



## A NEW SPECIES OF *BYTHINELLA* MOQUIN-TANDON, 1855 (CAENOGASTROPODA: TRUNCATELLOIDEA) FROM NAXOS ISLAND, GREECE

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**ABSTRACT:** A new species of *Bythinella*: *B. walensae* is described from Aria spring at Naxos Island, Greece. Cytochrome oxidase subunit I (*COI*) sequences of mtDNA, as well as internal transcribed spacer (*ITS-1*) of nuclear ribosomal DNA indicate distinctness of *B. walensae*. The shell, female reproductive organs and penis are described. The most characteristic of this species are: slender shell, narrow aperture, J-shaped bursa coupled with bulky and spherical receptaculum, and the length ratio of penis arms. Simple anatomy coupled with wide variation ranges overlapping between the species are emphasised.

**KEY WORDS:** molecular distinctness, mtDNA, cytochrome oxidase subunit I (*COI*), *ITS-1*, morphological distinctness, shell, penis, female reproductive organs

### INTRODUCTION

The genus *Bythinella* Moquin-Tandon, 1855 is a group of freshwater, dioecious, oviparous snails inhabiting springs and subterranean waters (GIUSTI & PEZZOLI 1977, FALNIOWSKI 1987). Its wide range extends from the Iberian Peninsula to western Asia, and from southern Poland to southern Greece. The diversity of *Bythinella* has mainly been studied in western, southern and central Europe (BOETERS 1973, 1998, GIUSTI & PEZZOLI 1977, FALNIOWSKI 1987), but the studies have mostly focussed on the external morphology and anatomy, initially of just the shell and, later, of the soft parts. It has been demonstrated, however, that morphology alone cannot be used for unequivocal species delimitation due to the limited number of taxonomically useful characters and their wide variation (FALNIOWSKI 1987, 1992, SZAROWSKA 2006, BICHAIN et al. 2007a, b, FALNIOWSKI et al. 2009a). More recent studies using molecular data have made it possible to distinguish several *Bythinella* species and also to synonymise a few nominal species (BICHAIN et al. 2007a, b, HAASE

et al. 2007, BENKE et al. 2009, FALNIOWSKI et al. 2009a, b, 2012b, FALNIOWSKI & SZAROWSKA 2011, 2012).

Several species of *Bythinella* have been described across Europe, mainly in western and central parts of the continent (e.g. RADOMAN 1976, 1983, BICHAIN et al. 2007a, b). In contrast, relatively few *Bythinella* species have been identified in Greece and those that have been were initially identified based on morphological characters (RADOMAN 1976, 1983, SCHÜTT 1980, REISCHÜTZ et al. 2008). More recent research combining morphology and genetic markers, cytochrome oxidase subunit I (*COI*) and internal transcribed spacer (*ITS-1*), revealed eight putatively distinct species in continental Greece: two in the Peloponnese, one in the Attica and Parnassos Mts (Central Greece), one on Lefkas Island and four in northern Greece (FALNIOWSKI & SZAROWSKA 2011, 2012). During our research on the Aegean *Bythinella* (SZAROWSKA et al. 2016) we found seven more molecularly distinct clades, of presumably species level.

The most distinct was the one inhabiting two springs at Naxos Island. For COI the  $p$ -distances between the clade inhabiting Naxos and the other clades were within 0.065–0.090, mean 0.080 for Naxos and the

other Aegean clades, and 0.741 for Naxos and all the Greek *Bythinella* (SZAROWSKA et al. 2016). For ITS-1 the mean values were 0.036 and 0.043, respectively (SZAROWSKA et al. 2016).

## MATERIAL AND METHODS

About ten specimens of *Bythinella* were collected at locality N01 (Fig. 1): small spring at Aghio Kyriaki, 37°04'09.6"N, 25°26'48.9"E. About twenty specimens were collected at locality N02 (Fig. 1), in a somewhat bigger spring Aria (Aria Pygi), 37°02'11.1"N, 25°29'37.8"E. Snails were collected by hand or sieve, and fixed with 80% ethanol. The shells and soft parts were photographed with a CANON EOS 50D digital camera, under a NIKON SMZ18 microscope with dark field and phase contrast. Five males and five females were dissected, the drawings were made from the photographs. A NIKON DS-5 digital camera measurement system was used to measure seven shell morphometric parameters (SZAROWSKA 2006, FALNIOWSKI et al. 2012b). For definitions of character states see HERSHLER & PONDER (1998), for the molecular data, and the results of Principal Component Analysis see SZAROWSKA et al. (2016).

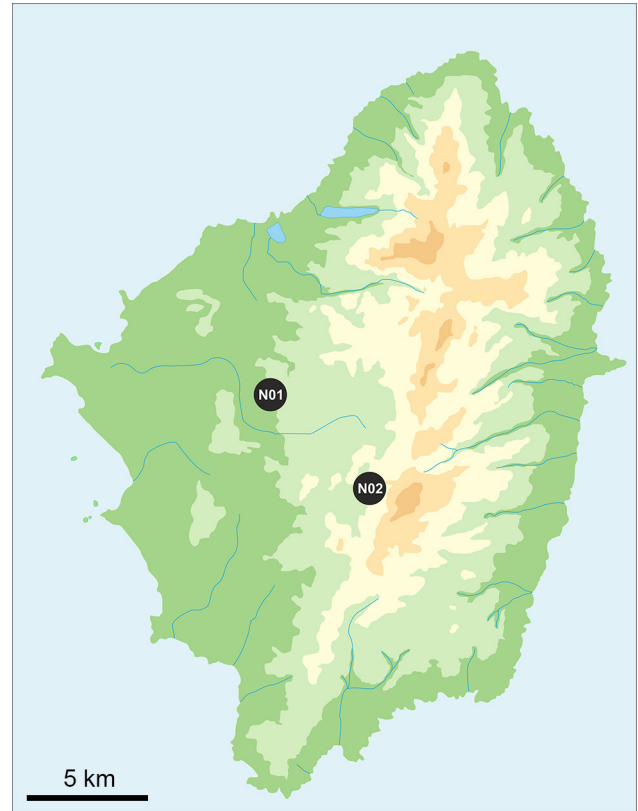


Fig. 1. Map of Naxos Island showing two localities inhabited by *Bythinella walensae* n. sp.

## SYSTEMATIC PART

### Family: Bythinellidae Germain, 1930

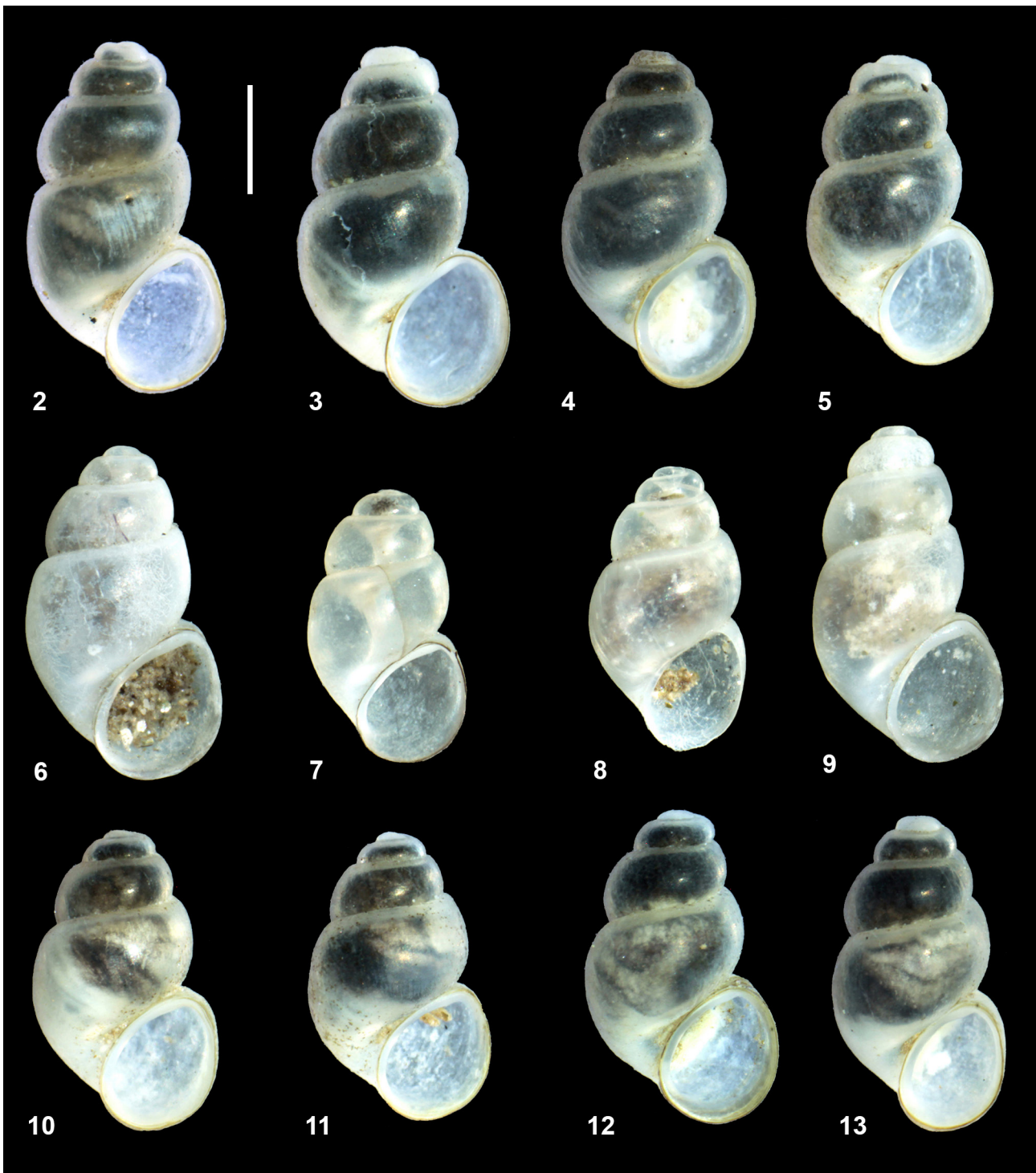
Genus: *Bythinella* Moquin-Tandon, 1855

#### *Bythinella walensae* sp. n.

**Types.** Twelve ethanol-fixed specimens from spring Aria (Aria Pygi) N02, 37°02'11.1"N, 25°29'37.8"E (type locality), September 2013, holotype: ZMUJ-M.1982, paratypes: ZMUJ-M.1983 – ZMUJ-M.1993; a few empty shells from a small spring at Aghio Kyriaki, N01; 37°04'09.6"N, 25°26'48.9"E, September 2013. The other ten paratypes destroyed for DNA extraction. Ten sequences of cytochrome oxidase subunit I (COI), GenBank numbers: KT353699-KT353700 from locality N01 (Aghio Kyriaki); KT353701-KT353708 from the type locality N02 (Aria Pygi).

Ten sequences of internal transcribed spacer ITS-1, GenBank numbers: KT353614-KT353615 from locality N01 (Aghio Kyriaki), and KT353616-KT353623 from the type locality N02 (Aria Pygi) (SZAROWSKA et al. 2016).

**Diagnosis.** Shell ovate-conical, with relatively high spire and simple outer lip, soft parts pigmented black, female reproductive organs with big J-shaped bursa copulatrix, big spherical receptaculum seminis, and long and narrow loop of renal oviduct, penis with flagellum and the arm containing vas deferens of similar length, narrow, especially the arm containing vas deferens. The most characteristic of this species are: slender shell, narrow aperture, J-shaped bursa coupled with bulky and spherical receptaculum, and the length ratio of penis arms.



Figs 2–13. Shells of *Bythinella walensae* n. sp. from the type locality: 3 – holotype; bar equals 1 mm

**Description.** Shell (Figs 2–13) ovate-conical with relatively high spire, up to 3.16 mm high, with ca. 4.5 whorls, spire height approximately 30% of shell height, and 59% of body whorl width. Teleoconch whorls moderately convex, evenly rounded, growing regularly in diameter. Aperture ovoid, outer lip simple, parietal lip complete, umbilicus slit-like. Teleoconch glossy, with delicate growth lines, periostracum whitish or white. Shell parameters for a

series of paratypes are given in Table 1. Operculum smooth on its inner and outer surface. Soft parts densely pigmented black. Female reproductive organs (Figs 14–15) with big, broad, J-shaped bursa copulatrix with moderately long duct, one big, spherical receptaculum seminis in the position of  $rs_1$ , and long and narrow loop of renal oviduct. Penis (Figs 16–21) with moderately long penial gland (Figs 17, 17a, 21), its arms of similar length, narrow, es-



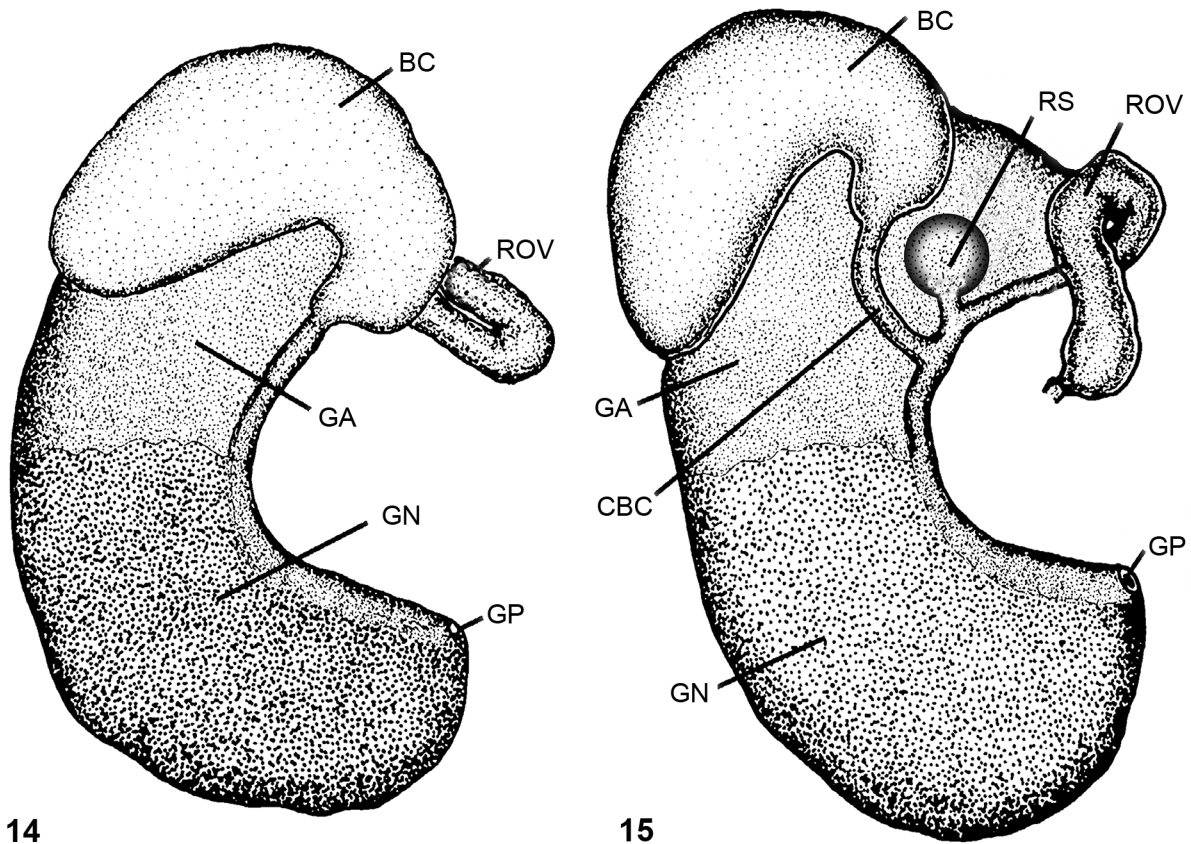
Table 1. Shell biometry of the holotype and paratypes (n=10) of *Bythinella walensae* from Naxos Island: a – shell height, b – body whorl width, c – aperture height, d – spire height, e – aperture width,  $\alpha$  – apex angle,  $\beta$  – angle between body whorl suture and horizontal surface (see Fig. 23); M – mean, SD – standard deviation, max – maximum, min – minimum. The a – e in mm,  $\alpha$  –  $\beta$  in degree angle

	a	b	c	d	e	$\alpha$	$\beta$
Holotype	3.16	1.64	1.30	1.02	1.15	117	17
Paratypes (n = 10)							
M	2.94	1.49	1.26	0.88	1.10	129.1	18.12
SD	0.21	0.11	0.08	0.13	0.05	11.41	1.73
max	3.16	1.64	1.37	1.05	1.17	145	21
min	2.51	1.32	1.10	0.65	1.01	117	16

pecially the arm containing the terminal part of vas deferens.

**Etymology.** Named in honour of President Lech Wałęsa, a Polish national hero and co-creator of our independence.

**Distribution and habitat.** Known from the type locality (Aria spring), but also from another locality at Naxos Island (spring at Aghio Kyriaki).



Figs 14–15. Renal and pallial section of female reproductive organs of *Bythinella walensae* n. sp.: 14 – bursa and coil of oviduct in natural position, 15 – bursa and coil bent, to show seminal receptacle (BC – bursa copulatrix, CBC – duct of bursa copulatrix, GA – albumen gland, GN – capsule gland, GP – gonoporus, ROV – coil of oviduct, renal oviduct, RS – seminal receptacle)



Figs 16–21. Penes of *Bythinella walensae* n. sp.: 17 and 21 – with penial gland, 17a – penial gland of specimen 17 (P – penis, FL – flagellum, PG – penial gland); scale bar equals 0.5 mm

## DISCUSSION

Species boundaries in *Bythinella* are still unclear, despite hundreds of sequences now available in the GenBank (FALNIOWSKI 1987, 1992, BICHAIN et al. 2007a, b, SZAROWSKA & FALNIOWSKI 2008, BENKE et al. 2009, FALNIOWSKI & SZAROWSKA 2011, 2012, SZAROWSKA et al. 2016). The simple and highly variable morphology is not sufficient even for species determination, thus molecular data are necessary. In Greece there are several molecularly discernible

clades, most of them of species level. However, they have not been described so far, thus we cannot present formal differential diagnoses between *B. walensae* and its closest relatives. *B. walensae* is molecularly more distinct than the other still unnamed clades (Fig. 22).

The shell height, spire height and aperture height had the highest loadings in PC1, and the angle between the body whorl suture and the horizontal sur-

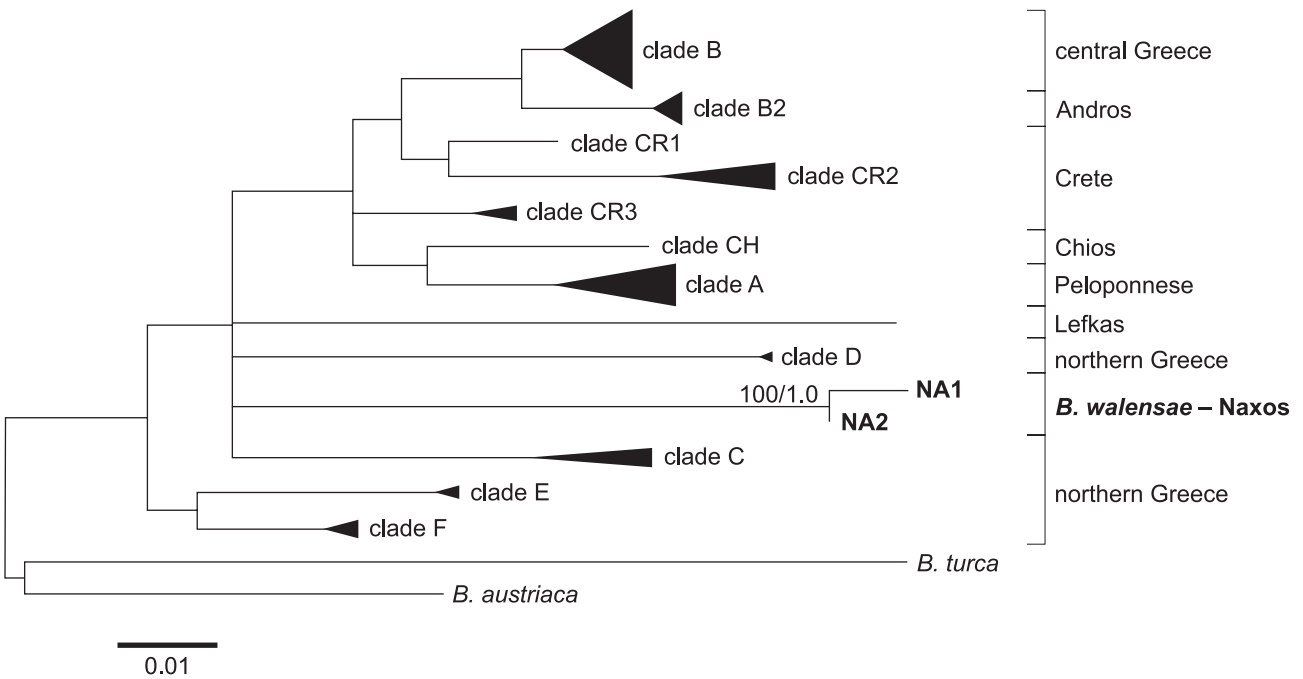


Fig. 22. Maximum likelihood tree for COI for the main clades of the Greek *Bythinella*, modified from SZAROWSKA et al. 2016; triangles indicate groups of several close haplotypes

face had the highest loading in PC2. Thus, as could be seen in Fig. 23, the shells of *B. walensae* could be distinguished based on their height, spire height and aperture height, thus their slenderness, although the variability range overlapped the ones of some other

clades. The same overlap is true of the female reproductive organs and penes. This is, however, a normal picture within the Truncatelloidea (FALNIOWSKI 1987, 1992, FALNIOWSKI et al. 2012a, b). One could either consider one or a few specimens of each species

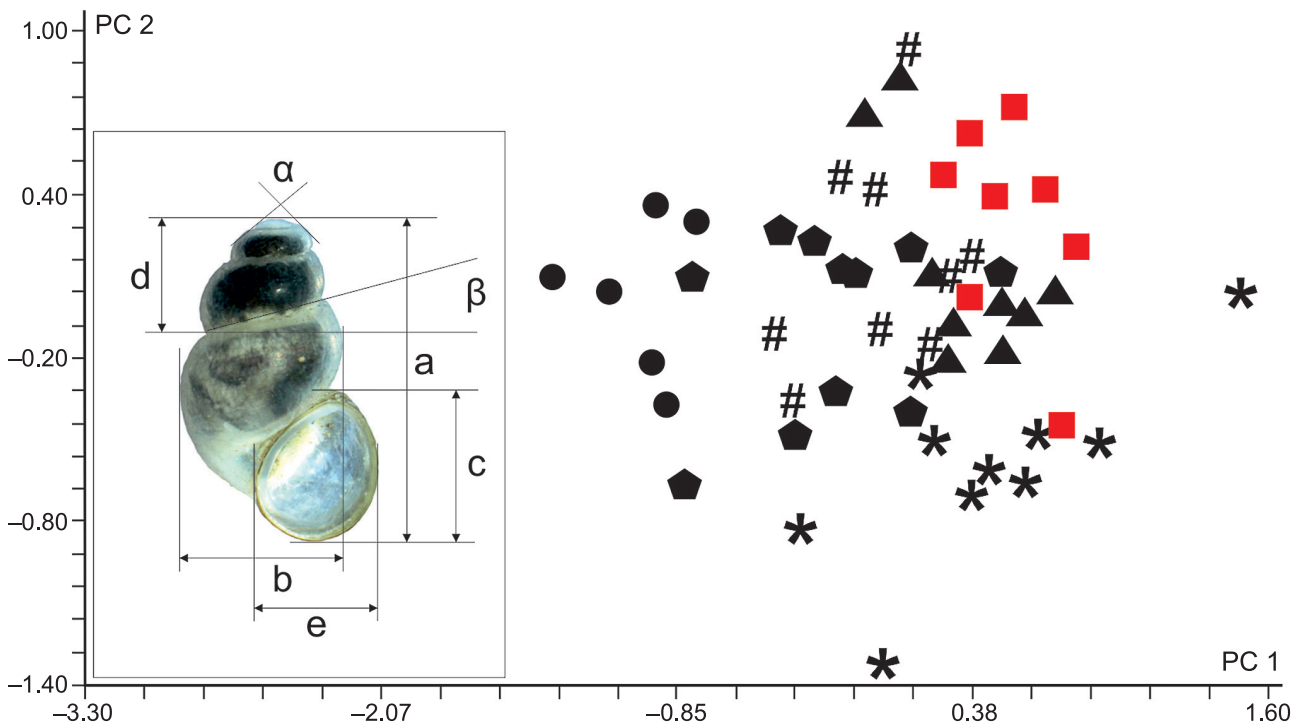


Fig. 23. PCA on the seven shell morphometric parameters, defined on the shell shown at the left side (for characters and mean values see Table 1); red squares represent *Bythinella walensae*, black symbols – the other clades from the Aegean; based on SZAROWSKA et al. 2016





and, then, discover evident differences between the species, or study numerous specimens of each nominal taxon – and see the (nearly) continuous variation. Morphological characters within the Truncatelloidea usually lack the states which are unique, or characteristic at least, the ranges of variation are nearly always wide and overlap between the species or even genera (e.g. FALNIOWSKI 1987, SZAROWSKA & FALNIOWSKI

2008, FALNIOWSKI & SZAROWSKA 2011, FALNIOWSKI et al. 2012a, SZAROWSKA et al. 2016). On the other hand, species identification with DNA sequences is always certain, although species boundaries remain disputable, especially with one locus and small divergence. In the case of *B. walensae*, however, there are two loci, and the p-distances are high (SZAROWSKA et al. 2016).

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*Received: March 3rd, 2016*

*Revised: June 17th, 2016*

*Accepted: June 23rd, 2016*

*Published on-line: July 19th, 2016*

