands only) and North Africa (Algeria). New research has led to redefine the distribution range of *P. angusta*, identifying new populations in Spain (Balearic Islands), continental France (Var department), southern Italy (Campania), Sardinia and Greece (Kerkyra Island) and to discover new sites of *P. placida* on Pantelleria island and of *Plagyrona* spp. in Zannone island and southern Italy. The specific determination of these and others populations by examination of recent literature from Sardinia, Campania and Calabria, was carried out on a morphometric basis, but, for some populations, the variability of the diagnostic characters and the limited number of available specimens, do not allow a precise assignment. On the other hand, the existence of two distinct species is not evident at all, at least in the Mediterranean countries.

INTRODUCTION

The genus *Plagyrona* Gittenberger, 1977, is one of the six European genera of small terrestrial molluscs belonging to the circumglobal family of the Valloniidae Morse, 1864, which includes about 65 species (Schileyko 1998). The genus *Plagyrona* includes two species only: *P.*

placida (Shuttleworth, 1852) and *P. angusta* Holyoak and Holyoak, 2012.

Plagyrona placida has a fairly large but fragmented distribution (Cianfanelli et al. 2013, Holyoak and Holyoak 2012, Bank and Neubert 2017), having been reported from the Canary Islands (El Hierro, La Gomera, La Palma, Tenerife) (Wollaston 1878, Ibáñez et al. 2001,

Bank et al. 2002, Brito and Fraga 2010), Madeira (Wollaston 1878, Waldén 1983, Bank et al. 2002, Cameron et al. 2006, Seddon 2008) and Porto Santo island (as fossil only; Cameron et al. 2006), Portugal (Servain 1880, Silva and Castro 1887, Locard 1899, Gittenberger 1977, Oliveira 2008, 2009, 2010, Torres and Oliveira 2010), Corsica (Ripken and Bouchet 1998, Falkner et al. 2002), Albania (Reischhütz et al. 2008, Ferhér and Eróss 2009), Italy, in the Tuscan Archipelago and Sardinia included (Cianfanelli et al. 2013, Manganelli et al. 2015, 2017) and Greece, in the Ionian Islands of Kerkyra, Ithaki and Kefhalonia (Gittenberger 1989); moreover, it is also known from Algeria (north-western Africa) (Bourguignat 1863, 1864, Gittenberger 1977). *Plagyrona angusta*, on the other hand, has been described and reported in Portugal only (Holyoak and Holyoak 2012).

In Cianfanelli et al. (2013) a synthesis of the knowledge on the distribution of *P. placida* was carried on, reporting the species collected for the first time in several Italian localities (Sardinia, Campania and Calabria); subsequently Manganelli et al. (2015, 2017) discovered populations of *Plagyrona* in the Tuscan Archipelago (Capraia, Elba, Montecristo, Giglio islands) and from Monte Argentario in Tuscany, always identifying them as *P. placida*. This research has ascertained a wider distribution for this genus, which was found in Balearic Islands in Spain (Mallorca Island), in southern France (Var department), and in some small Italian islands (Zannone and Pantelleria). The characters that distinguish the two species are not constant and therefore a morphometric study has been carried out for the specific attribution, in order to verify the diagnostic characters and update the geographical distribution of these taxa.

MATERIALS AND METHODS

Shells were collected in Mediterranean areas of Spain, France, Italy and Greece, from alluvial debris or litter; they were sieved using

decreasing mesh sieves and the specimens were visually separated or through a binocular microscope. The photographs of the shells were taken with the light microscope and related software. All dimensions (H, shells height; D, shells diameter; h, aperture height; d, aperture diameter; U, umbilicus diameter) were measured using a micrometer on the light microscope. For the analysis of morphometric data (ratios between the dimensions of the shell for describing the differences in shape between the two species: H/D, h/d, U/d, U/H) the Excel software 2016 (for linear regression curves, for the purpose of verifying the isometric or allometric model of growth, verifying the best regression curve in order to apply a correction value to the ratios of the parameters for the specific determination on the basis of the results in literature) and PAST 3.19 (for Principal Components Analysis, PCA, for the purpose of verifying the weight of the diagnostic parameter and how clustering the specimens/populations, and Non-metric multidimensional scaling, NMDS, for grouping together the objects (or variables), based on their similarity or correlation, so that the elements of a group are as similar as possible to each other) were used (Hammer et al. 2001). Data for PCA and NMDS were normalized.

The collection data are listed as follows: locality, collection site, altitude, municipality and province/department/region in parentheses, UTM coordinates (ED 50), denomination of the SCI (a Site of Community Importance, as defined in the European Commission Habitats Directive, 92/43/EEC), if the locality is inside a protected area, collectors and dates, number of shells and collection in parentheses. Names of the localities were taken from the following maps: for Italy, from the Istituto Geografico Militare 1:25,000 chart; for France, from Institut Géographique National 1:25,000; for Spain, Mallorca, from Kompass 1:75,000 map; for Greece, from the Greek Atlas 1:300,000, Studio F.M.B. Bologna 1991/92 edition; UTM coordinates were taken from the same maps or detected by GPS.

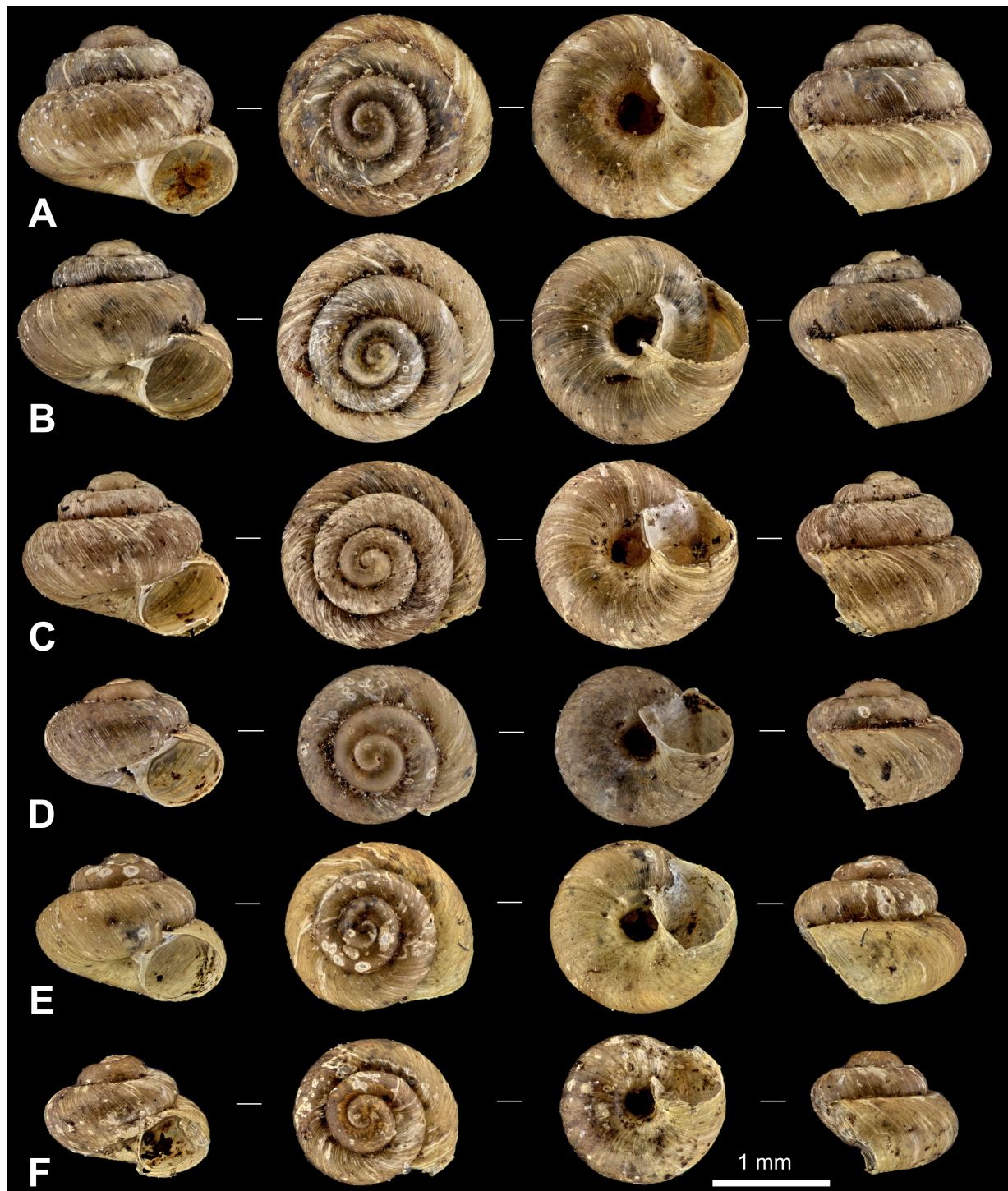


Figure 1. Shells of *Plagyrona angusta* Holyoak and Holyoak, 2012, from France (A), Greece (B-C), Spain (D) and Italy (E-F). A, Gapeau River, downstream the dam near La Clapières, upstream L'Oratoire (Hyères, Var, Provence-Alpes-Côte d'Azur), 32T KN6881, M. Bodon & S. Cianfanelli leg. 29/12/2016 (MZUF GC/52475); B-C, small valley from Lakones to Paleokastritsa (Paleokastritsa, Kerkyra, Ionian Islands), 34S CJ8992, E. Talenti leg. 05/07/1993 (MZUF GC/45670); D, Torrent dels Camps, near Can Corlet (Pollenca, Mallorca Island, Balearic Islands), 31S EE0015, M. Bodon & S. Cianfanelli leg. 30/12/2014 (MZUF GC/57153); E, gorge of Bianco River, La Montagnola (Buccino, Salerno, Campania), 33T WE3395, S. Cianfanelli & E. Talenti leg. 18/05/2014 (MZUF GC/46610); F, mouth of Codula de Sisine (Baunei, Ogliastra, Sardinia), 32T NK5348, E. Talenti leg. 5/07/2017 (MZUF GC/57303).

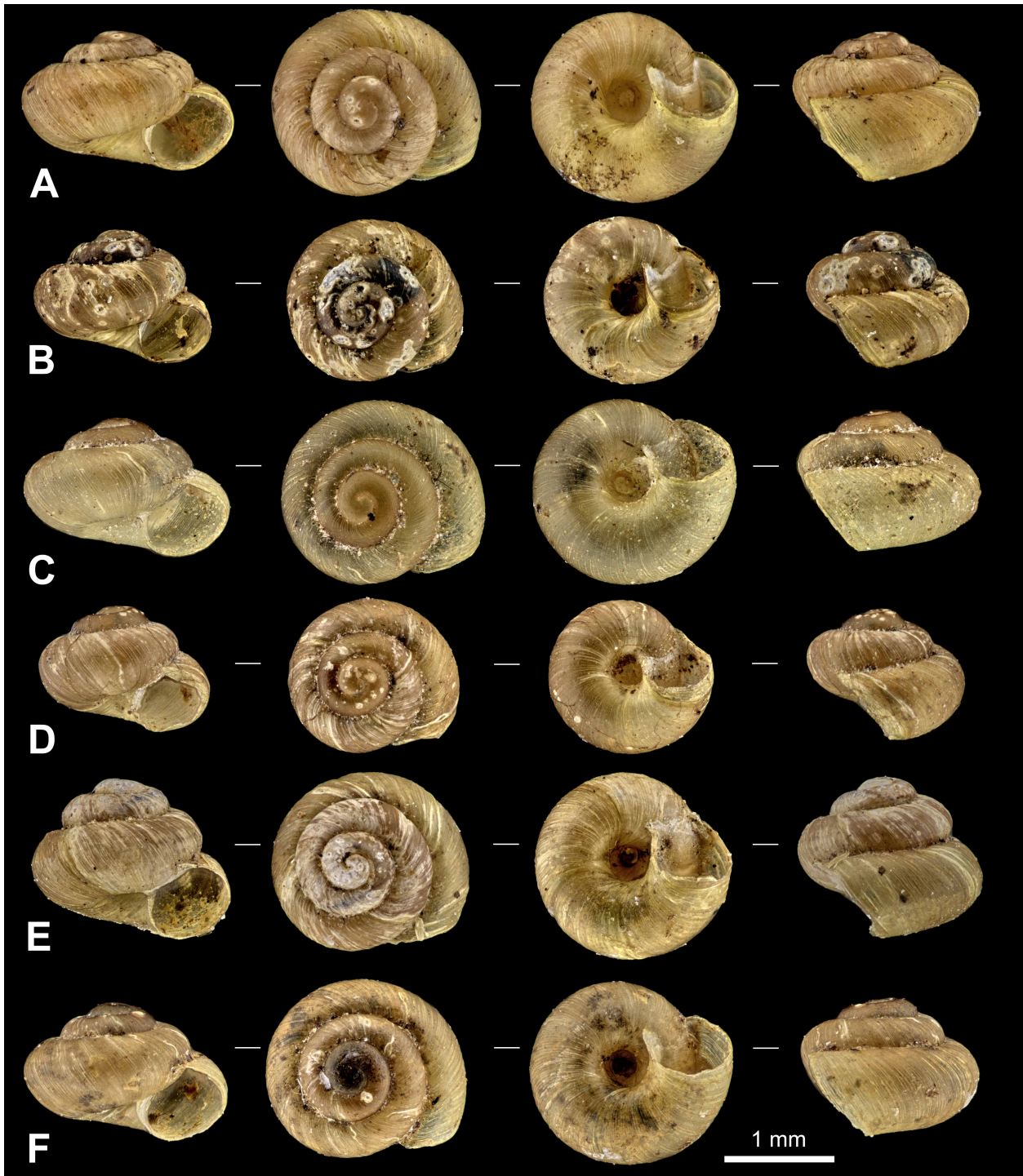


Figure 2. Shells of *Plagyrona placida* (Shuttleworth, 1852) (A) and *Plagyrona* sp. (B-F) from Italy. A, Passo del Kherch near the cave of Brigante, Montagna Grande, Pantelleria Island (Pantelleria, Trapani, Sicily), 33S TA3274, S. Cianfanelli & E. Talenti leg. 30/04/2013 (MZUF GC/43241); B, SSE of Zannone Island above Punta di Levante (Ponza, Latina, Latium); 33T UF3637, S. Cianfanelli & E. Talenti leg. 22/05/2014 (MZUF GC/54665); C-D, Argentino River near Orsomarso (Orsomarso, Cosenza, Calabria), 33S WE7705, S. Cianfanelli & E. Talenti leg. 27/04/1995 (MZUF GC/57182); E, Bussento River upstream the confluence of Sciarapotamo Stream, 3 km E Torre Orsaia (Morigerati, Salerno, Campania), 33T WE4342, M. Bodon & S. Cianfanelli leg. 05/01/2013 (MZUF GC/42579); F, Tanagro River 700 m SW from Auletta (Auletta, Salerno, Campania), 33T WE3589, S. Cianfanelli & E. Talenti leg. 21/04/1996 (MZUF GC/48110).

The examined material is presently preserved in the following collections: Museo di Storia Naturale dell'Università di Firenze, Sezione Zoologica "La Specola", Via Romana 17, Florence, Italy (MZUF); M. Bodon, Via delle Eliche 100/8, Genoa, Italy (MBC); S. Cianfanelli, Via Monferrato 3, Florence, Italy (SCC); E. Talenti, Piazza Parri 4, Figline e Incisa Valdarno, Florence, Italy (ETC).

RESULTS AND DISCUSSION

Systematics

Plagyrona angusta Holyoak and Holyoak, 2012.

Plagyrona angusta, Holyoak and Holyoak 2012: 153-165, Fig. 1 D-F.

Plagyrona angusta, Holyoak et al. 2014: 45.

Plagyrona angusta, Cadevall and Orozco 2016: 166.

Plagyrona angusta, Bank and Neubert 2017: 56.

Plagyrona angusta, Holyoak et al. 2019: 127.

Description

Shell (Fig. 1) very small (0.8-1.6 mm in height (H); 1.1-1.9 mm in diameter (D); 0.2-0.4 mm in umbilicus diameter (U); mean ratio H/D = 0.729 and mean ratio U/D = 0.164; according to Holyoak and Holyoak 2012, fig. 2), with three to four convex and slowly expanded whorls, separated by a very deep suture; spire rather elevated; last whorl not wide and slightly descending near the aperture. Protoconch not protruding, with the surface covered by many thin spiral striae and spiral grooves, crossed with more spaced and less marked growth lines; teleoconch covered by dense periostracal ribs, clearly visible and equal to each other, and by thin spiral lines, sometimes scarcely visible. Aperture roundish, with oblique outer peristome, not thickened and not reflected, interrupted in the parietal portion. Umbilicus small, corresponding to 1/6 of the maximum shell diameter. Periostracum light brown in colour, with weakly whitish bands, more evident in not fresh shells.

Plagyrona placida (Shuttleworth, 1852)

For literature before 2012, see Cianfanelli et al. (2013).

Plagyrona placida, Welter-Schultes 2012: 205.

Plagyrona placida, Holyoak and Holyoak 2012: 153-165, Figs. 1 A-C, 3 E-F.

Plagyrona placida, Cadevall and Orozco 2016: 167.

Plagyrona placida, Bank and Neubert 2017: 56.

Plagyrona placida, Welter-Schultes 2018.

Plagyrona placida, Holyoak et al. 2019: 127.

Description

Shell (Fig. 2) very small (0.7-1.6 mm in height (H); 1.0-2.3 mm in diameter (D); 0.2-0.6 mm in umbilicus diameter (U); mean ratio H/D = 0.651 and mean ratio U/D = 0.229; according to Holyoak and Holyoak, 2012, fig. 2), with three to three and a half convex and slowly expanded whorls, separated by a deep suture; spire not elevated; last whorl little wide and slightly descending near the aperture. Protoconch not protruding, with the surface covered by many thin spiral striae and spiral grooves, crossed with more spaced and less marked growth lines; teleoconch covered by dense periostracal ribs, clearly visible and equal to each other, and by thin spiral lines, sometimes scarcely visible. Aperture roundish, with oblique outer peristome, not thickened and not reflected, interrupted in the parietal portion. Umbilicus large, corresponding to 3/10 of the maximum shell diameter. Periostracum light brown in colour, with weakly whitish bands, more evident in not fresh shells.

New examined material

Spain

- *P. angusta*: Torrent dels Camps, near Can Corlet, alluvial debris, 80 m a.s.l. (Pollença, Mallorca, Balearic Islands), 31S EE0015, M. Bodon & S. Cianfanelli leg. 30/12/2014 (2 shells, MZUF GC/57153, Fig. 1 D; 7 shells, 2 young shells, SCC).

- *P. angusta*: Torrent de Son Vic, below La Granja, debris, 220 m a.s.l. (Esporles, Mallorca, Balearic Islands), 31S DD6291, M. Bodon & S. Cianfanelli leg. 01/01/2015 (1 shell, MBC).

France

- *P. angusta*: Gapeau River, downstream the dam near La Clapières, upstream L'Oratoire, Hyères, alluvial debris, 4 m a.s.l. (Hyères, Var, Provence-Alpes-Côte d'Azur), 32T KN6881, M. Bodon & S. Cianfanelli leg. 29/12/2016 (2 shell, MZUF GC/52475, Fig. 1 A; 2 shells, MBC; 1 shell, SCC).
- *P. angusta*: Gapeau River, downstream the bridge between Hyères and La-Londe-les-Maures, alluvial debris, 2 m a.s.l. (Hyères, Var, Provence-Alpes-Côte d'Azur), 32T KN6979, M. Bodon & S. Cianfanelli leg. 29/12/2016 (1 shell, MBC; 2 shells, MZUF GC/52475).

Greece

- *P. angusta*: small valley from Lakones to Paleokastritsa, litter in holm-oak forest at the base of a limestone cliff, 85 m a.s.l. (Paleokastritsa, Kérkira, Ionian Islands), 34S CJ8992, E. Talenti leg. 05/07/1993 (3 shells, MZUF GC/45670, Fig. 1 B-C; 1 shell, 1 young shell, MBC; 1 shell, SCC; 13 shells, 16 young shells, ETC).

Italy

- *Plagyrona* sp.: at SSE of Zannone Island, above Punta di Levante, litter in holm-oak, 150 m a.s.l. (Ponza, Latina, Latium); 33T UF3637, SCI "Isola di Palmarola e Zannone" (IT6040020), S. Cianfanelli & E. Talenti leg. 22/05/2014 (5 shells, 8 young shells MZUF GC/54665, Fig. 2 B; 2 shells, SCC).
- *P. angusta*: near the cave of San Michele Arcangelo, litter near limestone rocks in Mediterranean maquies, 500 m a.s.l. (Sant'Angelo a Fasanella, Salerno, Campania), 33T WE2878, M. Bodon & S.

Cianfanelli leg. 01/01/2013 (1 shell, MZUF GC/42503; 7 shells, SCC).

- *Plagyrona* sp.: Tanagro River, 700 m SW from Auletta, alluvial debris, 180 m a.s.l. (Auletta, Salerno, Campania), 33T WE3589, SCI "Fiume Tanagro e Sele" (IT8050049), S. Cianfanelli & E. Talenti leg. 21/04/1996 (1 shell, MZUF GC/48110, Fig. 2 F).
- *Plagyrona* sp.: left bank of Landro River, il Ponte, near San Marco, litter in Mediterranean maquies with limestone rocks, 240 m a.s.l. (Salvitelle, Salerno, Campania), 33T WE3994, S. Cianfanelli & E. Talenti leg. 18/05/2014 (1 shell, MZUF GC/46601).
- *Plagyrona* sp.: Calore River near the Cave of Castelcivita, alluvial debris, 70 m a.s.l. (Auletta, Salerno, Campania), 33T WE1782, SCI "Alta Valle del Fiume Calore Lucano (Salernitano)" (IT8050002), S. Cianfanelli & E. Talenti leg. 13/10/1994 (1 shell, MZUF GC/50149).
- *Plagyrona* sp.: Calore River in the bend between Case Lucia and Doto, alluvial debris, 75 m a.s.l. (Castelcivita, Salerno, Campania), 33T WE1781, SCI "Alta Valle del Fiume Calore Lucano (Salernitano)" (IT8050002), S. Cianfanelli & E. Talenti leg. 26/04/2013 (3 shells, MZUF GC/49084).
- *P. angusta*: gorge of Bianco River, in locality La Montagnola, litter in Ilex forest, 200 m a.s.l. (Buccino, Salerno, Campania), 33T WE3395, S. Cianfanelli & E. Talenti leg. 18/05/2014 (8 shells, MZUF GC/46610, Fig. 1 E).
- *Plagyrona* sp.: near the cave of Santa Croce, above Ottati, litter in Ilex forest, 620 m a.s.l. (Ottati, Salerno, Campania), 33T WE2679, SCI "Monti Alburni" (IT8050033), M. Bodon & S. Cianfanelli leg. 01/01/2013 (3 shell, MZUF GC/42383, 10 young shells, SCC).
- *Plagyrona* sp.: left bank of Mingardo River, Ponte Mingardo, Mediterranean maquies with limestone rocks, 35 m a.s.l. (Celle di

Bulgheria, Salerno, Campania), 33T WE3037, SCI “Fiume Mingardo” (IT8050013), M. Bodon & S. Cianfanelli leg. 05/01/2016 (1 shell, SCC).

- *Plagyrona* sp.: Bussento River upstream the confluence of Sciarapotamo stream, 3 km E Torre Orsaia, alluvial debris, 45 m a.s.l. (Morigerati, Salerno, Campania), 33T WE4342, SCI “Basso corso del Fiume Bussento” (IT8050007), M. Bodon & S. Cianfanelli leg. 05/01/2013 (1 shell, MZUF GC/42579, Fig. 2 E; 3 shells, SCC).
- *Plagyrona* sp.: Argentino River near Orsomarso, alluvial debris, 100 m a.s.l. (Orsomarso, Cosenza, Calabria), 33S WE7705, SCI “Valle del Fiume Argentino” (IT9310023), S. Cianfanelli & E. Talenti leg. 27/04/1995 (2 shells, MZUF GC/57182, Fig. 2 C-D).
- *P. angusta*: mouth of Codula de Sisine, alluvial debris, 0 m a.s.l. (Baunei, Ogliastra, Sardinia), 32 T NK5348, E. Talenti leg. 5/07/2017 (1 shell, MZUF GC/57303, Fig. 1 F).
- *P. placida*: Passo del Kherch near the Brigante cave, Montagna Grande, Pantelleria Island, litter in *Ilex* forest, 820 m a.s.l. (Pantelleria, Trapani, Sicily), 33S TA3274, SCI “Isola di Pantelleria: Montagna Grande e Monte Gibebe” (ITA010019), S. Cianfanelli & E. Talenti leg. 30/04/2013 (1 shell, 1 young shell, MZUF GC/43241, Fig. 2 A).

Habitat

The research was conducted by collecting organic substrate (litter or alluvial debris), where no alive animals were found, but only empty shells were extracted. Despite this, it was ascertained that the material was freshly deposited. The shells originated from the habitats near the collection sites, even if it was not possible to define with certainty the microhabitat where the specimens lived. The

literature reports cases where the species have been found alive (Holyoak and Holyoak 2012): in rocky habitats or trees, trunks and branches covered by mosses, in the Mediterranean scrub, in holm oak forests or in mesophilic deciduous forests. Even in Italy, *Plagyrona* has almost always been found in intact environments, some of which are included in Sites of Community Interest (SCI), in Campania, in the Alburni Mountains and in the Cilento, in the Mediterranean scrubs with vast limestone rocky outcrops, and mostly in the mature holm oaks both on Zannone and Pantelleria islands. For example, on the highest elevation in Pantelleria, Montagna Grande (Fig. 3), there is a wet holm oak, with a microhabitat formed by mosses and lichens abundant on the trunks and branches, which recalls that of the laurel forests in Macaronesia islands.



Figure 3. Collecting site of *Plagyrona placida* (Shuttleworth, 1852) in Pantelleria Island, Sicily, Italy.

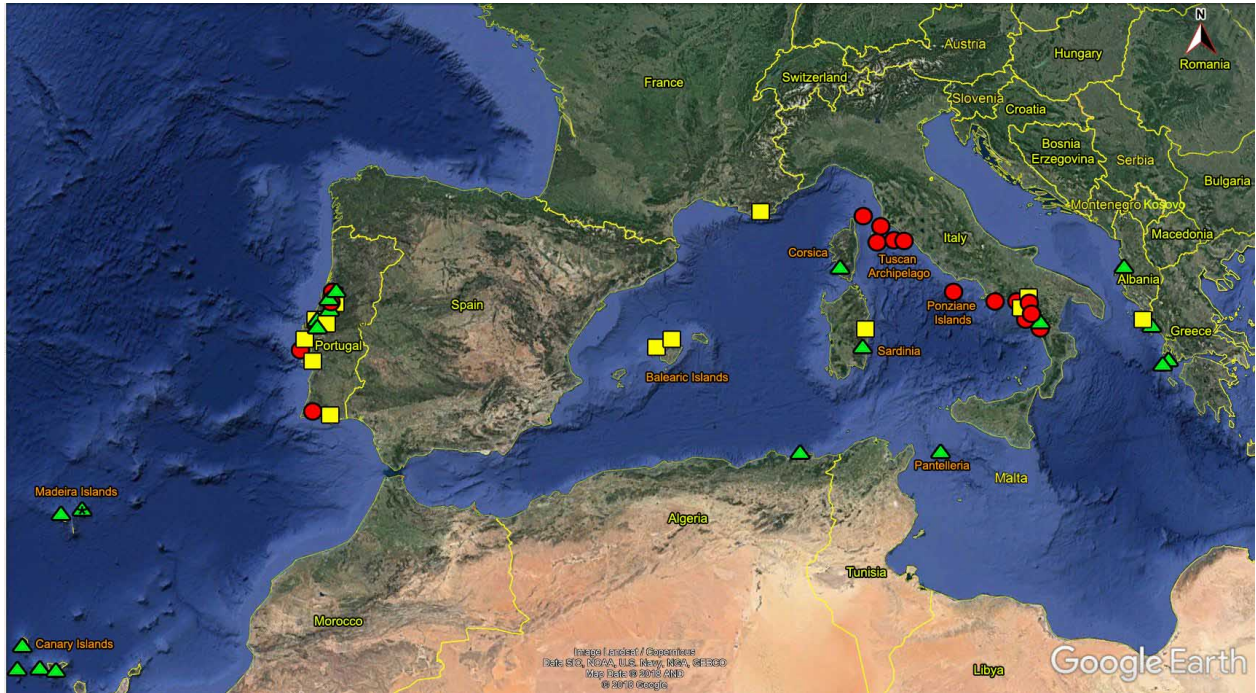


Figure 4. Distribution of populations of *Plagyrona* spp. in Macaronesia, Europe and northern Africa. *Plagyrona placida* (Shuttleworth, 1852), recent (green triangles), fossil (green triangles with asterisk); *Plagyrona angusta* Holyoak and Holyoak, 2012 (yellow squares); *Plagyrona* spp. (red dots).

Distribution and biogeography

The new data extend the known distribution of the genus *Plagyrona* already recorded in Cianfanelli et al. (2013) and confirm a Mediterranean distribution, stretching west to Macaronesia (chorotype according to Vigna Taglianti et al. 1999) (Fig. 4). From a biogeographic point of view, its presence in the islands of the Tuscan Archipelago and in Monte Argentario (Manganelli et al. 2015, 2017) and, based on our data, on Mallorca, in south France, and in Zannone and Pantelleria islands is very interesting. In fact, the presence of *Plagyrona* in islands - especially of small dimensions - implies a good ability to colonize new areas; this genus is probably transported by birds, as already demonstrated for the genus *Balea* Gray, 1824 (Preece and Gittenberger 2003), with species that shares the same habitat of *Plagyrona*, i.e., trunks of big trees and branches covered with mosses.

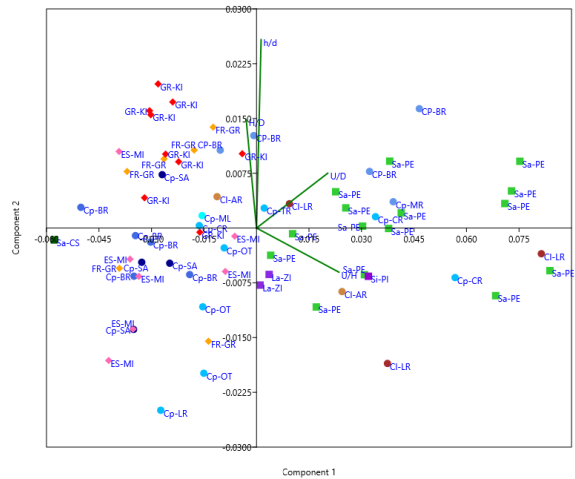


Figure 5. Principal Components Analysis of the ratios between the morphometric parameters of the shells in the populations of *Plagyrona* spp. For populations code see Table 1.

Remarks about species identity

While for the historical notes we refer to Holyoak and Holyoak (2012) and to Cianfanelli et al. (2013), a thorough examination of the data given by Holyoak and Holyoak (2012) and a comparison of the specimens from the various populations for a definition of the diagnostic characters, which may allow distinguishing the two species, is necessary.

Holyoak and Holyoak (2012) affirm that the shells of the two species are always identifiable if exceeding 1.5 mm in breadth and the two species can live sympatrically without showing any intermediate specimens.

According to Holyoak and Holyoak (2012), the discriminating characteristics between the shells of the two species are:

P. placida:

- D max 2.30 mm (largest shell in diameter);
- H/D= 0.586-0.511; 0.651 average (spire more flattened);
- U/D= 0.171-0.290; 0.229 average (wider umbilicus);
- less deeper sutures.

P. angusta:

- D max 1.95 mm (smaller shell in diameter);
- H/D= 0.658-0.854; 0.729 average (higher spire);
- U/D= 0.125-0.198; 0.164 average (narrower umbilicus);
- deeper sutures.

Due to the lack of an evident growth stop in adult shells, the D max character is useful to recognize adult shells of *P. placida*, since this species reaches larger dimensions; for *P. angusta* this parameter is less useful and can be used only if it is possible to examine several shells, probably including adult specimens. The depth of the sutures is difficult to quantify, while the relationships between H, D and U are characters that are useful for the morphometric analysis.

By processing the relations with the Principal Components Analysis, the percentage of the variance is 88.1 on the first axis, 6.4 on the second and 5.4 on the third. The parameters that most influence the PCA are the ratios U/D (loading = 0.6501) and U/H (loading = 0.75279) on the first axis (perhaps may be weakly correlated with the dimensions of the shells, see below, regression curve), h/d (loading = 0.82443) on the second axis and H/D (loading = 0.68041) on the third axis. Therefore, the ratios relating to the diameter of the umbilicus would be the parameters of greater importance than the ratios relating to the height/diameter of the shell or of the opening. Figure 5 shows a prevalent distribution of the points on positive values of the first axis (broad umbilicus with respect to the diameter or height of the shell), such as those from the Perdasdefogu population in Sardinia and Lao River in Calabria, while on negative values of PC1 and positive of PC2 (narrow umbilicus and greater height of the mouth or of the shell) are located the populations from Kerkyra, Greece and from Var, France. However, for the populations represented by a certain number of specimens, a strong dispersion is noted and therefore variability in relationships. Moreover, there is no clear concentration of the points that can confirm the existence of two or more distinct species. Even through Non-metric Multidimensional Scaling, the result is similar.

Based on the data of Holyoak and Holyoak (2012), the H/D and H/U ratios do not exactly follow a linear regression curve, but the equation that best correlates the trend is represented by an exponential curve (R^2 always higher than a straight line, in both cases, for both species) (Fig. 6). Therefore, the H/D and H/U ratios are not constant, but vary with the size of the shell, according to the exponential formula: $H = 0.3088e^{0.8224D}$ and $H = 0.0531e^{0.9879U}$ for *P. angusta*, $H = 0.3526e^{0.6578D}$ and $H = 0.0756e^{0.9286U}$ for *P. placida*. For each specimen, calculating on the basis of these exponential formulas, from the parameters “diameter D” and “umbilicus U”, the theoretical

dimension H for *P. angusta* and for *P. placida*, and comparing this value with the real dimension H, it is possible to evaluate the deviation respecting the theoretical regression curves, and therefore have an indicative value, useful for the attribution of the specimen to one or the other species. In Table 1 the deviation refers to the middle point between the two curves and it is expressed in percentage (%), with respect to the distance between the curve that represents the average between *P. angusta* and *P. placida*, with the curves of the respective species, with positive values if closer to *P. placida*, negative if closer to *P. angusta*. So a value of 0% means that the parameter H (or the parameter U) falls exactly in the middle between the curves of the two species, + 50% if it falls closer to the curve of *P. placida* ¼ than compared to ¾ from *P. angusta*, + 100% if it falls exactly in the curve of *P. placida*, + 200% if it falls beyond the curve of *P. placida* at a distance equal to twice the distance between the two curves (and therefore much more distant from the curve of *P. angusta*). Similarly, but on negative % values, if the H and U parameters are closer to the *P. angusta* curve.

Examining the results (Table 1), since the H/D and H/U correlations do not follow a linear trend but vary with size, some relationships give a very different result based on the exponential correlation. For example, the average H/D between the populations of Mallorca and Sardinia (Perdasdefogu) has the same value, while the result, based on the exponential correlation, attributes the two populations to two different species. However, there is a strong variability within populations, with values often close to the value limit between the two species or also discordant between the two series of H/D and H/U ratios. Whereas the specimens are quite numerous, and there are no strong variations in the parameters, it is possible to attribute them: to *P. angusta* for populations from Var, Kerkyra, Mallorca (although these are a little less high) and for some specimens from Campania (San Michele Arcangelo and Bianco River); to *P. placida* for

Perdasdefogu (Sardinia) and Lao River (Calabria) specimens. Where the values of the two parameters denote a concordance and therefore the attribution to one of the two species, on the other side, in many instances, the variability or the intermediate situation do not allow the certain identification. In these cases, it would be necessary to examine additional specimens in order to better study the variability or to check whether the two distinct taxa are present in sympatry.

However, it is important to note that both the PCA analysis and the analysis using the regression curves have provided comparable results. It would also be important to verify other populations, such as those of the Tuscan Archipelago, where, at least for Capraia island, based on the published illustrations (Manganelli et al. 2015), the shape of the shell would seem to belong to *P. angusta* rather than *P. placida*.

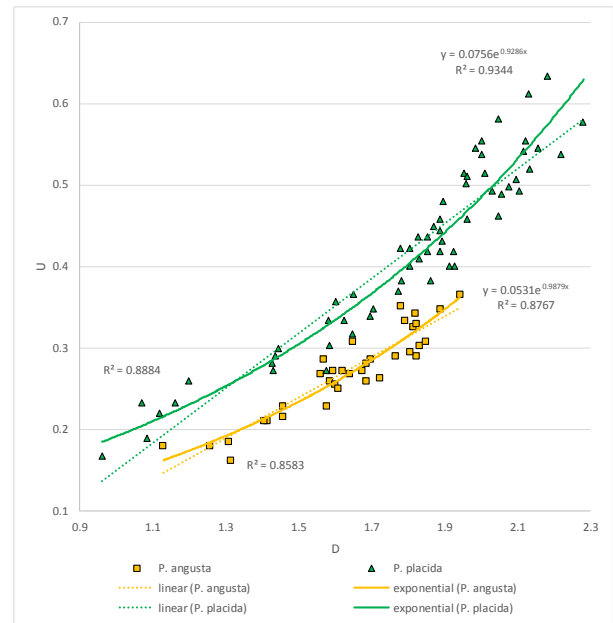


Figure 6. Linear and exponential regression curves based on H/D (7 A) and H/U (7 B) ratios based on the data from Holyoak and Holyoak (2012), fig. 2, with the relative value of R2 and the equations of the exponential curves.

Table 1. Code and site of the populations of *Plagyriona* listed in this paper (*: listed in Cianfanelli et al. 2013), with the mean and \pm standard deviation of H, D, h, d, U, H/D, U/D and the % of mean shifting H and U (and \pm standard deviation shifting), the species attribution and the number of measured shells. The % mean shifting is referred to % distance of H or U from the mean of regression exponential line between *P. placida* and *P. angusta* (0%) and the regression exponential line of *P. placida* (100%) or *P. angusta* (-100%), calculated by data of D on the bases of regression exponential line by data of Holyoak and Holyoak (2012).

Code	Site	H	D	h	d	U	H/D	U/D	% shifting H	% shifting U	Species	N° shells
ES-MI	Spain Mallorca Island	1.02 ±0.07	1.49 ±0.07	0.59 ±0.06	0.70 ±0.06	0.24 ±0.02	0.69 ±0.03	0.16 ±0.02	-52% ±86%	-74% ±70%	<i>P. angusta</i>	7
FR-GR	France Gapeau River	1.23 ±0.25	1.64 ±0.18	0.63 ±0.06	0.73 ±0.09	0.29 ±0.07	0.74 ±0.08	0.18 ±0.02	-119% ±95%	-57% ±56%	<i>P. angusta</i>	6
GR-KI	Greece Kerkyria Island	1.35 ±0.14	1.75 ±0.13	0.71 ±0.04	0.78 ±0.06	0.31 ±0.03	0.77 ±0.03	0.18 ±0.01	-183% ±42%	-65% ±49%	<i>P. angusta</i>	9
La-ZI	Lazio Zannone Island	1.20 ±0.04	1.69 ±0.02	0.58 ±0.04	0.73 ±0.03	0.34 ±0.00	0.71 ±0.01	0.20 ±0.00	-53% ±25%	+53% ±3%	<i>P. sp.</i>	2
Cp-ML	Campania Massa Lubrense*	1.16	1.68	0.70	0.77	0.30	0.69	0.18	-12%	-53%	<i>P. sp.</i>	1
Cp-SA	Campania Sant'Angelo a F.	1.13 ±0.06	1.58 ±0.06	0.63 ±0.03	0.76 ±0.04	0.26 ±0.02	0.71 ±0.01	0.16 ±0.01	-92% ±20%	-83% ±17%	<i>P. angusta</i>	4
Cp-TR	Campania Tanagro River	1.28	1.93	0.79	0.84	0.38	0.66	0.19	+78%	-62%	<i>P. sp.</i>	1
Cp-LR	Campania Landro River	0.91	1.53	0.61	0.79	0.23	0.59	0.15	+196%	-135%	<i>P. sp.</i>	1
Cp-CR	Campania Calore River	1.39 ±0.11	1.98 ±0.22	0.76 ±0.08	0.89 ±0.11	0.46 ±0.13	0.70 ±0.03	0.23 ±0.04	+24% ±101%	+43% ±83%	<i>P. sp.</i>	3
Cp-BR	Campania Bianco River	1.19 ±0.10	1.62 ±0.10	0.62 ±0.05	0.75 ±0.07	0.26 ±0.03	0.73 ±0.02	0.16 ±0.01	-124% ±35%	-102% ±54%	<i>P. angusta</i>	5
Cp-OT	Campania Ottati	1.15 ±0.15	1.61 ±0.11	0.62 ±0.03	0.82 ±0.11	0.30 ±0.04	0.71 ±0.05	0.18 ±0.01	-75% ±89%	-3% ±27%	<i>P. sp.</i>	3
Cp-MR	Campania Mingardo River	1.26	1.85	0.79	0.86	0.45	0.68	0.24	+36%	+158%	<i>P. sp.</i>	1
Cp-BR	Campania Bussento River	1.35 ±0.22	1.87 ±0.22	0.72 ±0.08	0.75 ±0.07	0.42 ±0.11	0.72 ±0.06	0.22 ±0.3	-18% ±100%	60% ±63%	<i>P. sp.</i>	4
Cl-AR	Calabria Argentino River	1.24 ±0.04	1.77 ±0.23	0.67 ±0.03	0.79 ±0.05	0.37 ±0.08	0.71 ±0.07	0.21 ±0.02	-48% ±192%	38% ±3%	<i>P. sp.</i>	2
Cl-LR	Calabria Lao River*	1.25 ±0.05	1.95 ±0.13	0.71 ±0.08	0.82 ±0.01	0.46 ±0.11	0.64 ±0.02	0.24 ±0.04	114% ±41%	97% ±103%	<i>P. placida</i>	3
Sa-PE	Sardinia Perdasdefogu*	1.40 ±0.11	2.04 ±0.17	0.77 ±0.07	0.88 ±0.06	0.51 ±0.10	0.69 ±0.03	0.25 ±0.03	+59% ±63%	+56% ±90%	<i>P. placida</i>	15
Sa-CS	Sardinia Codula Sisine	1.03	1.50	0.56	0.64	0.19	0.70	0.13	-91%	-216%	<i>P. angusta</i>	1
Si-PI	Sicily Pantelleria Island	1.34	1.98	0.73	0.88	0.46	0.68	0.23	+68%	+62%	<i>P. placida</i>	1

CONCLUSIONS

The discovery in Italy of new *Plagyrona* sites, with the first report of *P. placida* (Shuttleworth, 1852) for a new locality (Sicily), and the first reports of *P. angusta* Holyoak and Holyoak, 2012, in Spain, France, Greece and Italy (Campania and Sardinia), are the result of researches on the distribution of Palaeartic malacological fauna, not targeted but extended to many Italian and European areas.

The fact that the distribution of these two species was underestimated due to their small size, their habitat, their mimicry and to the fact of being rare and localized, had already been highlighted by Cianfanelli et al. (2013). Moreover, the species can be confused with other small terrestrial gastropods, such as those of the genera *Pyramidula* Fitzinger, 1833, *Paralaoma* Iredale, 1913, or *Acanthinula* Beck, 1847; also for this reason only recently the genus *Plagyrona* has been reported in Italy and in other Mediterranean European countries. For example, in the Alburni Mountains (Campania, Italy), a recent and detailed research on the terrestrial molluscs of this area (Maio et al. 2017), does not report its presence, when, instead, *Plagyrona* is present.

The doubt that *P. placida* may be a cryptogenic species, accidentally introduced by man in Italy, was expressed in Cianfanelli et al. (2013), but the new collected data are further and increasingly substantial elements to consider this species as autochthonous, thus excluding a passive anthropic diffusion as already occurred for other small terrestrial species. For example, *Paralaoma servilis* (Shuttleworth, 1852), *Lucilla scintilla* (Lowe, 1852), *Lucillaingleyana* (Pilsbry, 1890) are common species almost everywhere in the alluvial debris along the rivers (Cianfanelli et al. 2007), but if we exclude a site near a cave (Sant'Angelo a Fasanella), where *Paralaoma servilis* is present, no alien species were collected in all the other litter samples. Many of these sites are intact environments, far from inhabited sites, where the chance of non-native

species introductions is very unlikely. Therefore, on the basis of the collected elements, *Plagyrona* species are considered indigenous elements in all the collection sites.

The specific identification of the various populations has been carried out on a morphometric basis, but, for some populations (see Table 1), the variability of the discriminating characters and the limited number of available specimens, are facing a difficult certain assignment. On the other hand, from the analysis carried out, confirmation of the existence of two distinct species is not at all evident, at least in the Mediterranean countries. The genital tract of *Plagyrona* is still unknown; in the Valloniidae the aphyllia is frequent (Giusti et al. 1995, Schileyko 1998). In this case, the reproduction by parthenogenesis could express distinct morphological clones; a genetic analysis could therefore clarify the present status of the European populations. Unfortunately, the failure to find living specimens has so far prevented to carry out anatomical and genetic research on these species.

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