MULTIVARIATE ANALYSIS OF SHELL CHARACTERISTICS IN *AGATHYLLA EXARATA* (ROSSMÄSSLER 1835) (GASTROPODA: CLAUSILIIDAE) POPULATIONS AND ITS TAXONOMIC RELEVANCE.

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Abstract *Important conchological features (number of whorls, shell height and width, aperture height and width, number of ribs over the penultimate whorl and the cervix) of* Agathylla exarata *(Rossmässler 1835) populations were examined using the material of the Hungarian Natural History Museum and the Hungarian Geological Institute. Our multivariate analysis of variance (MANOVA) and discriminant function analysis (DFA) studies revealed that, on the basis of these quantitative characteristics, the* A. exarata *forms* mostarensis *(Brancsik 1889) and* denegabilis *(Küster 1847) can only be regarded as size variations and, hence, synonyms of the nominate subspecies. By contrast,* neumensis *Nordsieck 1970, having more distant ribs, was found well separable from the other population groups and could be confirmed as a distinct subspecies.*

Key words *Clausiliidae, taxonomy,* Agathylla, *multivariate analysis, Balkans*

INTRODUCTION

To our present knowledge, the genus *Agathylla* H. & A. Adams 1855 is distributed along the eastern coast of the Adriatic Sea from southern Dalmatia to northern Albania, and also in certain inland areas of Herzegovina, Albania and Epirus up to 60 kilometres from the sea. Toward the south, *Agathylla* becomes more sporadic and rare, so its populations in central and southern Albania, as well as Epirus, were discovered only in the previous decade (Nordsieck 1996, Gittenberger 1998, Erőss, Fehér & Szekeres 1999). This is an obligate petrophilic genus, and on limestone rocks they can often be found in large quantities.

Agathylla exarata (Rossmässler 1835) inhabits the northernmost part of the genus's distribution area: the nominate subspecies was described from the delta of the Neretva river; *mostarensis* (Brancsik 1889) is believed to live in Herzegovina between Mostar and Počitelj, and *neumensis* Nordsieck 1970 was found near Badžula, south of the Neretva delta. A further form, *denegabilis* (Küster 1847) was mentioned from the Velež mountain over Mostar, and from Mljet island (Küster 1847, Brancsik 1889). More recently, *denegabilis* was regarded only a synonym of the nominate form (Zilch 1977), thus the latest comprehensive study on the genus (Nordsieck 1972) deals only with the first three forms (fig 1., table 1.).

During this study some conchological parameters were analysed in the *Agathylla exarata* material of the Hungarian Natural History Museum and the Hungarian Geological Institute. The aim of our work was to find variables of taxonomic value; ascertain which *A. exarata* forms can reasonably be distinguished as subspecies, and elaborate an objective method for their identification.

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TABLE 1

The described *Agathylla exarata* forms and their mentioning in the literature.

"Names" and "Sampling sites or distribution areas" are taken from the referred publications using the original spelling. Currently used geographic names are given in square brackets.

Form	Literature data	Name	Sampling site or distribution area
exarata	Rossmässler 1835	*Clausilia exarata Z.*	Macarsca in Dalmatien
	Küster 1847	*Clausilia exarata Ziegler*	in Dalmatien bei Fort Opus (Narenta)
	Charpentier 1852	*Clausilia exarata Ziegl. Rssm.*	Ragusa; Narenta Dalm.
	Westerlund 1884, 1901	*Cl. exarata* (Z.) R..	Dalmatien, Herzegowina
	Zilch 1977	*Agathylla (Agathylla) exarata exarata* (ROSSMÄSSLER 1835)	Narenta bei Fort Opus [=Opuzen]
	Nordsieck 1972	*Agathylla (A.) exarata exarata* (ROSSMÄSSLER 1835)	Bagalovići bei Metković
denegabilis	Küster 1847	Var. A. minor, apice fusco. *Clausilia. denegabilis* Ziegler	bei Lago auf der Insel Meleda [=Mljet]
	Charpentier 1852	β. Minor, gracilior, apice fusco. Küst.	Ins. Meleda [=Mljet]
	Westerlund 1884, 1901	Forma 1.) minor	---
	Brancsik 1889	*Agathylla exarata Zgl. v. minor Chrp.*	in altissimis decliviis montis Veleš prope Mostar
	Jaeckel, Klemm & Meise 1957	*A. e. minor West.* 1884 *A. e. denegabilis (Küst.)* 1847	both from Mljet Island
	Zilch 1977	*Clausilia exarata* [var. A. *minor; Clausilia denegabilis*], Küster [synonim for the nominate subspecies]	Dalmatien: (bei Lago auf der) Insel Meleda [=Mljet]
mostarensis	Brancsik 1889	*Agathylla exatara Zgl. v. Mostarensis*	in antiquis muris urbis Mostar praecipue prope celebrem pontem
	Nordsieck 1972	*Agathylla (A.) exarata mostarensis* BRANCSIK 1889	Počitelj bei Čapljina
	Zilch 1977	*Agathylla (Agathylla) exarata mostarensis* BRANCSIK 1889	"in antiquis muris urbis Mostar praecipue prope celebrem pontem".
neumensis	Wagner 1918	*Agathylla narentana*	S-Dalmatien: Bačula [= Badžula] a.d. Straße Metkovi - Neum, Bagalovići bei Metković
	Nordsieck 1970	Agathylla (s. s.) exarata neumensis nom. nov. für *narentana* (A. J. WAGNER 1918) [non A. SCHMIDT]	Badžula bei Neum
	Nordsieck 1972	*Agathylla (A.) exarata neumensis* NORDSIECK 1970	Mislina bei Metković, Klek bei Neum
	Zilch 1977	*Agathylla (Agathylla) exarata neumensis* H. NORDSIECK 1970	S-Dalmatien: Bačula (= Badžula bei Neum), a.d. Straße Metkovic - Neum.

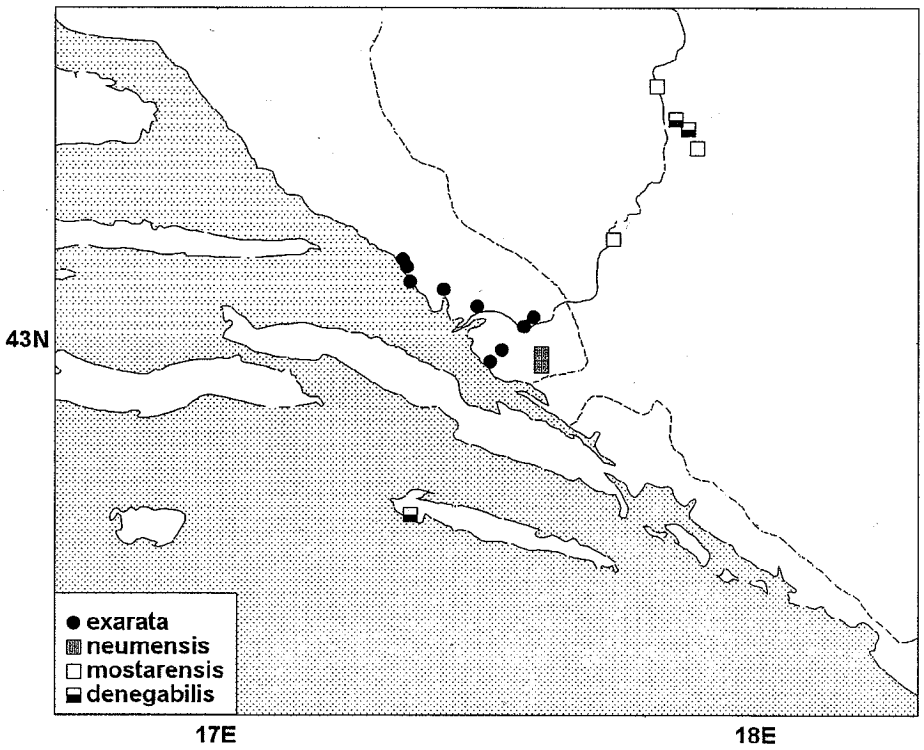

Fig.1 Geographic distribution of *Agathylla exarata* (Rossmässler 1835)

MATERIAL AND METHODS

Materials of the Hungarian Natural History Museum, Budapest (NHMB), and the Hungarian Geological Institute, Budapest (HGIB) were examined. Both collections contained material from the collections of T. Kormos and R. Streda, which had been donated by K. Brancsik. Those labelled as "*Clausilia exarata Zgl. var. mostarensis*" or "*Agathylla exarata mostarensis*" were considered as syntypes, and those designated as "*Clausilia exarata Zgl. f. minor*" were regarded the form to which the former one was compared in the original description (Brancsik 1889). The following samples were examined:

TABLE 2
Abbreviations for shell morphological parameters used in the text

H	shell height (mm)
W	spire width (mm)
H_A	height of the aperture
W_A	width of the aperture
N	number of whorls
RP	number of ribs on the penultimate whorl
RCS	number of ribs on the cervix
RD	rib density

Mostar area **Blagaj, Buna spring, near Mostar**: NHMB 10124/141 (13.11.1976. leg. Gy. Topál); NHMB 31077/5 (14.08.1966. leg. H. Nordsieck); NHMB 84820/2 (leg. A.J. Wagner / ex Museum für Naturkunde der Humboldt-Universität, Berlin ?); NHMB 84821/10 [10] (01.04.1999. leg. Erőss, Fehér, Kovács). **Gnojnice, near Mostar, over the village**: NHMB 43194/10 [8] (13.07.1985. leg. Kiss, Pintér). Podvelež, 3 km E of Donji Gnojnice: NHMB 84822/11 [10] (01.04.1999. leg. Erőss, Fehér, Kovács). **Počitelj, in the valley of the Neretva river**: NHMB 84824/4 (02.08.1981. leg. Z. Erőss); NHMB 84824/10 [10] (01.04.1999. leg. Erőss, Fehér, Kovács). **Mostar**: NHMB 09647/1 [1]; NHMB 31079/3 [3] (leg. K. Brancsik, SYNTYPUS "*mostarensis*"); NHMB 31635/5 [5]; NHMB 31674/9 [7] (ex R. Streda, ex K. Brancsik SYNTYPUS "*mostarensis*"); NHMB 64802/12 [12] (ex K. Brancsik SYNTYPUS "*mostarensis*"); NHMB 84825/4 [4] (ex R. Streda); HGIB-R4094 (ex T. Kormos, leg. K. Brancsik, SYNTYPUS "*mostarensis*"); HGI-R4095; HGI-R4096; NHMB 31675/3 [3] (ex K. Brancsik, "f. *minor*"); NHMB 84826/15 [15] (ex R. Streda, "f. *minor*").

Mljet island **"Dalmatia, Meleda" (=Mljet):** NHMB 10180/2 (leg. Tinter). **Mljet, Sv. Marija National Park:** NHMB 24382/8 (17.08.1976. leg. J. Podani); NHMB 42392/101 [11] (17.08.1976. leg. J. Podani, determined as *A. exarata exarata* by H. Nordsieck).

Neretva-delta **"Dalmatia":** NHMB 10181/1; HGIB-R4092 (ex T. Kormos, labelled as "*f. minor*"); HGIB-R4093. **"Narenta-valley"**: HGIB-R4091. **2 km E of Kremena:** NHMB 41179/165 [10] (12.07.1985. leg. Kiss, Pintér). **Rogotin**: NHMB 43195/134 [10] (12.07.1985. leg. Kiss, Pintér). **7 km SE of Gradač, over Bačinsko Jezero**: NHMB 84827/10 [10] (01.04.1999. leg. Erőss, Fehér, Kovács). **3 km NW of Ploče:** NHMB 84828/10 [10] (01.04.1999. leg. Erőss, Fehér, Kovács). **8 km NW of Klek (N of the conjunction to Raba):** NHMB 84829/10 [10] (02.04.1999. leg. Erőss, Fehér, Kovács). **Lovorje (near Neum):** NHMB 84830/8 [8] (03.04.1999. leg. Erőss, Fehér, Kovács). **2 km SE of Grada :** NHMB 84831/10 [10] (29.07.1998. leg. Z. Fehér). **"Gravosa" (=Gruž) (?):** HGIB-R4090 (1908. leg. Gagyi). **"Ragusa" (=Dubrovnik) (?):** HGI-R4089 (1905. leg. Weiss, ex T. Kormos).

Metković area **"Metković":** NHMB 85193/9 [9] (1936.09. leg. Knipper); HGI-R4088. **5,3 km N of Neum towards Badžula, at a monument**: NHMB 84832/2 (1982.09.01. leg. W. Fauer, type locality for "*neumensis*").

Conchological features of 177 specimens of 21 lots (underlined above) were involved in the statistical analyses. The numbers of examined specimens per lot are indicated in square brackets. 32 specimens of six different lots from Mostar were merged (Mostar I.), and those, labelled as "f.*minor*" (NHMB 31675/3, NHMB 84826/15) were also drawn together (Mostar II.), thus the examined specimens belonged to 15 groups (namely: Blagaj, Gnojnice, Podvelež, Počitelj, Mostar I., Mostar II. , Mljet, Kremena, Rogotin, Bačinsko Jezero, Ploče, Klek, Lovorje, Grada and Metković).

Several conchological features, including those, which were considered as important for this species (Rossmässler 1835, Brancsik 1889, Nordsieck 1972), were examined. Shell height (H), spire width (width of the widest whorl, W), aperture height (H_A) and aperture width (W_A) were measured by slide-callipers with 0,1 mm accuracy. As a consequence of the shell dimensions, the percentage of error is 5-7 times higher for the spire width and apertural sizes then for that of the shell height. The number of whorls (N) was measured with $^1/_4$ whorl accuracy. The number of ribs on the penultimate whorl (RP) and the cervix (RCS) was measured by the method of Welter-Schultes (2000). The rib density on the penultimate whorl (RD) was calculated as $RP/W*\pi$ (table 2.). Other shell characters that are difficult to measure, like the colouration and the structure of the clausilial apparatus, were examined and compared conventionally.

Mean, median, maximum, minimum, 10% and 90% percentile values were calculated

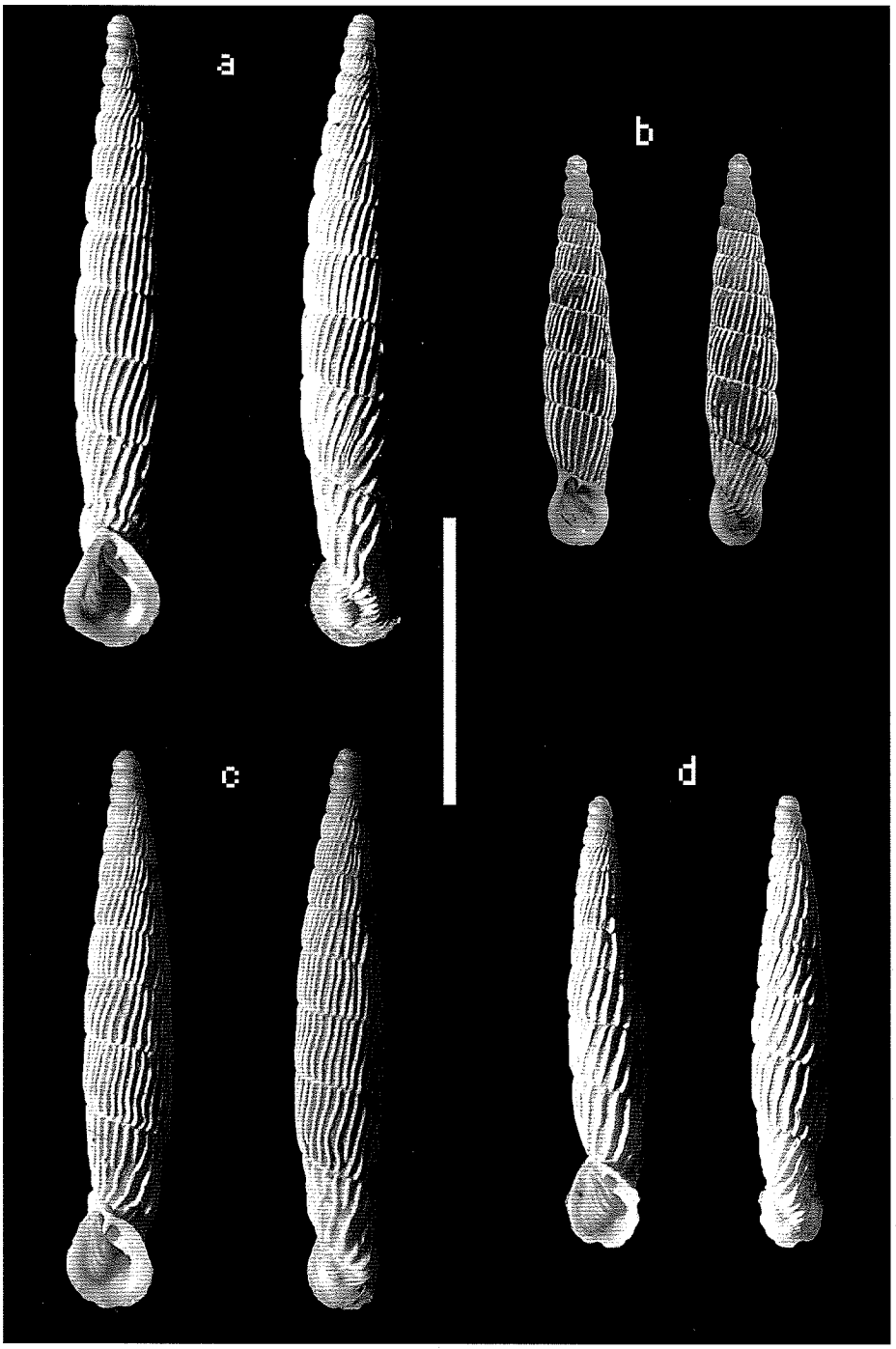

Fig. 2 Shell variations in the examined populations of *Agathylla exarata* (Rossmässler 1835) **a** Neretva delta region, which is the area typica of the nominate subspecies (Ploče, NHMB 84828) **b** Velež mountain (Podvelež, 3 km E of Donji Gnojnice, NHMB 84822) **c** a syntype specimen of *mostarensis* (Mostar, NHMB 64802) **d** a specimen from the type locality of *neumensis*. (5,3 km N of Neum towards Badžula, NHMB 84832). Scale bar = 10mm.

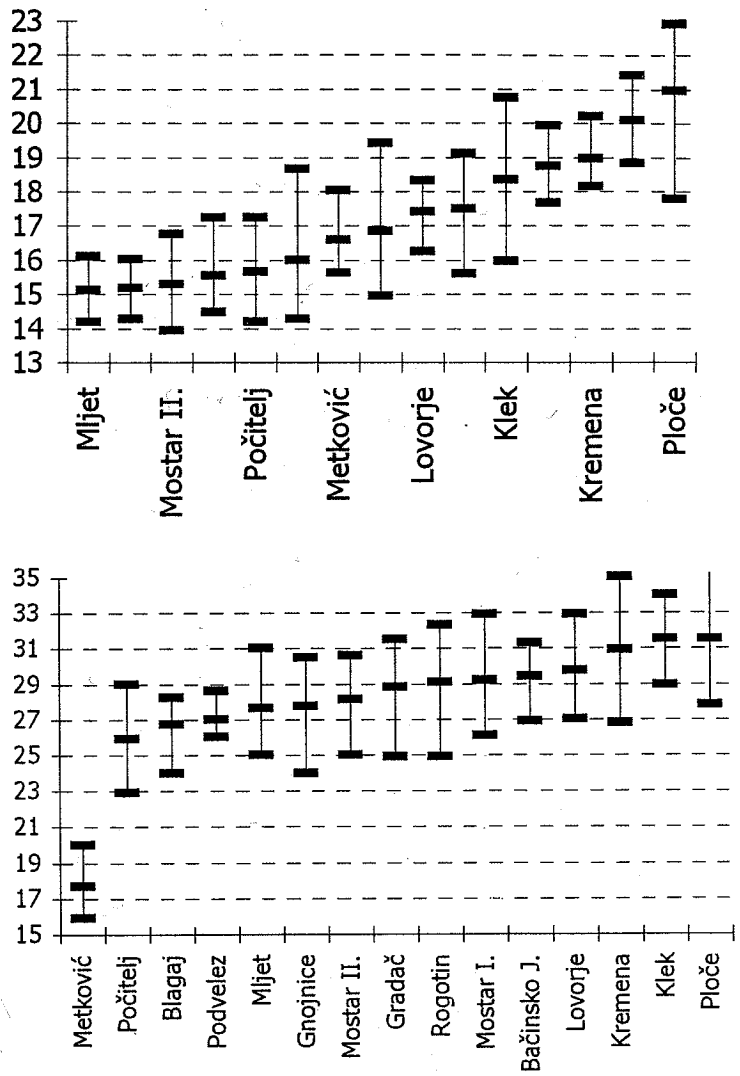

Fig. 3 Variation of shell characters in various *Agathylla exarata* populations. Median, 10% and 90% percentile values are plotted to ignore extreme values. **a** shell height (mm) **b** number of ribs over the penultimate whorl.

for each population and the homogeneity of variances was checked. The correlations between the variables were calculated. The populations were ordered by the mean values of each variable, and each "neighbours" were compared by t-test analysis (Hajtman 1971).

Discriminant function analysis (DFA) combined with multivariate analysis of variance (MANOVA) were performed in order to determine (i) which variables (shell characters) contribute to the discrimination between described subspecies and (ii) which subspecies a certain specimen or a population belongs to (Podani 2000).

Three preconceptual groups were designated, representing the subspecies, which were dealt with by Nordsieck (1972). The *exarata*-group consisted of ten randomly selected specimens from the populations of the Neretva-delta region (Gradač, Bačinsko Jezero,

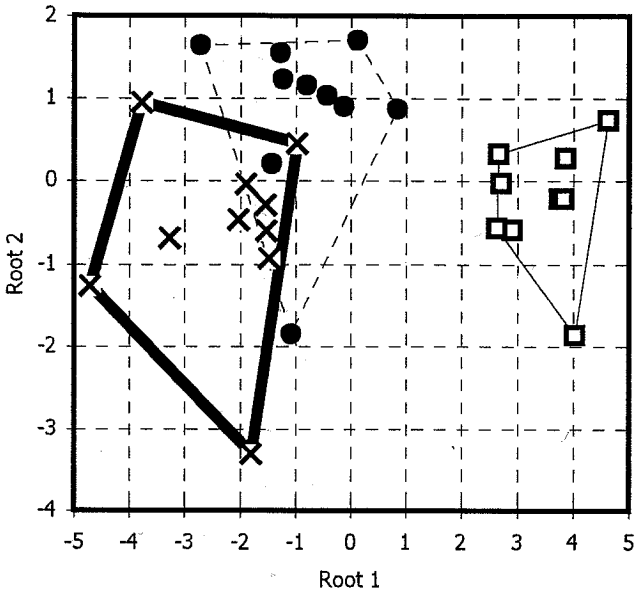

Fig. 4 Three described subspecies of the *Agathylla exarata* separated by discriminant function analysis (DFA). 29 specimens of three subspecies were selected - *exarata* (x), *neumensis* (empty squares) *mostarensis* (filled circles) - and their canonical scores were plotted for the two discriminant functions (roots). The 1st root, which pactically represents the ribbing of the shells, discriminates between *neumensis* and the others, and the 2nd root, which represents shell dimensions, discriminates between *exaratata* and *mostarensis* groups. The overlap of the points of the latter ones and the weak significance of the 2nd root makes their discrimination questionable.

Ploče, Klek, Lovorje), which is the area typica of the nominate form. The *neumensis*-group consisted of nine specimens from Metković, and the *mostarensis*-group consisted of ten randomly selected syntype specimens from Mostar. During these analyses four shell characters were used (H, W, N and RP) as original variables. During the DFA the original variables are replaced by "artificial" variables, called discriminant functions or roots, which are independent from each other, and the number of variables are reduced. In this case this was 2 (the number of the groups minus one). The first discriminant function provided the most overall discrimination between groups, more than any original variable alone. Canonical scores of the specimens in the preconceptual groups (i.e. their position in the space defined by the two discriminant functions) were calculated and plotted (fig. 4).

The overall discrimination of the three subspecies-groups was described by Wilks' λ statistics, which was computed as the ratio of the determinant of the within-groups variance/covariance matrix over the determinant of the total variance/covariance matrix. (Its value generally ranges from 0, meaning perfect discrimination, to 1, meaning no discrimination). The pair-wise discriminations of the groups were characterised by squared Mahalanobis distances (D^2), which are the squares of the distances between the group centroids. (A group centroid is the average of the canonical scores of specimens belonging to the same group.) The significance of the roots' contribution to the discrimination between the groups was tested by sequential Chi^2 test. Structure coefficients - correlations between the original variables and the discriminant functions - were then computed to see how the original variables contribute to the discrimination of the subspecies. Finally, the same discriminant function coefficients were used to compute the canonical scores for the rest of the 177 specimens and the group centroids

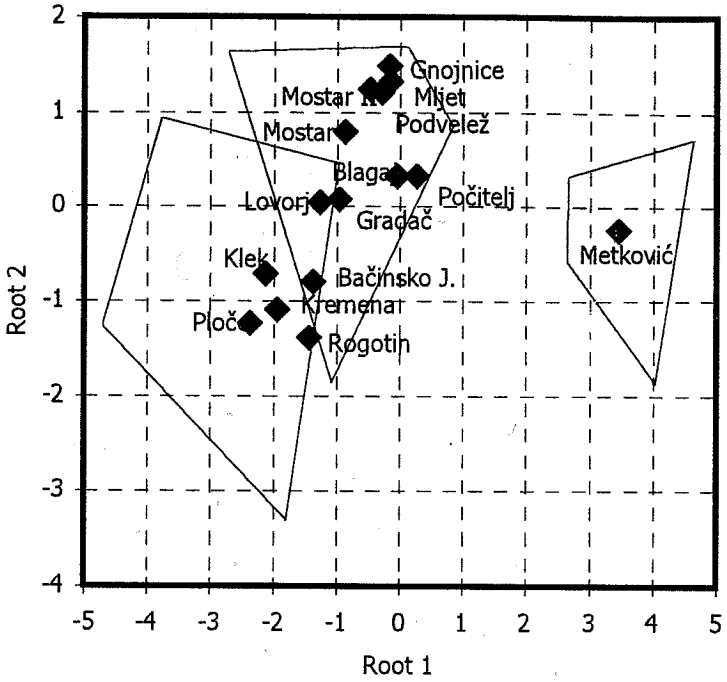

Fig. 5 Group centroids of the examined populations. First a discriminant function analysis was performed on three preconceptual groups, then the resulting canonical scores were applied to each specimen. These values were averaged within each population group, resulting group centroids. Except that of the *neumensis*-group, other centroids scattered more or less homogeneously along the 2nd root.

of every population, too. These scores were plotted in order to predict which subspecies a particular population belongs to (fig. 5).

RESULTS

Among the measured specimens shell height ranges between 13.2 mm (Počitelj) and 23 mm (Ploče), while spire width between 2.4 mm (Podvelež) and 3.7 mm (Klek). The number of whorls varies between $10^1/_4$ (Počitelj, Mostar) and $14^1/_4$ (Klek, Ploče). When comparing the populations by these variables, it is worth to note, that specimens in the Neretva delta are generally larger, while in the Velež mountain (Podvelež, Gnojnice, Mostar II.) and Mljet they are generally smaller than the average (fig. 2.). A considerable overlap could be seen between the populations ordered by the mean values of these variables (fig 3.). Comparing the neighbours of these series by t-test gave no significant differences. These results, however, should be interpreted carefully, since the groups are not independent (Hajtman 1971). H_A and W_A values varied from 2.5 to 4.1 mm and from 2.0 to 3.2 mm, respectively. Significant correlations ($p < 0.001$) were generally found between shell dimensions (H, W, H_A, W_A), the strongest ones being between H–N (0.87), H_A–W_A (0.84) and H–W (0.76).

Rib density was the highest in the specimens from the Velež mountain and Mljet island, the number of ribs, due to the larger circumference, is the highest in those of the Neretva delta region. Both the number of ribs and the rib density are the lowest in

the Metković population. Excepting this latter population, the others are homogenous statistically, while Metković population differ significantly from all of them (RCS: 6–8; RP: 15–20; RD 1.6–2.4 vs. RCS: 8–14; RP: 22–37; RD: 2.3–4.3; $p < 0.0000001$) (Fig. 2.). However ribs were not counted in the upper whorls, but the Metković population is ribbed apparently sparser in the whole shell. Furthermore, ribs of the Metković specimens seem to be somewhat sharper and higher than those of the others. Regarding the RP and RCS values, the rest of the populations seem to be homogenous, without significant differences between the neighbours of the series (fig 3.).

Neither the colour of the shell nor the structure of the clausilial apparatus seemed to differentiate between the groups, therefore these characters were not dealt with further on. Considering that aperture dimensions showed correlation with the shell dimensions ($r \approx 0.6$), and the higher percentage of error of their measurements, H_A and W_A were excluded from the further analyses too.

It was shown by the MANOVA that at least one of the three preconceptual groups differs significantly from the others (Wilks' $\lambda = 0.094$, $F(8,46) = 13.0$, $p < 0.0000001$). The most important variable differentiating them is RP ($F(2,26) = 79.2$, $p < 0.0000001$), but effects of other variables are also significant (p-levels for W, H and N are 0.001, 0.007 and 0.038 respectively).

Pair-wise analysis of group distances resulted in significant squared Mahalanobis distances (D^2) between each pair (*mostarensis - exarata*: $D^2 = 4.8$, $p = 0.0058$, *mostarensis - neumensis*: $D^2 = 21.6$, $p < 0.0000001$, *exarata - neumensis*: $D^2 = 37.0$, $p < 0.0000001$). The 1st discriminant function (Root 1) separates *neumensis* group from the others, while the 2nd function separates the *exarata* and *mostarensis* groups from each other (fig. 4). Sequential Chi^2 test revealed that the 1st discriminant function contributes to the discrimination of the groups in a large extent ($x^2 = 57.9$, $df = 8$, $p < 0.0000001$). The contribution of the 2nd discriminant function however is less significant ($x^2 = 8.9$, $df = 3$, $p = 0.031$). Structure coefficients - the correlations between the original variables and the discriminant functions - showed that RP determines the 1st function almost exclusively ($r = -0.97$), and the 2nd function is determined mainly by W and H ($r = -0.61$ for both). If we would like to assign substantive "meaningful" labels to the discriminant functions, the 1st function can be labelled as "ribbing" and the 2nd one as "shell dimensions".

Individual canonical scores of each specimen and centroids of each population were calculated, then plotted in order to predict which subspecies a particular population belongs to. Except for Metković, the populations did not separate clearly; their sets of points overlap to a large extent; populations of the Velež mountain and Mljet (*denegabilis* form) grouped on the opposite side, compared to some populations of the Neretva delta (typical *exarata* form), while other populations of the Neretva delta and the Mostar area have intermediate positions (fig. 5).

DISCUSSION

In the latest review of the genus three distinct subspecies of *A. exarata* were distinguished, and the number of ribs over the penultimate whorl was assigned as the main distinguishing feature. The mean values of rib number per 2 mm were given as 8.0 for *exarata*, 7.8 for *mostarensis* and 5.7 for *neumensis* (Nordsieck 1972). Apart from the methodical problems of Nordsieck's rib-count (Welter-Schultes 2000), neither the range, nor the variance values were given in this study, so it is impossible to assess if the given mean values differ significantly.

This study shows that the Metković population (*neumensis*) can be distinguished from the others both by the number and/or density of the ribs. From a practical point of view, the number of ribs over the penultimate whorl seems to be the best distinguishing mark,

which ranges between 15 and 20 for *neumensis* and 22–37 for the other groups. In this case only one variable can satisfactorily distinguish a subspecies, thus the application of a multivariate method is unnecessary.

By contrast, due to the overlapping characters, difficulties were experienced during the classification of the other populations and designation of the boundaries of *mostarensis*. Unfortunately, Brancsik's original description (1889) does not help to distinguish *mostarensis* from the nominate form. It compares *mostarensis* to "*Agathylla exarata Zgl. v. minor Chrp.*" living in the high regions of the Velež mountain, and from which *mostarensis* differs by its larger shell and aperture dimensions, and sharper and denser ribs. The form *minor*, described not by Charpentier (1852) but Küster (1847), is a synonym of *Clausilia denegabilis* (Küster 1847) from Mljet (table 1.). The large distance between the Velež mountain and Mljet makes highly questionable any closer relationship between these populations, but the result of the DFA in the present study confirmed Brancsik's opinion, namely that these populations are morphologically very similar. Regarding the rib density, *mostarensis* is believed to be somewhere between *denegabilis* and *exarata*, since *mostarensis* is ribbed denser and sharper than *denegabilis* (Brancsik 1889), and *exarata* is ribbed denser than *mostarensis* (Nordsieck 1972). According to the shell dimensions given by various authors (Rossmässler 1835, Küster 1847, Westerlund 1884 and 1901, Brancsik 1889) *mostarensis* also stands between *denegabilis* and *exarata*. This intermediate position of *mostarensis* could be confirmed by the present study (fig 5.), indicating that *mostarensis* could only be considered a distinct subspecies if *denegabilis* was that too.

However the considerable overlap of quantitative conchological features made a clear separation of these groups impossible even by using multivariate methods. Consequently, our present conchological analyses strongly suggest that the whole *exarata - mostarensis - denegabilis* group should be considered as a continuous range of geographical variations, therefore *mostarensis* and *denegabilis* forms have no subspecific value, they are synonyms of the nominate subspecies.

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