Available online at www.sciencedirect.com
SCIENCE DIRECT®
Zoologischer Anzeiger 244 (2005) 97–113
ZOOLOGISCHER ANZEIGER
www.elsevier.de/jcz

The *Euscorpius tergestinus* (C.L. Koch, 1837) complex in Italy: Biometrics of sympatric hidden species (Scorpiones: Euscorpiidae)

Valerio Vignoli[a,*], Nicola Salomone[a], Tancredi Caruso[b], Fabio Bernini[a]

[a]*Department of Evolutionary Biology, University of Siena, Via A. Moro 2, 53100 Siena, Italy*
[b]*Department of Environmental Science, University of Siena, Via Mattioli 4, 53100 Siena, Italy*

Received 20 October 2004; received in revised form 29 April 2005; accepted 5 May 2005

Corresponding editor: Andrew Parker

Abstract

This work stems from the results of a recent phylogenetic investigation on the *Euscorpius carpathicus* species complex from the Italian peninsula (Salomone et al. 2004. Phylogenetic relationships between the sibling species *Euscorpius tergestinus* and *E. sicanus* (Scorpiones, Euscorpiidae) as inferred from mitochondrial and nuclear sequence data. In: Proceedings of the16th Congress of Arachnology, August 2–7, 2004, Ghent University, Belgium, 268pp.; Salomone et al. in prep.). Molecular investigation produced interesting and unexpected findings on the scorpion *Euscorpius tergestinus* (C.L. Koch, 1837). Both nuclear and mitochondrial sequence data provided evidence of substantial genetic differentiation in specimens identified as *Euscorpius tergestinus* according to recent taxonomical changes (Fet and Soleglad 2002. Morphology analysis supports presence of more than one species in the "*Euscorpius carpathicus*" complex (Scorpiones: Euscorpiidae). Euscorpius 3, 51pp.). These specimens clearly belong to two well-differentiated evolutionary lineages. Molecular results highlighted the need for a new morphological investigation. The present study undertook the morphological analysis of specimens belonging to both genotypes with the aim of identifying morphological characteristics able to discriminate between the two taxa. The analysis of trichobothria patterns, morphometric ratios, granulation patterns and the observation of the pectinal sensilla confirm the difficulty in distinguishing these two genotypes and the high polymorphism of the subgenus *Euscorpius* Thorell, 1876. The length of pedipalp segments and dorsal patellar spurs (DPS), as well as femur leg granulation, are the main diagnostic characters; other ratios together with body color also help to distinguish the different genotypes. This study confirms the presence in Italy of two different cryptic species belonging to the "*Euscorpius tergestinus*" complex. *Euscorpius tergestinus* is a reddish, slender euscorpiid with a large dorsal patellar spine (DPS). A darker and generally squat phenotype with a short DPS, which corresponds to *Euscorpius carpathicus concinnus* sensu Caporiacco (1950), is elevated to the species level: *Euscorpius concinnus* (C.L. Koch, 1837). These two species are sympatric in several Italian regions, and their distribution pattern is possibly determined by intraguild predaction interaction.

Keywords: Scorpions; Morphology; Biometrics; Multivariate analysis; ANOVA

*Corresponding author. Tel.: +39 0577 234393.
E-mail address: vignoli@unisi.it (V. Vignoli).

0044-5231/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jcz.2005.05.002

1. Introduction

The systematics of the genus *Euscorpius* Thorell, 1876 (Scorpiones: Euscorpiidae) has undergone several taxonomic changes since 1999, after the first molecular investigation (Gantenbein et al. 1999). *Euscorpius* (*Euscorpius*) *carpathicus* (Linnaeus, 1767) is now considered a complex of at least 7 sibling species. *Euscorpius carpathicus* in sensu stricto remains valid but only for the populations of the type locality in Romania (Fet and Sissom 2000; Fet and Soleglad 2002). Some of these species are characterized by localized distributions such as the Balearic *E. balearicus* Caporiacco, 1950, the Crimean *E. tauricus* (C.L. Koch, 1837) and the Greek *E. koschewnikowi* Birula, 1900, which were elevated to the species level after both molecular and morphological analyses (Gantenbein et al. 2001; Fet 2002; Fet and Soleglad 2002). *Euscorpius hadzii* Caporiacco, 1950, is considered a Balkan species with a widespread geographic distribution (Fet and Soleglad 2002). Two other "new" species with a relatively wide distribution area are *E. sicanus* (C.L. Koch, 1837) and *E. tergestinus* (C.L. Koch, 1837); they are the only known taxa belonging to the "*carpathicus*" complex that are present in Italy. The geographical distribution of *E. sicanus* on the Italian Peninsula is more southern, including Sicily and Sardinia (Fet et al. 2003), while *E. tergestinus* is widely distributed from north to south (the exact geographic range is not yet well defined) and in the countries bordering northern Italy (Fet and Soleglad 2002; Vignoli and Crucitti 2003). The high phenotypic variability within these scorpions led different authors (Koch 1837, 1841; Caporiacco 1950) to classify several different species and subspecies. Recent studies (Fet and Soleglad 2002), based on the morphological re-examination of a large number of specimens, conclude that 9 "old" taxa actually belong to the same species and are synonymous with *E. tergestinus* (Fet and Soleglad 2002). These taxonomical changes are based on the presence of a "standard" character, i.e. the trichobothrial formula of the pedipalp patella external aspect, with $eb = 4/4$. The high morphological variability within and between populations was attributed to intraspecific polymorphism.

A recent study based on nuclear and mitochondrial sequence data on the "*E. carpathicus*" complex from the Italian peninsula confirmed that *E. sicanus* is a well-differentiated evolutionary lineage (Fet et al. 2003). With respect to specimens determined on the basis of the trichobothrial pattern, such as *E. tergestinus* ($eb = 4/4$), genetic data clearly identified two deeply divergent lineages (Salomone et al. in prep.). The observed sequence divergence between these two assemblages was large enough to be comparable to that separating other *Euscorpius* species, e.g. *E. flavicaudis* (De Geer, 1787) and *E. italicus* (Herbst, 1800). These results demonstrate that although populations of *E. tergestinus* from Italy are morphologically very similar, they do indeed represent different evolutionary lineages. DNA analysis led to the fascinating discovery of unexpected, hidden or cryptic species, as previously found for other animal groups (Trewick 1998; Taylor et al. 1998; Lessios et al. 1999; Colborn et al. 2001; Wellborn and Cothran 2004), including arachnids such as pseudoscorpions (Zeh and Zeh 1994; Wilcox et al. 1997). We here focus on these two different genotypes of "*E. tergestinus*". The extensive intraspecific polymorphism exhibited by *E. tergestinus* has been highlighted by several authors (Huber et al. 2001; Fet and Soleglad 2002); however, we noted that in general, upon closer examination, our sequenced specimens were easy to distinguish through qualitative characters such as color and general morphology. We aimed to morphologically distinguish the two different genotypes with the typical *E. tergestinus* trichobothrial pattern. We report morphological data on all specimens included in the molecular analysis (Salomone et al. in prep.). Note that some non-sequenced specimens belonging to the same populations of the sequenced specimens were included in this study in order to equilibrate the data set.

2. Materials and methods

2.1. Materials

A total of 167 specimens, all collected in Italy, were analyzed (see Appendix A). The complete collection of scorpions labeled "*Euscorpius carpathicus*" within the Società Romana di Scienze Naturali (69 specimens) was included in this study together with some of Caporiacco's specimens preserved in MZUF (12 specimens). The samples are preserved in the private collection of the first author in the Dipartimento di Biologia Evolutiva, University of Siena, Italy, with exclusion of the MZUF specimens.

2.2. Morphological analysis

Starting from the specimens that were analyzed in the molecular study, 8 adult specimens were randomly selected to obtain a total of 10 adult individuals (5 males and 5 females) for each genetic group (see Appendix A and Table 1). The selection criteria were age and sampling locality. Only adults and, when possible, specimens from the same population as the sequenced one were considered. We analyzed both the patella trichobothria series (Pv, Pe) and pectinal tooth counts of all the sequenced "*tergestinus*" specimens. All morphometric ratios and carination quotients were determined for the selected material. The conventions

Table 1. Trichobothrial counts, sex and Dp of 20 selected specimens belonging to *Euscorpius tergestinus* (C.L. Koch, 1837) complex

	VVZC code	Locality	m/f	Dp		Pv		Pe															
				L	R	L	R	*et*		*est*		*em*		*esb*		eb_a		*eb*		Pe total		Pv + Pe	
								L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
Black type	246	Volterra (PI), Tuscany	m	8	8	8	8	6	6	4	4	4	4	2	2	4	4	4	4	24	24	32	32
	260	Poggibonsi (SI), Tuscany	m	8	8	8	8	6	6	4	4	4	4	2	2	4	4	4	4	24	24	32	32
	310	Brenna (SI), Tuscany	m	8	8	9	9	6	6	4	4	4	4	2	2	4	4	4	3	24	23	33	32
	304	Siena (SI), Tuscany	m	8	9	8	8	6	6	4	4	4	4	2	2	4	4	4	4	24	24	32	32
	101	Barberino del Mugello (FI), Tuscany	m	7	8	8	8	6	6	4	4	4	4	2	2	4	4	4	4	24	24	32	32
	106	Lucca (LU), Tuscany	f	7	7	8	8	6	6	4	4	4	4	2	2	4	4	4	4	24	24	32	32
	102	Montaione (FI), Tuscany	f	7	8	9	8	6	7	4	4	4	4	3	2	4	4	4	4	25	25	34	33
	46	Elba Island, Tuscany	f	7	7	9	8	6	7	4	4	4	4	2	2	4	4	3	4	23	25	32	33
	95	Bologna (BO), Emilia Romagna	f	7	7	8	8	6	7	4	4	4	4	2	2	4	4	4	4	24	25	32	33
	89	Pavia (PV), Lombardy	f	7	7	8	8	6	6	3	4	4	4	2	2	4	4	4	5	23	24	31	32
Red type	96	Sistiana (TS), Friuli V. Giulia	f	5	5	9	9	6	6	4	4	4	4	2	2	3	4	4	4	23	24	32	33
	243	Siena (SI), Tuscany	f	7	7	9	8	6	6	4	4	4	4	2	2	4	4	4	4	24	24	33	32
	38	Rome (RM), Latium	f	7	7	9	8	6	5	4	4	4	4	2	2	4	4	4	4	24	25	33	33
	219	Rieti (RI), Latium	f	7	7	9	9	6	6	4	4	4	4	2	2	4	4	4	4	24	24	33	33
	86	Rome (RM), Latium	f	7	7	9	9	6	6	4	4	4	4	2	2	4	4	4	4	24	24	33	33
	172	Rome (RM), Latium	m	8	9	9	9	6	5	4	4	4	4	2	2	4	4	4	4	24	23	33	32
	62	Carapelle Alvisio (AQ), Abruzzo	m	8	9	9	9	6	6	4	4	4	4	2	2	4	4	4	4	24	24	33	33
	114	Rome (RM), Latium	m	8	8	9	9	6	6	4	4	4	3	2	2	4	4	4	4	24	23	33	32
	246C	Rome (RM), Latium	m	8	8	9	9	6	6	4	4	4	4	2	2	4	4	4	4	24	24	33	33
	112	Rome (RM), Latium	m	8	8	9	9	6	6	4	4	4	4	2	2	4	4	4	4	24	24	33	33

of Vachon (1974) were followed for trichobothrial notations. All measurements are in millimeters (mm) and follow Stahnke (1970) and Sissom (1990). Biometric measurements were taken with a micrometric ocular lens applied to an optical Wild Heerbrugg stereomicroscope. We followed the method used by Fet and Soleglad (2002); 26 measurements of each specimen were taken and subsequently used for statistical analysis. Only the measurement of pedipalp chela depth followed Sissom (1990). Granulation and carination, of metasoma, pedipalps and legs, were also observed with a stereomicroscope. To analyze the variation of carinae we employed the "carinae development quotient" (Fet and Soleglad 2002) in order to provide a general distinction. The chelal carinae configuration follows the diagram of Soleglad and Sissom (2001: Fig. 54). The pectinal tooth shape was investigated and the right pecten of four specimens was cut and analyzed under a scanning electron microscope (SEM). Scanning electron micrographs were prepared from selected specimens stored in 75% ethyl alcohol. They were ultrasonically cleaned after chloroform treatment in order to remove the cerotegument. Finally, they were air-dried, placed on aluminum stubs and coated with 20 nm Au–Pd using a Balzers MED 010 sputter-coater before observation under a Philips XL 20 SEM.

Key to museum and collection acronyms: VVZC collection – V. Vignoli, Dipartimento di Biologia Evolutiva, University of Siena, Italy; VF collection – private collection of V. Fet; MZUF – Museo Zoologico "La Specola", Sezione del Museo di Storia Naturale, University of Florence, Italy.

Abbreviations: DPS – dorsal patellar spur; Pv – patella ventral; Pe – patella external; m – male; f – female; Dp – pectinal teeth; L – left; R – right; *et* – external terminal; *est* – external subterminal; *em* – external median; *esb* – external suprabasal; eb_a – external basal-a; *eb* – external basal.

2.3. Biometrics

Two factors were analyzed in a crossed design: genetic group (2 fixed levels: 1 = black type and 2 = red type) and sex (2 fixed levels: males and females). A multivariate matrix was constructed with the 20 selected individuals as objects and 27 biometrical measurements as variables, following Fet and Soleglad (2002), with the addition of the DPS length. After normalization, the data set was analyzed using principal component analysis (PCA) ordination to detect the main morphological group (Chatfield and Collins 1980). A variable discriminant analysis was performed using the eigenvector scores that indicate the weight of each variable on the first 2 principal components (PCs). A two-way crossed analysis of variance (2-way ANOVA) was used to test univariate differences between genetic groups and sex for all variables (Underwood 1997). A logarithmic transformation was used when data lacked the assumption of variance homogeneity. The 2-way ANOVA was also applied to the following indices obtained from the ratio between the main biometric variables: pedipalp chela width/pedipalp chela length (ChelW/ChelL); fifth metasomal segment length/fifth metasomal segment width (metasoma V length/metasoma V width); dorsal patellar spine (DPS) length/pedipalp patella length; pedipalp femur length/pedipalp femur width (femur length/femur width).

3. Morphological results

To avoid confusion, instead of considering the numerous known subspecies, we chose to divide specimens into two distinct morphotypes according to their phenotypic aspect.

"*Red*" *morphotype*: Medium–large euscorpiid with carapace length of 5.4–6.1 mm. Orange–brown body with yellowish chelicerae, legs and telson. All analyzed specimens are similar in color. The elongated chelas give this morphotype a slender appearance (Fig. 1b). Tubercles on leg femur are crenulated on both dorsal and ventral surfaces (Figs. 2b and d).

"*Black*" *morphotype*: Medium-sized euscorpiid with short pedipalp segments (Fig. 1a). Considerable polymorphism is evident in both the color and the size of the sexually mature specimens analyzed. The carapace length ranges from 3.9 to 5.8 mm. Dark brown–blackish body with clear maculation pattern. Legs, telson and chelicerae are yellowish and show the same pattern. The extremities of the chela fingers are reddish or pale brown. Granulation on leg femurs is present but weak (Figs. 2a and c).

3.1. Comparisons between "red" and "black" morphotypes

3.1.1. Trichobothria pattern and number

Type C, major additive neobothriotaxic on patella. All specimens have trichobothria statistical ranges typical of the *Euscorpius* subgenera (ventral chela (V): 4/4; ventral patella (Pv) > 6). The trichobothria of the patella ventral surfaces range from 8 to 9, with higher values in the "red" morphotype (9/9) than in the "black" one (see Table 1). The patella external region shows the typical number of the recently re-described *E. tergestinus*. Patella external formula: $eb = 4/4$, $eb_a = 4/4$, $esb = 2/2$, $em = 4/4$, $est = 4/4$, $et = 5-7$ (6). Even the pattern of patellar external trichobothria is identical (see Fig. 60 in Fet and Soleglad 2002). Pedipalps were all carefully examined, and the *em* series

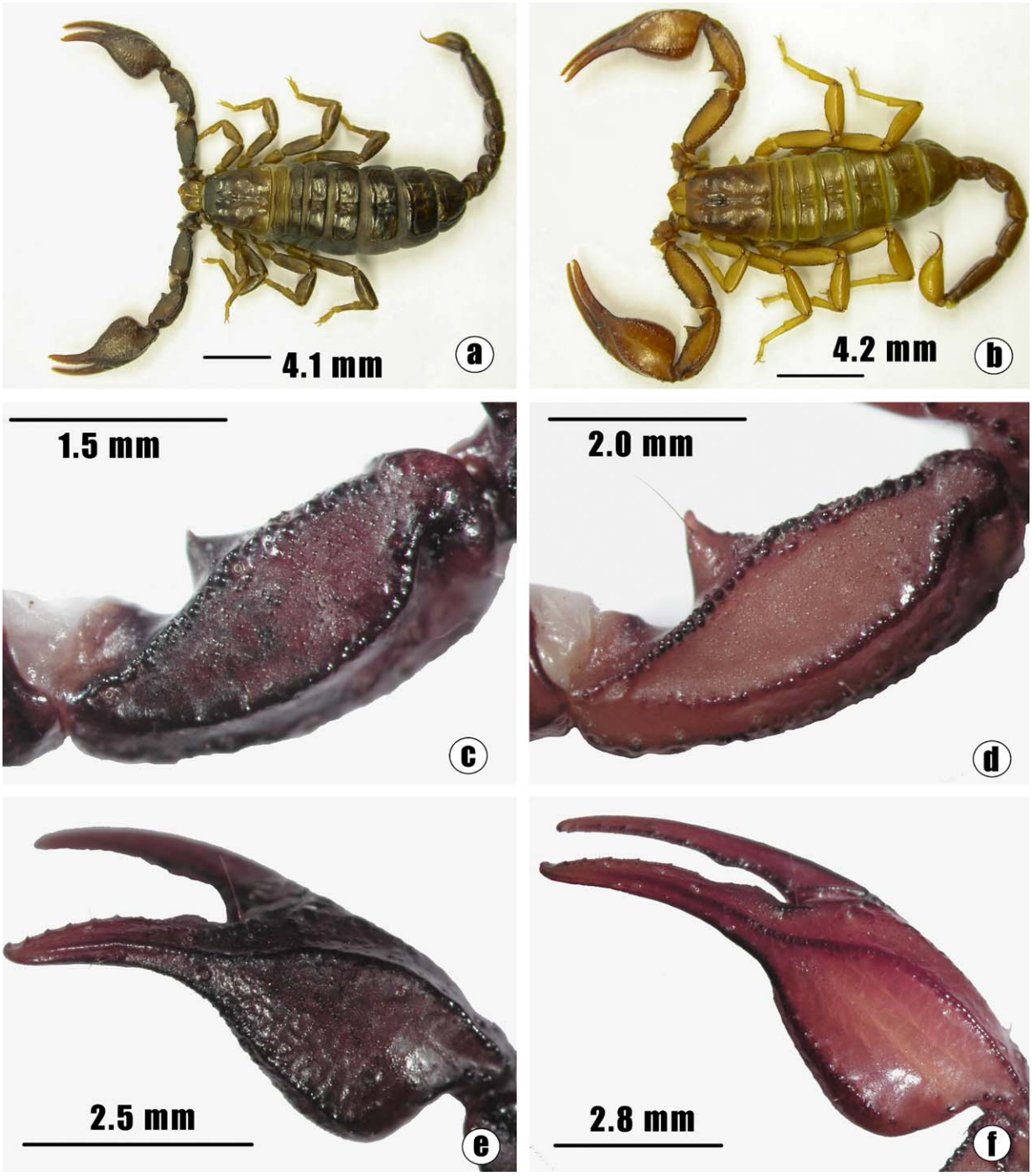

Fig. 1. *Euscorpius tergestinus* (C.L. Koch, 1837): "black" morphotype (VVZC Eut516: a, c, e) and "red" morphotype (VVZC Eut243: b, d, f). (a, b) Dorsal view of adult female; (c, d) dorsal aspect of right patella; (e, f) dorsal aspect of pedipalp chela.

seemed to differ between the two morphotypes. While the disposition of the four trichobothria in "red" specimens is more compact, that of "black" specimens is more "open". The angle formed between trichobothria 1–2 and 3–4 is larger in the "black" than in the "red" specimens. Although the single position of each trichobothria could change from specimen to specimen, and in some cases between different pedipalps of the same scorpion, only the distance between trichobothria 3 and 4 differs somewhat; however, the general pattern in the studied material is always as described above. Other differences in trichobothria patterns are the position of *d* and *i* on the pedipalp femur. In the "red" specimens *d* is more distant from *i*, while the

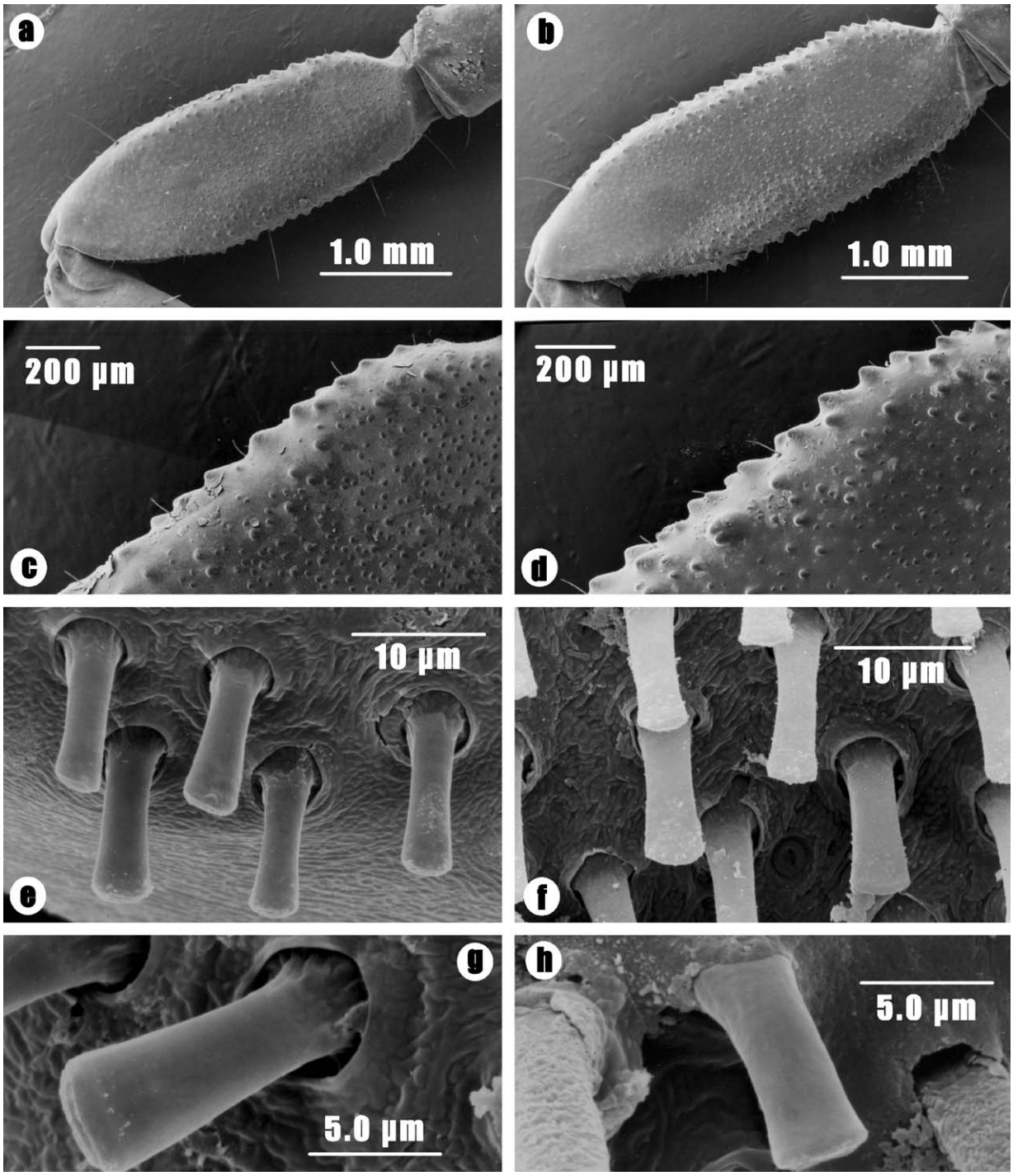

Fig. 2. *Euscorpius tergestinus* (C.L. Koch, 1837): "black" morphotype (VVZC Eut304: a, c; Eut102: e, g) and "red" morphotype (VVZC Eut96: b, d, h; Eut243: f). (a, b) lateral view of leg femur (× 30); (c, d) detail of the dorsal aspect of leg femur (× 90); (e, f) microstructure of peg sensilla on tooth (× 3200); g, h. detail of peg sensilla (× 6400).

position of *e* on the dorsoexternal carina is similar in both. Even the position of trichobothria V_4 is different: it is situated in a dimple on the ventral external carina (V1) in "black" specimens, whereas it is on the external surface of the other morphotype.

3.1.2. Morphometric ratios

Our morphometric dataset indicated that some ratios are representative while others are not, due to the high polymorphism of "black" specimens. The ratio obtained between the total length and width of chela (chela length/

chela width) statistically highlights the slender appearance of the "red" type. This is evident only when females and males are considered separately. The exceptional development of the DPS spine in "red" specimens is clear (Fig. 1d), without having to exclude either sex, in the plot that takes into account the ratio DPS/patella length (Fig. 4d).

3.1.3. Pectinal tooth count and shape

As shown in Table 1, this character does not help to distinguish our morphotypes. Pectinal tooth counts vary between specimens, and sexual dimorphism is evident. Values range from 5/5 to 7/7 in females, while they are more constant, predominantly 8/8, in males. The shape of pectines sensilla of the "black" specimens (VVZC Eut102-260) is similar to that of the "red" morphotypes (VVZC Eut96-243) and is identical to the drawing of "*E. carpathicus*" in Bonacina (1980) (Figs. 2e–h). SEM images of the pectines sensilla did not reveal morphological differences, confirming that these characters are only useful at higher taxonomically levels (Valle 1975).

3.1.4. Carinae development: metasoma, pedipalps and legs

Results show that metasoma carination is more developed in the "red" morphotypes (carinae development quotient: 375) than in the "black" morphotypes (carinae development quotient: 223). Moreover, while the lateral keel of the first segment is never present in the "black" specimens, it is always present, though sometimes faint and smooth, in the other morphotype. Another finding of metasoma observations was the lack of the inferior median carina in the first 3 segments and, in some cases, also in the fourth segment. In the "red" type this carina is always present in the fourth segment and sometimes also in the third metasomal segment. Observation of pedipalp carinae development revealed that carination is more marked and "organized" in "red" morphotypes than in "black" morphotypes (Figs. 1d and f), and is similar in all analyzed "red" specimens. The most apparent differences in pedipalp carination between the 2 morphotypes are: on the femur, dorsal carinae are granulate in the "black" types but crenulate in the "red" types; granules on the ventral surface of the latter are also larger and more numerous. The patella of the 2 morphotypes are not very different (Figs. 1c and d). Most of the chelal carinae are similar; however, carinae D3 and D4 are rough and weakly granulate in the "black" types and smooth, like in the dorsal intercarinal spaces, in the "red" types (Figs. 1e and f). The "black" specimens are more polymorphic; some carinae are less developed, and granules are often unequal in size and irregularly distributed. In addition, we considered the dorsal carination of leg femurs. Each leg was analyzed, and results show that carination is much stronger in the "red" specimens (171) than in the "black" specimens (85) (Figs. 2a–d). The ventral carination of the same segment is also stronger in the "red" specimens.

Table 2. Eigenvalues, percentage (%) variation and cumulative percentage (cum. %) variation for the first 4 principal components (PCs) of the multivariate data set

PC	Eigenvalues	% Variation	Cum. % variation
1	19.65	72.8	72.8
2	1.83	6.8	79.6
3	1.67	6.2	85.7
4	1.10	4.1	89.8

4. Statistical results

In the PCA analysis the first 2 components account for 79.6% of cumulative variance (Table 2). The first component (PC1) distinguishes morphotype 1 ("black") from morphotype 2 ("red") with some overlap (Fig. 3). Eigenvector coefficients reveal that, compared to individuals with high positive scores, individuals with high negative values along PC1 all have higher biometric values; PC1 therefore generally separates the 2 genetic groups according to general dimensions, although some individuals lie in an intermediate position. PC2 reveals some contrasts between several variables: pedipalp patella, femur and chela length, pedipalp length (femur + patella + chela), DPS length and carapace length increase with positive scores along PC2. Other variables, on the other hand, in particular femur and palm width, palm depth, and the width of all metasomal segments, increase with negative scores. Because the 2 morphotypes are also separated along PC2, this second axis should be considered an index of form in which the features of morphotype 2 are more elongated than those of morphotype 1. The 2-way factorial ANOVA on each variable of the same data set confirmed multivariate results. Because the major source of variation in the PCA was explained by general dimensions, in all cases the average value of a variable was significantly greater in morphotype 2 ("red") than in morphotype 1 ("black"). Therefore, as an example, only the tests for 3 of the analyzed variables are reported in Table 3. ANOVA on the indices of form also yielded significant results (Table 3), confirming several differences in the morphology of the two genetic groups (Fig. 4).

5. Discussion

5.1. Taxonomy

From a taxonomic standpoint, the 2 studied phenotypes belong to different described subspecies. The most

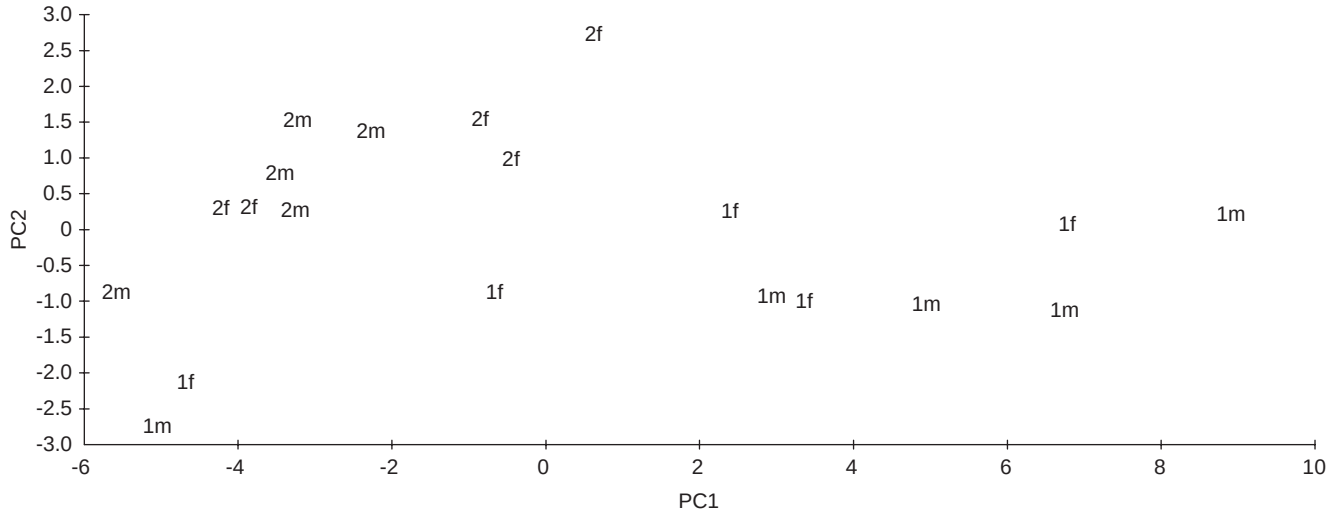

Fig. 3. Principal component analysis plot. Codes: 1 = morphotype 1 (“black”); 2 = morphotype 2 (“red”); f = female; m = male.

Table 3. Two-ways ANOVA table

	Mean ± SD		Morphotype		Sex	
Variable/index	1	2	*F*	*p*	*F*	*p*
Carapace length	4.9 ± 0.7	5.7 ± 0.2	20.2	***	3.55	n.s.
Chela length	8.2 ± 1.4	10.3 ± 0.5	23.9	***	2.81	n.s.
DPS	0.33 ± 0.07	0.72 ± 0.09	119.5	***	0.32	n.s.
Chela length/chela width	2.5 ± 0.2	2.9 ± 0.1	42.63	***	2.20	n.s.
Femur length/femur width	2.4 ± 0.5	2.9 ± 0.3	9.09	**	0.26	n.s.
Metasoma V length/metasoma V width	2.8 ± 0.5	3.2 ± 0.1	7.16	*	0.02	n.s.
DPS/patella length	0.08 ± 0.01	0.15 ± 0.02	162.7	***	2.62	n.s.

The value of the *F*-statistic is reported together with the *p*-level of significance for factors Morphotype and Sex. For the factor Morphotype, mean values (±SD) are reported for each variable pooling males and females data. Data are expressed in mm. Codes are: n.s. = not significant; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; 1 = morphotype (“black”); 2 = morphotype (“red”)

recent taxonomical revision of the *Euscorpius carpathicus* complex (Fet and Soleglad 2002) considers 9 different old taxonomical entities as synonyms of *E. tergestinus* (C.L. Koch, 1837). Three of these were described by C.L. Koch: *Scorpius aquilejensis* (Koch 1837), *S. concinnus* (Koch 1837) and *S. niciensis* (1841). Four subspecies were described by Caporiacco (1950): *E. carpathicus apuanus*, *E. c. corsicanus*, *E. c. picenus* and *E. c. oglasae*. The 2 other taxa are: *S. tergestinus var. austriacus* (Ferrari, 1872) and *E. c. mesotrichus* Hadži, 1929; since the latter is a homonym of *E. italicus mesotrichus* Hadži, 1929, this taxon cannot be used. The reason for this attribution of synonymy was the insufficiency of diagnostic morphological characters and the presence of one character, external pedipalp patella series *eb* = 4/4, in all the above-mentioned taxa. Our “red” types undoubtedly coincides with the *E. tergestinus* neotype accurately re-described by Fet and Soleglad (2002). Comparative analysis was necessary to determine the taxonomical position of our “black” types. The study of Caporiacco’s material provided a better understanding of his described subspecies and allowed the comparison of “black” specimens with Caporiacco’s specimens. Our black phenotypes clearly correspond to *E. c. concinnus* re-described in the manuscript on Italian euscorpiids by Caporiacco (1950). The author considered this scorpion as the most widely distributed taxon, with diagnostic characters such as medium size, dark color and an oligotrichous trichobothrial pattern. Other morphological aspects are also identical: the pectinal tooth count, 7/7 in females and 8/8 in males, Pe = 24 and Pv = 8. While comparing 4 of Caporacco’s specimens from central Italy (Emilia Romagna, Umbria), it was possible to determine other ratios and characteristics that were not taken into account by Caporiacco. The DPS is short and the pedipalp chela is stout, while chela carinae D3 and D4 are rough and the leg femur granulation is weak.

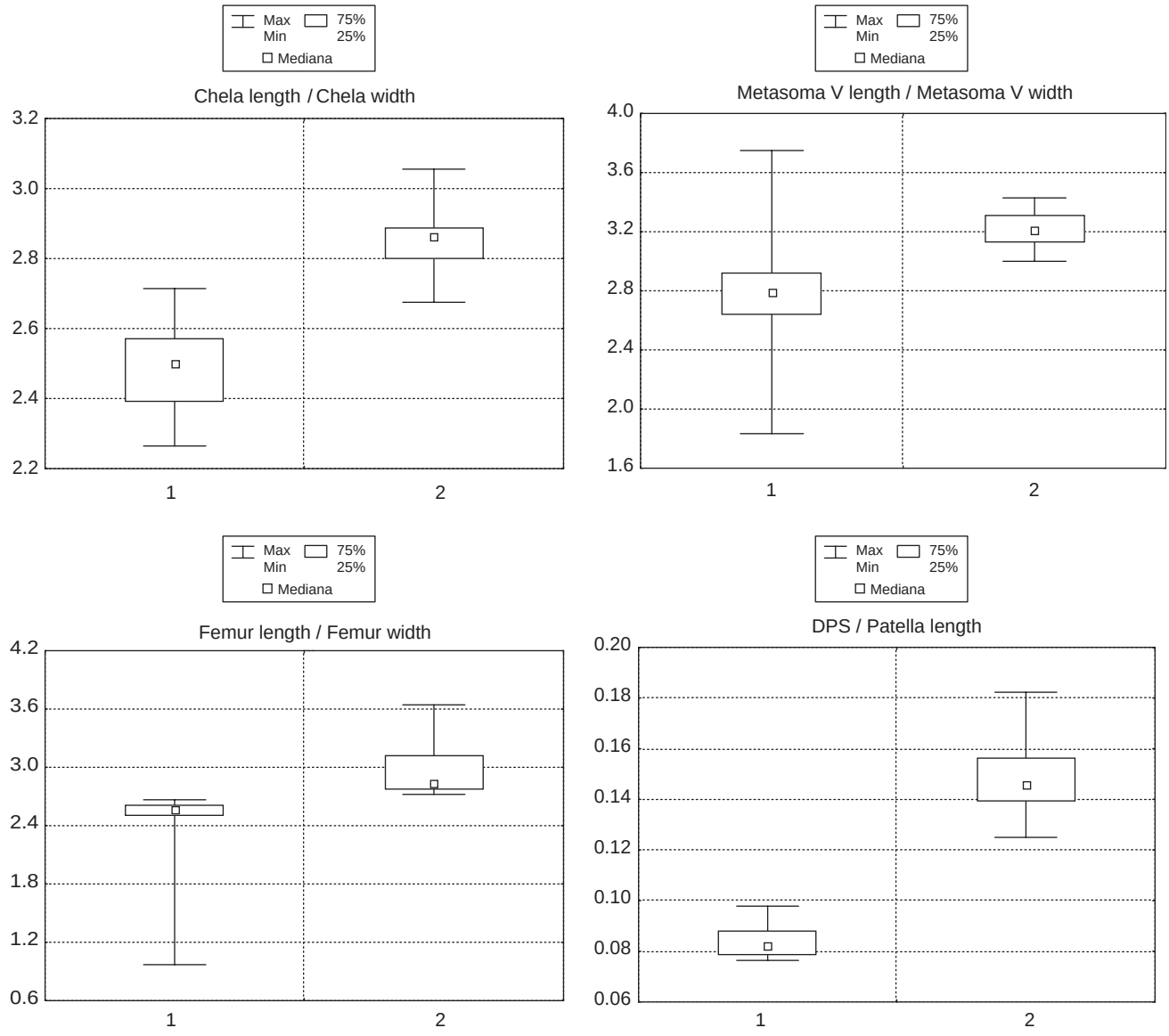

Fig. 4. Average ± SD for the 4 indices of form calculated for morphotypes 1 (“black”) and 2 (“red”) (including males and females).

The angle between trichobothria 1 and 4 of the *em* series is slightly variable, particularly the position of number 4, but all 8 patterns are more “open” than the narrow pattern of *E. tergestinus*. It would be unwise to consider this difference diagnostic; intraspecific variability of the position (and number) of trichobothria is not rare. This kind of polymorphism was observed for the same series (*em*) in euscorpiids such as *E. hadzii* Caporiacco, 1950 by Fet and Soleglad (2002) and *S. niciensis* C.L. Koch, 1837 by Lacroix (1991). The color may be compared with that of the most recent of Caporiacco’s specimens from Umbria, but not with that of specimens from Emilia Romagna, which are too old and have lost their pigmentation. As explained above, this qualitative character is variable but similar between specimens collected in central and southern Tuscany. Two specimens (VVZC Lucca, Eut106; Bologna, Eut95) are totally black, chelicerae and telson included, except for the dark yellow tarsus and the slightly reddish apix of chelae fingers. The specimen from Elba Island (VVZC Eut46) is totally dark reddish brown, except for the lighter-colored legs that show no maculation. Very similar is the specimen from Pavia (VVZC Eut89). The unnamed specimens from Elba Island (Tuscany Archipelago) belonging to an $eb = 4/4$ morphotype were noted by Valle (1975) and considered as *E. tergestinus* (Fet et al. 2003). They show all characters of the “black” phenotype. *Scorpius aquilejensis* (C.L. Koch, 1837), which was described on the basis of only one specimen and represented in Koch’s famous paper, “Die Arachniden” (1837), as a “yellow” *Euscorpius* with crenulate metasomal keels and ventral patellar series 8/8. Our

dataset contains 1 unusual "yellow" specimen (Eut278) collected in Rome; given that molecular and morphological aspects of this specimen are the same as those of our "red" type (= *E. tergestinus*), 1 hypothesis is that *S. aquilejensis* was defined on the basis of 1 anomalous specimen which was light in color and lacked some trichobothria on the patellar ventral series. Caporiacco (1950) analyzed specimens from several localities in the vicinity of Trieste (*locus typicus*), and gave the ventral patella series (9/9) and the external patella (24/24) as diagnostic trichobothrial characters. These values, together with the color description and geographic correspondence (we also have 1 specimen from Sistiana, Trieste), indicate that our "red" morphotype corresponds even to *S. aquilejensis* (*E. c. aquilejensis* sensu Caporiacco 1950). We also analyzed an additional specimen from Trieste (Nabresina) deposited in MZUF. This specimen shows a polytrichous trichobothrial pattern (Pe: $eb = 4/4$, $eb_a = 4/4$, $esb = 2/2$, $em = 4/4$, $est = 4/4$, $et = 8 - 8$; Pv = 10/11) and provides evidence of the high polymorphism of the complex; besides, the geographic position of Trieste is a crucial point for dispersal between the Balkans and the Italian Peninsula. No type specimens of this taxon are available for study (Fet and Soleglad 2002); although it remains an enigmatic entity, we agree with the authors that it should be considered synonymous with *E. tergestinus*. *Euscorpius c. picenus* was described by Caporiacco (1950) as a *Euscorpius* subspecies extremely similar to *E. c. concinnus*, but with a higher trichobothrial number (Pv = 9/9) and darker color. We analyzed 3 specimens from Abruzzo (Caramanico negli Abruzzi) of Caporiacco's material. Two show the same morphology as *E. tergestinus*, while 1 has the features of our "black" type (pedipalp chela and DPS short, Pv = 8/8). As previously noted by Fet and Soleglad (2002), the specimens extremely similar to *E. tergestinus* have a different metasomal carination, which is less granulate. To avoid confusion we decided to consider *E. c. picenus* synonymous with *E. tergestinus*, as in Fet and Soleglad (2002). The last subspecies to be examined and compared was *E. c. apuanus* Caporiacco, 1950. This was considered a taxonomical entity principally on the basis of its yellow color; the author also gave other morphological characteristics, but they are insufficient to distinguish it from other taxa, which are very similar. To distinguish this subspecies from the other "yellow" euscorpiids, Caporiacco highlighted the fainter granulation of the leg femurs. Two females from Levigliani, in the Apuan Alps, were studied. Their general aspect is similar to that of *E. tergestinus*, with elongated pedipalp chelae, granulation of the pedipalp patella and femora, long total length and similar pigmentation; nevertheless, they also show some intermediate characters. The dorsal surface of the pedipalp chela and D1–D4 carinae are weakly granulate; moreover, the granulation of both ventral and dorsal metasomal carinae is sparse, as in our analyzed *E. tergestinus* specimens. As we do not have this phenotype in our molecular dataset we cannot come to any taxonomical conclusions; we therefore follow the taxonomy proposed by Fet and Soleglad (2002), which considers *E. c. apuanus* synonymous with *E. tergestinus*. During this study we analyzed some specimens from Montecristo Island, a small island situated in the central Tyrrhenian Sea; *E. c. oglasae* was defined as an oligotrichous euscorpiid similar to the subspecies (*E. c. corsicanus*) described by the same author in Corsica, France, but lighter in color (Caporiacco 1950). As we only have juveniles, and especially because we lack molecular data, we do not discuss their taxonomy but follow Fet and Soleglad (2002). Finally, during the investigation of the material preserved in MZUF, a unique specimen from Monte Pisano, Pisa (Tuscany), which Caporiacco (1950, p. 186) considered similar to *E. c. canestrinii* (Fanzago 1872), was found and analyzed. The damaged specimen presents typical characteristics of a "pale-colored" *E. tergestinus*, such as the pedipalp patella trichobothria formula ($eb = 4/4$, $eb_a = 4/4$, $esb = 2/2$, $em = 4/4$, $est = 4/4$, $et = 6/6$; Pv = 9/9), rather than those of the "*E. sicanus*" complex to which this Sardinian endemic morphotype is ascribed (Vachon 1978; Fet et al. 2003). In keeping with the results of this morphological and statistical analysis, molecular results (Salomone et al. in prep.), indicate that the "red" and "black" morphotypes belong to the *E. tergestinus* complex and may be distinguished as separate forms. Although multivariate analysis revealed the high polymorphism of the "black" morphotype, the 2 morphotypes are certainly distinct and belong to 2 different morphological groups (Fig. 3). The 2-way factorial ANOVA analysis confirmed multivariate results and provides further indication of morphological differentiation between the two studied types. The plots that provide the best evidence of differentiation (Figs. 4a–c) mainly highlight the more slender structure (chela length/chela width; femur length/femur width; metasoma V length/metasoma V width) of the "red" type with respect to that of the "black" type. The DPS/patella length ratio only highlights the shape of a specific structure, i.e. the DPS spine (Fig. 4d). The large DPS spine, the marked leg granulation and the elongated chelae, especially in females, are the most important features distinguishing the "red" morphotype from the "black" morphotype. Although the color pattern is considered in this paper, this parameter is highly qualitative and cannot be considered alone. We can split the Italian "*E. tergestinus*" complex into 2 valid species: *E. tergestinus s.s.* (C.L. Koch, 1837) and the "black" type which corresponds to *E. c. concinnus sensu* Caporiacco (1950). Based on our comparative morphological analysis, which is sustained by molecular

results (Salomone et al. 2004 in prep.), we elevate this taxon to the species level.

***Euscorpius concinnus* (C.L. Koch, 1837)**

(Figs. 1a, c and e; Table 4)

Scorpius concinnus C.L. Koch, 1837: 105-103, pl. CVI, Fig. 246.

References (selected):

Scorpius concinnus: C.L. Koch, 1850: 86; Pavesi, 1876: 430.

Euscorpius carpathicus concinnus: Caporiacco 1950: 190–194. *Note*: Caporiacco (1950 p. 194) used this subspecies name since it did fit Koch's description illustration. Since Koch's holotype is lost and the type locality is not known, we maintain this name as valid and fix neotype for *E. concinnus* here.

Euscorpius tergestinus: Fet and Sissom 2000: 372; Fet and Soleglad 2002: 16–24.

Type material: Holotype: 1 female (lost), type locality unknown. Neotype: Adult female (VVZC Eut516) collected in the locality of Ponte a Bozzone (N 43°21′01.2″, E 11°23′10.1″), Castelnuovo Berardenga (SI), Tuscany, central Italy; under tree bark, pine wood, 273 m a.s.l., 13 October 2003 (V. Vignoli & F. Ciccognardi coll.). Type specimen will be deposited in MZUF.

Diagnosis: Medium-sized euscorpiid with general squat aspect (Fig. 1a; Table 4). Blackish–dark brown body, with reddish chela and pale brown legs and chelicerae. The pedipalp patellar external trichobothria number and pattern are typical of the "*tergestinus*" complex (Fet and Soleglad 2002). The dorsal patellar spur (DPS) is quite developed, as is granulation on leg femurs (Figs. 1c, 2a and c). Pectinal tooth: males 8–8; females 7–7.

Description of neotype: *Color*. Carapace, mesosoma, metasoma and telson vesicle all brown (Fig. 1a). Pedipalp femur is the same color as the body, while the patella and chelae are more reddish. The extremities of chela fingers are paler. The chelicerae are pale brown, with maculate pigmentation, and are similar to the telson aculeus. *Carapace.* Characterized by a slight, uniform roughness. *Mesosoma*. All tergites are slightly rough, like the carapace, and do not show any carinae. Ventral side is yellow–brown with smooth sternites. Stigmata are small and hardly visible. *Metasoma*. Generally squat structure. Intercarinal spaces of segments I–IV are smooth; only ventral intercarinal space of segment V presents slightly enlarged granules. Segment I: dorsal and dorsal lateral carinae partially granulate; lateral, inferior lateral and inferior median obsolete. Segment II: similar to segment I. Segment III: dorsal carina weakly granulate, inferior lateral and inferior median smooth but evident; other carinae are absent. Segment IV: similar to segment III. Segment V: dorsal lateral weakly granulate; lateral carina obsolete; inferior lateral and median, weakly granulate. *Telson*. Squat shape and smooth surface. Vesicle with 8 long ventral setae, and 2 long setae in the region between vesicle and aculeus. Aculeus with strong curvature. *Pectines*. Pectinal tooth count: 7/7. Middle lamellae: 5/5. The peg sensilla shows the typical shape of the "*carpathicus* complex" drawn by Bonacina (1980) for *E. carpathicus*. Length: 1.9; width: 1.0. *Genital operculum*. Separated most of length. Length: 0.6; width: 1.7. *Sternum*. Pentagonal shape. Length: 1.4; width: 1.5. *Chelicerae*. Movable finger: ventral edge typically smooth with brush-like setae on the entire inner part; dorsal edge: two subdistal denticles. Fixed finger: standard configuration. *Pedipalps*. The general structure is stocky. Femur. Dorsal carinae granulate, ventral

Table 4. Meristic data for the neotype (female), and an adult male (VVZC Eut304) of *Euscorpius concinnus*

		Neotype	Male
Total	Length	28.7	29.9
Carapace	Length	4.2	4.5
	Posterior width	4.2	4.6
Metasoma	Length	9.3	11.4
Segment I	Length	1.2	1.4
	Width	1.5	1.8
Segment II	Length	1.4	1.7
	Width	1.4	1.6
Segment III	Length	1.6	2.0
	Width	1.4	1.5
Segment IV	Length	2.0	2.4
	Width	1.3	1.3
Segment V	Length	3.1	3.9
	Width	1.2	1.3
Telson	Length	3.4	4.5
Vesicle	Length	2.0	3.2
	Width	1.2	1.8
	Depth	1.2	1.9
Aculeus	Length	1.4	1.1
Pedipalp	Length	13.9	15.1
Femur	Length	3.3	3.6
	Width	1.3	1.4
Patella	Length	3.5	3.7
	Width	1.5	1.6
Chela	Length	7.1	7.7
Palm	Length	3.4	3.7
	Width	2.7	3.4
	Depth	1.8	2.3
Movable finger	Length	4.1	4.6
Pectinal teeth		7/7	8/9

Measurements are in millimeters.

crenulate and intercarinal spaces partially granulate. Trichobothria *d* and *i* are situated at the same distance on the femur. Patella. Dorsal carinae are granulate (Fig. 1c) while the ventral carinae are weakly granulate, like the intercarinal spaces. DPS short: 0.28 mm. Ventral patellar spine reduced to a little tubercle. Trichobothria pattern, type C neobothriotaxic (major additive) (Vachon, 1974); Patella external (Pe) formula: $et = 7/7$, $est = 4/4$, $em = 4/4$, $esb = 2/1$, $eb_a = 4/4$, $eb = 4/4$; patella ventral (Pv): 8/9. Chela. Carinae granulation: dorsal carinae D1 and D4 are weakly granulate; D3 and D5 are rough. Dorsal intercarinal surface rough. Ventral carina V1 and V2 are weakly granulate; V2 smooth. External (E) carina weakly granulate. Dorsal surface between carina D3 and D4 is rough (Fig. 1e). Trichobothria V_4 is situated on the ventral carina V1. *Legs*. Two pedal spurs without spines. Tarsus: median ventral row with 8–9 stout spinules (leg III); tarsal setae (adjacent to the median ventral spinule row), flanking pairs: 3; terminal pairs: 2. Basitarsus: 2–3 small proventral spinules on legs I–II; 1 spinule on leg III, absent on leg IV. *Male*. Specimen similar to the neotype in color and body granulation. Total body length: 29.9 mm; carapace length: 4.5 mm. Telson vesicle swollen and aculeus more strongly curved than female one. Pectinal tooth count: 8/9. DPS: 0.32 mm.

Materials examined: Besides the material from VVZC and the specimens from the Società Romana di Scienze Naturali, the first author analyzed some of Caporiacco's specimens preserved in MZUF labeled as *E. c. concinnus* (C.L. K.) and as *E. carpathicus* (see Appendix A).

Variations: The diagnostic characters are reasonably constant in all examined populations. Although we noted the variability of the carinae on metasomal segments, especially of the inferior median carinae, this characteristic was not considered in the morphological discussion because it has already been widely documented (Soleglad and Sissom 2001; Fet and Soleglad 2002). The metasomal ventral granulation of three specimens from three close localities (Barberino del Mugello, Bologna, Lucca) is different despite the molecular similarity (Salomone et al. in prep.). The granulation of the dorsal surface between carinae D3 and D4 of the pedipalp chela is another variable character. The dorsal surface of chela in two specimens, one from Emilia Romagna (VVZC Bologna, Eut95) and the other from Latium (VVZC Cori, Eut105), is smoother than usual. Body color and total length vary among different populations. The southern populations, from Monte Semprevisa and National Park of Circeo (Latium), are entirely black (vesicle, legs, tarsus and basitarsus included). Two males from eastern Tuscany (Castell'Azzara (GR) and Arezzo) are similar; the specimen from Arezzo (VVZC Eut142), with a carapace length of 6.2 mm, is the largest studied specimen (total length: 45 mm). Specimens from the Marche region are brown, with yellowish legs and vesicle, and larger than the other specimens; the carapace length of these large specimens is: 4.9 (VVZC Eut512), 5.0 (VVZC Eut515), 5.3 (VVZC Eut513).

Fig. 5. Map indicating sampling sites of investigated specimens of *Euscorpius tergestinus* (Δ) and *Euscorpius concinnus* (*).

Distribution and ecology: *Euscorpius concinnus* has a wide geographic distribution (Fig. 5); *E. c. concinnus* was considered 1 of the most common and widely distributed "*carpathicus*" subspecies in Italy (Caporiacco, 1950). We analyzed specimens belonging to different populations collected in an area of more than 650 km (Bergamo-Salerno) throughout the Italian Peninsula, which comprises 9 regions (Lombardy, Liguria, Friuli V. Giulia, Emilia Romagna, Tuscany, Marche, Umbria, Latium and Campania). Their distribution extends from the Tyrrhenian coast to the Adriatic coast, and includes insular populations such as those of Palmaiola and Elba Island (Tuscany Archipelago). This species seems to be tolerant (eurytopic) of a wide range of habitats and altitudes, from sea level to 1500 m, where they have principally corticolous and lapidicolous habitus. Nevertheless, a preference for natural rather than anthropogenic habitats was observed.

5.2. Ecology

Cryptic taxa can not only show small different morphological features but also ecological and behavioral

differences (Colborn et al. 2001; Muster et al. 2004; Wellborn and Cothran 2004); these could be the result of an adaptation, given that the phenotypic similarity of similar species also implies limited scope to occupy different habitats (Wellborn and Cothran 2004). We observed ecological differences between the 2 similar species, which are sympatric but not syntopic; *E. concinnus* is more common in natural habitats, such as woods, whereas *E. tergestinus* was prevalently found in anthropogenic habitats. We suppose that the distribution of these 2 euscorpiids in different microhabitats (not really different habitats as Wellborn and Cothran (2004) confirm) could be the result of strong interspecific interaction (intraguild predation interaction) instead of "anthropotolerance" (i.e. major or minor tolerance to anthropogenic habitats (Crucitti et al. 1998a, b)) or "anthropophily" (Vachon 1983; Lacroix 1990). Intraguild predation interaction is thought to greatly affect scorpion distribution patterns (Polis and McCormick 1987), and this competition could promote their coexistence. Size differences between sympatric scorpion species seem to be important in determining this interaction, and larger species occupy the most favorable microhabitats (Polis and McCormick 1987). Wall crevices protect specimens from extremes in temperature (Benton 1992) and predators, and ensure relatively constant humidity during the dry Mediterranean summer; these are all reasons why man-made environments could be considered favorable habitats. The susceptibility to climatic conditions, such as precipitation, which are correlated to relative humidity and appear to determine the distribution of euscorpiids (Fet et al. 2001), sustains this hypothesis. *Euscorpius concinnus* and *E. tergestinus* were never found together; the larger species (*E. tergestinus*) was present in anthropogenic habitats, while the smaller (*E. concinnus*) occupied natural habitats. The same distribution pattern has been observed in Italy for other pairs of sympatric euscorpiids, such as *E. italicus* (Herbst 1800) (larger) and *E. concinnus* (smaller) in northeast Tuscany (V. Vignoli and J. Ove Rein pers. obs.) and Latium, and *E. sicanus* (C.L. Koch, 1837) (larger) and *E. concinnus* (smaller) in central Tuscany. The unusual recovery of *E. tergestinus* under bark in a pine wood of Sistiana, near Trieste, instead of in a rural construction could be due to the presence of *E. italicus* in the same region; the latter, larger species is known to colonize anthropogenic habitats (Braunwalder 2001; Crucitti et al. 1998a, b).

We do not exclude behavioral differences between these cryptic taxa, and mating tests will help determine whether there are stable reproductive barriers and, if so, whether they are pre- or post-zygotic.

6. Conclusions

The most important outcome of our study, based on the observation of 167 specimens, is that in Italy there are at least 2 different valid species belonging to the "*E. tergestinus*" complex. *Euscorpius tergestinus* is a slender, reddish euscorpiid with a big dorsal patellar spine. *Euscorpius concinnus* is generally darker and squat (see Table 5 for a summary of characters used to distinguish the 2 taxa). Moreover, the latter species is characterized by a heavy metasoma, relatively short pedipalp segments, a short dorsal patellar spine (DPS), and weak granulation on leg femurs. The 2 taxa are sympatric in several Italian regions (Lombardy, Friuli V. Giulia, Latium, Tuscany, Umbria). Intraguild predation interaction could be the principal cause of the specific distribution in different microhabitats, establishing an equilibrium that makes coexistence of these cryptic species possible. The distribution of *E. tergestinus*, previously limited to northern Italy (Fet and Soleglad 2002), has been extended to include Siena and Rome, both in central Italy.

Further studies will be useful to complete knowledge of this polymorphic scorpion group in Italy. In particular, relationships among the endemic subspecies of Montecristo Island, *E. c. oglasae* Caporiacco, 1950,

Table 5. Diagnostic characters distinguishing *Euscorpius tergestinus* from *Euscorpius concinnus*

Character	*Euscorpius tergestinus*	*Euscorpius concinnus*
Pedipalp patella ventral (Pv) trichobothria number	9/9	8/8
Dorsal patellar spine length	Long	Short
Pedipalp chela length	Long	Short
Chela carinae D3 and D4	Smooth	Rough
Color	Orange, reddish	Black, dark brown
Legs femur dorsal granulation	Crenulate	Granulate
Angle between trichobothria 1 and 4 of *em* series (Pe)	Narrow	Large
Metasoma and pedipalp granulation	Emphasized, organized, crenulated	Irregular, weakly granulate to granulate

and particular morphotypes (specimens with Pe = eb_a: 5/5, $eb = 4/4$ and specimens from the Apuan Alps, *E. c. apuanus* Caporiacco, 1950), as well as their geographic distribution must be further investigated. We cannot exclude the presence of other cryptic species in Italy, especially in the mountainous regions or on the islands, where gene flow is absent or reduced due to natural barriers. Last but not least, without molecular evidence and morphological congruence, it would not have been possible to complete this study according to modern scientific standards.

Acknowledgements

We are grateful to Gaia Pigino, Massimo Migliorini, Francesco Cicconardi, Jan Ove Rein, Andrea Petrioli, Marco Bastianini, Diego Facheris, Marco Colombo, and Maria Vittoria Di Giovanni for collecting zoological material used in this study. We also thank Pietro Lupetti and Eugenio Paccagnini for assistance with microscope images. Particular thanks go to Sarah Whitman for loaning specimens preserved in the Museo Zoologico "La Specola" of Florence, and to Pierangelo Crucitti for the generous loan of his entire euscorpiid collection, preponderant for the results of this manuscript. Useful comments and suggestions of Victor Fet (Marshall University) greatly improved the final version of the manuscript. We also thank two anonymous reviewers for comments that improved the manuscript.

Appendix A

All specimens were collected in Italy and, where not otherwise indicated, the samples are preserved in the private collection of the first author in the Dipartimento di Biologia Evolutiva, University of Siena, Italy. Habitat indications are reported only when known. The following list of materials includes all the sequenced specimens (Salomone et al. in prep.) with evidence of the typical "*tergestinus*" morphology. ***Euscorpius tergestinus*** **(C.L. Koch, 1837)**: Tuscany: 1 adult female (VVZC Eut243), Loc. Vico Alto, Siena (SI), house, 30 October 2002 (R. Dallai coll.); 1 adult female (VVZC Eut35), Siena (SI), house, 09 October 2000 (G. Innocenti coll.), in VF collection. Latium: 1 subadult female (VVZC Eut278), Rome (RM), house, 19 February 2003 (P. Crucitti coll.). Friuli V. Giulia: 1 adult female (VVZC Eut96), Sistiana (TS), under tree bark, pine wood, 18 m, 11 March 2002 (V. Vignoli coll.). ***Euscorpius concinnus*** **(C.L. Koch, 1837)**: Lombardy: 1 female (VVZC Eut89), Brallo di Pregola (PV), under stone, beach wood, 1500 m, 31 July 2001 (D. Facheris coll.). Tuscany: 1 adult male (VVZC Eut101), Barberino del Mugello (FI), under stone, beach wood, 29 June 2002 (V. Vignoli coll.); 1 adult female (VVZC Eut102), Montaione (FI), under stone, pine wood, 27 June 2002 (J. Ove Rein coll.); 1 adult female (VVZC Eut106), Loc. Tereglio, Lucca (LU), under stone, beach wood, 02 May 2002 (A. Petrioli coll.); 1 adult male (VVZC Eut260), Poggibonsi (SI), under tree bark, holly oak wood, 19 December 2002 (V. Vignoli & N. Salomone coll.); 1 adult male (VVZC Eut304), Loc. Ponte a Bozzone, Castelnuovo Berardenga, Siena (SI), under tree bark, pine wood, 05 May 2003 (V. Vignoli coll.); 1 adult male (VVZC Eut310), Brenna (SI), under tree trunk, deciduous wood, 03 May 2003 (F. Cicconardi coll.); 1 adult female (VVZC Eut46), Marciana, Elba Island (LI), under chestnut tree bark, 620 m, 11 March 2001 (V. Vignoli coll.); 1 adult female (VVZC Eut256), Castellina in Chianti (SI), under pine tree bark, 08 December 2002 (V. Vignoli coll.); 1 adult female (VVZC Eut265), Badia al Cerreto (SI), under bark, holly oak wood, 19 December 2002 (V. Vignoli & N. Salomone coll.); 1 adult female (VVZC Eut36), Castello di Brolio, Gaiole in Chianti (SI), 01 October 2000 (G. Manganelli coll.); 1 subadult female (VVZC Eut245), Loc. Pecchiaiola, Volterra (PI), under tree bark, cypress wood, 10 November 2002 (V. Vignoli coll.); 1 adult female (VVZC Eut311), Loc. Montalbuccio, Siena (SI), under chestnut tree bark, 07 May 2003 (V. Vignoli coll.); 1 adult female (VVZC Eut266), Gambassi Terme (FI), under tree trunk, deciduous wood, 19 December 2002 (V. Vignoli & N. Salomone coll.); 1 adult female (VVZC Eut43), Marina di Campo, Elba Island (LI), under stone, pine wood, 10 March 2001 (V. Vignoli coll.); Emilia Romagna: 1 adult female (VVZC Eut95), Loc. S. Luca, Bologna (BO), under tree bark, holly oak wood, 16 March 2002 (V. Vignoli coll.); Liguria: 1 adult female (VVZC Eut88), Loc. Le Grazie, Porto Venere (SP), 12 June 2001 (D. Facheris, coll.), in VF collection. Latium: 1 adult female (VVZC Eut267), Campo di Montelanico, Monti Lepini (RM), under stone, beach wood, 900 m, 04 January 2003 (A. Petrioli coll.); 1 adult female (VVZC Eut105), Foresta di Cori, Cori (LT), 500 m, 28 May 2002 (A. Petrioli coll.).

Several specimens (69) belonging to the zoological collection of the Società Romana di Scienze Naturali, labeled as *E.* "*carpathicus*", and used in a study on the Latium scorpiofauna (see Crucitti et al., 1998a for detailed data), were morphologically reanalyzed. In particular, the study undertook the comparative biometric analysis of the following 8 selected adult specimens. ***Euscorpius tergestinus*** **(C.L. Koch, 1837)**: Latium: 3 females (VVZC Eut219C-38C-86C) Antrodoco, Rieti (RI); Via Leonina 7, Rome (RM); Via del Corso, Rome (RM); 4 males (VVZC Eut/172C-112C-114C-246C), Via dei Greci, Rome (RM); Via Margotta, Rome (RM); Via dei Panieri, Rome (RM); Via G. Giolitti, Rome (RM).

Abruzzo: 1 male (VVZC Eut62C), Carapelle Alvisio, (AQ).

Additional material: ***Euscorpius tergestinus* (C.L. Koch, 1837)**: Tuscany: 2 adult females (syntypes, MZUF 125/5938-5945), Monte Corchia, Vallecchia, Pietrasanta, Levigliani, Apuan Alps, Lucca (LU), 03 August 1875 (Del Prete & G. Cavanna coll.); 1 female (VVZC Eut521), Chiusi (FI), house, 10 August 1996 (F. Vignoli coll.); 2 subadults (VVZC Eut218-219), Montecristo Island (LI), 13 July 1968 (L. Lazzeroni coll.); 1 female (VVZC Eut520), Siena, April 1996 (F. Vignoli coll.); 1 adult male (MZUF 305/9490), Monte Pisani, Pisa (PI), ? (Canara coll.). Abruzzo: 3 adult females (syntypes, MZUF 163/5997-6002-6009), Falde of Mt. Morone, Caramanico negli Abruzzi, Pescara, 27 July 1876–04 August 1878 (G. Cavanna coll.). Lombardy: 1 female (VVZC Eut522), Presezzo (BG), 11 November 1998 (D. Facheris coll.); 1 female (VVZC Eut144), Mapello, Bergamo (BG), 28 April 2002 (D. Facheris coll.); 1 male (VVZC Eut338), Ambivivere (BG), 06 May 2003 (D. Facheris coll.). Umbria: 1 female (VVZC Eut519), Loc. S. Marco, Perugia (PG), 15 November 2003 (E. Di Giovanni coll.); 1 female (VVZC Eut518), Coreiano, Perugia (PG), 11 November 2003 (Goretti coll.). Latium: 1 female (VVZC Eut281C), Via Donizzetti, Rome, 10 February 1998, (G. Amori coll.). Friuli V. Giulia: 1 subadult male (MZUF 187/6275), Nabresina, Trieste (TS), August 1879 (Paulucci coll.). ***Euscorpius concinnus* (C.L. Koch, 1837)**: Tuscany: 1 female (MZUF 135/5699), Resceto, Massa Carrara, 500 m, 10 June 1879, (Del Prete coll.); 1 female (VVZC Eut147), Barberino del Mugello (FI), under stone, deciduous wood, 25 June 2002, (V. Vignoli & J. Ove Rein coll.); 1 female (VVZC Eut524), Padule di Fucecchio, Pistoia, pine wood, 05 March 1994 (V. Vignoli coll.); 1 female (VVZC Eut523), Brenna (SI), deciduous wood, 03 June 1996 (V. Vignoli coll.); 1 male (VVZC Eut257), Castellina in Chianti (SI), under tree bark, pine wood, 08 December 2002 (V. Vignoli coll.); 1 female (VVZC Eut303), Loc. Ponte a Bozzone, Castel Nuovo Berardenga (SI), under tree bark, pine wood, 05 May 2003 (V. Vignoli coll.); 2 males (VVZC Eut502-503), Colognole, Livorno (LI), holly oak wood, 09 November 2003, (M. Migliorini coll.); 1 female (VVZC Eut289), Gaiole in Chianti, Siena (SI), under stone, beach wood, 01 May 2003 (V. Vignoli coll.); 2 females (VVZC Eut290-291), Radda in Chianti, Siena (SI), under tree bark, pine wood, 01 May 2003 (V. Vignoli coll.); 1 female (VVZC Eut288), Gaiole in Chianti, Siena, (SI), under stone, beach wood, 01 May 2003 (V. Vignoli coll.); 1 female (VVZC Eut197), 04 May 1970, Loc. Fungaia, Siena (SI) (Gatti coll.); 1 male (VVZC Eut528), Castell'Azzara (GR), 01 November 2003 (M. Bastianini coll.); 1 female (VVZC Eut229), Montieri (GR), 29 April 1981 (F. Giusti coll.); 1 female (VVZC Eut210), Montalbuccio (SI), 19 August 1966 (C. Baroni coll.); 1 female (VVZC Eut182), Palmaiola Island (LI), 12 July 1969 (F. Giusti coll.); 1 male (VVZC Eut261), Poggibonsi, Siena (SI), under tree bark, oak wood, 19 December 2002 (V. Vignoli & N. Salomone coll.); 1 male (VVZC Eut237), Marciana, Elba Island (LI), under tree bark, oak wood, 11 February 2001 (V. Vignoli coll.); 3 subadults (VVZC Eut239-240-241), 1 female (VVZC Eut238), Marina di Campo, Elba Island (LI), under stone, pine wood, 10 February 2001 (V. Vignoli coll.); 1 female (VVZC Eut200), Marina di Campo, ibidem, 22 August 1967 (F. Giusti coll.); 1 male (VVZC Eut99), Loc. Cercina, Florence (FI), on stone wall 14 June 2002 (V. Vignoli coll.); 1 male (VVZC Eut179), Arezzo (AR), June 1998 (? coll.); 1 adult male (VVZC Eut246), Loc. Pecchiaiola, Volterra (PI), under tree bark of cypres wood, 10 November 2002 (V. Vignoli coll.). Abruzzo: 1 subadult (VVZC Eut525), Venere, Aquila (AQ), under stone, 750 m, 08 June 1998 (V. Vignoli coll.). Campania: 2 adult females (VVZC Eut223), 700 m, Corleto Monforte, Salerno (SA), 16 June 1998 (C. Pignataro coll.); 1 female (VVZC Eut526), ibidem, 07 June 1998 (C. Pignataro coll.). Marches: 2 females (VVZC Eut512-513), ibidem, 15 August 2003 (E. Di Giovanni coll.); 1 male (VVZC Eut515), Loc. Ponte Nativo, Roccafluvione, Ascoli Piceno (AP), neglected house, 31 July 2003 (E. Di Giovanni coll.). Latium: 2 males (VVZC Eut250-251), 1 female (VVZC Eut249), Loc. Cerasella, National Park of Circeo, Latina (LT), under tree bark, oak wood, 13 November 2002 (F. Cicconardi coll.); 1 subadult (VVZC Eut143C), ibidem, humus under *Quercus pubescens*, 05 May 2002, (F. Cicconardi coll.); 4 males and 1 female (VVZC Eut506-510), Loc. San Felice, ibidem, under tree bark, oak wood, 08 November 2003, (F. Cicconardi coll.); 1 male (VVZC Eut230), Poggio Bustone, Mt. Reatini (RI), wood, 08 August 1969 (F. Giusti coll.); 1 male (VVZC Eut228), Vallonina Bassa, Terminillo, (RI), 19 August 1967 (F. Giusti coll.); 1 female (VVZC Eut527), Mt. Semprevisa, Bassiano, Mts. Lepini (RM), 1100 m, 19 March 2000 (M. Bastianini coll.); 1 female (VVZC Eut529), 1 subadult (VVZC Eut530), ibidem, 14 December 2003 (A. Petrioli coll.). Marches: 1 male (VVZC Eut199), Sirolo, Ancona (AN), 31 August 1967 (B. Urbani coll.). Friuli V. Giulia: 2 females (VVZC Eut226-227), Trieste (TS), 13 April 1967 (F. Giusti coll.); 1 subadult (VVZC Eut141), Doberdò del Lago (TS), under tree bark, deciduous wood, 10 March 2002 (V. Vignoli coll.). Liguria: 1 female (VVZC Eut292), Pignone (SP), rock wall, 24 April 2003 (M. Colombo coll.); 1 female (VVZC Eut90), Rio Maggiore (SP), 10 June 2001 (D. Facheris coll.). Emilia Romagna: 1 female (MZUF 138/5653), Modena, 1874 (Carruccio coll.), labeled as *E. c. concinnus* (C.L. K.); 1 male (MZUF 139/5621), Casinalbo, Formigine, 1880 (Fiori coll.), labeled as *E. c. concinnus* (C.L. K.). Umbria: 1 female (MZUF 158/5661), Marzano, Lippiano, Perugia, , VII.1925 (A. Andreini

coll.), labeled as *E. carpathicus*; 1 male (MZUF 157/5639), Lippiano, Perugia, 09.X.1934 (? coll.), labeled as *E. carpathicus*.

References

Benton, T.G., 1992. The ecology of the scorpion *Euscorpius flavicaudis* in England. J. Zool. London 226, 351–368.

Bonacina, A., 1980. Sistematica specifica e sottospecifica del complesso "*Euscorpius germanus*" (Scorpiones, Chactidae). Riv. Mus. Civ. Sci. Nat. "Enrico Caffi" (Bergamo) 2, 47–100.

Braunwalder, M.E., 2001. Scorpions of Switzerland: summary of a faunistic survey. In: Fet, V., Selden, P.A. (Eds.), Scorpions 2001. In Memoriam Gary A. Polis. British Arachnological Society, Burnham Beeches, Bucks, pp. 279–286.

Caporiacco, L.Di., 1950. Le specie e sottospecie del genere "*Euscorpius*" viventi in Italia ed in alcune zone confinanti. Mem./Accad. naz. Lin. (ser. 8) 2, 159–230.

Chatfield, C., Collins, A.J., 1980. Introduction to Multivariate Analysis. Chapman and Hall, London.

Colborn, J., Crabtree, R.E., Shaklee, J.B., Pfeiler, E., Bowen, B.W., 2001. The evolutionary enigma of bonefishes (*Albula spp.*): cryptic species and ancient separations in a globally distributed shorefish. Evolution 55 (4), 807–820.

Crucitti, P., Buccedi, S., Malori, M., 1998a. Il Genere *Euscorpius* nell'Italia Centrale. La distribuzione nel Lazio (Scorpiones, Chactidae). Boll. Assoc. rom. Ent. 53, 1–17.

Crucitti, P., Malori, M., Rotelli, G., 1998b. The scorpions of the urban habitat of Rome (Italy). Urban Ecosyst. 2, 163–170.

Fet, V., 2002. The Crimean scorpion, *Euscorpius tauricus* (C.L. Koch, 1837) (Scorpiones: Euscorpiidae): an endemic species supported by mitochondrial DNA evidence. Arth. Selecta 11 (4), 271–276.

Fet, V., Sissom, W.D., 2000. Family Euscorpiidae Laurie, 1896. In: Fet, V., Sissom, W.D., Lowe, G., Braunwalder, M.E. (Eds.), Catalog of the scorpions of the world (1758–1998). New York Entomological Society, New York, NY, pp. 355–380.

Fet, V., Soleglad, M.E., 2002. Morphology analysis supports presence of more than one species in the "*Euscorpius carpathicus*" complex (Scorpiones: Euscorpiidae). Euscorpius 3 51pp.

Fet, V., Kuntner, M., Sket, B., 2001. Scorpions of Slovenia: a faunistic and biogeographical survey. In: Fet, V., Selden, P.A. (Eds.), Scorpions 2001. In Memoriam Gary A. Polis. British Arachnological Society, Burnham Beeches, Bucks, pp. 255–265.

Fet, V., Soleglad, M.E., Gantenbein, B., Vignoli, V., Salomone, N., Fet, E.V., Schembri, P., 2003. New data on the "*Euscorpius carpathicus*" species complex (Scorpiones: Euscorpiidae) from Italy, Malta, and Greece: evidence from mitochondrial DNA and morphology. Rev. Suisse Zool. 110 (2), 355–379.

Gantenbein, B., Fet, V., Largiadèr, C.R., Scholl, A., 1999. First DNA phylogeny of *Euscorpius* Thorell, 1876 (Scorpiones, Euscorpiidae) and its bearing on taxonomy and biogeography of this genus. Biogeographica (Paris) 75, 49–65.

Gantenbein, B., Soleglad, M.E., Fet, V., 2001. *Euscorpius balearicus* Caporiacco, 1950, stat. nov.: molecular (allozymes and mtDNA) and morphological data support the existence of an endemic scorpion species on the Balearic Islands (Scorpiones: Euscorpiidae). Organisms Diversity Evolut. 1, 301–320.

Huber, D., Gantenbein, B., Fet, V., Sherabon, B., 2001. *Euscorpius carpathicus* (L.) from Austria (Scorpiones: Euscorpiidae): phylogenetic position clarified by mitochondrial DNA analysis. In: Fet, V., Selden, P.A. (Eds.), Scorpions 2001. In Memoriam Gary A. Polis. British Arachnological Society, Burnham Beeches, Bucks, pp. 273–278.

Koch, C.L., 1837. Die Arachniden. Nürnberg, C. H. Zeh'sche Buchhandlung 3 (6), 105–115.

Koch, C.L., 1841. Die Arachniden. Nürnberg, C. H. Zeh'sche Buchhandlung 8, 1–114.

Lacroix, J.B., 1990. Faune de France; Arachnida: Scorpionida. 4e note. Genus *Euscorpius* Thorell, 1876. Arachnides 6, 16–29.

Lacroix, J.B., 1991. Faune de France; Arachnida: Scorpionida. 5e note. Sub-genus (*Euscorpius*) Thorell, 1876. Arachnides 8, 17–36.

Lessios, H.A., Kessing, B.D., Robertson, D.R., Paulay, G., 1999. Phylogeography of the pantropical sea urchin *Eucidaris* in relation to land barriers and ocean currents. Evolution 53 (3), 806–817.

Muster, C., Schmarda, T., Blick, T., 2004. Vicariance in a Cryptic Species Pair of European Pseudoscorpions (Arachnida, Pseudoscorpiones, Chthoniidae). Zool. Anz. 242, 299–311.

Polis, G.A., McCormick, S.J., 1987. Intraguild predation and competition among desert scorpions. Ecology 68 (2), 332–343.

Salomone, N., Vignoli, V., Frati, F., Bernini, F., 2004. Phylogenetic relationships between the sibling species *Euscorpius tergestinus* and *E. sicanus* (Scorpiones, Euscorpiidae) as inferred from mitochondrial and nuclear sequence data. In: Proceedings of the16th Congress of Arachnology, August 2–7, 2004, Ghent University, Belgium, 268pp.

Salomone, N., Vignoli, V., Frati, F., Bernini, F. (in prep.): Species boundaries and phylogeography of the "*Euscorpius carpathicus* complex" (Scorpiones: Euscorpiidae) in Italy.

Sissom, W.D., 1990. Systematics, biogeography and paleontology. In: Polis, G.A. (Ed.), The Biology of Scorpions. Stanford University Press, Stanford, pp. 64–160 587pp.

Soleglad, M.E., Sissom, W.D., 2001. Phylogeny of the family Euscorpiidae Laurie, 1896: a major revision. In: Fet, V., Selden, P.A. (Eds.), Scorpions 2001. In memoriam Gary A. Polis. British Arachnological Society, Burnham Beeches, Bucks, pp. 25–112.

Stahnke, H.L., 1970. Scorpion nomenclature and mensuration. Ent. News 81, 297–316.

Taylor, D.J., Finston, T.L., Hebert, P.D.N., 1998. Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. Evolution 52, 1648–1670.

Trewick, S.A., 1998. Sympatric cryptic species in New Zealand, Onychophora. Biol. J. Linn. Soc. 63 (3), 307–329.

Underwood, A.J., 1997. Experiments in Ecology. Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge, UK.

Vachon, M., 1974. Étude des caractères utilisés pour classer les familles et les genres de Scorpions (Arachnides). 1. La trichobothriotaxie en Arachnologie, Sigles trichobothriaux et types de trichobothriotaxie chez les Scorpions. Bull. Mus. Nat. Hist. Nat. (Paris) 140, 857–958.

Vachon, M., 1978. Remarques sur *Euscorpius carpathicus* (Linné, 1767) *canestrinii* (Fanzago, 1872) (Scorpionida, Chactidae). Ann. Hist. Nat. Mus. Nat. Hung. 70, 321–330.

Vachon, M., 1983. La repartition, en France métropolitaine, des Scorpions appartenant au genre *Euscorpius* Thorell 1876 (Famille des Chactidae). Bull. Sci. de Bourg. 36, 25–41.

Valle, A., 1975. Considerazioni intorno alle sottospecie di *Euscorpius carpathicus* (L.) (Scorpiones, Chactidae). Aten. Parmense 11 (1), 209–234.

Vignoli, V., Crucitti, P., 2003. Aggiornamento alla checklist delle specie della fauna italiana. Fascicolo 21 – Arachnida, Scorpiones, Palpigradi, Solifugae, Opiliones. Boll. Soc. Entomol. Ital. 134 (3), 279–288.

Wellborn, G.A., Cothran, R.D., 2004. Phenotypic similarity and differentiation among sympatric cryptic species in a freshwater amphipod species complex. Freshwater Biol. 49, 1–13.

Wilcox, T.P.W., Hugg, L., Zeh, J.A., Zeh, D.W., 1997. Mitochondrial DNA sequencing reveals extreme genetic differentiation in a cryptic species complex of neotropical pseudoscorpions. Mol. Phylogenet. Evol. 7 (2), 208–216.

Zeh, D.W., Zeh, J.A., 1994. When morphology misleads: interpopulation uniformity in sexual selection masks genetic divergence in harlequin beetle-riding pseudoscorpion populations. Evolution 48, 1168–1182.