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Morphometric and meristic diversity of the species *Scolopendra cingulata* Latreille, 1829 (Chilopoda: Scolopendridae) in the Mediterranean region

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Abstract

Scolopendra cingulata Latreille, 1829 is the commonest scolopendromorph species in the Mediterranean area. However, its morphological and geographic variation has not been examined so far, and therefore robust hypotheses about the factors that have shaped that variability are lacking. We examined, using multivariate methods for 19 morphometric and meristic characters, the morphological variation of that species using a comprehensive sample of 503 adult specimens from 130 restricted geographic localities. The localities were distributed in three major geographic areas (Balkans, Asia Minor-Middle East, and Italy) in order to discern biological entities and to estimate the morphological relationships between populations and geographic regions. Results showed significant differences between the three geographic groups. Characters such as the distance between the paramedian sulci of the 7th and 8th tergite, the number of antennal segments, the number of spines on the dorsal side of 21st prefemur and number of spines on the 21st prefemoral process significantly discriminated populations of *S. cingulata* along a west–east geographic gradient. Both eastern (Balkan) and easternmost populations (Asia Minor-Middle East) showed higher mean values of antennal articles and spines of 21st prefemur than the Italian populations. No significant morphological variation was discovered between the sexes of *S. cingulata* apart from cephalic width and distance between the two paramedian sulci of the 7th and 8th tergite. Based on certain morphometric differences among the three major geographic regions, we suggest that *S. cingulata* originated in the east and colonized south European area from two different geographic routes. It is suggested that west–east morphological trend of *S. cingulata* is related either to the prominent palaeogeographic events of the area or to the competition with *S. oraniensis* Lucas, 1846 in the western Mediterranean.

Key words: Asia Minor; Balkan; Geographic pattern; Intraspecific variation; Italy; Middle East; Morphological discrimination; Scolopendromorpha

1. Introduction

Morphological variation is often attributed to differential adaptations to diverse habitats. Therefore, the study of intraspecific morphological variation is a necessary tool to understand the diversity of life, and to examine if morphological variability can be attributed to selection by ecological and environmental factors or is the result of palaeogeographic history (Adams et al., 2004; Kamilari and Sfenthourakis, 2009).

The evolution of a dynamic geological system such as that of the Mediterranean region has affected the distribution of many terrestrial animals. Mediterranean is characterized

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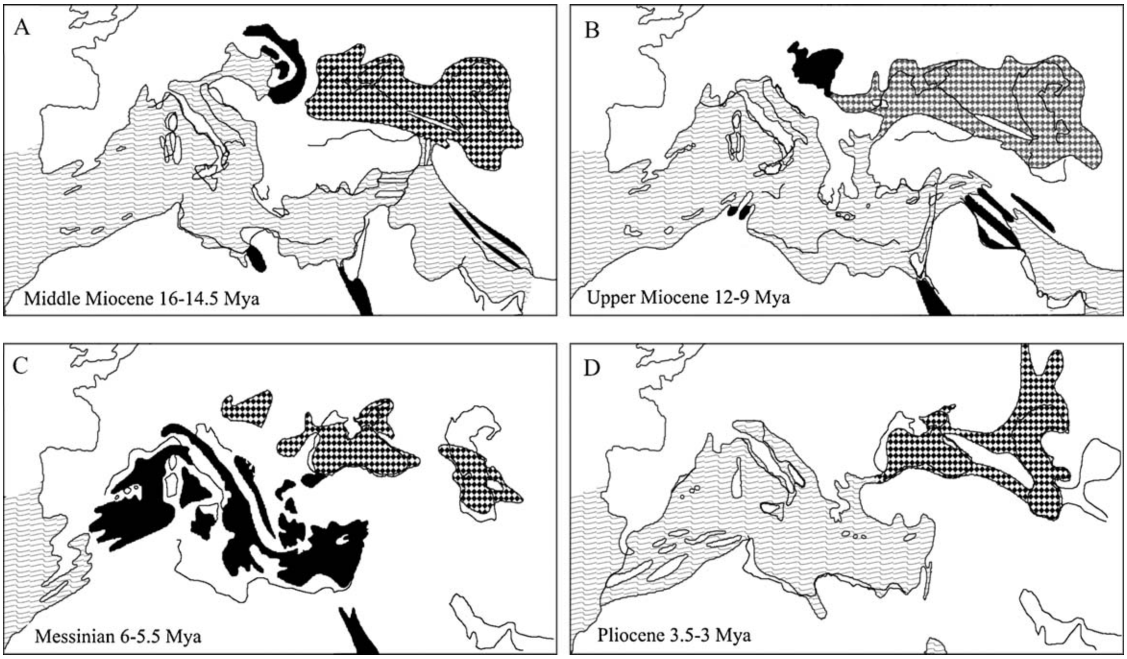

Fig. 1. Schematic view of the palaeogeographic evolution of the Mediterranean and the Paratethys regions from middle Miocene (16–14.5 Mya) to Pliocene (3.5–3 Mya) (Roegl and Steininger, 1983).

by numerous and occasionally complex geographic events in the past (Roegl and Steininger, 1983) (Fig. 1). Therefore, in modern phylogeographic studies, the Mediterranean represents a model area from which to infer intra- and inter-population variation among geographically distinct areas, such as the Balkan, Anatolian, Middle Eastern and Italian peninsulas. In particular, spatially and temporally fragmented habitats (e.g. islands, mountainous peaks) are used to understand evolutionary processes that might provide answers on species and/or population variability. There are numerous works using molecular data in conjunction with the palaeogeography of the Mediterranean area to study population differentiation among land snails (Douris et al., 1995; Parmakelis, 2003), scorpions (Parmakelis et al., 2006) and vertebrates (Poulakakis et al., 2003; Kasapidis et al., 2005; Kornilios et al., 2005; Poulakakis et al., 2005; Thanou et al., 2005; Tryfonopoulos et al., 2008). However, morphological phylogeographic studies are few although multivariate morphometric analysis has been shown to demonstrate complex, multidimensional patterns of variation and has been proved to be a powerful and reliable method for analyzing intraspecific relations. There are few such works, dealing with beetles (Chatzimanolis et al., 2003), butterflies (Dennis et al., 2000), snails (Giokas, 2000) and vertebrates (Tsekoura et al., 2002; Fraguedakis-Tsolis et al., 2009).

Based on the Chilobase (an electronic database, publicly and freely available through the World Wide Web – http://chilobase.bio.unipd.it/, last updated 2006), the order Chilopoda comprises around 3500 valid species out of an estimated global fauna of approximately 8000 species (Adis and Harvey, 2000). There are nearly 700 species of scolopendromorph centipedes worldwide (Chilobase, 2006). The Scolopendridae is one of the largest families in the Order Scolopendromorpha, including approximately 10% of the world's centipede diversity (Menon et al., 2003). The family has been the subject of research around the world (for example Schileyko and Minelli, 1998; Shelley and Kiser, 2000; Chagas-Jr, 2001; Shelley, 2002, 2006; Shelley et al., 2005). The commonest genus within the family Scolopendridae (Newport, 1894) is the genus *Scolopendra* (Linnaeus, 1758). Species of *Scolopendra* Linnaeus, 1758 are widespread in all tropical, subtropical and warm temperate areas (Lewis, 1981). Until recently, the taxonomy of *Scolopendra* was based exclusively on external morphology. Würmli (1978, 1980) produced a comprehensive taxonomic revision of *Scolopendra* species (*canidens* group), examining thousands of specimens and identifying, for the first time, 21 qualitative and quantitative morphological characters. However, more recent studies have concentrated on the systematics and distribution of certain *Scolopendra* species (Shelley, 2002, 2006; Shelley et al., 2005). Simaiakis and Mylonas (2008) have shown that there are nine species distributed in the Mediterranean region. Among them *Scolopendra cingulata* (Latreille, 1829) is the most widely distributed species, and it also occurs in western Asia.

The main aim of this work was the investigation and explanation of the patterns of morphological variation of *S. cingulata* in the southern Mediterranean region, in relation to the geography and palaeogeography of that area. We used sample material collected from sites that provide comprehensive coverage of the main distributional range of *S. cingulata* in the Mediterranean. We examined these samples using, for first time in that species, multivariate analyses for a series of established and new quantitative morphological characters

Table 1. Number of individuals used for the present study (per region and per Natural History Museum). All samples stored in Natural History Museums and Scientific Institutions. NHMC: Natural History Museum of Crete, ZMUC: Zoological Museum of Copenhagen, HNHM: Hungarian Natural History Museum, UPAD: University of Padova. MAE: Minor Asia and Middle East, BAL: Balkan peninsula, ITA: Italian peninsula. Immatures were excluded from the analysis. Number of sampling sites per region is in brackets.

	NHMC	ZMUC	HNHM	UPAD
Females	163	50	50	16
Males	131	41	39	13
Immatures	35	–	–	–
Sum	329	91	89	29
	BAL (89)	MAE (25)	ITA (16)	
Females	116	118	44	
Males	110	74	41	
Immatures	35			
Sum	261	192	85	

(morphometric and meristic). We sought to improve the statistical accuracy and reliability and also to identify the major morphological characteristics that differentiate geographic groups within that species, and to look for possible morphological adaptations related to the diversification processes in this taxon.

2. Materials and methods

2.1. Sample collection

Samples of *S. cingulata* were collected by hand or by pitfall traps during the annual wet periods (September–December and March–May) from three major geographic areas: (a) the Balkan peninsula (89 restricted sites), (b) Minor Asia and Middle East (25 restricted sites) and (c) the Italian peninsula (16 restricted sites) (Appendix A). The geographic units were grouped based on the palaeogeography of the Mediterranean area (see Fig. 1). Specimens were mainly found under stones, among leaf litter, on the soil surface and under fallen tree trunks. In total, we examined 538 individuals (Table 1) from 130 restricted geographic areas (Appendix A). While morphometric analysis has been in part performed on age-related characters (e.g. body length), and because it could be risky any control for age, specimens were selected randomly from all above-mentioned restricted geographic areas. 35 out of 538 specimens were excluded from further statistical analysis because we could not determine their sex. With respect to Lewis (2010), specimens of less than 40 mm, with less than 17 antennomeres, were excluded from further statistical analysis. Material was preserved either in 95% or 75% ethyl alcohol and deposited in the Natural History Museum in Crete (NHMC), the Hungarian Natural History Museum in Budapest (HNHM), the Zoological Museum in Copenhagen (ZMUC) and the University of Padova (UPAD) (Table 1).

2.2. Morphometrics

Morphometric characters are continuous characters representing linear distances between body landmarks. Apart from the usual key taxonomical characters used by scientists in the past for scolopendrid species (Lewis, 1968, 1981, 1985, 2003, 2010; Würmli, 1978, 1980), we also analyzed new morphometric variables (for further details see below) related to specific body landmarks of *S. cingulata* (J.G.E. Lewis and G. Edgecombe, pers. comm.). All characters were measured with an ocular micrometer using a Leica MZ6 stereomicroscope at a magnification of 10× or 20×. Data are given in millimetres (mm). Sex was determined under a stereomicroscope based on the description of the reproductive system and the external genitalia figured by Jangi (1966). To check the repeatability of individual measurements, we randomly double-measured four out of twelve morphometric characters in every specimen. The twelve morphometric measurements are listed below, and nine out of twelve are shown in detail in Fig. 2:

- Body length (BL) from the anterior margin of the head shield to the end of the telson was measured with a ruler accurate to one millimetre.
- Cephalic plate width (CW) measured maximum width.
- Cephalic plate length (CL) measured from the anteriormost point of the clypeal margin to the mid-point of the occipital margin.
- 7th tergite (T7D), measured the distance between the paramedian sulci of the seventh tergite close to the posteriormost margin.
- 8th tergite (T8D), measured the distance between the paramedian sulci of the eighth tergite close to the posteriormost margin.
- 21st Tergite width (T21W) measured maximum width of tergite.
- 21st Tergite length (T21L) measured maximum length of tergite.
- 21st Sternite width (S21W) measured maximum width of sternite.
- 21st Sternite length (S21L) measured maximum length of sternite.
- 21st Prefemur width (21PFW) measured dorsally at base of the right prefemur.
- Median suture on 21st Tergite (T21SL) measured length of suture from anteriormost mid-point of tergite margin to the end of suture in the middle of tergite.
- 21st Coxal pore area width (PAW) measured maximum width of coxal pore field ventrally.

2.3. Meristics

All meristic traits are presented in detail in Fig. 2 and were counted using a Leica MZ6 stereomicroscope as follows:

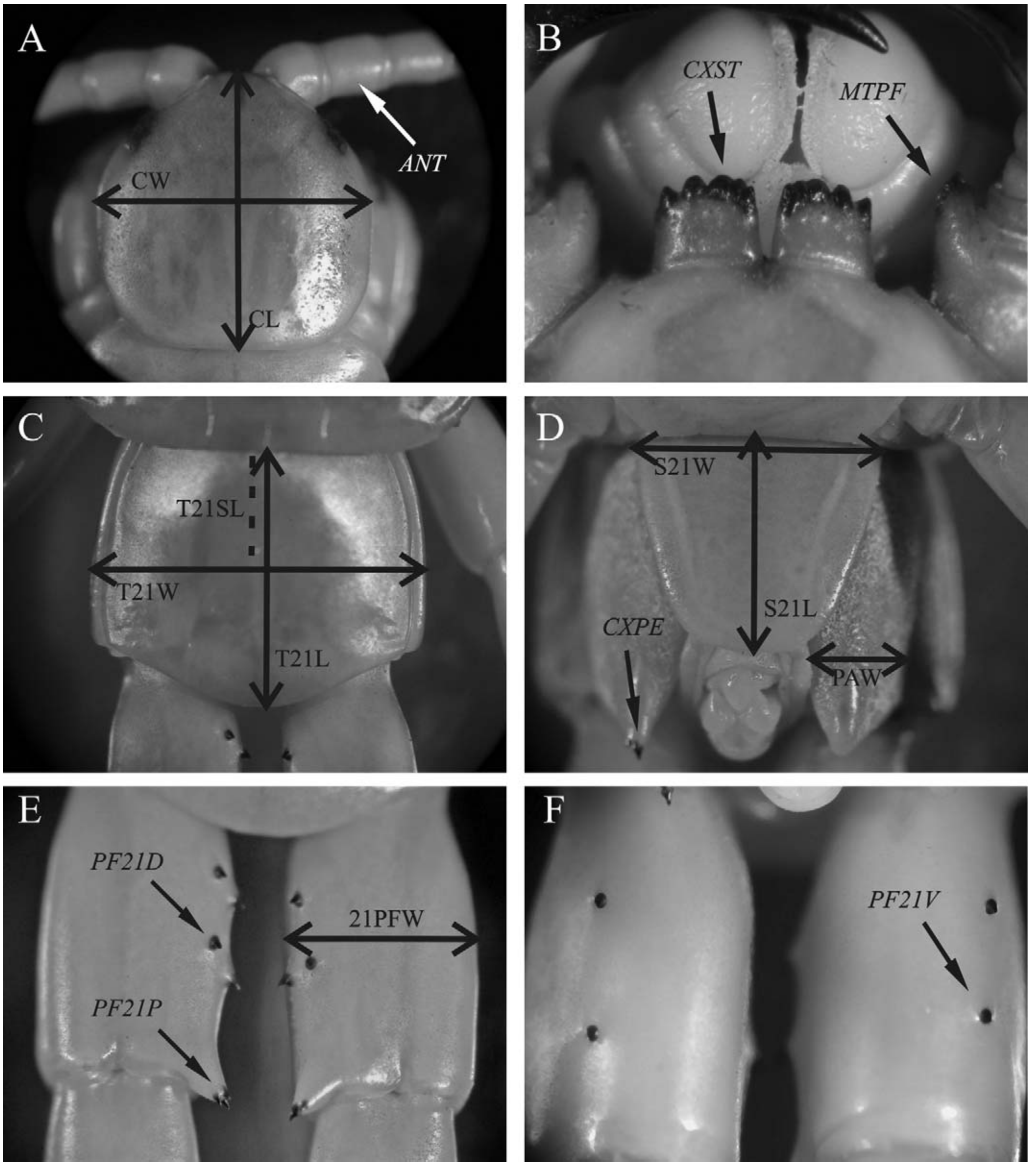

Fig. 2. Stereomicroscope photographs showing 16 out of 19 morphometric and meristic characteristics examined in *Scolopendra cingulata* (for further details see Section 2). (A) Head plate (dorsal view): Cephalic plate width (CW), cephalic plate length (CL), antenomeres (*ANT*). (B) Coxosternum (ventral view): Coxosternal teeth (*CXST*), trochanteroprefemoral process (*MTPF*). C) 21st tergite (dorsal view): 21st tergite width (T21W), 21st tergite length (T21L), median suture length on 21st tergite (T21SL). D) 21st sternite (ventral view): 21st sternite width (S21W), 21st sternite length (S21L), coxopleural end (*CXPE*). E) Prefemur of last pair of legs (dorsal view): 21st prefemur dorsal spines (*PF21D*), 21st prefemur spines on process (*PF21P*), 21st prefemur width (21PFW). F) Prefemur of last pair of legs (ventral view): 21st prefemur ventral spines (*PF21V*). Meristic characters are indicated with italics.

- Antennomeres (ANT), the number of articles on both antennae.
- Coxosternal teeth (CXST), the number of teeth on both coxosternal tooth plates.
- Trochanteroprefemur process (MTPF), the number of teeth on the process of both forcipular femuroid.
- Coxopleural end (CXPE), the number of spines on both coxopleural processes.
- 21st Prefemur ventral (PF21V), the number of spines on prefemora of both terminal legs ventrally.
- 21st Prefemur dorsal (PF21D), the number of spines on prefemora of both terminal legs dorsoventrally.
- 21st Prefemur process (PF21P), the number of spines on prefemoral processes of both terminal legs dorsally.

2.4. Statistical analysis

Sampling localities from the same broader geographic area were pooled and three major geographic areas (groups) (1: Balkan peninsula, 2: Asia Minor and Middle East, 3: Ital-

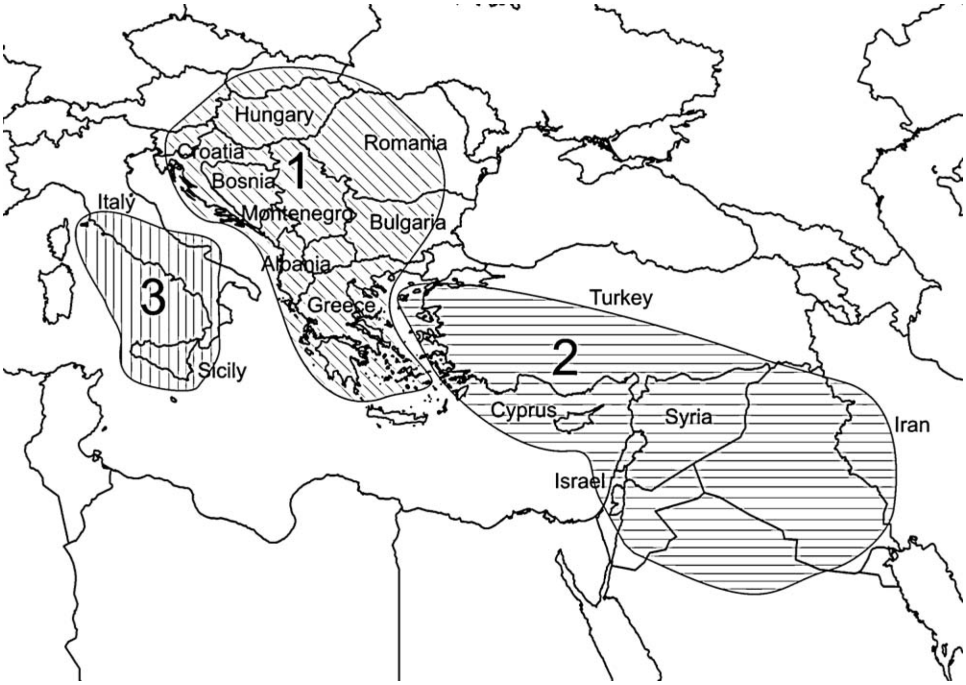

Fig. 3. Map showing the three main geographic regions compared in this study. 1: Balkan peninsula, 2: Asia Minor and Middle East, 3: Italian peninsula.

ian peninsula) were used (Fig. 3). The selection of these broader geographic areas was based on the available information concerning the palaeogeographic history of the southern Mediterranean (Roegl and Steininger, 1983). These, areas (according to our knowledge) have a distinct paleageographic history and for that reason their *a priori* delineation is justified.

We analyzed the morphometric and the meristic data separately, because these variables differ both statistically and biologically and because both types of variable may respond in different ways to environmental and genetic conditions (Hair et al., 1996; Lawing et al., 2008); therefore the two types of data are different. In order to study the size-free shape differences between those groups (i.e. to remove the effect of a within-population size factor from the between-population group morphometric comparison), the morphometric data were analyzed through the allometric Burnaby's size correction method (Burnaby, 1966; Humphries et al., 1981; Rohlf and Bookstein, 1987) using the programme BURNABY PCA. That method projects the set of measured distances onto a space orthogonal to the first principal component. Burnaby's method may remove isometric size from the data, for further "size-free" data analysis. The allometric Burnaby's method log-transforms the data prior to projection, and thus conceivably removes also allometric size-dependent shape variation from the data.

In the BPCA approach size and shape components are separated and a multivariate analysis of shape is accomplished eliminating the contribution of size on the second and following principal components (shape components), thus restricting the size component to PC1 (PC, principal component). This procedure is considered to be the most effective technique in morphometric analysis of linear distance data for isolating shape from size variation (Rohlf and Bookstein, 1987). Furthermore, variability among groups was tested using Canonical Variates Analysis (CVA) with cross-validation classification on the Burnaby adjusted data set. For the meristic data we used the square root transformation (Quinn and Keough, 2002), analyzed with PCA and CVA with a cross-validation classification.

Morphological relationships between groups were also evaluated by Mahalanobis distances between centroid means (Dillon and Goldstein, 1984) of the adjusted morphometric and meristic data. In order to reveal possible geographic patterns the morphometric and meristic squared Mahalanobis distances derived from CVA were used for a cluster analysis (UPGMA) of the pooled populations of each group. The goodness of fit of the cluster analysis (UPGMA trees) to the data was examined by an analysis of cophenetic values (Rohlf and Sokal, 1981), using NTSYS (Rohlf, 2005). Finally, a Mantel-test was used to examine possible correlation and congruence between the derived morphometric and the meristic Mahalanobis distances (between the broader distributional geographic areas used) for each group, using PAST (Hammer et al., 2001).

MANOVA and ANOVA tests, using the transformed morphometric and meristic data, and the resultant shape PCs were used to identify possible differences between groups. MANOVA tests, using also the resultant shape PCs, were further used to examine the effect of sexual dimorphism and geographic location for each group. For the statistical methods used in this study see Reyment et al. (1984),

Table 2. Eigenvector elements and percentage of total variance of principal components of the morphometric variables (highly significant loadings presented in bold). 21PFW and PAW were excluded from the principal component analysis due to low extraction values.

Variables	PC1	PC2
BL	0.208	**0.713**
CW	**0.867**	0.314
CL	**0.808**	0.378
T7D	**0.927**	0.030
T8D	**0.937**	−0.035
T21W	**0.815**	0.349
T21L	0.516	0.544
S21W	0.695	0.470
S21L	0.023	**0.827**
T21SL	**−0.869**	−0.485
% Variance	63.52	12.78

Table 3. Standardized canonical discriminant function coefficients for the morphometric variables. *PAW failed to pass tolerance test due to high collinearity and excluded from the discussion. Significant variables in bold.

Variable	CV1	CV2
T8D	**0.516**	0.244
T7D	**0.464**	0.433
T21L	0.185	0.097
S21W	0.301	**0.668**
T21W	0.297	**0.626**
PF21W	−0.042	**0.605**
T21SL	−0.206	**−0.537**
S21L	−0.443	**0.519**
CL	0.088	**0.400**
CW	0.196	**0.398**
BL	−0.020	0.160
PAW*	−0.058	0.106

Morrison (1990), and Quinn and Keough (2002). Prior to the analyses data were examined for departures from statistical assumptions (normality and deviation from equality of variances), and were adjusted when necessary (logarithmic and square root transformations). All analyses were performed with SPSS for Windows v.17.

3. Results

Supplementary material (Appendix B) summarizes for each geographic region all measured morphological characteristics of the 503 *S. cingulata* samples (sex ratio is also given).

3.1. Morphometric

Through the Burnaby principal component analysis of size-corrected morphometric variables two eigenvector elements (PC) were extracted that accounted for 76.31% of the total variance (Table 2). The first principal component (PC1) accounted for more than 63% and was positively correlated with all variables, except for T21SL. PC1 was especially correlated with T8D, T7D, T21SL, CW, CL and T21W (Table 2). The size-free PC2 accounted for approximately 13% of the total variance and was positively correlated with more than half of the variables. It was most correlated with S21L and BL (Table 2). PC1 and PC2 scores of individuals overlapped notably between the different geographic areas.

Size-free CVA evidently discriminated populations of *S. cingulata* from the three major geographic compartments (Balkan peninsula, Asia Minor and Middle East, Italian peninsula). Standardized canonical coefficients of the first and second canonical variate are given in Table 3. Regarding the morphometric variables, the plot of the first size-free canonical variate (CV1) versus the second (CV2) is given in Fig. 4a. Along the first discriminant axis (CV1) a notable morphological discrimination between the populations of *S. cingulata* can be observed across the three major geographic units that are well separated. In Table 3, CV1, accounting for 93.3% of the variation, discriminated eastern populations (Balkan peninsula, Asia Minor and Middle East) from the western population (Italian peninsula) and revealed the most important characters responsible for this divergence namely T7D and T8D (see Fig. 4a). The CV2, accounting for 6.7% of the within-population variation, discriminated the Balkan populations from the Asia Minor and Middle East populations (Fig. 4a). The most important characters for this separation were S21W, T21W, PF21W, T21SL, S21L, CL and CW (Table 3).

Results of the multivariate analysis of variance showed that morphometric characters were significantly different among the three major geographic areas as well as between sexes (Table 4). However, there was no significant interaction between geographic area and sex.

Table 4. Results of multivariate analysis of variance that tested the effect of geographic region (REG; 1: Balkan peninsula, 2: Minor Asia and Middle East, 3: Italian peninsula), sex, and interactions on size-adjusted morphometric variables (A) and on square root transformed meristic variables (B).

Effect	Pillai's trace	*F*	Degrees of freedom	Probability
(A)				
REG	0.495	14.573	22	0.000
SEX	0.134	6.845	11	0.000
REG * SEX	0.041	0.939	22	0.542
(B)				
REG	0.367	7.789	28	0.000
SEX	0.012	0.432	14	0.964
REG * SEX	0.062	1.104	28	0.324

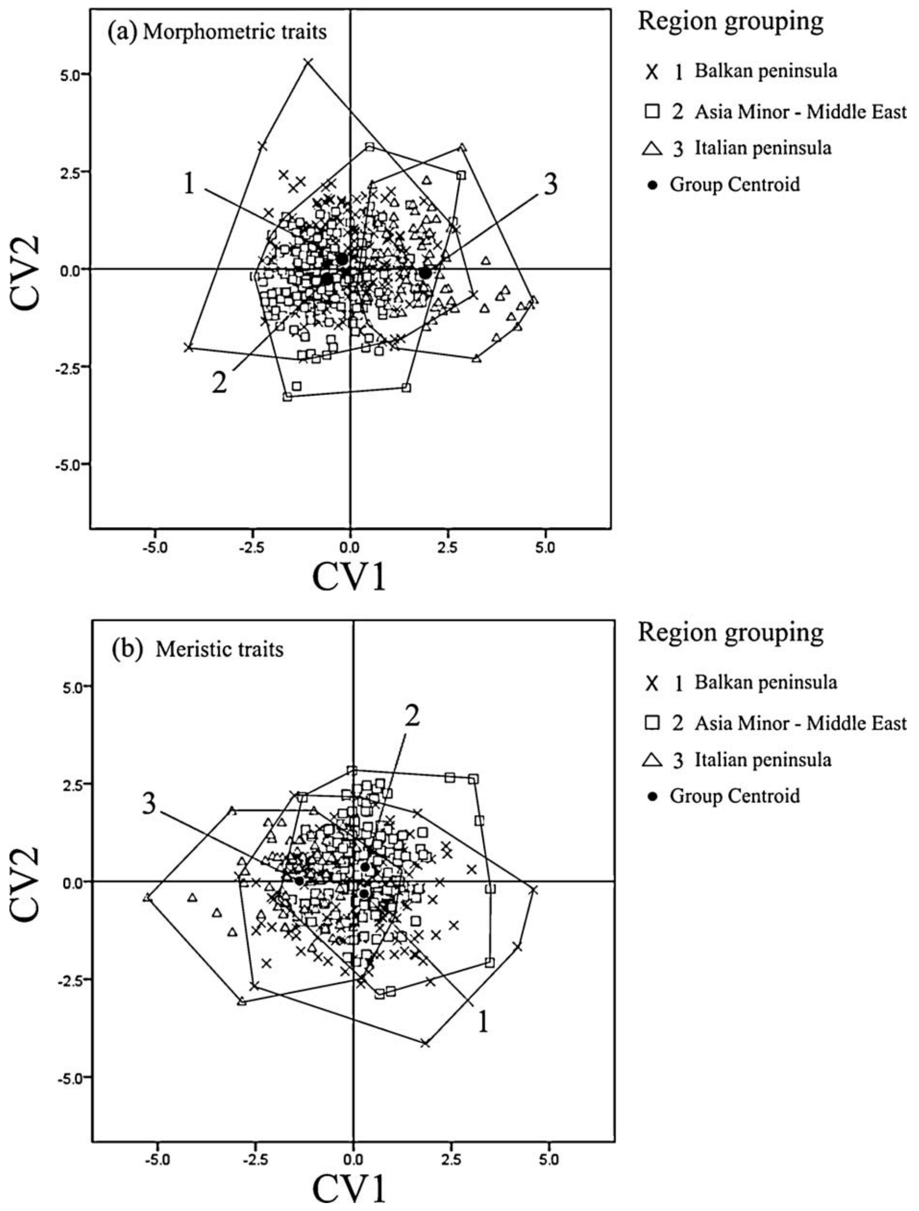

Fig. 4. CVA plots for the adjusted morphometric (a) and meristic (b) data of *Scolopendra cingulata* for the three main geographic regions studied (1: Balkan peninsula, 2: Asia Minor and Middle East, 3: Italian peninsula) as indicated by within-group correlation of variables to the canonical variates. Boundary of each geopgraphic group is added to the plots.

Mahalanobis distances between centroid means of the adjusted morphometric variables indicated that the populations of *S. cingulata* from the Anatolian peninsula and Balkans were the most related ($d = 0.41$). Morphometric squared Mahalanobis distances were further used for a cluster analysis of the pooled populations of each geographic compartment (results are not presented here).

As far as it concerns variability among the three geographic groups, results of correct classification of CVA on the morphometric variables are shown in Table 5. In total, cross-validation classification of *S. cingulata* individuals to correct geographic compartment was more than 60% (Table 5). In detail, individuals from the Balkan and the Italian peninsula were highly correctly classified (64.6% and 67.1% respectively), whereas classification accuracy of *S. cingulata* specimens from Asia Minor and Middle East to the correct geographic area was 52.6% (Table 5).

3.2. Meristic

The first principal component (PC1) produced by the square-root transformed variables accounted for 30.86% of the total variance, the second principal component (PC2) accounted for more than 23%, while the third principal component (PC3) accounted for almost 21% (Table 6). PC1 was positively correlated to PF21D and PF21P. PC2 was most correlated to CXST and MTPF whereas PC3 was correlated with

Table 5. Classification of *Scolopendra cingulata* to geographic region using adjusted (A) morphometric and (B) meristic variables (bold values indicate correct classifications). 1: Balkan peninsula, 2: Minor Asia and Middle East, 3: Italian peninsula.

	Classified as 1	Classified as 2	Classified as 3	Sum	% Correct
(A)					
1	**146**	61	19	226	64.6
2	81	**101**	10	192	52.6
3	25	3	**57**	85	67.1
Total	252	165	86	503	
60.4% of cross-validated grouped cases correctly classified					
(B)					
1	**147**	61	18	226	65.0
2	92	**88**	12	192	45.8
3	21	13	**51**	85	60.0
Total	260	162	81	503	100.0
56.9% of cross-validated grouped cases correctly classified					

Table 6. Eigenvector elements and percentage of total variance of principal components of the meristic variables (highly significant loadings presented in bold). ANT and CXPE were excluded from the principal component analysis due to low extraction values.

Variables	PC1	PC2	PC3
CXST	−0.020	**0.800**	−0.074
MTPF	−0.060	**0.769**	0.116
PF21V	−0.022	0.032	**0.969**
PF21D	**0.866**	−0.038	0.194
PF21P	**0.837**	−0.055	−0.253
% Variance	30.86	23.46	20.72

PF21V (Table 6). Due to the low proportion of the variance in the original variables that is accounted for by the factor solution (less than 0.50), ANT and CXPE were excluded from the analysis.

CVA on the square-root transformed meristic data indicated a significant discrimination of population variation among the three major geographic compartments (Balkan peninsula, Asia Minor and Middle East, Italian peninsula) because of the high cumulative explained variance of CV1 (80.2%). Standardized canonical coefficients of CV1 and CV2 were given in Table 7. With regard to the meristic variables, the plot of the first size-free canonical variate (CV1) versus the second (CV2) is given in Fig. 4b. In detail, western populations of *S. cingulata* (Italian peninsula) were significantly discriminated from eastern populations (Balkan peninsula, Asia Minor and Middle East) along the first discriminant axe. According to CV1, the most important variables responsible for this divergence were ANT, PF21P and PF21D. On the other hand, CV2, accounting for 19.8% of the variation, discriminated Balkan populations from easternmost population of Asia Minor and Middle East (Fig. 4b). The most important character for this separation was MTPF (Table 7).

Table 7. Standardized canonical discriminant function coefficients for the meristic variables. Significant variables in bold.

Variable	CV1	CV2
sqrt(ANT)	**0.734**	0.448
sqrt(PF21P)	**0.580**	0.071
sqrt(PF21D)	**0.574**	−0.144
sqrt(PF21V)	0.084	−0.154
sqrt(MTPF)	−0.286	**0.715**
sqrt(CXPE)	−0.123	0.381
sqrt(CXST)	−0.150	0.180

Multivariate analysis of variance showed that there was no significant interaction between geographic area and sex. Meristic variables were significantly different among the three major geographic areas but not between sexes (Table 4). Mahalanobis distances between centroid means of the square-root transformed meristic variables indicated that eastern populations of *S. cingulata* (Balkans and Asia Minor – Middle East) were the most related ($d = 0.47$).

With regard to variability among the three geographic regions, results of CVA with cross-validation classification on the square-root transformed meristic data were shown in Table 5. The overall cross-validated grouped cases correctly classified into their original geographic region were almost 57% (Table 5). Pairwise discriminant functions indicated that individuals of *S. cingulata* can be classified to the Balkan and the Italian peninsula with 65% accuracy and 60% accuracy, respectively (Table 5). In contrast, the classification accuracy of *S. cingulata* specimens from Asia Minor and Middle East to the correct region was only 45.8% (Table 5).

Finally, Mantel-test detected significant correlation between the morphometric and the meristic Mahalanobis distances ($r = 0.972$, $P = 0.1664$).

3.3. Sexual dimorphism

In order to check for sexual dimorphism in meristic or morphometric characters we used a CVA. It is interesting to

Table 8. Sexual dimorphism as indicated by univariate *F* statistic and canonical correlation of adjusted morphometric variables (highly significant loadings are presented in bold).

	Univariate *F*	Canonical correlation
BL	0.001	0.003
CW	10.574	**−0.372**
CL	6.646	−0.295
T7D	11.832	**−0.393**
T8D	14.122	**−0.430**
T21W	1.547	0.142
T21L	2.580	0.184
S21W	0.223	−0.054
S21L	0.561	0.086
21PFW	6.926	0.301
T21SL	1.171	0.124
PAW	0.008	0.010

note that no meristic variable supported sex differentiation. Similarly, most of the morphometric variables indicated no discrimination between sexes. However, three morphometric characters, namely, cephalic width (CW) and the distances between the two paramedian sutures of the seventh (T7D) and eighth tergite (T8D), significantly discriminated females from males. In particular, CVA revealed that males have relatively a smaller cephalic width as well as shorter distance between the two median sulculs of the 7th and 8th tergite. *F* values and standardized canonical coefficients were presented in Table 8. Nevertheless, when we performed separate size-adjusted CVAs for each gender to avoid the effect of sexual dimorphism there were no geographic differences between the three major groups (Balkan peninsula, Asia Minor and Middle East, Italian peninsula).

4. Discussion

4.1. Previous biometrical studies

Multivariate quantitative analysis of morphology has not been used for centipedes before. In particular, while there are numerous studies on scolopendrids emphasizing systematics (Lewis, 1989, 2003; Shelley, 2002), checklists (Khanna, 2001; Lewis, 2001a; Shelley, 2006) as well as faunistic and distributional aspects (Szalay, 1956; Lewis, 1985, 1996, 2001b; Lewis and Gallagher, 1993; Zapparoli, 2002; Akkari et al., 2008; Simaiakis and Mylonas, 2008), there appears to be little information on the morphological differentiation of *Scolopendra* species at a large spatial scale. For instance, Jangi (1955, 1959) worked with traditional morphometrics to describe certain morphological aspects on *S. morsitans* from India, while Lewis revised previous knowledge and presented comparable results on variation in key taxonomic characters between *S. amazonica* and *S. morsitans* from Africa (Lewis, 1969), Sudan (Lewis, 1966, 1967) and Nigeria (Lewis, 1968, 1970). Some years later, Würmli (1978, 1980) presented a taxonomic revision of the "*canidens*" *Scolopendra* species examining thousands of specimens for 21 quantitative and qualitative morphological characters using multivariate morphometrics. Recently, Korsós et al. (2006) reported notes on morphological differentiation of *S. cingulata* from Hungary. Apart from these studies, there was no modern approach on that subject and many questions about the morphological variation along the distributional range of *S. cingulata* remained unanswered. This work has provided new evidence for the geographic patterns of intraspecific variation of *S. cingulata* in the Mediterranean region.

4.2. Discrimination pattern along the west–east cline

There are useful insights with sound results regarding the geographic discrimination of *S. cingulata* in the Mediterranean region (see Tables 2, 3 and Fig. 4). It is also interesting to note that all meristic and most of the morphometric variables indicated no sexual dimorphism. Overall results obtained both from morphometric and meristic characters support two or three morphologically differentiated groups of *S. cingulata*. On the one hand there is a clear differentiation of eastern (Balkan peninsula) and far south-eastern populations (Asia Minor and Middle East) from the western Mediterranean population (Italian peninsula). On the other hand, there are characters supporting the discrimination between Balkan and Asia Minor – Middle East populations. Most of the variables distinguishing eastern (Balkan) from far south-eastern samples (Asia Minor-Middle East) were key characters for *S. cingulata* (S21W, T21W, 21PFW, T21SL, S21L, CL and CW; J.G.E. Lewis, pers. comm.).

The most valuable morphometric characters for separating eastern from western populations of *S. cingulata* along the Mediterranean region have been found to be mainly the distance between the two median sulci of the 7th and 8th tergite (T7D and T8D), while the most significant variables discriminating Balkan from far eastern samples were the width of 21st sternite and tergite (S21W and T21W), the width of 21st prefemur (21PFW), the length of median suture on the 21st tergite (T21SL), the length of 21st sternite (S21L) as well as the cephalic length and width (CL and CW). Length of the median suture on the 21st tergite (T21SL) has been previously used by Würmli (1980) as a key taxonomic character of the "*canidens*" group of *Scolopendra*. In *S. cingulata*, it seems that T21SL follows a similar pattern with easternmost samples having a much longer sulcus (Appendix B).

With regard to meristic characters, the number of spines on the dorsal side of 21st prefemur (PF21D), the number of spines on the 21st prefemoral process (PF21P) and the number of antennal segments (ANT) significantly discriminated eastern from western form of *S. cingulata* in the Mediterranean area. Differentiation between the Balkan and the easternmost populations was significantly supported by the number of teeth on the trochanteroprefemoral process (MTPF).

4.3. Secondary sexual characters

S. cingulata usually has four dorsolateral spines (PF21D) and an equal number of spines on the prefemur process (PF21P) in each leg. Data showed a significant difference between western and eastern samples, with more spines in material from the eastern and south-eastern Mediterranean populations. According to Lewis (1985), if spines on the prefemur of the last legs are used in specific discrimination prior to mating, it would not be expected for two sympatric species to have the same spine arrangement. Apart from this theory, we suggest that there is a trend for an increased number of last leg spines towards the east–south-eastern parts of *S. cingulata* distribution.

With regard to palaeogeographic events across the Mediterranean region, we support the idea that *S. cingulata* populations from eastern (Balkans) and far south-eastern (Asia Minor and Middle East) were in continuous connections at the middle Miocene (Roegl and Steininger, 1983) and later during the Messinian period (see Fig. 1). On the other hand, Italy retains the most isolated populations during the same period.

This is also in accordance with previous palaeogeographic events, supporting the opinion that the ancestral species of *S. cingulata* colonized south Europe from the easternmost parts of its distributional range (Asia Minor and Middle East), following either the Anatolian-Balkan path or the northern African migration route (for further details see also Simaiakis and Mylonas, 2008). The latter suggestion is in accordance with the palaeogeographic history of the Mediterranean area (for further discussion on the Aegean palaeogeography see Poulakakis et al., 2005 and Parmakelis et al., 2006) revealing continuous connections of Anatolia-Middle East region with the Balkan peninsula (see Fig. 1).

4.4. The antennae

A similar pattern was observed in relation to the number of antennal segments (ANT). The majority of Italian specimens had 19 articles, some 20, while the easternmost specimens had 20, some 21 and 22. It is interesting to know that the number of antennal segments are significantly different and right and left antennae frequently have different numbers of segments in the same individual (Lewis, 1966). In our study, there was no considerable bilateral variation in the number of antennomeres. Due to the fact that antennal articles are able to increase during post-larval development, after damage or loss, thus leading to atypically high numbers (Lewis, 1978), this could explain minimum numbers of 14, 15 or 16 given in Appendix B. Moreover, despite the low intraspecific variation in the number of antennal segments in *S. cingulata*, this character significantly differentiated the eastern (Balkan) and far south-eastern population (Asia Minor and Middle East) from the western populations (Italian peninsula). Any other attempt to find characters that further separate eastern and easternmost populations from western populations have so far proved unsuccessful.

4.5. Body length differences

In the sample of 503 specimens, body length (BL) showed no differentiation between Balkan and Asia Minor-Middle East populations, but significant discrimination from the Italian populations. What could be the explanation for the widely distributed *S. cingulata*? In numerous species mean body size is known to increase with latitude, a trend known as Bergmann's Rule. We suggest that body length appears to exhibit a similar trend in the same direction as the number of antennal segments, but this is fractionally outside formal significance. Furthermore, should body length differences respond to geographic and environmental characteristics then we cannot overlook the idiosyncratic *S. cingulata* populations (Lewis, 1978).

S. cingulata mainly coexists with *S. oraniensis* in mainland Italy and Sicily (Zapparoli, 2009). In terms of character displacement, one might expect interspecific competition in the Italian peninsula to lead to *S. cingulata* diverging in size from its putative competitor (*S. oraniensis*). Here, we did not observe a pattern like this and we believe that this is might be due to common adaptations. On the other hand, *S. cingulata* is widespread in mainland (Zapparoli, 2002) and insular Greece (Simaiakis et al., 2005). However, it is absent from Crete and its satellite islands (Simaiakis and Mylonas, 2008) with few sympatrically distributed congeneric species (e.g. *S. canidens* in western Kyklades and *S. dalmatica* in few localities in western part of Greece; for more details see Simaiakis and Mylonas, 2008). It is remarkable that intraspecific variation in size was high in the Balkan region (that includes most of the Aegean islands in the analysis) where some individuals were gigantic (e.g. *S. cingulata* with 156 mm body length on a small islet close to Skyros Isl.).

Further analysis on the morphological variation in polymorphic species such as *S. cingulata* would be fundamental to biological research in order to reveal cryptic geographic or ecological trends. As Lewis (1978) pointed out, there is a need for additional reliable characters for use in scolopendrid taxonomy and biometrical analysis. Edgecombe and Koch (2008, 2009) followed this prompt and presented new character evidence applied to morphology-based phylogenetics of Scolopendromorpha. As far as the Mediterranean populations are concerned, it would be useful to add new morphometric data from the Mediterranean coast of northern Africa suitable for comparative statistical treatment, to include, apart from quantitative, key qualitative characters and to compare common set of variables among sympatrically distributed species of the widespread genus of *Scolopendra*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcz.2010.11.006.

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