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Morphological variability in and distributional data on *Phaleria bimaculata* populations from islands of the Central Mediterranean area

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Abstract

This study aimed to collect new taxonomic and distribution data on *Phaleria bimaculata* (L.) (Coleoptera Tenebrionidae) from Sicily (southern Italy), the circum-Sicilian islands and the Maltese archipelago. Particular attention was paid to the diagnostic morphological features observed in the populations sampled, focusing on the subspecies *Phaleria bimaculata marcuzzii* Aliquò. *P. bimaculata* individuals were collected from 20 different locations, of which 2 were located in the Sicilian mainland, 9 in circum-Sicilian islands and 9 in the Maltese Islands. In addition, four *Phaleria acuminata* Kuster populations were also recorded along southern Sicilian shores. The two *Phaleria* species were never recorded in sympatry within the current study. Geometric morphometrics were deployed to quantify and analyze the intra-specific differentiation in the shape of two external anatomical structures, i.e. the pronotum and the elytra. The elytra of the individuals from the Aeolian island of Vulcano, *locus typicus* of the ssp. *marcuzzii*, were more elongated and elliptical in comparison to the Maltese and the Sicilian individuals, underscoring the degree of genetic differentiation within different populations of the species.

Key words: *Phaleria bimaculata*, *Phaleria bimaculata marcuzzii*, sandy beaches, Central Mediterranean, morphology, Geometric morphometrics.

Introduction

The genus *Phaleria* Latreille (Coleoptera Tenebrionidae) includes several species of psammophilic polysaprophagous and saprophagous tenebrionids. In Europe, nine *Phaleria* species have been recorded to date (de Jong, 2010), with many subspecies of uncertain systematic value.

In Sicily and the Maltese Islands, two *Phaleria* species are known to occur, i.e. *Phaleria bimaculata* (L.) and *Phaleria acuminata* Kuster, whilst the presence of *Phaleria reveillierei* Mulsant et Rey, reported only from poorly-preserved specimens (collected from Sicily) and found in the Natural History Museum of Budapest (Canzoneri, 1968; Aliquò and Soldati, 2010), needs to be confirmed.

P. bimaculata s.l. is a polymorphic species with a Mediterranean chorotype (Löbl and Smetana, 2008), which is restricted to beaches characterized by a coarse grain size ranging from coarse sand to pebbles and cobbles (Canzoneri, 1968; Fattorini, 2008). Within the Italian populations, the following subspecies have been described: *Phaleria bimaculata adriatica* Rey along the Adriatic coast of peninsular Italy (Canzoneri, 1968; Bonometto and Canzoneri, 1970); *Phaleria bimaculata jonica* Canzoneri along the Ionian coast of the Italian regions of Calabria and Apulia (Canzoneri, 1968); and *Phaleria bimaculata marcuzzii* Aliquò in the Aeolian Islands (*locus typicus*: Vulcano; Aliquò, 1993). In addition, *P. bimaculata* populations from the Egadian Islands are considered by Canzoneri (1970) to be different from the nominal subspecies.

Within the Maltese archipelago, previous entomological surveys (Mifsud and Scupola, 1998; Deidun *et al.*, 2010) reported the sympatric occurrence of *P. bimaculata* and the congeneric *P. acuminata* on two sandy beaches (Golden Bay and Mgiebah). Such sympatry had already been reported in different areas of the Mediterranean (e.g. in Morocco by Colombini *et al.*, 2008). According to Deidun *et al.* (2010), the two species normally occupy different beach zones; for instance, at Golden Bay and Mgiebah (Malta) *P. bimaculata* had a more seaward distribution than *P. acuminata*.

The current study was conceived in order to collect new taxonomic and distribution data on *P. bimaculata* in Sicily, the circum-Sicilian islands and Maltese archipelago, paying special attention to diagnostic morphological features encountered in the populations sampled, especially regarding the subspecies *P. bimaculata marcuzzii*, whose *locus typicus* was included in the sampling area.

Materials and methods

Sampling

A total of 49 sandy beaches on different Central Mediterranean island archipelagos (Maltese, Pelagian, Egadian, Aeolian) and on the Sicilian mainland were sampled once over the March-October 2010 period. The full list of sampled sandy beaches is given in table 1, whilst figure 1 gives the location of the sampled sites. The supralittoral zone of these beaches was sampled by means of individual pitfall traps or by means of constellations of pitfall traps, each of which consisted of five

Table 1. List of the sampled localities. s: single traps, c: constellation traps; A: *P. acuminata*, B: *P. bimaculata*, n.c.: no *Phaleria* collected; §: first record of the genus *Phaleria* on the island.

Code	Island	Beach	Coordinates (WGS84)	Sampling date	Traps deployed	Species collected
1	Malta	Golden Bay	14°20'37"E - 35°56'00"N	6.II.10	3c	B
2	Malta	Ghajn Tuffieha	14°20'38"E - 35°55'44"N	6.II.10	2c	B
3	Malta	Gnejna	14°20'35"E - 35°55'13"N	6.II.10	2c	B
4	Malta	Mellieha Bay	14°21'03"E - 35°58'10"N	13.II.10	6s	n.c.
5	Malta	Mgiebah	14°22'55"E - 35°58'05"N	13.II.10	6s	B
6	Malta	Armier	14°21'26"E - 35°59'21"N	13.II.10	6s	B
7	Malta	Little Armier	14°21'34"E - 35°59'23"N	13.II.10	6s	B
8	Malta	White Tower Bay	14°21'53"E - 35°59'31"N	13.II.10	2c	n.c.
9	Malta	Birzebbuga	14°31'47"E - 35°49'29"N	6.III.10	6s	n.c.
10	Malta	St. George's Bay	14°29'18"E - 35°55'33"N	6.III.10	4s	n.c.
11	Malta	Qarraba Bay	14°20'34"E - 35°55'36"N	6.II.10	4s	n.c.
12	Malta	Tà Lazzenin	14°23'58"E - 35°56'59"N	13.II.10	2s	n.c.
13	Gozo	Ramla l-Hamra	14°17'03"E - 36°03'41"N	3.IV.10	3c	B
14	Gozo	San Blas	14°18'03"E - 36°03'25"N	3.IV.10	5s	B
15	Gozo	Dahlet Qorrot	14°19'00"E - 36°02'57"N	3.IV.10	3s	n.c.
16	Gozo	Hondoq ir-Rummien	14°19'26"E - 36°01'39"N	3.IV.10	3s	n.c.
17	Gozo	Mgarr ix-Xini	14°16'16"E - 36°01'12"N	3.IV.10	2s	n.c.
18	Gozo	Qbajjar	14°15'09"E - 36°04'35"N	3.IV.10	2s	n.c.
19	Comino	Santa Marija Bay	14°20'14"E - 36°01'00"N	20.III.10	6s	B
20	Comino	San Niklaw	14°19'48"E - 36°01'00"N	20.III.10	2s	n.c.
21	Comino	Cominotto Beach	14°19'22"E - 36°00'46"N	20.III.10	2s	n.c.
22	Lampedusa	Spiaggia dei Conigli	12°33'26"E - 35°30'47"N	3.V.10	2c	B
23	Lampedusa	Guigtgia	12°36'00"E - 35°29'55"N	3.V.10	2c	B
24	Lampedusa	Cala Francese	12°37'29"E - 35°29'44"N	3.V.10	3s	B
25	Lampedusa	Le palme	12°37'24"E - 35°30'17"N	3.V.10	3s	B
26	Linosa	Cala Pozzolana di Ponente	12°51'04"E - 35°52'02"N	5.V.10	3s	B§
27	Linosa	Cala Pozzolana di Levante	12°52'32"E - 35°51'21"N	5.V.10	3s	n.c.
28	Favignana	Porto	12°19'32"E - 37°55'44"N	16.X.10	2c	B
29	Favignana	Lido Burrone	12°20'17"E - 37°55'06"N	16.X.10	2c	n.c.
30	Favignana	Cala Grande	12°16'49"E - 37°55'48"N	16.X.10	4s	n.c.
31	Favignana	Cala Rotonda	12°17'03"E - 37°55'27"N	16.X.10	4s	n.c.
32	Favignana	Preveto	12°18'09"E - 37°54'55"N	16.X.10	2s	n.c.
33	Vulcano	Vulcanello Ponente	14°57'49"E - 38°24'42"N	11.X.10	4c	B
34	Vulcano	Gelso	14°59'45"E - 38°22'08"N	11.X.10	8s	B
35	Lipari	Canneto	14°57'56"E - 38°29'16"N	12.X.10	6s	B
36	Lipari	Marina Lunga	14°57'19"E - 38°28'18"N	12.X.10	6s	n.c.
37	Lipari	Quattrocchi	14°56'07"E - 38°27'31"N	12.X.10	10s	n.c.
38	Sicily	Foce del fiume Belice	12°52'13"E - 37°34'53"N	10.V.10	2c	A
39	Sicily	Capo San Marco	13°01'09"E - 37°29'46"N	10.V.10	2c	n.c.
40	Sicily	Torre di Gaffe	13°51'18"E - 37°07'21"N	11.V.10	2c	A
41	Sicily	Torre di Manfria	14°06'55"E - 37°06'11"N	11.V.10	2c	A
42	Sicily	Foce del Fiume Ippari	14°26'22"E - 36°52'34"N	11.V.10	2c	n.c.
43	Sicily	Kamarina	14° 27'09"E - 36°50'50"N	11.V.10	2c	A
44	Sicily	Oliveri	15°05'10"E - 38°07'12"N	13.X.10	8s	B
45	Sicily	San Giorgio	14°56'59"E - 38°10'11"N	13.X.10	8s	n.c.
46	Sicily	Marina di Patti	14°58'11"E - 38°09'09"N	13.X.10	8s	n.c.
47	Sicily	Foce del torrente Agrò	15°21'26"E - 37°55'53"N	13.X.10	8s	B
48	Sicily	Letojanni	15°18'22"E - 37°52'41"N	13.X.10	8s	n.c.
49	Sicily	Nizza di Sicilia	15°24'59"E - 37°59'41"N	13.X.10	8s	n.c.

plastic cups (diameter = 7.5 cm) buried with their mouth flush with the surface of the sand and connected by means of thin wooden walkways resting on the surface of the sand. Such walkways increase the efficiency of the traps as they divert any wandering animal that makes contact with the walkways into the traps. Gauci *et al.* (2005) give an illustration of the layout of such pitfall trap constellations. Where substrate type (e.g. too coarse a sediment) or beach attributes (e.g. too narrow a beach) precluded the deployment of pitfall trap constellations, individual, non-connected traps (plastic cups) were used instead. Mildly-concentrated vinegar was

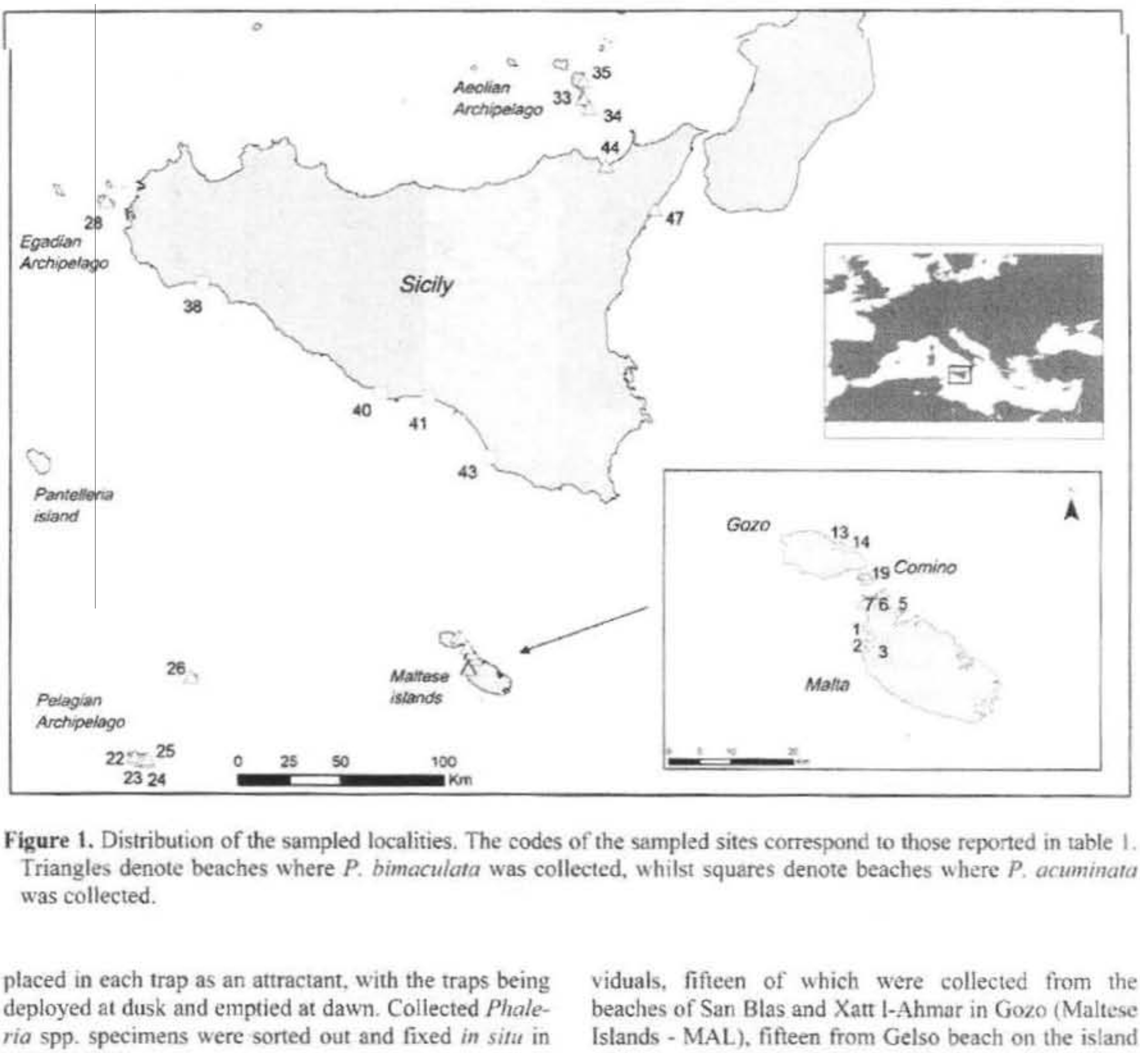


Figure 1. Distribution of the sampled localities. The codes of the sampled sites correspond to those reported in table 1. Triangles denote beaches where *P. bimaculata* was collected, whilst squares denote beaches where *P. acuminata* was collected.

placed in each trap as an attractant, with the traps being deployed at dusk and emptied at dawn. Collected *Phaleria* spp. specimens were sorted out and fixed *in situ* in 80% ethanol.

Identification of the collected specimens was carried out under a stereomicroscope according to the morphological characteristics listed in Canzoneri (1968). When necessary, selected specimens were dissected in order to study the form of the aedeagus.

Geometric morphometrics

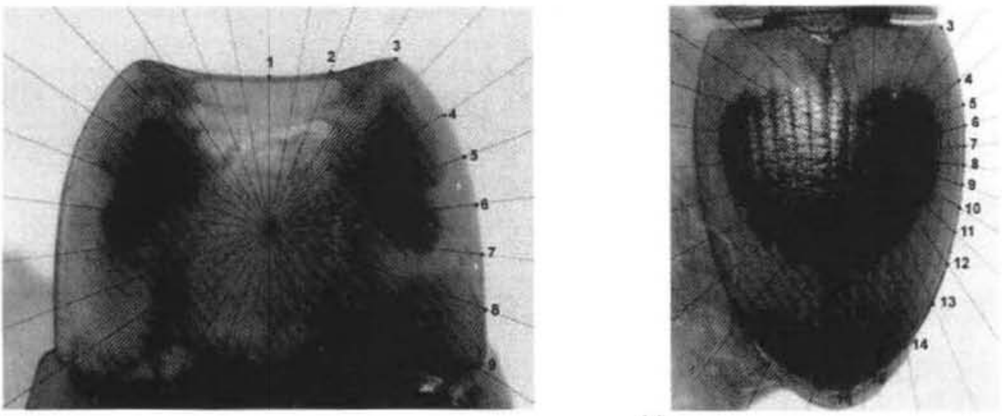
Geometric morphometrics techniques, nowadays a standard protocol in morphological research and described in Rohlf and Marcus, 1993; Adams *et al.*, 2004 and Zelditch *et al.*, 2004, were used to analyse the intra-specific differentiation in the shape of pronotum and the elytra in three populations of *P. bimaculata*. We focused on these two body parts because the shape of pronotum and elytra is considered of diagnostic interest in the genus *Phaleria*. Moreover, previous studies in tenebrionid beetles showed that pronotum shape is particularly suitable for geometric morphometric analyses (Palmer, 2002a; 2002b; Taravati *et al.*, 2009). Statistical variations in the shape of the pronotum and the right elytra in different *P. bimaculata* individuals from different populations were analyzed using multivariate statistics.

Analyses were performed on 45 *P. bimaculata* individuals, fifteen of which were collected from the beaches of San Blas and Xatt l-Ahmar in Gozo (Maltese Islands - MAL), fifteen from Gelso beach on the island of Vulcano (Aeolian Islands - VUL), and fifteen from Oliveri beach (SIC), along the northern coast of Sicily. The head and forelegs were dissected from the specimens to be used for future molecular analyses. After dissection, the remaining body parts were mounted on a card.

The specimens were positioned along a horizontal plane; for each individual, the right portion of the body was examined. Dorsal imagines were digitized using a Leica D-LUX 3<<LMS>> camera mounted on the optical stereomicroscope Wild M3.

The digital imagines of the pronotum and the elytra were processed separately with MakeFan6 software (Sheets, 2003). Within the pronotum, the cartesian x, y coordinates of four landmarks and six semi-landmarks were recorded. In the elytra, the cartesian x, y coordinates of four *landmarks* and twelve *semi-landmarks* were recorded. The position of the pronotum *landmarks* and *semi-landmarks* adopted in this study is shown in figure 2a, while the position of elytra *landmarks* and *semi-landmarks* is shown in figure 2b.

The bi-dimensional coordinates of the anatomical *landmarks* and *semi-landmarks* on the outline of the dorsal view of the pronotum and the right elytra were



a) b)

Figure 2. a) The *landmarks* for pronotum (N = 4: 1-3-9-10) and the *semi-landmarks* for pronotum (N = 6) and **b)** adopted for the *P. bimaculata* individuals studied. The *landmarks* for elytra (N = 4: 1-2-3-16) and the *semi-landmarks* (N = 12) were digitized on half of each structure to remove the variability introduced by an eventual asymmetry.

collected and digitized by means of the tpsDIG 2 software (Rohlf, 2004). In order to better perform statistical analysis, the *landmarks* and *semi-landmarks* were successively recognized with tpsUtil 1.45 (Rohlf, 2008). The resulting coordinates were subjected to a Generalized Procrustes Analysis (GPA), which removes all the information unrelated to shape (Rohlf and Slice, 1990).

For the comparison of the configurations of *landmarks* and *semi-landmarks*, the Relative Warps Method was used (Bookstein, 1991; Rohlf, 1993). The relative warps are principal component vectors of the partial warps, variables generated for thin-plate spline transformations (Bookstein, 1989), and these were used to describe the major trends in shape variation among specimens within the sample (Rohlf, 1993; 1996). Thin-plate spline deformation grids were generated to facilitate description of shape variation. The analyses were performed by means of Relative Warps 1.39 software (Rohlf, 2004).

In order to single out a putative relationship between site and shape, a clustering of the studied specimens was performed on the basis of their geographical position, through the Canonical Variate Analysis (CVA). The CVA of the morphometric data was performed using MorphoJ 1.01 software (Klingenberg, 2011) and used to simplify the identification of differences between predefined group means (Zelditch *et al.*, 2004).

Results and discussion

P. bimaculata individuals were recorded at 20 different locations, of which 2 were located in Sicily, 9 in circum-Sicilian islands and 9 in the Maltese Islands. In addition, three *P. acuminata* populations were also recorded along southern Sicilian shores (table 1). The two *Phaleria* species were never recorded in sympatry within the current study. Such an observation was unexpected for the Maltese beaches of Golden Bay and Mgiebah where a previous sympatry between the two *Phaleria* species was reported (Deidun *et al.*, 2010). A possible reason for such a discrepancy might be the snapshot nature of the sampling conducted in the present study which might have resulted in *P. acuminata* not being represented in collections, although there are no records of significant phenological differences between the two species.

Major body shape differences seem to be probably related to geometric differences in elytra, as evident from deformation grids (figure 3): the elytra of the individuals from Vulcano (points 31-45 in the plot) were more elongated and elliptical in comparison to the Maltese individuals (1-15) and the Sicilian individuals (16-30), with the latter being positioned in the central area of the plot.

The CVA showed a cumulative variance in the first two axis of 100%, for both pronotum and elytra analysis. In the CVA scatterplot, the three groups, Vulcano, Malta, Sicily, can be clearly distinguished (figure 4). The Mahalanobis distances (table 2) showed, in all pairwise comparisons, strong significant differences (p < 0.001) in body shape, highlighting the differentiation of elytra-shape between the site Vulcano and the rest of samples.

The observed inter-population shape differentiation clustered the populations into three groups, according to a spatial structure pattern, which could be described as "site-specific". A model of isolation by distance cannot

Table 2. Mahalanobis distances among groups. Below elytra, above pronotum analysis.

	Malta	Sicily	Vulcano
Malta		3.1369***	3.0108***
Sicily	3.9024***		3.1567***
Vulcano	6.8089***	5.8927***	

P-values from permutation tests (10000 permutation rounds); ***P < 0.001.

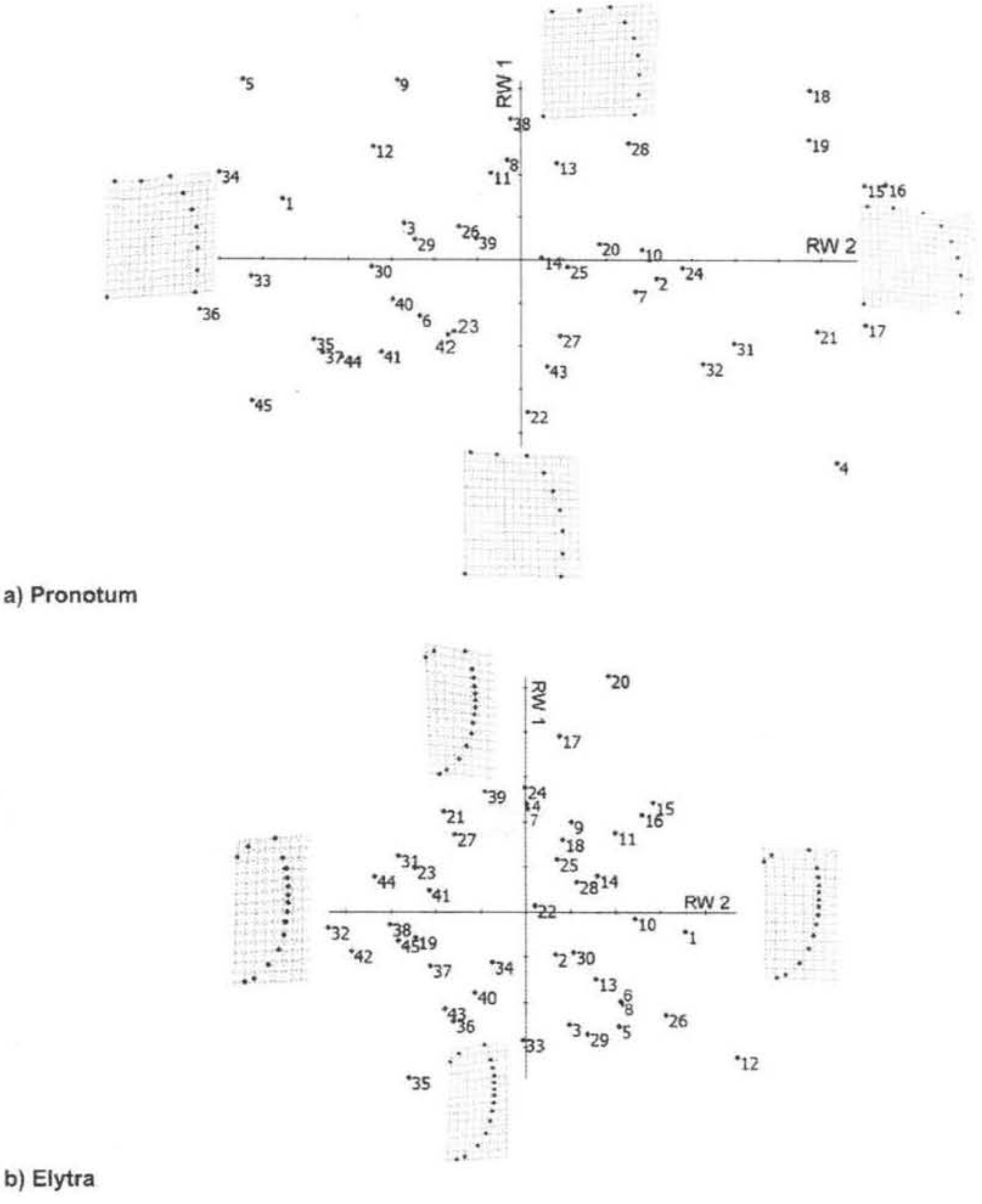


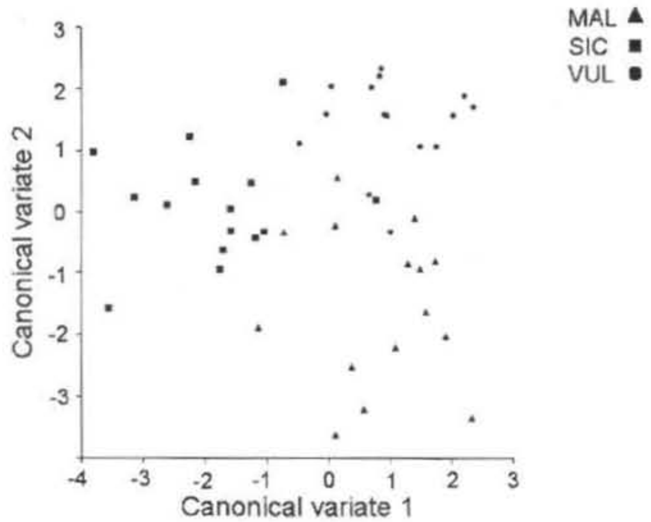
Figure 3. Scatterplots of the two first relative warps scores obtained from the Relative Warp Analysis (RWA) of the shape of two external morphological structures. Plots represent the analysis of the shape and show deformation grids relative to the axis; **a)** pronotum: RW1 + RW2 accounted for a total of 81.41%, **b)** elytra: for a total of 79.62%. MAL, Malta, points 1-15 in the plot; SIC, Sicily, points 16-30; VUL, Vulcano, points 31-45.

be excluded and it could be hypothesized that inter-population shape differentiation arose because populations are geographically isolated and live under a different set of environmental conditions.

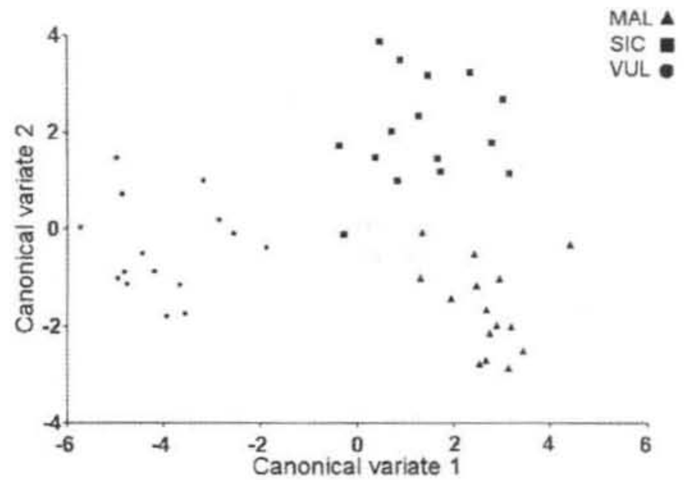
On the basis of subtle morphological differences, Canzoneri (1968) ascribed some Sicilian populations to a differentiated, infra-subspecific taxon (natio *concii*).

However, morphological comparisons of individuals from two Sicilian mainland localities (Foce del Torrente Agrò and Oliveri beach), characterized by coarse sand and small pebbles, with those collected in the circum-Sicilian islands and Maltese archipelago, revealed no such differentiation.

With reference to the *P. bimaculata* populations from the beaches of the Egadian archipelago, Canzoneri (1970) considered the populations for this species recorded on Favignana and Marettimo to be systematically distinct from the nominal subspecies in view of a darker dorsal coloration, narrower elytra and markedly convex elytral intervals. Canzoneri (1970) also stated



a) Pronotum



b) Elytra

Figure 4. Plot of canonical variates 1 against 2. **a)** Represents the analysis of the pronotum shape in relation to the sites; **b)** represents the analysis of the elytra shape in relation to the sites. MAL, Malta; SIC, Sicily; VUL, Vulcano.

that specimens from the Egadian Islands were distinct from all other *P. bimaculata* specimens coming from France and western Italy and grossly similar to those from the Pelagian Islands. *P. bimaculata* populations sampled within this study at Favignana did not exhibit morphological features markedly different from those of the nominal subspecies, although such an observation must necessarily be substantiated by a more comprehensive sampling effort of the *P. bimaculata* populations in question.

From a morphological perspective, all the *P. bimaculata* populations sampled on the Sicilian mainland (i.e. those sub natio *concii*, *sensu* Canzoneri, 1968), Lampedusa, Favignana and the Maltese Islands exhibited the features typical of the nominal subspecies.

In contrast, the populations sampled in Vulcano and Lipari corresponded to the description for the subspecies *P. bimaculata marcuzzii*, which is distinguished from the nominal subspecies through the morphology of its larger aedeagus and its darker dorsal coloration. *P. bimaculata marcuzzii* is considered by Marcuzzi (1996) as simply a melanic form of the nominal subspecies. Aliquò *et al.* (2006) and Aliquò and Soldati (2010) subscribe to such a hypothesis, and in fact synonymise such a subspecies with the nominal one. Löbl and Smetana (2008) consider the subspecies *marcuzzii* as a valid one, differently from de Jong (2010). The observation and the morphometric analysis of the *P. bimaculata* specimens sampled within the context of this study in Vulcano and Lipari support the systematic validity of assigning these populations to a separate subspecies in view of their consistent morphological divergence from *Phaleria bimaculata bimaculata* (L.).

In relation to the results of the geometric morphometric analysis, major body shape differences seem to be probably related to geometric differences in elytra, as evident from deformation grids (figure 3): the elytra of the individuals from Vulcano (points 31-45 in the plot) were more elongated and elliptical in comparison to the Maltese individuals (1-15) and the Sicilian individuals (16-30), with the latter being positioned in the central area of the plot.

In conclusion, our study confirms the high degree of inter-population morphological variability within *P. bimaculata* in the surveyed geographical area, and our results are compatible with the possible sub-specific taxonomic status of the Aeolian populations (ssp. *marcuzzii*). It is recommended that the degree of such inter-population morphological variability and the phylogeographic implications alluded to in this study should be further assessed through the application of appropriate molecular techniques, in order to further define the taxonomic status of the various *P. bimaculata* populations.

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