EUSCORPIUS NAUPLIENSIS (C. L. KOCH, 1837) (SCORPIONES: EUSCORPIIDAE) FROM GREECE: ELEVATION TO THE SPECIES LEVEL JUSTIFIED BY MOLECULAR AND MORPHOLOGICAL DATA

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Abstract

New molecular (allozyme and 16S mtDNA sequence) and morphological data on *Euscorpius italicus* (Herbst, 1800) (Scorpiones: Euscorpiidae) reveal two groups of populations. One including *E. italicus* from Italy, Switzerland, and Pindos Mts (Greece), and another, Greek populations from the Zakynthos Island (formerly described as *E. i. zakynthi* Caporiacco, 1950) and the Peloponnese (formerly described as *Scorpius naupliensis* C. L. Koch, 1837). The genetic divergence between these two groups was similar or higher to that observed between other congeneric species, i.e. fixed for private alleles at eight out of 18 allozyme loci and ~5% mtDNA sequence divergence. A morphological study of the material covering the entire range of *E. italicus* (Italy, Switzerland, Slovenia, Croatia, Albania, Greece, Turkey, Georgia, and Russia) is consistent with genetic data. *Euscorpius naupliensis* (C. L. Koch, 1837) (=*E. i. zakynthi* Caporiacco, 1950, **syn. n.**) from Greece is restored from synonymy and elevated to the rank of species. It is diagnosed by a number of morphological features, i.e. the absence of the esb_a external trichobothrial series on the pedipalp patella, position of trichobothria on the pedipalp fixed finger, and morphometric ratios.

Key words: Scorpions, *Euscorpius*, morphology, allozymes, 16S mtDNA

Taxonomy:

Euscorpius naupliensis (C. L. Koch, 1837), **restored name.**
Euscorpius naupliensis (C. L. Koch, 1837)
= *Euscorpius italicus zakynthi* Caporiacco, 1950, **syn. n.**

Euscorpius naupliensis (C. L. Koch, 1837) (Scorpiones: Euscorpiidae) de Grecia: elevación al rango de especie basada en datos moleculares y morfológicos.

Resumen

Nuevos datos moleculares (secuencia de alozimas y 16S mtDNA) sobre *Euscorpius italicus* (Herbst, 1800) (Scorpiones: Euscorpiidae) revelan la existencia de dos grupos de poblaciones. Uno incluye a *E. italicus* de Italia, Suiza y los montes Pindos (Grecia), y el otro las poblaciones griegas de la isla de Zakynthos (descrita como *E. i. zakynthi* Caporiacco, 1950) y el Peloponeso (descrita como *Scorpius naupliensis* C. L. Koch, 1837). La divergencia genética entre estos dos grupos era similar o mayor que la observada entre otras especies congenéricas, i.e. fijada para alelos privados en 8 de 18 loci de alozimas y ~5% de divergencia en la secuencia de mtDNA. El estudio morfológico del material que cubre todo el área de *E. italicus* (Italia, Suiza, Eslovenia, Croacia, Albania, Grecia, Turquía, Georgia y Rusia) concuerda con los datos genéticos. Se restituye *Euscorpius naupliensis* (C. L. Koch, 1837) (= *E. i. zakynthi* Caporiacco, 1950, **syn. n.**), de Grecia, anulando la sinonimia previa, y se eleva al rango de especie. Su diagnosis viene definida por una serie de rasgos morfológicos, i.e. la ausencia de la serie tricobotrial externa esb_a en la patela pedipalpal, la posición de los tricobotrios en el dedo fijo del pedipalpo, y proporciones morfométricas.

Palabras clave: Scorpions, *Euscorpius*, morfología, alozimas, 16S mtDNA

Taxonomía:

Euscorpius naupliensis (C. L. Koch, 1837), **nombre restituido**.
Euscorpius naupliensis (C. L. Koch, 1837)
= *Euscorpius italicus zakynthi* Caporiacco, 1950, **nueva sinonimia**

REVISTA IBÉRICA de Aracnología

ARTÍCULO:
Euscorpius naupliensis (C. L. Koch, 1837) (Scorpiones: Euscorpiidae) from Greece: elevation to the species level justified by molecular and morphological data

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Revista Ibérica de Aracnología
ISSN: 1576 - 9518.
Dep. Legal: Z-2656-2000.
Vol. **6**, 31-XII-2002
Sección: Artículos y Notas.
Pp: 13–43.

Edita: **Grupo Ibérico de Aracnología (GIA)**
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Introduction

Scorpions of the genus *Euscorpius* Thorell, 1876 (Scorpiones: Euscorpiidae) are very abundant in southern Europe and are ecologically diverse. They occupy a variety of habitats from xeric to mesic, from the Mediterranean shoreline to the high altitudes of the Alps and Balkans. The taxonomy of *Euscorpius* has been very confusing since numerous subspecies have been described but poorly defined both in morphology and geographic range (Hadñi, 1929; Caporiacco, 1950; , ur...if, 1972; Bonacina, 1980, 1982; Fet & Sissom, 2000). It also has been unknown whether hybridisation occurs between some of these subspecies (Kinzelbach, 1975; Fet & Braunwalder, 2000; Gantenbein *et al.*, 2001). The recent application of genetic markers opens new possibilities to address phylogenetic relationships among and within species (Gantenbein *et al.*, 1999; Scherabon *et al.*, 2000) but also allows to address the genetic population structure of these scorpions (Gantenbein *et al.*, 1998, 2000, 2001). Moreover, a careful re-evaluation of numerous museum collections and the analysis of trichobothrial patterns (especially on the external aspect of the pedipalp patella) revealed that this character correlates well with the major phylogenetic lineages defined by genetic analysis in *E. carpathicus* (L.) (Fet & Soleglad, 2002). Especially powerful is the combination of multiple genetic markers (nuclear and mitochondrial) with morphological data; this approach supported our view of highly diverged lineages, which deserve species status (Gantenbein *et al.*, 2000, 2001; Scherabon *et al.*, 2000).

A large, conspicuous *Euscorpius (Polytrichobothrius) italicus* (Herbst, 1800) has been known to the arachnologists for 200 years, and to the humankind for millennia. It is commonly found in many localities in Italy and Greece, being an especially common species in human habitations (Braunwalder, 2000, 2001). This species is found from French Riviera to the northern and eastern shores of the Black Sea (Fig. 26). *E. italicus* prefers xeric microclimate (Birula, 1917a, 1917b; Braunwalder & Tschudin, 1997; Braunwalder, 2001; Fet *et al.*, 2001). In Italy, this species is locally very abundant and usually synanthropic; in the north it is limited by the southern Alpine valleys in Italy and Switzerland (Crucitti, 1993; Braunwalder, 2001); in Turkey and Caucasus, it also does not venture into the high mountains (Birula, 1917a, 1917b; Crucitti & Cicuzza, 2001). The species' altitudinal preference seems to range from 0 to 500m, while reported well-isolated "island" populations above 500 m could be attributed to recent human-mediated range expansion (Braunwalder & Tschudin, 1997). The species has been reportedly introduced by humans to many places outside its continuous range (Vachon, 1952, 1983; Fet & Gruodis, 1987), in some cases establishing reproducing populations (e.g. in Yemen; Birula, 1937).

Several species described in the 19th century have been synonymized to *E. italicus*. Further, several subspecies were described in this species by a number of authors (Caporiacco, 1950) but are currently not recognized (Kinzelbach, 1975; Vachon, 1981; Bonacina, 1982; Fet & Sissom, 2000). However, the existence of possible "good" biological species (Mayr, 1942), "hidden" within *E. italicus*, is likely considering its wide geographic range and reported morphological variation. A detailed taxonomic history of *E. italicus* is given below.

The species *Scorpius naupliensis* C. L. Koch, 1837 from Peloponnese was for many years considered a synonym of *E. italicus* (Fet & Sissom, 2000). Another taxon from Greece, the subspecies *E. italicus zakynthi*, was described by Caporiacco (1950) from the Zakynthos (=Zante) Island in the Ionian Sea, and a small Pelouzo (=Peluso) Island nearby. Vachon (1981) listed *E. i. zakynthi* as a synonym of the nominotypical *E. italicus*. However, Vachon (1981, Fig. 13) also noticed that specimens from Peloponnese differ from all other *E. italicus* by the absence of the esb_a series of trichobothria on external aspect of pedipalp patella, which is quite an important diagnostic character for *E. italicus*. Recently, Crucitti (1995, 1999b) and Crucitti & Bubbico (2001) collected numerous of specimens from various localities in the southwestern Peloponnese, and confirmed that these populations of "*E. italicus*" differed in morphology from those in northern Greece (Pindos Mts.) and Italy.

Following indications of Vachon (1981), we observed that the populations from Peloponnese and Zakynthos are morphologically differentiated from all the rest of *E. italicus* specimens, collected throughout the entire geographic range of the latter. These two groups, existence of which also is confirmed by our genetic data, are treated below as *bona fide* species; justification for such treatment is provided within the Results sections. The senior synonym *E. naupliensis* (C. L. Koch, 1837) is the name that applies to those southern Greek populations, and is therefore removed from synonymy of *E. italicus* (Herbst, 1800). We give a detailed redescription of both species, followed by their morphological comparison, and results of the genetic analysis (variation of 18 allozyme loci and 16S rRNA mtDNA sequences).

Material and Methods

Material

For morphological analysis we used extensive preserved collections deposited in several zoological museums, in total 132 specimens of *E. italicus* and 95, of *E. naupliensis* (label data see below, under "Material studied").

For both allozyme and DNA analysis, *E. flavicaudis* (DeGeer, 1778) was used as an outgroup. For allozyme analysis, we used new specimens for *E. italicus* from Tortoreto, Italy (n=10) and Brissago, Switzerland (n=10), and *E. naupliensis* from Zakynthos, Greece (n=2) and Itylo, Greece (n=11) (label data see below for DNA specimens). We also used comparative data from Gantenbein *et al.* (1998, 2001) which include *E. italicus* from Coglio, Switzerland (n=10) and Vico Morcote, Switzerland (n=10), and the outgroup *E. flavicaudis* from Lauris, France (n=49).

For DNA analysis, we used eight specimens belonging to *E. italicus* and *E. naupliensis*; Three DNA sequences used in earlier studies (Gantenbein *et al.,* 1999, 2001) were extracted from the GenBank nucleotide sequence database, *i.e*., the sequences *Ei*BR, *Ei*TO1, and *Ef*LA1. Identical haplotypes were not considered in further analyses. Four new sequences were deposited in the EMBL database (www.ebi.ac.uk); the accession numbers for all DNA sequences are listed below.

Specimens used for DNA analysis: *E. italicus*: *Ei*BR (AJ389378), Brissago, Ticino, Switzerland, 25 May 1996 (B. Gantenbein); *Ei*TO1 (AJ298067), Tortoreto, Abruzzo, Italy, 7 October 1997 (M. Bellini); *Ei*MV1 (AJ506152), Metsovo, Epirus, Greece, 13 May 2001 (V. Fet); *Ei*SL1 (AJ512752), Brje, Dobravlje, Aidovš…ina, Slovenia, 7 August 2000 (B. Sket). *E. naupliensis*: *En*ZA1=*Ei*ZA2 (AJ506153), Zakynthos Island, Greece, 20 August 1999 (K. Palmer); *En*IT1 (AJ506154), *En*IT2 (AJ506155), Itylo, Peloponnese, Greece, 14 March 1998 (I. & B. Gantenbein). Outgroup *E. flavicaudis*: *Ef*LA1 (AJ389381), Lauris, Vaucluse, France, 1997-1999 (A. Scholl).

Morphological analyses

All measurements (i.e., morphometrics) presented in this paper are in millimeters (*mm*). For meristic and morphometric statistical data presented in this paper the following conventions are used:

min - max (mean) (±SD) [n]: {cmin - cmax} z cv

for the above *statistical data group*, *min* = minimum value, *max* = maximum value, *SD* = standard deviation, *n* = number of samples, *cmin* = corrected minimum (*mean-SD*), *cmax* = corrected maximum (*mean+SD*), *cv* = coefficient of variability (*SD/mean*). The range established by the corrected minimum and maximum is referred to as the *plus/minus standard error* range. Each statistical data group represents a dataset based on some specified partitioning (i.e., a species, a subspecies, a genus, gender, etc.).

Approaches to morphometrics applied in this study and special terminology used to describe the hemispermatophore of the male scorpion are described and illustrated in Fet & Soleglad (2002). Terminology describing chelal finger dentition and pedipalp ornamentation follows that described and illustrated in Soleglad & Sissom (2001).

Allozyme analysis

All specimens were killed by deep-freezing and stored at –80 EC prior to allozyme starch electrophoresis. Traditional horizontal starch gel electrophoresis of allozymes was carried out using the same buffer systems and conditions as in earlier studies (Gantenbein *et al.*, 1998, 1999). We scored the same 18 allozyme loci and compared the relative mobility of the electromorphs with the most frequent allele (mobility=100) of a reference population of *E. flavicaudis*. Due to high resolving power, differences in electromorph mobility could be traced up to 1 mm.

DNA analysis

We used a standard protocol as described in Gantenbein *et al.* (1999). For DNA analyses, genomic DNA was extracted from fresh or preserved (94-98% ethanol) muscle tissue (usually pedipalp or metasoma) using a standard phenol/chloroform precipitation method (Sambrook *et al.*, 1989) or the Qiagen™ DNeasy extraction kit. Extracted DNA was amplified by the polymerase chain reaction (PCR) in a Perkin Elmer 2400 or in an MJ-Research PTC-100 thermocycler using conditions and primers as described in Gantenbein *et al.* (1999). The mitochondrial LSU (large ribosomal subunit) 16S rRNA PCR primers corresponded to the positions 11,173-11,190 and 11,625-11,606 in the *Limulus polyphemus* mitochondrial genome (Lavrov *et al.*, 2000). The forward primer is a scorpion-specific version of the "universal" primer 16Sbr, or LR-J-12887, while the reverse primer has a scorpion-specific sequence designed by one of the authors (V.F.). The resulting PCR product was verified on 1% agarose electrophoretic gel and purified by Ultrafree MC 30000 cellulose filters (Millipore, Inc.) or using Cycle sequencing was performed using the 16Sbr as the sequencing primer and using the cycling conditions given in Gantenbein *et al.* (1999). The fragments were then resolved on the automated sequencer (LI-COR model 4200) and all sequences were checked manually for sequencing errors.

Statistical analyses of molecular data

Allele frequencies at allozyme loci and the observed and excepted heterozygosity (Nei, 1978) were calculated using GENETIX 4.1 (Belkhir *et al.,* 1996). We calculated chord distances (Cavalli-Sforza & Edwards, 1967) and used them as an input for the construction of a phenogram by the Neighbor-Joining algorithm (NJ) (Saitou & Nei, 1987). This method is considered to be highly consistent in phylogenetic inference and relaxes the molecular clock assumption (Li, 1997). Taking into account the low within-population variability that was observed (see results section), we included the allele frequency data of all samples with size *N* $ 2 individuals in the analyses. These calculations were performed using PHYLIP (Felsenstein, 1995).

Ten ingroup mtDNA sequences and one outgroup sequence of *E. flavicaudis* (*Ef*LA1, AJ389381) were aligned using CLUSTAL X (Thompson *et al.*, 1997). The software package PAUP* Version 4.0b10 (Swofford, 1998) was used for sequence analysis to perform phenetic (Felsenstein, 1984) and cladistic phylogenetic analyses. We are aware that these methods are based on different assumptions but all of these are expected to estimate the "true" phylogeny in the absence of long-branch attraction (Li, 1997): genetic distance calculation using Maximum Likelihood (ML), Maximum Parsimony (MP), and ML analyses. For ML, we have chosen the HKY+Γ DNA substitution model (Hasegawa *et al.*, 1985) with a rate heterogeneity among sites was assumed to follow a gamma distribution (shape parameter α was ML-estimated) with four categories, each represented by its mean (Yang, 1996). Search for most likely topology was carried out with the branch-and-bound algorithm. For MP analysis the tree space was explored by 100 heuristic tree searches and by randomizing the order of the sequence input in PAUP*. The three parsimony-informative gaps in the alignment were treated as the fifth base (McGuire *et al.*, 2001). Confidence limits of individual nodes for all trees were assessed using non-parametric bootstrapping (1,000 pseudoreplicates) (Felsenstein, 1985).

Abbreviations

BG, private collection of Benjamin Gantenbein; BMNH, Natural History Museum, London, UK; MES, private collection of Michael E. Soleglad; MNNH, Muséum National d'Histoire Naturelle, Paris, France; MZUF, Museo Zoologico "La Specola" dell'Università de Firenze, Florence, Italy; Natural History Museum Berne, Berne, Switzerland; NMW, Naturhistorisches Museum Wien, Vienna, Austria; PAN, Polish Academy of Science, Warsaw, Poland; SRSN, Società Romana di Scienze Naturali, Rome, Italy; VF, private collection of Victor Fet, Huntington, West Virginia, USA; UL, University of Ljubljana, Slovenia; USNM, United States National Museum (Smithsonian Institution), Washington, DC, USA; ZISP, Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia; ZMH, Zoologisches Museum Universität Hamburg, Hamburg, Germany; ZMMSU, Zoological Museum, Moscow State University, Moscow, Russia.

Results

Taxonomy and Morphological Analysis

***Euscorpius italicus* (Herbst, 1800)**
(Figs. 1-5, 9-14, 21, 25 and Table I)

Scorpio italicus Herbst, 1800: 70, Tab. I, Fig. 2.
Notes. Herbst's type material (Italy) is lost (Fet & Sissom, 2000). However, the species is well distinguished from other *Euscorpius* and there is no current taxonomic problem involved with its recognition, at least within Italy. Therefore, it is not necessary to designate a neotype for *E. italicus* (ICZN, 1999; Article 75).

Synonyms

Scorpius provincialis C. L. Koch, 1837: 114, Pl. CIV, Fig. 243 (synonymized by Thorell, 1876: 212). Types lost; Marseille, France.
Scorpio awhasicus Nordmann, 1840: 731, Pl. I, Fig. 4 (synonymized by L. Koch, 1878: 38).
Syntypes lost; Sukhumi and Poti, Abkhazia, now Georgia.
Euscorpius italicus polytrichus Hadñi, 1929: 33-35, Fig. 1-4 (synonymized by Kinzelbach, 1975: 38). Syntypes: two males (depository unknown), Greece.
Euscorpius italicus mesotrichus Hadñi, 1929: 35-36 (synonymized by Caporiacco, 1950: 170).
Syntypes: one male, one female (depository unknown), type locality unknown.
Euscorpius italicus oligotrichus Hadñi, 1929: 36 (synony mized by Kinzelbach, 1975: 38).
Holotype: female (depository unknown), type locality unknown.
Euscorpius italicus etruriae Caporiacco, 1950: 172, 224 (synonymized by Vachon, 1981: 196).
Syntypes: seven adult males, five adult, three subadult, one juvenile females (MZUF 488), Lippiano, upper Tiber Valley, Perugia, Umbria, Italy, July-October 1935 (A. Andreini).

References (selected; for detailed reference list see Fet & Sissom, 2000: 373-374).

Scorpius italicus: C. L. Koch, 1837: 95-101, Tab. CIV, Figs. 241-243; Fanzago, 1872: 80-81, Fig. 2.
Scorpius provincialis: Fanzago, 1872: 81-82, Fig. 3.
Euscorpius italicus: Simon, 1879: 107-108; Simon, 1884: 351; Kraepelin, 1899: 163 (in part); Birula, 1900: 15, 17; Werner, 1902: 604 (in part); Zykoff, 1912: 209; Birula, 1917a: 105-122, Figs. 6-8; Birula, 1917b: 173-192, Pl. III, Figs. 1-6, 9, Pl. IV, Fig. 3-4; Hadñi, 1929: 33; Birula, 1937: 107-108; Caporiacco, 1950: 164-173 (in part); Vachon, 1951: 342; Vachon, 1952: 362, Fig. 541; , ur...if, 1972: 83-88; Kinzelbach, 1975: 38-40, Fig. 18 (in part); Vachon, 1975: 637-643, Fig. 12-14, 21-22, 26-28; Vachon, 1981: 196-202 (in part), Fig. 4-6, 9-10, 14-16; Bonacina, 1982: 3-15; Vachon, 1983: 77; Fet & Gruodis, 1987: 42-45, Fig. 1; Fet, 1989: 129-132; Michalis & Dolkeras, 1989: 262; Lacroix, 1991: 14-25, Figs. 126-150 (in part); Crucitti, 1993: 291-293, Fig. 3; Braunwalder & Tschudin, 1997: 9-15, figs; Crucitti & Malori, 1998: 129; Gantenbein *et al.*, 1998: 33-39; Crucitti, 1999a: 87; Gantenbein *et al.*, 1999: 49-65; Fet & Braunwalder, 2000: 20, Fig. 4 (in part); Fet & Sissom, 2000: 373-375 (in part); Braunwalder, 2001: 279-286; Kovaĺík, 2002: 13-14 (in part).
Euscorpius awhasicus: Birula, 1900: 15.
Euscorpius provincialis: Birula, 1900: 15.
Euscorpius italicus awhasicus: Caporiacco, 1950: 165, 223; Vachon, 1951: 343; Tolunay, 1959: 366.
Euscorpius italicus italicus: Caporiacco, 1950: 170, 224; Vachon, 1975: 641; Vachon, 1981: 198; Lacroix, 1991: 14-25, Figs. 126, 130, 133, 136, 142, 144, 148.
Euscorpius italicus oligotrichus: Caporiacco, 1950: 172, 224; Vachon, 1975: 641; Lacroix, 1991: 23.
Euscorpius italicus polytrichus: Caporiacco, 1950: 224; Vachon, 1975: 641; Lacroix, 1991: 23.
Euscorpius italicus mesotrichus: , ur...if, 1971: 93-95, Fig. 1; Vachon, 1975: 641.
Euscorpius italicus etruriae: Vachon, 1975: 641; Bartolozzi *et al.*, 1987: 296; Lacroix, 1991: 23.
Euscorpius italicus avhasicus (incorrect spelling): Vachon, 1975: 641; Lacroix, 1991: 14-25, Figs. 127, 129, 131, 137, 141, 143.

Taxonomic history

This conspicuous *Euscorpius* (the largest scorpion species found in Italy) was recognized by the zoologists long before its formal description (Braunwalder, 2000).

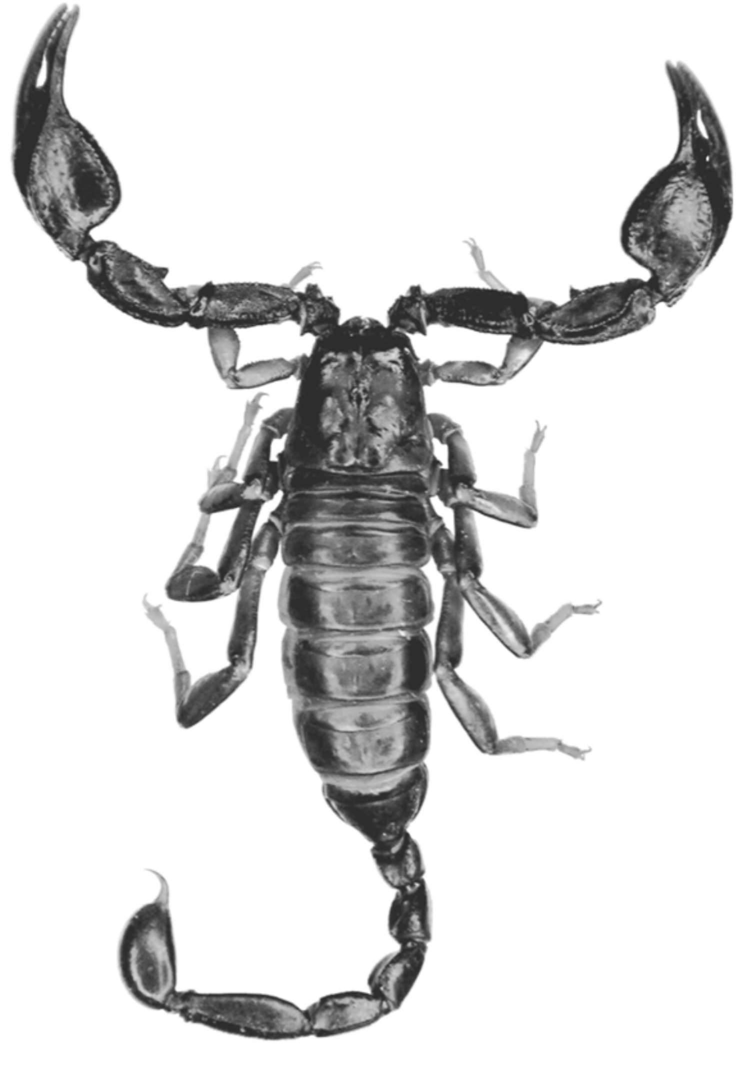


Fig. 1. Dorsal view of male *Euscorpius italicus* (Herbst), Tortoreto, Abruzzo, Italy.

Linnaeus used the name "Scorpio italicus" in pre-1758 publications (Linnaeus, 1748) but did not list this species in 1758 or 1767 editions of "Systema Naturae"; the identity of 1748 species is unclear (Fet *et al.*, 2002). Another unmistakable pre-1758 record of this species (with a wonderful illustration), also addressed as "Scorpio italicus" is found in Roesel (1755, Tab. LXVI). The first author of the available name *Scorpio italicus*, however, is Herbst (1800: 70), who gave a very brief description and a single illustration (Tab. I, Fig. 2), and did not designate type locality other than "Italy".

C. L. Koch (1837) redescribed in detail and illustrated *Scorpius italicus* from Trieste, Italy. In the same work, he also described *S. provincialis* from Marseille, France (currently a synonym of *E. italicus*) and *S. naupliensis* from Peloponnese, Greece, which is discussed in the present paper in detail (see below). At the same time, Nordmann (1840) described *Scorpio awhasicus* from Caucasus (modern Georgia), which is now also a synonym of *E. italicus*.

Birula (1917a, 1917b) provided a very detailed description of *E. italicus*; he compared the Caucasian form, *E. i. awhasicus* (Nordmann, 1840) to the typical (Italian) populations and noted that they do not differ (Note that Kraepelin (1899) mistakenly listed *E. awhasicus* as a synonym of *E. carpathicus*). It is important to note that Birula (1917a), in order to accommodate *E. italicus*, created a special monotypic subgenus *Polytrichobothrius*, diagnosed by the high number of ventral chelal trichobothria ("6-8 or more", as opposed to 3-4 in other subgenera).

Three subspecies ("races") of *E. italicus* (*E. i. polytrichus*, *E. i. mesotrichus* and *E. i. oligotrichus*) were described by Hadži (1929) and used in his subsequent works (Hadži, 1930). These taxa were delineated on the basis of total number of pedipalp patellar trichobothria, but did not distinguish separate series within the trichobothria of external aspect.

Caporiacco (1950), who also based his taxonomy on total counts of external patellar trichobothria, distinguished five subspecies in *E. italicus*. He noticed that Hadži's "racial" division does not leave place for a nominotypical *E. italicus italicus*, and, as a first reviser, declared *E. i. mesotrichus* Hadži, 1929 its junior synonym. At the same time, Caporiacco (1950) retained *E. i. polytrichus* Hadži, 1929 (described from an unknown locality in Greece) and *E. i. oligotrichus* Hadži, 1929 (no type locality; later assigned by Caporiacco to

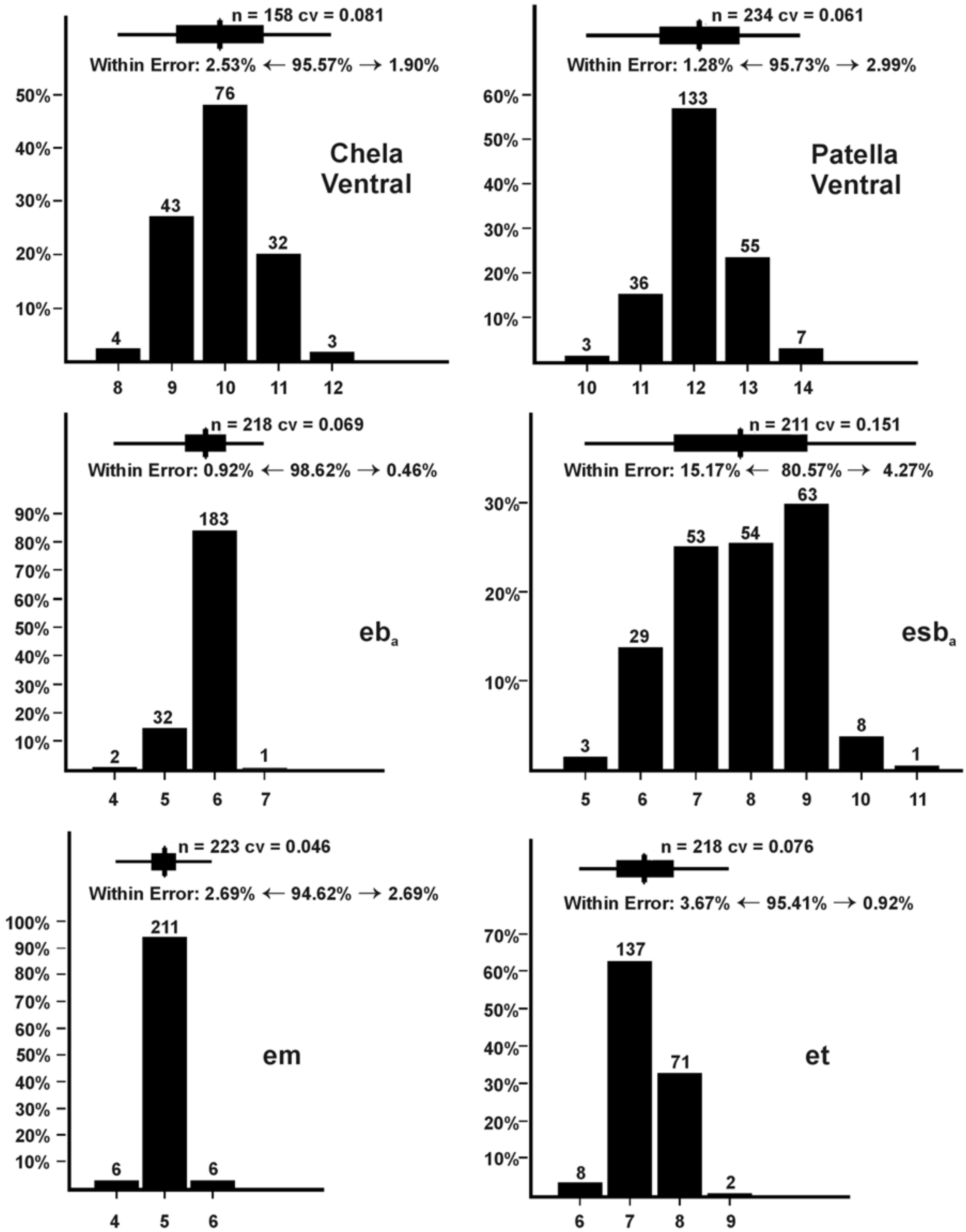


Fig. 2. Statistical data for pedipalp patella trichobothrial counts of *Euscorpius italicus*. eb_a = external basal-a, esb_a = external suprabasal-a, *em* = external median, *et* = external terminal.

Table I
Morphometrics (mm) of *Euscorpius italicus* (Herbst). *E. i. etruriae* Caporiacco is shown for comparison.

	Tortoreto, Italy		Ioannina, Greece		Lippiano, Italy (*E. i. etruriae*)	
	Male	Female	Male	Female	Male	Female
Total length	48.25	37.75	43.30	41.25	45.60	42.40
Carapace length	6.80	6.60	6.80	6.70	6.75	6.80
Mesosoma length	17.90	11.00	12.05	10.90	15.70	14.65
Metasoma length	16.95	15.20	17.85	17.35	16.90	15.80
Metasomal segment I						
length	2.20	2.00	2.45	2.30	2.25	2.05
width	2.30	2.25	2.35	2.35	2.35	2.40
Metasomal segment II						
length	2.70	2.40	2.85	2.7	2.60	2.45
width	2.10	1.95	2.00	2.1	2.15	2.10
Metasomal segment III						
length	2.95	2.60	3.05	3.05	2.90	2.65
width	2.00	1.85	2.00	2.05	2.05	1.90
Metasomal segment IV						
length	3.45	3.15	3.65	3.55	3.50	3.30
width	1.90	1.80	1.90	1.90	1.95	1.85
Metasomal segment V						
length	5.65	5.05	5.85	5.75	5.65	5.35
width	1.95	1.75	1.90	1.95	2.05	1.85
Telson length	6.60	4.95	6.60	6.30	6.25	5.15
Vesicle length	4.90	3.65	5.05	4.80	4.95	3.55
width	2.25	1.55	2.20	2.35	2.40	1.70
depth	2.70	1.75	2.60	2.90	2.80	1.60
Aculeus length	1.70	1.30	1.55	1.50	1.30	1.60
Pedipalp length	23.05	22.55	23.85	22.95	23.35	22.95
Femur length	5.45	5.45	5.75	5.55	5.70	5.55
width	2.30	2.20	2.20	2.30	2.30	2.30
Patella length	5.55	5.45	5.85	5.55	5.75	5.65
width	2.60	2.60	2.90	2.50	2.85	2.70
Chela length	12.05	11.65	12.25	11.85	11.90	11.75
Palm length	6.00	5.90	6.30	6.10	6.20	6.15
width	4.00	3.65	4.20	3.90	4.25	4.05
depth	4.60	4.10	4.80	4.50	4.90	4.40
Movable finger length	7.10	6.40	7.00	6.85	6.85	6.75
Pectines						
teeth	10-10	8-8	10-10	10-x	10-10	8-8
middle lamellae	6-6	4-5	6+-6+	- - -	6+-6+	4-3

the Italian Alpine populations) as separate subspecies. Caporiacco (1950) also established two new subspecies, *E. i. etruriae* from Italy (Umbria) and *E. i. zakynthi* from Greece (Zakynthos); he mentioned but did not discuss the Caucasian form, *E. i. awhasicus*. Kinzelbach (1975) synonymized all known subspecies to the nominotypical *E. italicus*, although he did not address intraspecific variability, and did not analyze external patellar trichobothria. Later, however, he (Kinzelbach, 1985) listed the Caucasian *E. i. awhasicus* as a separate subspecies, without a justification.

Vachon (1981) briefly acknowledged the intraspecific variability in *E. italicus*; he studied specimens from Italy, Switzerland, Greece, and Turkey, and was the first to notice a peculiar trichobothrial features of the Peloponnese specimens (see below under *E. naupliensis*). At the same time, Vachon (1981: 196) listed all previously described forms as synonyms, thereby effectively declaring that subspecies within *E. italicus* are not diagnosable. Bonacina (1982) studied several hundred specimens of *E. italicus* from Italy, and demonstrated a wide variation of number of ventral chelal and patellar trichobothria.

Fig. 26 presents geographic range of *E. italicus*. Detailed maps and/or locality data on the species' distribution in Italy is given by Caporiacco (1950) and Crucitti (1993); in France, by Vachon (1983) and Lacroix (1991); in Switzerland, by Braunwalder (2001); in Slovenia, by Fet *et al.* (2001); in other parts of ex-Yugoslavia, by Hadñi (1930) and ‚ur…iƒ (1971); in Georgia and Russia, by Fet (1989). The distribution in Turkey (Black Sea coast) is less well documented and only a few coastal sites are known between Istanbul and Rize (Vachon, 1951; Tolunay, 1959; Crucitti & Cicuzza, 2001). In general, *E. italicus* did not enjoy such complicated and overly detailed taxonomic treatment as did other species of *Euscorpius* (see e.g. Caporiacco, 1950; Kinzelbach, 1975; Bonacina, 1980; Fet & Sissom, 2000; Fet & Soleglad, 2002).

There are currently no valid subspecies in *E. italicus* (Fet & Sissom, 2000). Still, several taxonomic problems remain unresolved, concerning the status of some Italian, Greek, Turkish, and Caucasian populations. We analyzed the types of *E. i. etruriae* Caporiacco, 1950 from Italy (see below) and concluded that this taxon is not valid. Further data should be obtained on Italian populations to address validity of *E. i. oligotrichus* Hadñi, 1929 as accepted by Caporiacco (1950). The existence of a separate polytrichous form ("*E. i. polytrichus* Hadñi, 1929", diagnosed with 14-17 trichobothria ventrally and 38-45 externally on patella) allegedly described from Greece, has not been yet confirmed. Kinzelbach (1975) assumed that specimens of Hadñi (1929) were deviant; however, we analyzed a specimen from Ioannina (Epirus, Greece), which had 14 trichobothria ventrally and 38 externally on patella (Fig. 14), thus falling within limits of Hadñi's original diagnosis. A specimen from Georgia that we analyzed in PAN collection (with only 12 ventral trichobothria on patella) was labeled by Hadñi himself as "*E. i. polytrichus*"; therefore, Hadñi's treatment of subspecies was inconclusive. There is definitely no record of any "polytrichous" population of *E. italicus* in Peloponnese as , ur...if (1971: 87, Fig. 4) (hypothetically?) showed in his map, or as stated by Lacroix (1991: 23).

In Greece, *E. italicus* s.str. has been reported from Corfu Island (Vachon, 1975), and from a number of localities in coastal and inland Epirus (Kinzelbach, 1975; Michalis & Dolkeras, 1989; Lacroix, 1991; Crucitti & Malori, 1998; Kovaĺík, pers. comm. 2002; our data, see below). The easternmost known record of this species in Greece is from western Thessaly (Trikala); it appears that *E. italicus* is not found in mainland Greece east of 22EE (Fig. 26 – map); and it has never been reported from any Aegean islands. Southern Greek populations formerly listed under *E. italicus* are classified here as *E. naupliensis* (C. L. Koch, 1837) (see below).

Diagnosis

A large *Euscorpius* species, generally dark brown to almost black in overall coloration. Dorsal metasomal carinae granulated, inferior median carina present on segment IV and sometimes on segment III; inferior carinae of segment V crenulate. Pedipalp trichobothrial patterns: chelal ventral series found on ventral-external surfaces, 8–12 (10); patellar ventral surface, 10–14 (12); patellar external surface, *eb* = 4, eb_a = 6, *esb* = 2, esb_a = 5-13 (7–9), *em* = 5, *est* = 4, *et* = 6–9 (7); distance between chelal fixed finger trichobothria *dsb-est* is greater than or equal to distance between *est-et*. Pectinal tooth counts: female, 7–9 (8); male, 8-12 (10). Variable neobothriotaxy of the chela ventral aspect exceeding 6 trichobothria and the presence of the unique patellar external esb_a series exceeding 4 accessory trichobothria are key diagnostic characters of this species.

MALE. Redescription based on an adult male (BG-27-06 #405) from Tortoreto, Abruzzo, Italy, 7 October 1997 (M. Bellini) (NMHB). Measurements of this male and other material are provided in Table I. Dorsal view of a sexually mature male is shown in Fig. 1.

COLORATION. Basic overall color dark brown. Carapace darkest anteriorly; pedipalps somewhat darker than body exhibiting black carinae; metasoma dark with fuscous patterns; telson orange exhibiting fuscous patterns dorsally and laterally; legs dark orange with subtle fuscous patterns on femur and patella; sternites and pectinal area dark yellow.

CARAPACE. Generally rough at 10x; slightly granulose on lateral aspect just below lateral eyes; anterior edge straight, lacking setae, extending anteriorly from lateral eyes. Two lateral eyes, anterior eye largest; median eyes and tubercle situated anterior of middle with following length and width formulas: 293|680 (anterior edge to median tubercle center|carapace length) and 100|576 (width of median tubercle|width of carapace at that point).

MESOSOMA. Tergites smooth to rough at 10x, slight traces of median carinal pair proximally on segment VII; sternites smooth and shiny, carinae absent on segment V; stigmata small, slit-like to sub-oval.

METASOMA. Carinae — Segments I-IV: dorsal granulated; dorsal lateral rounded and granulose on I, rounded and smooth on II–IV; lateral obsolete; inferior lateral obsolete to slightly smooth on I, smooth on II–IV; inferior median obsolete on I–II, slight traces on anterior one-half of III, smooth to slightly granulose on IV. Carinae — Segment V: dorsal lateral rounded and slightly granulose; lateral obsolete; inferior lateral and median crenulate. Posterior spine of dorsal carinae of segments I-IV slightly evident. Intercarinal areas of segments I–IV smooth, granulose on ventral aspect of V.

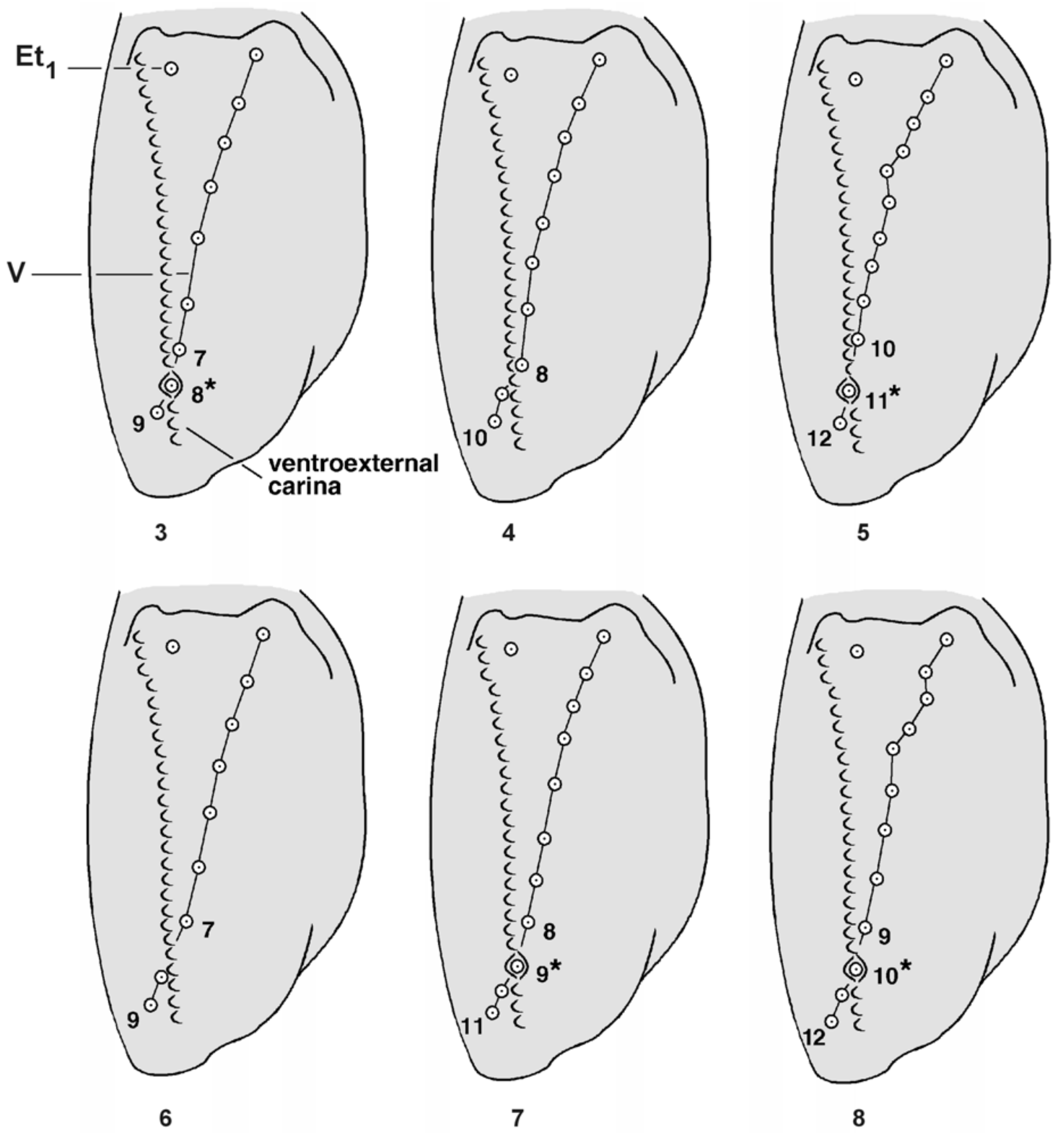
TELSON. Vesicle swollen both laterally and ventrally, lateral and dorsal aspects of vesicle quite rough in areas of fuscous pattern. Aculeus forming a short conspicuous curve from vesicle; 5–6 irregular pairs of setae at vesicle/aculeus juncture.

PECTINES. Length|width formula 314|152 (length taken at anterior lamellae|width at widest point including teeth). Pectinal tooth counts 10/10 and middle lamellae counts 6/6; fulcra well developed for entire pecten; numerous fine setae situated on anterior lamellae. Sensorial areas of teeth developed along approximately 1/2 – 2/3 their length. Basal piece anterior edge slightly concave, length|width formula 84|199.

GENITAL OPERCULUM. Separated most of length, genital papillae extends proximally.

STERNUM. Pentagonal, wider than long, length|width formula 210|225.

CHELICERAE. Movable finger: dorsal distal denticle considerably shorter than ventral distal denticle; dorsal edge with two subdistal denticles; ventral edge smooth, lacking serrulae, and covered with heavy brush-like setae for most of its length. Fixed finger: four denticles configured normally (basal two denticles conjoined on a common trunk).



Figs. 3-8. Ventral trichobothrial series of pedipalp chela showing various configurations distributed on ventral and external aspects. Numbers refer to terminal trichobothria on ventral, ventroexternal carina (found on carina), and external aspects, respectively. **Figs. 3-5.** *Euscorpius italicus*: **3 & 4.** Tortoreto, Italy. **5.** Agarone, Switzerland. **Figs. 6-8.** *Euscorpius naupliensis* (from Peloponnese, Greece): **6 & 7.** Selinitsa. **8.** between Kalamata and Kardarmili. *Trichobothrium found on ventroexternal carina. Et_1 = external terminal; *V* = ventral.

PEDIPALPS. Pedipalpal chelae exhibiting prominent scalloping at finger bases. Femur: dorsal and ventral carinae serrulate; dorsal and ventral surfaces granulose, internal surface with numerous enlarged granules. Patella: dorsal internal crenulate, dorsal external smooth to granulate; ventral external and internal carinae serrulate; exteromedian rounded and irregularly serrulate. All surfaces granulose; dorsal patellar spur (DPS) well development and sharp, ventral patellar spur (VPS) very weak, represented as small granule. Chela carinae: digital very strong and smooth with slight polished granulation at finger base; subdigital in relief, represented by a granule; dorsal secondary obsolete; dorsal marginal rounded, continuous and granulose; dorsal internal rounded and granulose; ventroexternal strong with polished granulation extending to external condyle of finger, external to trichobothrium Et_1; ventromedian essentially obsolete; ventrointerior rounded and granulose; and external secondary irregularly granulose. Chelal finger dentition: Median denticle row straight; 6/6–7 inner denticles, 6/7 outer denticles, and 4/5 inner accessory denticles for fixed and movable fingers respectively. Trichobothria patterns: Type C, neobothriotaxic (major additive) on patella and chela. Femur: trichobothrium *d* positioned proximal in relation to *i*, *e*, anterior to both, situated on dorsoexternal carina. Patella: ventral series number 12/13 and external series number *eb* = 4/4, eb_a = 6/6, *esb* = 2/2, esb_a = 6/7 *em* = 5/5, *est* = 4/4, and *et* = 7/7. Chela: Ventral trichobothrial series number 10/10, 2/2 on external aspect, 0/0 on ventroexternal carina and 8/8 on ventral surface; *et-est*/*est-dsb* ratio 0.846 and 0.840 for left and right chelae, respectively.

LEGS. Two pairs of pedal spurs present, tarsal spines absent. Tarsus III: ventral median spinule row formed by 9 stout spinules; one stout pair of ventral distal spinules. Basitarsus I-IV: ten proventral spinules on legs I, nine on II and 3 on III.

HEMISPERMATOPHORE. Well developed lamina with conspicuous basal constriction, tapered distally; truncal flexure present; capsular lobe complex well developed; ental channel emanating from the trunk, spinose distally, exhibiting 15 delicate variable sized spines (Fig. 21: male from Pridvor, Slovenia).

Female

Adult female (Tortoreto, Abruzzo, Italy), used for comparison. Sexually mature male specimens with well developed proximal scalloping on the chelal finger base. Mature females exhibit a much more subtle scalloping. Granulation of carapace, metasoma and pedipalps same as in male except as follows: for the female the inferior median carina of the metasoma is obsolete on segment III and only exists as a smooth carina on segment IV.

Morphometrics

We compared morphometrics of ten sexually mature males and females originating from localities in Switzerland, Italy, Croatia, Slovenia, and Greece. Males and females did not exhibit significant differences in overall size, carapace lengths ranged 5.80–7.45 (6.505) for males and 6.15–7.40 (6.665) for females. The metasoma of the male is slightly thinner than it is on the female, but only exhibiting very slight mean value differences when all segment length/width ratios are compared, a range of 0.7–6.7%; segment I exhibited the 6.7% difference. However, the considerably inflated telson vesicle of sexually mature males is quite conspicuous when compared to the thinner "teardrop" shaped telson of the female. This was dramatically illustrated using morphometrics. Morphometric ratios calculated from the carapace length divided by the vesicle width and depth showed considerable mean value differences and significant separation of plus/minus standard error ranges:

Carapace Length/Vesicle Width
Mean value difference = 42.9%; separation gap = 377%

Females
4.00-4.39 (4.187) (±0.120) [010]: {4.07-4.31} z 0.029
Males
2.68-3.46 (2.931) (±0.227) [010]: {2.70-3.16} z 0.078

Carapace Length/Vesicle Depth
Mean value difference = 67.9%; separation gap = 765.3%

Females
3.92-4.29 (4.098) (±0.131) [010]: {3.97-4.23} z 0.032
Males
2.32-2.60 (2.440) (±0.094) [010]: {2.35-2.53} z 0.038

Chelal fixed finger trichobothria ratio, *et-est*/*est-dsb*, is smaller in males with a mean value difference of 19%, implying the distance of *est-dsb* is relatively *larger* in males than females.

Genital operculum/genital papillae: On the female, the genital operculum is connected for its entire length by a membrane, whereas on males, it is separated for most of its length, exposing protruding genital papillae.

Pectinal tooth counts: The pectines are more prominent on the male, teeth longer as well as larger in number:

Male
8-12 (9.855) (±0.546) [117]: {9.309-10.400} z 0.055
Female
7- 9 (8.209) (±0.458) [139]: {7.751- 8.667} z 0.056

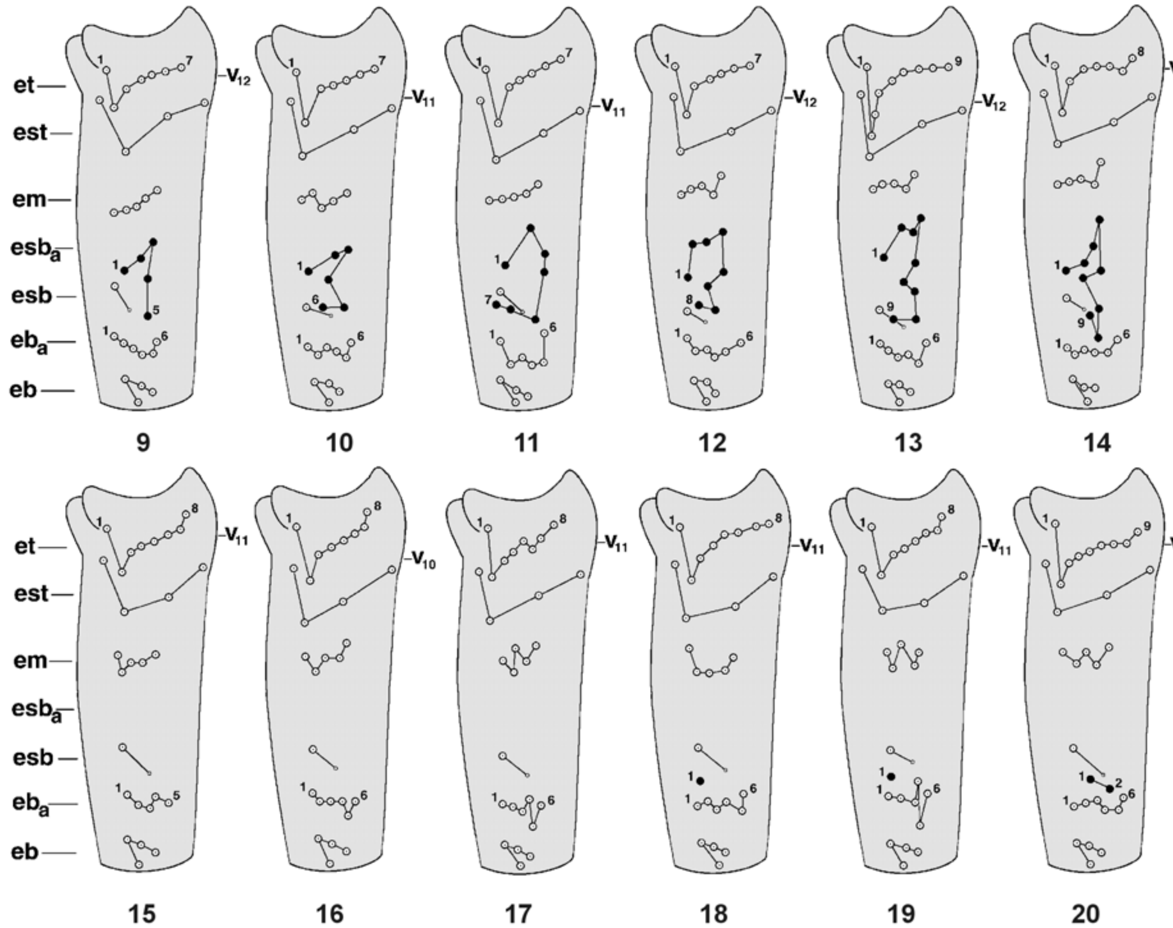
The mean value difference is 19.2%, roughly a 1.5 tooth difference between the male and female.

Variation within species

Besides the described male and female of *E. italicus* from Italy, we examined additional 130 specimens from Europe, Turkey, and Caucasus. In particular, we analyzed the statistical distribution of the pedipalp trichobothria. See Fig. 2 for the statistical ranges of the trichobothrial series of the chela and patella based on 140-230 samples per series. Of a special importance is the high support for external series *eb*=4 (98.2% of 225 samples), eb_a=6 (83.9% of 218 samples) and series *em*=5 (94.6% of 223 samples). Expected variability in the chelal ventral and patellar ventral and *et* series is present, all coefficients of variability (*cv*) under 9%. The chelal ventral trichobothria are located on both the ventral and external aspects of the palm. In most specimens one (rarely two) trichobothrium is found on the ventroexternal carina, situated in a "dimple" formed in the carina. For 158 samples 15 different configurations were detected in the numbers found on the external, ventroexternal carina, and ventral surfaces of the palm (Figs. 3-5). 89.9% of these samples exhibited trichobothria situated on the ventroexternal carina and 35.4% had two trichobothria on the external surface. The following six configurations were dominant accounting for 82.3% of the samples:

1+1+8 (external + ventroexternal carina + ventral)
= 10, 26.6%
1+1+7 = 9, 21.5%
2+1+7 = 10, 12.0%
2+1+8 = 11, 10.8%
2+0+8 = 10, 6.3%
1+2+8 = 11, 5.1%

The esb_a series exhibited the most variability showing a somewhat high *cv* of 15.1%, implying that in

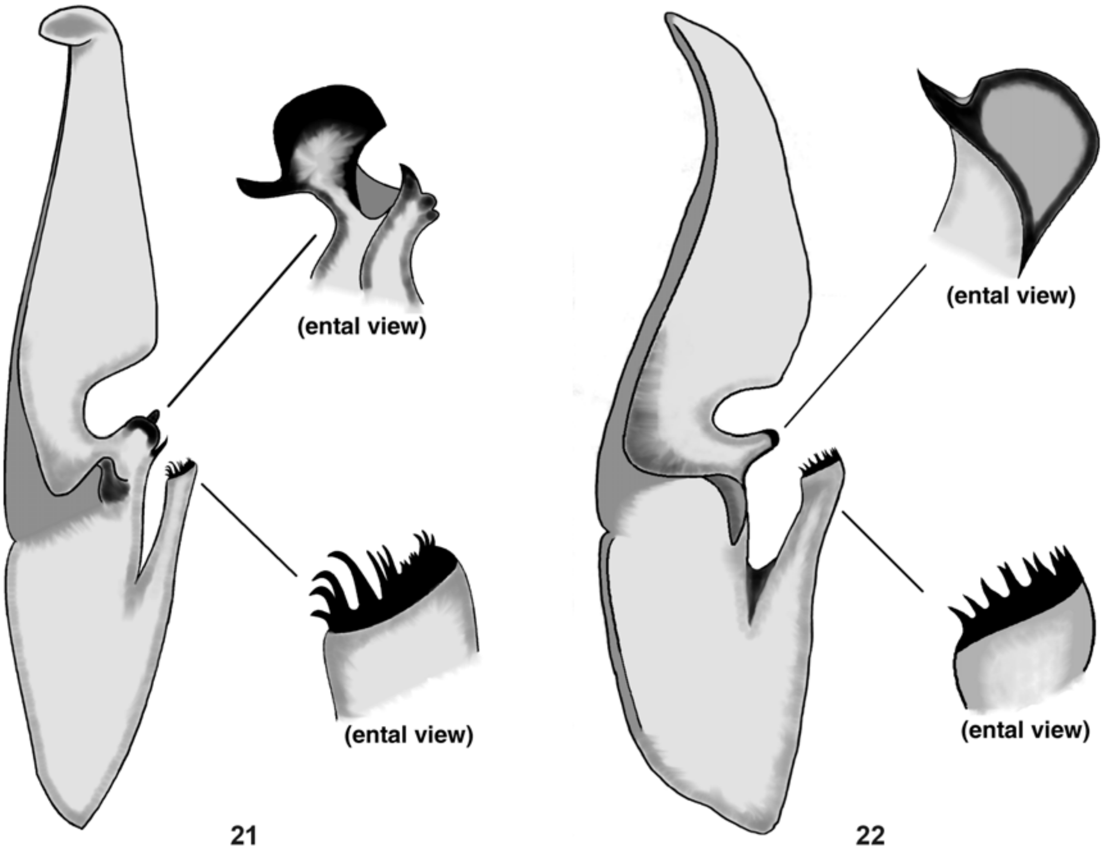


Figs. 9-20. Trichobothrial patterns of external aspect of pedipalp patella showing variability in configurations of variable series, eb_a, esb_a, *et* and *v*. Series esb_a represented as *filled in* circles. **Figs. 9-14.** *Euscorpius italicus*: **9 & 10.** Lippiano, Italy (*E. i. etruriae*). **11.** Tortoreto, Italy. **12.** Coglio, Switzerland. **13.** Brissago, Switzerland. **14.** Ioannina, Greece. **Figs. 15-20.** *Euscorpius naupliensis* (from Peloponnese or Zakynthos, Greece): **15.** Itylo. **16.** Zakynthos. **17.** Kalidona. **18.** Kurtaina. **19.** Zakynthos. **20.** between Kalamata and Kardamili. *eb* = external basal, eb_a = external basal-a, *esb* = external suprabasal, esb_a = external suprabasal-a, *em* = external median, *est* = external supraterminal, *et* = external terminal, *v* = ventral.

general this special series of accessory trichobothria is somewhat unstable in its number and also in its positional distribution. This is also apparent from the examination of specimens from diverse localities. Figs. 9-14 illustrate variability in this patellar external series.

The great variability in the esb_a series is discussed and illustrated elsewhere in this paper as it applies to the comparison of species *E. italicus* and *E. naupliensis*. Lacroix (1991: Figs. 126-145) also illustrated the variability found in the esb_a series. In our analysis, based on 211 samples, the esb_a series ranged in number from 5 to 11, with 7-9 being typical. In Lacroix's study esb_a counts were estimated as high as 13 (see his Fig. 144). Note that in this figure, as well as others, Lacroix created a second *esb* series based on, evidently, the petitness of another accessory trichobothrium. Of course, this designation is incorrect, since the actual *esb* series is composed of orthobothriotaxic trichobothria and is found in all scorpion species complying with the Type C pattern. Therefore, one must transfer this second "*esb* series" to esb_a in order to calculate the correct number of trichobothria in this series. Also, in Lacroix's Fig. 144, he designates only five trichobothria for series eb_a, this again is incorrect based on positional analysis. One of the basal esb_a trichobothria must be transferred to eb_a, thus reducing the esb_a number in this figure to 13.

The overall variability found in the esb_a series appears, in part, to be the cause for Caporiacco's (1950) creation of the subspecies *E. i. etruriae*, placing it in the "mesotrichous" range for the external aspect of the patella. We examined 16 syntypes of *E. i. etruriae* from Abruzzo, Italy and discovered that only the esb_a series varied statistically from the other specimens of *E. italicus*: The mean value difference for the esb_a series was 15.5%, roughly a single trichobothrium difference, *E. i. etruriae* with the smaller number. For the other series, MVD's only ranged from 0 to 5%, showing that the two subspecies are completely in compliance statistically. Interestingly, Caporiacco's key stated that



Figs. 21-22. Hemispermatophore, dorsal view. **21.** *Euscorpius italicus*, Pridvor, Slovenia. **22.** *Euscorpius naupliensis*, Zakynthos, Greece.

the total patella external trichobothria count ranged from 26-34 (28-32). Our examination of the sixteen syntypes contradicted this, exhibiting total ranges of 33-37 (35). Other trichobothria counts stated by Caporiacco (1950) were consistent with our findings. The pectinal tooth counts of the two subspecies were also statistically the same, exhibiting very small mean value differences: female, with a mean of eight, 0.8% difference, and for the male, mean of ten, 1.38% difference. We also measured an adult male and female (see Table I) and in general the derived morphometric ratios were consistent when compared to the other *E. italicus* material. Caporiacco also distinguished this subspecies by it being "darker" than the other subspecies, clearly an insignificant if not dubious diagnostic character. Since esb_a is quite variable in *E. italicus* in general, and the statistical difference between *E. i. etruriae* and *E. i. italicus* is essentially a single trichobothrium, we see no reason based on morphological grounds for Caporiacco's original designation of *E. i. etruriae* as a subspecies of *E. italicus*. Therefore we concur with Vachon (1981) who synonymized this subspecies with the nominotypical *E. italicus*.

Material examined

ALBANIA: 1 adult female (BMHN), Lescoik, 18 June 1933 (A. H. G. Alston & M. J. Sandwith); 1 adult female (NMW), Marmirojt, June 1914; 1 subadult female (BMNH), Voskopaj (now Voskopoja), 4 April 1933 (A. H. G. Alston & M. J. Sandwith). **CROATIA:** 1 adult female (UL), Karlobag (=Carlopago), 1898 (det. J. Hadñi as "*E. italicus mesotrichus*"); 1 subadult male, 2 adult, 1 subadult females (UL), Sali, Dugi Otok Island, 30 October 1937; 2 adult females (UL), Klimno (Krk Island), 21 September 1961; 1 subadult male, 2 adult females (NMW), Krk Island, May 1987 (H. L. Nemeschkal). **GEORGIA:** 1 male (ZISP 974), Batumi, 1906 (K. A. Satunin); 1 juvenile male (ZISP 982), Batumi, July 1909 (K. A. Satunin); 1 male (ZISP 971), Zelenyi Mys, Batumi, 8 February 1895 (B. Kislyakov); 1 female (ZISP 981), Zelenyi Mys, Batumi, 25 August 1907 (A. Silantyev); 1 male (ZISP 964), Gagry, June 1893 (G. I. Radde); 2 males (ZISP 973), Gagry, August 1904 (Kharazov); 1 male (ZISP 976), Gagry, 5-13 July 1904 (A. Skorikov); 1 female (ZISP 1840), 1912 (P. Stefan); 1 female (ZMMSU Tb-468), Gagry, October 1984 (V. E. Kurilenko); 1 male (PAN), Gagry (L. Kotowski) (det. J. Hadñi as "*E. italicus polytrichus*"); 1 male, 2 females (ZISP 972), 1 female (ZISP 966), Poti, 24 August 1875 (K. F. Kessler); 7 juvenile males, 4 juvenile females (ZISP 965), Poti, 28 August 1875 (K. F. Kessler); 2 females (ZMMSU Tb-20), Poti (P. R. Freiberg); 1 male (ZISP 983), Sukhumi, 29 August 1905 (M. Kalishevsky); 1 juvenile male (ZISP 988), Sukhumi, 31 August 1905 (M. Kalishevsky); 1 female (ZMMSU Tb-22), Sukhumi (Levshin); 1 female (ZISP 967), Sukhumi, 1880 (Chernyavsky); 4 females (ZISP 968), Sukhumi, 1879 (Chernyavsky); 1 female (ZMMSU Tb-177), Sukhumi, 1982 (S. V. Parin). **GREECE:** 1 adult, 1 subadult females (NMW), Platanousa, Xerovuni Mts, Epirus, 12-15

May 1932 (M. Beier); 1 adult female (NMW 2101), Luros River near Arta, Epirus, 1892; 3 adult males (UL), Ioannina, Epirus, 13 April 1927 (Komaven); 1 juvenile male, 3 adult, 1 subadult female, Metsovo, Epirus, 13 May 2001 (VF); 1 adult female (NMW 2682), 1882, Patras (Patra), Peloponnese. **ITALY:** 1 adult female (VF), Silvi Marini, Abruzzo, 10 June 2000 (F. KovaÍík); 1 adult male, 1 adult female (BG), Tortoreto, Abruzzo, 7 October 1997 (M. Bellini); 7 adult males, 5 adult, 3 subadult, 1 juvenile females (*E. i. etruriae* syntypes; MZUF 488), Lippiano, upper Tiber Valley, Perugia, Umbria, July-October 1935 (A. Andreini). **RUSSIA:** 1 male (ZISP 975), Novorossiisk (introduced?); 1 female (ZISP 987), Sochi, 13 July 1900 (A. Bykov); 1 female (ZISP 970), Uchdere (D. Glazunov). **SLOVENIA:** 1 adult female (VF), Brje, Dobravlje, Aidovscina, 7 August 2000 (B. Sket); 1 adult male, 1 adult female (UL), Galjevica, Ljubljana (M. Kuntner); 2 adult, 1 subadult males, 3 adult, 1 subadult, 1 juvenile females (UL), Pridvor (St. Anton), Dekani, Koper district, August 1995 (S. Toth); 1 adult male, Miren, Bilije (UL), 19 September 1973; 1 adult, 1 juvenile males, 1 adult, 1 juvenile females, (UL), Socerb, August 1995 (N. Dolenc); 1 adult male (UL), Secovlje, August 1973; 1 adult male, 1 adult female (UL), Siska, Ljubljana, 9 October 1992; 5 adult males, 5 adult females (UL), Tolmin, Soca valley. **SWITZERLAND:** 1 adult male, 3 adult females (BG), Brissago, Ticino, 25 May 1996 (B. Gantenbein); 3 adult males, 1 adult female (BG), Coglio, Ticino, 27 May 1996 (B. Gantenbein); 1 adult male (MES), Agarone, Ticino (M. E. Braunwalder). **TURKEY:** 1 adult female (BMNH), Bebek, 19 May 1951 (Burr); 1 adult, 1 juvenile males, 1 adult, 1 juvenile females (BMNH), Istanbul; 1 male (USNM), Istanbul (E. C. Trivette); 1 subadult male (BMNH), Trabzon, 16 July 1960.

Other source data. None.

Geographical distribution

Albania, Croatia (west), France (southeast), Georgia (Black Sea coast), Greece (west), Italy (north), Macedonia, Monaco, Romania (introduced?), Russia (Krasnodar Region, Black Sea coast), San Marino, Slovenia (west), Switzerland (south), Turkey (north, Black Sea coast), Yugoslavia (Montenegro). Introduced populations: Algeria, Iraq (Fet & KovaÍík, in press), Morocco, Yemen. See map in Fig. 26.

Euscorpius naupliensis (C. L. Koch, 1837)

(Figs. 6-8, 15-20, 22-25 and Tables II-III)

Scorpius naupliensis C. L. Koch, 1837: 93-95, Tab. CIV, Fig. 240.

Holotype (lost): female, [Nafplio, Peloponnese, 37E34’N, 22E48’E], Greece, coll. Schuch.

Note. The species is well distinguished from other *Euscorpius* and there is no current taxonomic problem involved with its recognition. Therefore, it is not necessary to designate a neotype for *E. naupliensis* (ICZN, 1999; Article 75).

Synonyms

Euscorpius italicus zakynthi Caporiacco, 1950: 172, 224, **syn. n**.

Lectotype (designated here according to the ICZN Article 74 from the syntype series).

Subadult female (MZUF 74), Pelouzo (=Peluso) Island [37E42’N, 20E56’E], near Zakynthos (=Zante) Island, Ionian Sea, Greece, [23 March 1936, J. Eiselt – see notes below]. Three labels contained in vial: (1) Syntype (typed); (2) four lines (typed and handwritten): “La Specola” – Firenze C74, *Euscorpius italicus zakynthi* di Cap., Grecia: Zakynthos, Peluso: det. Di Cap. 1947; (3) two lines (handwritten): *Euscorpius italicus zakynthi* di. Cap., Peluso presso Zante.

Paralectotypes (designated here). 1 adult male, 1 adult female (NMW), [23] March 1936 (J. Eiselt); Zakynthos (=Zante) Island, Ionian Sea, Greece (labeled by Vachon in 1982 as VA 2679).

Notes. The original syntype series was represented by five specimens, of which three originated from Pelouzo, and two, from Zakynthos. All five were taken from NMW collection (Caporiacco, 1950: 172). Only one specimen (lectotype) could be located in MZUF. Other four specimens are designated here as paralectotypes according to the ICZN Article 74. Matching localities with literature data (Werner, 1941), we can identify J. Eiselt as the collector, on 23 March 1936, of both Zakynthos and Pelouzo specimens; therefore, two Zakynthos specimens currently deposited in NMW belong to the syntype series. We designated the subadult female from Pelouzo as a lectotype since it is the only specimen which bears original Caporiacco’s label, and also since MZUF is the major depository of Caporiacco’s types (see ICZN Recommendation 74D). The current depository of two remaining paralectotypes from Pelouzo is unknown.

References (selected):

Scorpius naupliensis: C. L. Koch, 1842: 18-19, Tab. CCCXXX, Fig. 766 (description of a male).

Euscorpius naupliensis: Pavesi, 1877: 326; Simon, 1884: 351; Birula, 1900: 15.

Euscorpius italicus naupliensis: Birula, 1917a: 198; Birula, 1917b: 120; Vachon, 1975: 641; Lacroix, 1991: 23.

Euscorpius italicus: Werner, 1937: 153; Werner, 1941: 115; Kinzelbach, 1975: 38-40, Fig. 18 (in part); Michalis & Kattoulas, 1981: 110-111; Vachon, 1981: 196-202 (in part), Figs. 1-3, 8, 13; Lacroix, 1991: 14 (in part), Figs. 132, 134; Kritscher, 1993: 387-388; Crucitti, 1999b: 252-255; Fet & Braunwalder, 2000: 20, Fig. 4 (in part); Fet & Sissom, 2000: 373-375 (in part); Crucitti & Bubbico, 2001: 48-55, Figs. 1b, 5-9; KovaÍík, 2002: 14 (in part).

Euscorpius italicus zakynthi: Vachon, 1975: 641; Bartolozzi *et al.*, 1987: 298; Lacroix, 1991: 23.

Euscorpius cf. *italicus*: Crucitti, 1995: 95-96, Figs. 4-7.

Taxonomic history

C. L. Koch (1837: 93-95) described *Scorpius naupliensis* based on a female from Greece. Judging from the species’ name, the type locality is Nafplio (Nauplia, Navplio, Navplion) in the western Peloponnese, although this town is never mentioned explicitly in Koch (1837, 1842). Later, Koch (1842) he added a description of a male (from an unnamed locality in Greece). Koch (1837, 1842) specifically noticed that this species is close to *Scorpius italicus* (now *E. italicus* (Herbst)), which he also listed and redescribed from Italy; both species were diagnosed by Koch as having high number of ventral chelal trichobothria (8 to 9 for *S. naupliensis*, 9 for *S. italicus*). However, Koch (1842) paid a special attention to the differences between two species; he wrote that “the Greek scorpion is closely related [to *S. italicus*], but still differs in important features”, which he considered diagnostic, among them: "a thinner metasoma, with segment V ventrally with weaker carinae" and "…smooth, not granulated dorsal and ventral surfaces of pedipalp femur and patella, their carinae more weakly granulated, dorsal carina on femur smooth and not granulated". These subtle differences still hold (see our redescriptions of both species) and allow unmistakably to recognize the diagnosis of Koch's species as the only Peloponnese form of *Euscorpius* close enough to *E. italicus*.

Koch's name, however, was relatively forgotten after its description. Simon (1884: 351) mentioned this species as *Euscorpius naupliensis* "…which is unknown to me, appearing very close to *E. italicus*". Therefore Simon (1884) in fact did not formally synonymize this taxon with *E. italicus*, as stated by Fet & Sissom (2000: 373), and the Koch's name remained valid. Birula (1900) was last to quote *E. naupliensis* as a separate species, but he suggested (p. 18) that it might be a synonym of *E. italicus*. Later, he (Birula, 1917a, 1917b) addressed it as a possible subspecies of *E. italicus* and wrote (Birula, 1917a: 115): "…The only specimen of *Euscorpius italicus* originating from the Peloponnese (Taygetos Mountains) I have seen, has a much more finely granular pedipalp femur as compared with Italian and Caucasian specimens… If the pedipalp femur of the southern Greek specimens of this species is really constantly finely granular (which by the way can be inferred also from the description of *Scorpius naupliensis* C. Koch), the Peloponnese *Euscorpius italicus* should then be considered a separate race of this species". It is likely that the scorpion seen by Birula was the Langada specimen (NMW 2102) collected by Werner (1902: 604), then the only Taygetos specimen available in the European museums. Birula was closely familiar with the NMW scorpion collection, from which he analyzed a number of species. More specimens from Taygetos (Xechori, NMW 2195) were to be collected only years later by Werner (1937).

Caporiacco (1950) described the island subspecies *E. italicus zakynthi* from Pelouzo and Zakynthos in the Ionian Sea. Since its description, the population was never studied further. Also, Caporiacco (1950) studied a single specimen from Taygetos Mts. from MNHN, noting its low number of external patellar trichobothria (29) but did not assign it to any subspecies.

Kinzelbach (1975) and Michalis & Kattoulas (1981) reported new specimens collected from Peloponnese as *E. italicus*. Kinzelbach (1975: 38) listed both *Scorpius naupliensis* Koch and *E. italicus zakynthi* Caporiacco as synonyms of *E. italicus*; he studied Werner's Taygetos specimens from NMW but did not report the external patellar trichobothria. Kritscher (1993) published new records from Peloponnese (Taygetos, Tripoli), and Zakynthos (material in NMW, partially examined by us, see below) as *E. italicus*. None of these authors discussed deviant features of the Peloponnese and Zakynthos populations.

Vachon (1981) noticed that the form of *E. italicus* inhabiting Peloponnese (Taygetos Mts.) is morphologically different from Italian populations. He studied two Peloponnese specimens available in MNHN, RS 2969 from Langadia (in Arkadia in the central Peloponnese, between Tripoli and Olimbia; not Langada as in NMW 2102!) and RS 3828 from Taygetos. Vachon (1981, Figs. 1-6, 13-16) compared this Peloponnese form to specimens from Italy and Turkey, and indicated existence of six external patellar trichobothrial series instead of seven (i.e. absence of the typical for *E. italicus* series esb_a). In addition, Vachon (1981) paid attention to the difference in *et-est/est-dsb* ratio on the chelal fixed finger in the Peloponnese form as opposed to other *E. italicus* populations. Bonacina (1982), following Vachon's indication, analyzed that ratio in *E. italicus* from Italy, and reported that trichobothria *est* and *dsb* were remote from each other, while in the Peloponnese form (as could be judged from the figures in Vachon (1981)) they lied closer together. Bonacina (1982) did not analyze any new material from Greece. Vachon (1981) stopped short of assigning any taxonomic rank to the Peloponnese population, but noted that it could belong to the same taxon as *E. i. zakynthi* Caporiacco, 1950 from Zakynthos.

Lacroix (1991) reproduced figures from Vachon (1981) and also provided additional illustrations of *E. italicus*, including the MNHN Taygetos specimens; he followed Vachon's suggestion to distinguish two groups of *E. italicus*, with six versus seven external patellar series. Crucitti (1995) listed the western Peloponnese (Minthi Mts.) form as "*Euscorpius* cf. *italicus*". Crucitti (1999b), Fet & Sissom (2000) and Fet & Braunwalder (2000) also mentioned existence of the separate Peloponnese form under *E. italicus*. Kovařík (2002: 14) reported *E. italicus* from Vitina in central Peloponnese (between Langadia and Tripoli), which most likely also belongs to this form.

Most recently, Crucitti & Bubbico (2001) studied ecology and distribution of scorpions in the southwestern Peloponnese. They collected 99 adults and 72 juveniles of "*E. italicus*" from 19 localities, and again confirmed Vachon's observation that populations from the Peloponnese differ morphologically from other *E. italicus*. Crucitti & Bubbico (2001) did not formally assign the taxonomic status to the Peloponnese populations but they discussed the trichobothrial variation (especially noticing absence of the series esb_a) and a possibility that these populations may represent a separate taxon. They mentioned availability of the names *Euscorpius naupliensis* (for eastern Peloponnese population) and *E. italicus zakynthi* (for Zakynthos/Pelouzo population). Our morphological analysis of the numerous new specimens collected by P. Crucitti, the Caporiacco's types from Pelouzo and Zakynthos, and additional specimens from Zakynthos and Peloponnese, is presented below, and is further corroborated by genetic analysis. We confirm that these populations are sufficiently different from *E. italicus* to have a species rank as *Euscorpius naupliensis* (C. L. Koch, 1837), which is the senior synonym of *E. italicus zakynthi* Caporiacco, 1950, **syn. n.**

Fig. 23. Dorsal view of female *Euscorpius naupliensis* (C. L. Koch), Selinitsa, Peloponnese, Greece.

Diagnosis

A medium to large *Euscorpius* species, generally brown to dark brown in overall coloration. Dorsal metasomal carinae weakly granulated, inferior median carina essentially obsolete on segments I-IV; inferior carinae of segment V granulate**.** Pedipalp trichobothrial patterns: chelal ventral series found on ventral-external surfaces, 8-13 (9-10); patellar ventral surface 10-14 (11-12); patellar external surface *eb* = 4, eb_a = 5-6, *esb* = 2, esb_a = 0-2, *em* = 5, *est* = 4, *et* = 7-10 (8); distance between chelal fixed finger trichobothria *dsb*-*est* is considerably less than the distance between *est*-*et*. Pectinal tooth counts: female, 6-9 (7+); male, 8-11 (9). Variable neobothriotaxy of the chela venter exceeding 6 trichobothria and the complete or near absence of the unique esb_a series (if present, it is only represented by one or two basal trichobothria) are key diagnostic characters of this species.

Female

Redescription based on subadult female lectotype of *E. i. zakynthi* from Pelouzo Island, Greece. Measurements of female lectotype and other material provided in Table II. Dorsal view of a sexually mature female is shown in Fig. 23.

COLORATION. Overall basic color medium brown to mahogany. Carapace and pedipalps dark brown to mahogany in color, very subtle fuscous patterns on proximal lateral aspects of carapace; eyes and tubercles dark brown to black; pedipalpal carinae dark brown. Metasoma brown, telson a lighter tan-orange, aculeus dark brown. Mesosoma and legs a lighter brown-orange; chelicerae orange-yellow.

CARAPACE. Smooth and semi-glossy at 10x; anterior edge slightly convex, evenly from lateral eyes, lacking setae. Two lateral eyes, anterior eye largest; median

Table II
Morphometrics (mm) of *Euscorpius naupliensis* from Peloponnese and Zakynthos.
*lectotype of *E. italicus zakynthi* Caporiacco.

	Zakynthos		Gythio		Kiparissia	
	Female*	Male	Female	Female	Male	Male
Total length	30.10	38.05	42.25	41.20	42.85	41.00
Carapace length	4.85	5.80	5.95	5.75	5.85	5.20
Mesosoma length	11.30	12.35	18.00	17.80	16.45	16.55
Metasoma length	10.50	14.60	13.85	13.35	15.25	13.80
Metasomal segment I						
length	1.45	1.95	1.85	1.80	2.10	1.80
width	1.60	2.00	1.95	1.90	2.05	1.85
Metasomal segment II						
length	1.60	2.25	2.15	2.10	2.40	2.20
width	1.35	1.75	1.70	1.60	1.70	1.60
Metasomal segment III						
length	1.80	2.55	2.40	2.30	2.60	2.40
width	1.30	1.70	1.55	1.50	1.55	1.45
Metasomal segment IV						
length	2.15	3.05	2.85	2.85	3.15	2.90
width	1.20	1.55	1.45	1.40	1.45	1.45
Metasomal segment V						
length	3.50	4.80	4.60	4.30	5.00	4.50
width	1.20	1.55	1.45	1.50	1.50	1.45
Telson length	3.45	5.30	4.45	4.30	5.30	5.45
Vesicle length	2.25	3.90	3.15	3.15	4.20	4.20
width	1.15	1.95	1.45	1.35	2.00	1.85
depth	1.10	2.15	1.35	1.35	2.20	2.15
Aculeus length	1.20	1.40	1.30	1.15	1.10	1.25
Pedipalp length	15.90	20.20	20.55	19.10	20.15	18.90
Femur length	3.80	4.90	4.80	4.60	4.90	4.50
width	1.55	1.90	1.95	1.90	2.05	1.90
Patella length	3.95	5.05	5.05	4.65	5.05	4.75
width	1.85	2.25	2.20	2.20	2.40	2.25
Chela length	8.15	10.25	10.70	9.85	10.20	9.65
Palm length	4.45	5.55	5.75	5.45	5.55	5.35
width	2.60	3.75	3.80	3.65	4.05	3.35
depth	2.95	4.05	4.40	4.00	4.30	3.75
Movable finger length	4.55	5.90	5.80	5.45	5.75	5.45
Pectines						
teeth	7-7	10-10	8-8	7-7	10-10	10-10
middle lamellae	5-5	6-6	5-4	5-5	6-7	5+-6

Table III
Statistical distribution of pedipalp patella external trichobothria series eb_a and esb_a for *Euscorpius naupliensis* segregated into 13 populations distributed throughout Peloponnese. Populations are divided into three groups: (1) >60% of samples have lost one trichobothrium in series eb_a (i.e., eb_a = 5) and *no* trichobothria are present in series esb_a; (2) <10% of samples have lost one trichobothrium in series eb_a and <50% samples have one esb_a trichobothrium; (3) >85% samples with no lose of trichobothrium in series eb_a (i.e., eb_a = 6) and >60% samples with 1-2 esb_a trichobothria. eb_a = external basal-a, esb_a = external suprabasal-a. *N* = number of samples. * considered aberrant.

	N	eb_a counts (percentage%)			esb_a counts (percentage%)		
		4	5	6	0	1	2
Itylo	13	1 (7.7)*	12 (92.3)	-	13 (100.0)	-	-
Passavas	11	-	9 (81.8)	2 (18.2)	11 (100.0)	-	-
Sparti	4	-	3 (75.0)	1 (25.0)	4 (100.0)	-	-
Zerbitsis	8	-	6 (75.0)	2 (25.0)	8 (100.0)	-	-
Selinitsa	48	-	29 (60.4)	19 (39.6)	48 (100.0)	-	-
Zakynthos	14	-	1 (7.1)	13 (92.9)	8 (57.1)	6 (42.9)	-
Zacharo	18	-	1 (5.6)	17 (94.4)	12 (67.0)	6 (33.0)	-
Ambula	21	-	-	21 (100.0)	18 (85.7)	3 (14.3)	-
Nedontas	16	-	2 (12.5)	14 (87.5)	3 (18.75)	12 (75.0)	1 (6.25)
Kurtaina	8	-	-	8 (100.0)	3 (37.5)	4 (50.0)	1 (12.5)
Taygetos	9	-	-	9 (100.0)	2 (22.2)	4 (44.4)	3 (33.4)
Kiparissia	4	-	-	4 (100.0)	-	2 (50.0)	2 (50.0)
Kalamata	6	-	-	6 (100.0)	-	3 (50.0)	3 (50.0)
	180	1 (0.6)	63 (35.0)	116 (64.4)	130 (72.2)	40 (22.2)	10 (5.6)

eyes and tubercle small situated anterior of middle with following length and width formulas: 210|485 and 73|409.

MESOSOMA. Tergites smooth at 10x lacking carination on segment VII; sternites smooth, carinae absent on segment V; stigmata small, slit-like to sub-oval.

METASOMA. All segments somewhat polished in appearance. Carinae — Segments I-IV: dorsal rounded and smooth on I-III, slightly granulose on IV; dorsal lateral smooth anteriorly on I-II, round and smooth on III-IV; lateral obsolete; inferior lateral obsolete on I, round and smooth on II-IV; inferior median obsolete. Carinae — Segment V: dorsal lateral rounded and rough to granulate; lateral obsolete; inferior lateral and median carinae delicately granulate. Intercarinal areas smooth.

TELSON. Vesicle smooth and polished. Aculeus forming a gradual curve from the vesicle; 2 pairs of setae at vesicle/aculeus juncture.

PECTINES. Length|width formula 199|84. Pectinal tooth counts 7/7 and middle lamellae counts 5/5; fulcra well developed entire pecten; numerous fine yellow setae situated on anterior lamellae. Sensorial areas of teeth developed slightly on internal edge of teeth. Basal piece anterior edge lacking concave depression, length|width formula 52|168.

GENITAL OPERCULUM. Connected by membrane for its entire length.

STERNUM. Pentagonal, wider than long, length|width formula 155|170.

CHELICERAE. Movable finger: dorsal distal denticle considerably shorter than ventral distal denticle; dorsal edge with two subdistal denticles; ventral edge smooth, lacking serrulae, and covered with heavy brush-like setae for basal half of finger. Fixed finger: four denticles configured normally.

PEDIPALPS. Slight scalloping on chelal finger bases. Femur: dorsal and ventral internal carinae serrulate; dorsal external irregularly serrulate on proximal one-half; ventral external round and weakly granulate; dorsal and ventral surfaces smooth to rough, internal surface with numerous enlarged granules. Patella: dorsal and ventral internal carinae crenulate; dorsal external smooth and rounded; ventral external irregularly granulate; exteromedian rounded and irregularly granulate. Dorsal surface smooth to rough, ventral smooth; DPS well developed, VPS essentially obsolete represented by a small granule. Chela carinae: digital very strong and smooth, exhibiting subtle granulation; subdigital in relief, represented by two granules; dorsal secondary obsolete; dorsal marginal rounded, continuous and with slight granulation; dorsal internal rounded with scattered granulation; ventroexternal strong extending to external condyle of finger, external to trichobothrium Et_1, delicately crenulate; ventromedian essentially obsolete; ventrointerior rounded and slightly granulose; and external secondary strong and irregularly granulose. Chelal finger dentition: Median denticle row straight; 6/7 inner denticles, 6/7 outer denticles, and 4/5-6 inner accessory denticles for fixed and movable fingers respectively. Trichobothria patterns: Type C, neobothriotaxic (major additive) on chela and patella. Femur: trichobothrium *d* positioned proximal in relation to *i*, *e* distal to both. Patella: ventral series number 10/10 and external series number *eb* = 4/4, eb_a = 6/6, *esb* = 2/2, esb_a = 0/0, *em* = 5/5, *est* = 4/4, and *et* = 8/8. Chela: Ventral series number 10/10, 1/1 external, 1/1 on ventroexternal carina, and 8/8 ventral; *et-est*/*est-dsb* ratio 1.571 and 1.476 for left and right chelae, respectively.

LEGS. Two pairs of pedal spurs present, tarsal spines absent. Tarsus III: ventral median spinule row formed by 9+ elongated spinules; one pair of elongated ventral distal spinules. Spination of basitarsus undeterminable (see data for male below).

Male

Male from Selinitsa, Peloponnese, Greece used for comparison. Sexually mature male specimens possess well developed proximal scalloping on the chelal finger bases, mature females exhibiting a much more subtle scalloping. Granulation of carapace, metasoma and pedipalps same as in male. Basitarsus of legs I-IV on male exhibit 5, 3 and 2 proventral spinules on legs I-III, respectively.

HEMISPERMATOPHORE. Well developed lamina with conspicuous basal constriction, tapered distally; truncal flexure present; capsular lobe complex well developed; ental channel emanating from the trunk, spinose distally, exhibiting ten delicate spines (Fig. 22: male from Zakynthos Island, Greece).

Morphometrics

We compared morphometrics of ten sexually mature males and females from eight different localities from Peloponnese and Zakynthos. Males and females did not exhibit undue differences in overall size, carapace lengths ranged 4.60-5.85 (5.22) for males and 4.60-5.95 (5.03) for females. The metasoma of the male is thinner than it is on the female, but only exhibiting small mean value differences when all segment length/width ratios are compared, a range of 1.5 – 8%. However, the considerably inflated telson vesicle of sexually mature males is quite conspicuous when compared to the thinner telson of the female. This is illustrated using morphometrics. Morphometric ratios calculated from the carapace length divided by the vesicle width and depth showed considerable mean value differences and significant separation of plus/minus standard error ranges:

Carapace Length/Vesicle Width
Mean Value Difference = 42.7% Separation Gap = 417.5%

Females
3.75-4.45 (0.195) (±4.133) [010]: {3.94-4.33} z 0.047
Males
2.75-3.06 (0.111) (±2.897) [010]: {2.79-3.01} z 0.038

Carapace Length/Vesicle Depth
Mean Value Difference = 73.7% Separation Gap = 698.3%

Females
3.91-4.68 (0.208) (±4.327) [010]: {4.12-4.53} z 0.048
Males
2.38-2.71 (0.109) (±2.491) [010]: {2.38-2.60} z 0.044

Chelal fixed finger trichobothria ratio, *et-est*/*est-dsb*, is larger in males with a mean value difference of 28%, implying the distance of *est-dsb* is relatively *smaller* in males than females.

Genital operculum/genital papillae: On the female, the genital operculum is connected for its entire length by a membrane, whereas on males, it is separated for most of its length, exposing protruding genital papillae.

Pectinal tooth counts: The pectines are more prominent in males, teeth longer as well as larger in number:

Females
8-11 (9.313) (±0.656) [067]: {8.657-9.970} z 0.070
Males
6- 9 (7.415) (±0.631) [118]: {6.784-8.047} z 0.085

The mean value difference is 25.6%, roughly a 1.9 tooth difference between the male and female.

Variation within species

In addition to the female lectotype of *E. i. zakynthi* from Pelouzo and the male from Selinitsa, we examined 96 specimens from the Peloponnese. In particular we analyzed the distribution of accessory trichobothria found both on the chela and patella. Fig. 24 illustrates the statistical ranges of the trichobothrial series of the chela and patella based on over 175 samples per series. Of particular importance is the high support for external series *eb*=4 in 100% of all cases (179 samples), series *em*=5 (86.7% of 181 samples), and series eb_a=5 (35%) or 6 (64.4%) (however, this breakdown is population dependent; see below). Figs. 15-20 illustrate variability in the patellar external trichobothrial patterns. As expected, variability in the chelal ventral and patellar ventral and *et* series was present (i.e., variability in the latter two series is quite common throughout the genus, however, the former is exclusively *E. italicus* and *E. naupliensis* specific). The chelal ventral trichobothria are found on both the ventral and external aspects of the palm. In most samples one (rarely two) trichobothrium is found on the ventroexternal carina, situated in a "dimple" formed in the carina. For 179 samples 24 different configurations were detected in the numbers found on the external, ventroexternal carina, and ventral surfaces of the palm (Figs. 6-8). 86.4% of these samples exhibited trichobothria situated on the ventroexternal carina and 59.7% had two trichobothria on the external surface. The following six configurations were predominant, accounting for 75.5% of the samples:

1+1+7 = 9, 17.6%
2+1+6 = 9, 17.0%
2+1+7 = 10, 14.2%
1+1+8 = 10, 11.4%
2+1+8 = 11, 8.5%
2+0+7 = 9, 6.8%

During this study we originally assumed, following earlier workers such as Vachon (1981) and Lacroix (1991), that the esb_a series exhibited in *E. italicus* had been lost in this species (i.e., except for this loss, *E. naupliensis* generally matched *E. italicus* in all major characters). Consequently our initial statistical database compiled for *E. naupliensis* was based on this assumption. When compared to the database compiled for *E. italicus* we noticed the significant variability in the eb_a series which exhibited a coefficient of variability (*cv*) of 14.9% in contrast to that exhibited by *E. italicus* where the *cv* in this series was only 6.9%, reflecting a dominant 83.9% compliance for trichobothria numbers equal to six. However, in *E. naupliensis* we observed numbers ranging from 5-8, without any particular predominant values, 35.6% for eb_a= 5; 37.3% for eb_a= 6, 22% for eb_a= 7, and 4.6% for eb_a= 8. Although the counts of five certainly imply a loss of an accessory trichobothrium from the normal series as exhibited in *E. italicus*, we cannot, however, necessarily hypothesize a gain of one or two accessory trichobothria in this series for those cases where the numbers are seven or eight. An alternative hypothesis, which is more efficient with respect to required derivations (i.e., minimizes derivations), is to suggest that *not* all trichobothria in the esb_a series have been lost in all populations of *E. naupliensis*. That is, the one or two "new" accessory trichobothria assigned to the eb_a series are actually residual accessory trichobothria remaining from the esb_a series. Based on positional analysis of many specimens of *E. italicus* and *E. naupliensis*, it is clear that some basal esb_a trichobothria do occupy areas quite close to the eb_a series, although there is great variability in this (as discussed in length above for *E. italicus*). Consequently the trichobothria statistics presented in Fig. 24 reflect this hypothesis. Several comparative configurations of these series based on this hypothesis are illustrated for both *E. italicus* and *E. naupliensis* in Figs. 9-20. Note that Caporiacco (1950) characterized this species with a reduced number of external trichobothria on the patella, typically 29. It is the complete or near loss of the esb_a series that causes this reduction in trichobothria counts. Caporiacco did not deal with series level analysis, which is a more definitive approach originally introduced by Vachon (1963) following Caporiacco's work.

We analyzed statistically the trichobothrial distribution across six series of the pedipalp for the 98 *E. naupliensis* specimens, grouping them into 13 distinct populations (note that samples from some populations only contain two specimens while others have ten or more; 24 the largest, from Selinitsa). For the three trichobothrial series where we expect the most variability (the chelal ventral, patella ventral and *et* series), populations from three localities consistently exhibited the higher trichobothria numbers: a locality between Kalamata and Kardamili, Nedontas River (between

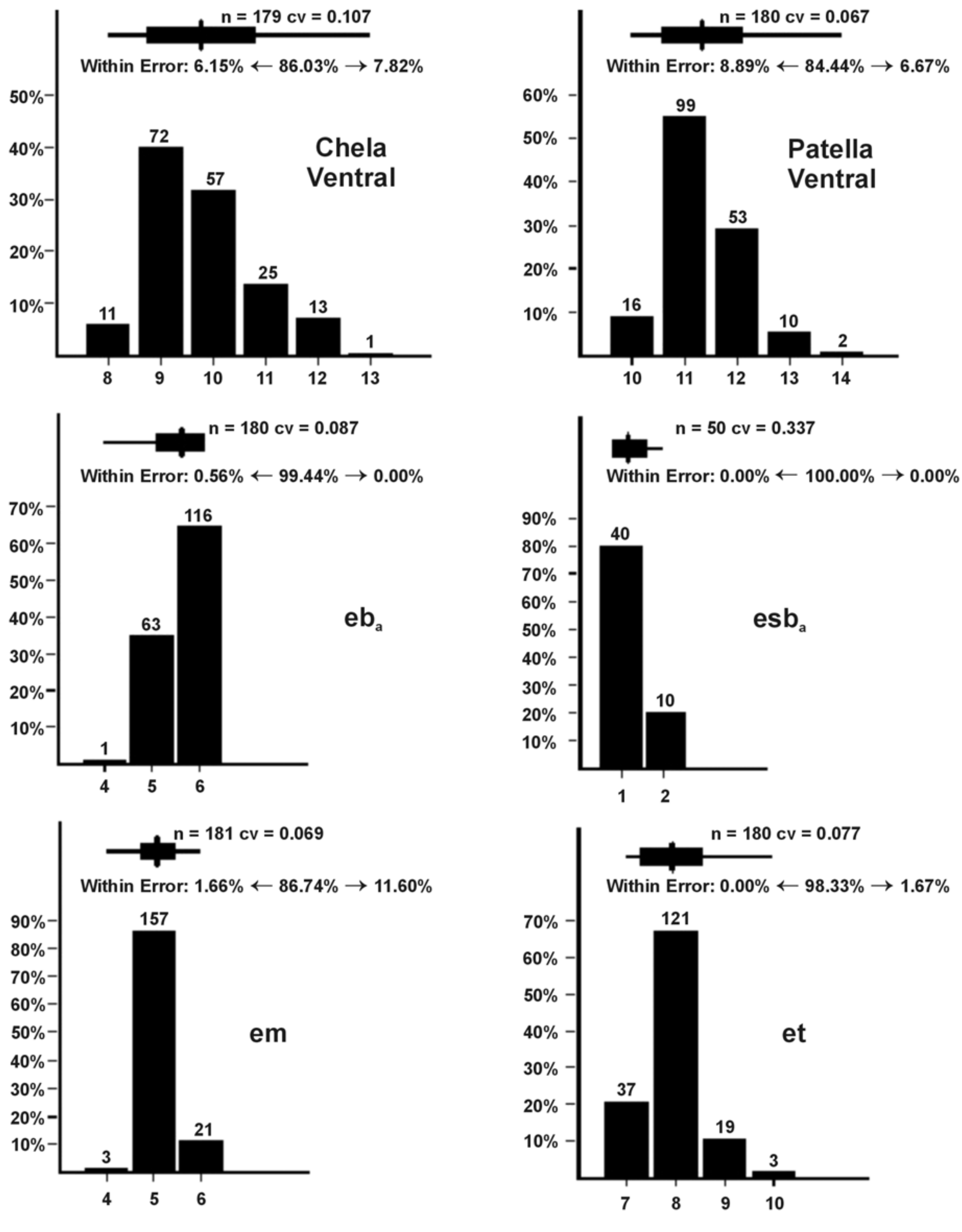


Fig. 24. Statistical data for pedipalp patella trichobothrial counts of *Euscorpius naupliensis*. eb_a = external basal-a, esb_a = external suprabasal-a, *em* = external median, *et* = external terminal.

Artemisia and Kalamata), and Artemisia –areas that are geographically close. On the other hand, Itylo, Kurtaina, and Passavas populations generally showed the lower counts in these three trichobothria series. The mean value differences between these population extremes were 20%, a two trichobothria difference, 9%, one trichobothrium difference, and 12%, one trichobothrium difference, for the chelal ventral, patellar ventral and *et* series, respectively. The *em* = 5 series number was essentially unvarying across all populations. Due to the importance of the eb_a and esb_a series as key diagnostic characters for this species we provide a detailed breakdown of percentages for these series for all populations (see Table III). What is interesting from this data is that, in general, in the specimens where the loss of a trichobothrium from series eb_a is predominant, the entire esb_a series is also absent. On the other extreme, populations that predominantly exhibited all six trichobothria in the eb_a series, also had one or two esb_a trichobothria present, always situated basally close to the eb_a series. For the latter situation those populations that always possessed trichobothria in the esb_a series, they were evenly split between one and two trichobothrium in this series. Specimens from Itylo possessed in general the least number of trichobothria in most of the series and also had lost the eb_a trichobothrium in all samples examined. Itylo is the southernmost population studied.

E. naupliensis is isolated on the Peloponnese and Zakynthos Island (with the nearby Pelouzo Island). We have only one record of E. *italicus* s.str. occurring on the Peloponnese, in its extreme northwest corner (a single specimen from Patras). Based on the instability of this unique esb_a series, composed entirely of accessory trichobothria, it is not unreasonable to suggest that the isolation imposed on *E. naupliensis* might have contributed, in part, to the loss of this series. Of even more interest, this observed instability in *E. naupliensis* accessory trichobothria has even occurred in the usually stable eb_a series in some populations (see Table III). Gantenbein *et al.* (2001, p. 311) recently reported the drastic reduction in accessory trichobothria in some populations of the species *E. balearicus* Caporiacco from the Balearic Islands off the coast of Spain. This reduction involved the patella *et* and ventral series which reflected a 20-25% reduction. Relevant to this discussion, the reduction in trichobothria was detected in populations from smaller islands and islets surrounding Mallorca, the largest island in the Balearic archipelago. Based on these observations for *E. balearicus*, one might expect populations from Zakynthos would show the most affect of isolationism of the thirteen populations studied (i.e., the most reduction in accessory trichobothria). In some situations, Zakynthos specimens did exhibit a somewhat reduced number of trichobothria, in general only differing by one-half trichobothrium from the particular population that contained the lowest number found in that series. However, for the eb_a series, Zakynthos specimens did in general have six trichobothria and approximately one half of the samples possessed a single esb_a trichobothrium (based on 14 samples). Most southern populations from the Peloponnese (e.g., Itylo) generally exhibited eb_a = 5, and no esb_a trichobothria, thus having in general the lowest number of trichobothria in these two series.

Material examined

GREECE. Peloponnese: 2 adult males, 5 adult, 1 subadult females (SRSN), Ambula (near Kalidona), 19 August 1994 – 13 April 1995 (P. Crucitti); 1 adult male, 5 adult, 3 subadult females (SRSN), Arini (near Zaharo), 16 April 1995 (P. Crucitti); 2 females (NMW 16.028/1-2), Artemisia, Taygetos Mts., 31 May 1984 (E. Kritscher); 2 adult, 1 subadut, 1 juvenile males, 2 adult, 1 subadult, 1 juvenile females (SRSN), Nedontas River, between Artemisia and Kalamata, 10 August 1993–29 July 1995 (P. Crucitti); 1 adult, 1 subadult, 1 juvenile males, 4 adult, 3 subadult, 1 juvenile females (BG), Itylo, between Gythio and Kalamata, 14 March 1998 (I. & B. Gantenbein); 1 adult, 1 subadult males, 1 subadult female (SRSN), between Kalamata and Kardamili, 11 August 1993 (P. Crucitti); 1 subadult female (MES), Kalidona, 1995 (P. Crucitti); 2 adult males (SRSN), Kiparissia, 17 April 1995 (P. Crucitti); 2 adult, 1 subadult males, 1 subadult female (SRSN), Kurtaina (near Kalidona), 20 August 1994 – 13 April 1995 (P. Crucitti); 1 female (NMW 2102), Langada, near Ladha, Taygetos Mts., April 1901 (F. Werner); 1 adult, 1 juvenile males, 1 adult, 1 subadult, 2 juvenile females (SRSN), Passavas (near Gythio), 16 August 1994 (P. Crucitti); 7 adult, 4 subadult males, 9 adult, 3 subadult, 1 juvenile females (SRSN), Selinitsa (near Gythio), 28 July 1993 – 11 August 1995 (P. Crucitti); 2 adults (VF), Sparti, 30 July 1993 (P. Crucitti); 1 adult female, 1 adult male, 1 juv. (NMW 2195) Xechori, Taygetos Mts., 7 June 1937 (F. Werner; labeled by Vachon in 1981 as VA 2636); 2 adult females (VF), Zacharo (near Kalidona), 13 April 1995 – 21 August 1996 (P. Crucitti); 1 adult male, 3 adult females (SRSN), Zerbitsis (near Dafni), 7 August 1993 (P. Crucitti). **Zakynthos (=Zante) Island:** 1 adult, 1 subadult females (BG), 20 August 1999 (K. Palmer); 1 adult female (NMW 2103; labeled by Vachon in 1981 as VA 2637); 1 adult male, 1 adult female (NMW), 23 March 1936 (J. Eiselt; labeled by Vachon in 1982 as VA 2679; paralectotypes of *E. i. zakynthi*); 1 adult male, 1 subadult female (NMW 16.029/1-2), Laganas, 7 June 1983 (Bilek). **Pelouzo (=Peluso) Island** (near Zakynthos): 1 subadult female (lectotype of *E. i. zakynthi*) (MZUF 74, formerly from NMW, undoubtedly 23 March 1936, J. Eiselt; see Werner, 1941).

Other source data. None.

Geographical distribution

Greece; restricted to Peloponnese and Zakynthos Island (with nearby Pelouzo Island) in the Ionian Sea. See map in Fig. 26.

Morphological comparison of two species

We compared *E. italicus* and *E. naupliensis* species from several morphological perspectives: trichobothrial patterns, morphometrics, pectinal tooth counts, carination, and coloration and patterns. In general, the first two characters provide the most significant diagnostic characters; the others are of lesser importance.

Caporiacco (1950) used trichobothrial counts, granulation of the metasoma and overall coloration to distinguish *E. italicus zakynthi* from other subspecies of *E. italicus*, (including the subspecies *E. i. etruriae*). In his diagnosis (contained in a key) *E. i. zakynthi* was distinguished by its lower trichobothria counts of the external surface of the patella, lesser granulation of the dorsal metasomal carinae of segments I-IV and ventral carinae of segment V, and a lighter coloration as compared to the darker *E. italicus*. All of Caporiacco's distinctions were confirmed in our analysis; some, of course, are more significant than others.

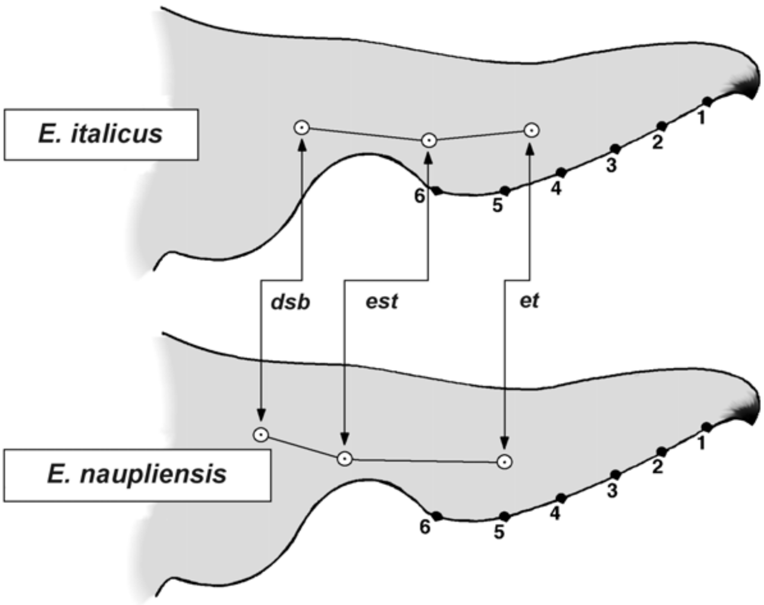


Fig. 25. Diagrammatic trichobothrial pattern (in part) illustrating *et-est*/*est-dsb* ratio for male *E. italicus* and *E. naupliensis*. Outer denticles of fixed finger are numbered.

Trichobothrial Patterns

Presented below are statistical comparisons of six trichobothrial series with accessory trichobothria for these two species. For the three variable series, we see small mean value differences between *E. italicus* and *E. naupliensis*: 1.4% for the chelal ventral series, 6.7% for the patella ventral series, and 8.6% for series *et*, all less than a single trichobothrium difference. For the *em* series only a 2% mean value difference was present. As discussed in detail elsewhere, the esb_a series shows the most variation between these two species, 0-2 trichobothria found in *E. naupliensis* and 5-11 (7-8) in *E. italicus*. Although the mean value difference shown for the eb_a series is small at 3.5%, the range of 5-6 for *E. naupliensis* is population dependent, a trend not found in *E. italicus*. The variability reflected in the eb_a series and the near loss of the esb_a series for *E. naupliensis* are key diagnostic characters separating these two species. Following are statistical data for these trichobothrial series:

Chela ventral: 1.4% mean value difference

italicus
8-12 (9.918) (±0.806) [158]: {9.112-10.723} z 0.081
naupliensis
8-13 (9.777) (±1.041) [179]: {8.735-10.818} z 0.107

Patella ventral: 6.7% mean value difference

italicus
10-14 (12.115) (±0.741) [234]: {11.375-12.856} z 0.061
naupliensis
10-14 (11.350) (±0.766) [180]: {10.584-12.116} z 0.067

Patella eb_a: 3.5% mean value difference

italicus
4-7 (5.839) (±0.404) [218]: {5.436-6.243} z 0.069
naupliensis
4-6 (5.639) (±0.493) [180]: {5.146-6.132} z 0.087

Patella esb_a: 552% mean value difference

italicus
5-11 (7.820) (±1.182) [211]: {6.638-9.001} z 0.151
naupliensis
1- 2 (1.200) (±0.404) [050]: {0.796-1.604} z 0.337

Patella *em*: 2.0% mean value difference

italicus
4-6 (5.000) (±0.232) [223]: {4.768-5.232} z 0.046
naupliensis
4-6 (5.099) (±0.351) [181]: {4.748-5.451} z 0.069

Patella *et*: 8.6% mean value difference

italicus
6- 9 (7.307) (±0.553) [218]: {6.754-7.861} z 0.076
naupliensis
7-10 (7.933) (±0.613) [180]: {7.321-8.546} z 0.077

As indicated by Vachon (1981) and Bonacina (1982), the chelal fixed finger trichobothria ratio, *et-est*/*est-dsb*, provides a good diagnostic character for separating these two species. We calculated this ratio from 20 samples per gender for each species, a total of 80 samples, representing wide spread geographical distribution of both. The separation of plus/minus standard error ranges of both genders is significant, exhibiting 177% and 260% for females and males, respectively. Interestingly, there is significant sexual dimorphism in this ratio, but this difference is not consistent across the two species: For *E. italicus*, the distance represented by *est-dsb* is relatively larger in males, whereas in *E. naupliensis*, it is smaller. The difference in this ratio between the two species can be attributed, in part, to the relatively shorter chelal fingers found in *E. naupliensis* (see discussion below on morphometric ratios of the pedipalp). Fig. 25 illustrates the relative position of these three trichobothria in context with the outer denticles of the fixed finger for sexually mature males. All three trichobothria are more basally situated on the finger of *E. naupliensis* than in

E. italicus. Trichobothrium *et* in *E. italicus* is situated either midpoint between outer denticles 4 and 5 or closer to 4. In *E. naupliensis*, this trichobothrium is more proximal, situated quite close to outer denticle 5. The displacement of trichobothrium *est* to the proximal aspect is quite exaggerated in *E. naupliensis*, effectively contributing the most to the differences found in this ratio. For *E. italicus* trichobothrium *est* is found at the distal base of the proximal scallop socket essentially aligned with outer denticle 6. In *E. naupliensis* this trichobothrium is found proximal of the socket, considerably basal to outer denticle 6. Below is statistical data representing this ratio:

Female: 56.9% mean value difference, separation gap = 176.6%

italicus
0.83-1.15 (0.983) (±0.085) [020]: {0.90-1.07} z 0.086
naupliensis
1.29-1.85 (1.542) (±0.176) [020]: {1.37-1.72} z 0.114

Male: 140.1% mean value difference, separation gap = 260.4%

italicus
0.69-1.07 (0.825) (±0.111) [020]: {0.71-0.94} z 0.134
naupliensis
1.36-2.89 (1.981) (±0.468) [020]: {1.51-2.45} z 0.236

Morphometric Ratios

We extracted 26 measurements from 20 sexually mature males and females (ten per each sex) for each of the two species (40 in all). All possible morphometric ratios were calculated and compared within males and females. The results of these comparisons uncovered four morphometric ratios that were relevant for *both* males and females. The specific morphometrics used in these four ratios were isolated by calculating the frequency the individual morphometric contributed to that ratio being larger or smaller as compared to the other sample. We isolated four such morphometrics, two relevant for *E. naupliensis*, the chelal palm width and the chelal palm length; and two for *E. italicus*, the chelal movable finger length and the telson vesicle depth. For morphometrics chelal palm width and length, ratios for *E. naupliensis* (both male and female) constructed with the other 25 morphometrics were “larger” in 49 out of 50 comparisons. For female *E. italicus* the morphometrics movable finger length and telson vesicle depth, ratios were larger in 48 out of 49 comparisons; for the male, the percentages were smaller in 34 comparisons out of 49. By constructing ratios with these four morphometrics we were able to maximize the mean value differences exhibited between the two species, for both males and females. Four morphometric ratios across both genders were identified: movable finger length divided by chelal palm width and length, and telson vesicle depth divided by chelal palm width and length. For the two ratios involving the movable finger length, there is good separation between the plus/minus standard error ranges, for ratios involving the telson vesicle depth, minimum range overlap is present (8-17%). From this data we can conclude that in general *E. naupliensis* has a larger chelal palm (length and width) with a shorter movable finger. Also, the telson is not as deep in this species as that found in *E. italicus*. Following are statistical data for these ratios:

Movable Finger Length/Chela Width:
Females: 9.8% mean value difference, separation gap = 11.4%

italicus
1.67-1.81 (1.740) (±0.047) [010]: {1.69-1.79} z 0.027
naupliensis
1.49-1.76 (1.585) (±0.097) [010]: {1.49-1.68} z 0.061

Males: 9.6% mean value difference, separation gap = 17.1%

italicus
1.62-1.79 (1.705) (±0.050) [010]: {1.66-1.76} z 0.029
naupliensis
1.43-1.66 (1.556) (±0.082) [010]: {1.47-1.64} z 0.053

Movable Finger Length/Chelal Palm Length:
Females: 9.5% mean value difference, separation gap = 61.8%

italicus
1.08-1.15 (1.110) (±0.024) [010]: {1.09-1.13} z 0.022
naupliensis
0.96-1.08 (1.013) (±0.043) [010]: {0.97-1.06} z 0.042

Males: 9.1% mean value difference, separation gap = 27.4%

italicus
1.08-1.19 (1.133) (±0.038) [010]: {1.10-1.17} z 0.033
naupliensis
0.98-1.11 (1.039) (±0.036) [010]: {1.00-1.08} z 0.035

Telson Depth/Chela Width:
Females: 9.7% mean value difference, overlap = 9.6%

italicus
0.39-0.46 (0.418) (±0.022) [010]: {0.40-0.44} z 0.052
naupliensis
0.36-0.42 (0.381) (±0.019) [010]: {0.36-0.40} z 0.050

Males: 10.8% mean value difference, overlap = 17.3%

italicus
0.62-0.74 (0.679) (±0.044) [010]: {0.63-0.72} z 0.065
naupliensis
0.55-0.64 (0.613) (±0.033) [010]: {0.58-0.65} z 0.054

Telson Depth/Chelal Palm Length:
Females: 9.3% mean value difference, overlap = 13.7%

italicus
0.25-0.28 (0.266) (±0.010) [010]: {0.26-0.28} z 0.039
naupliensis
0.22-0.28 (0.244) (±0.015) [010]: {0.23-0.26} z 0.062

Males: 10.3% mean value difference, overlap = 8.2%

italicus
0.42-0.51 (0.451) (±0.028) [010]: {0.42-0.48} z 0.062
naupliensis
0.39-0.44 (0.409) (±0.017) [010]: {0.39-0.43} z 0.041

Pectinal Tooth Counts

We analyzed the pectinal tooth counts of both species for males, 180+ samples, and females, 250+ samples. For males, the mean value difference is 6.2%, showing roughly a one-half tooth difference. The mean value difference between females is larger at 10.9%, approximately a one tooth difference. This slight reduction in

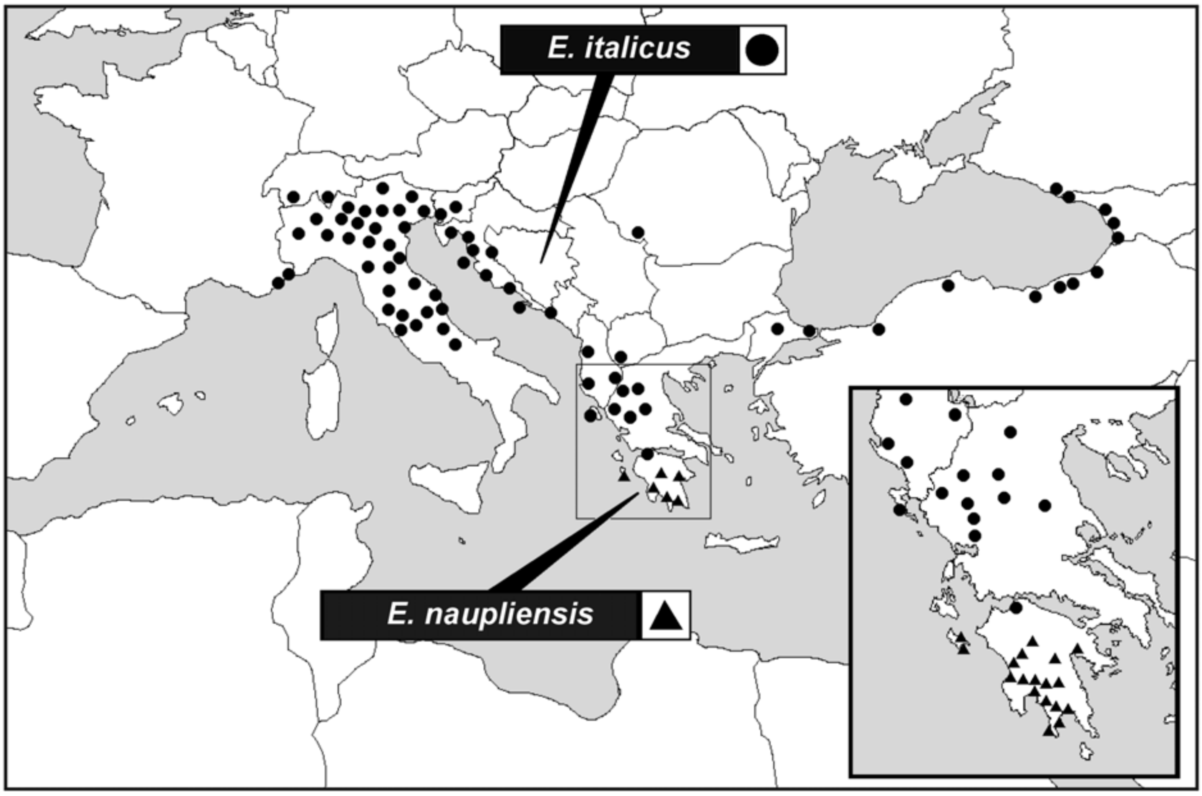


Fig. 26. Map of the Mediterranean area showing distribution of *Euscorpius italicus* and *E. naupliensis*. Boxed area shows blowup of Greece and Albania (partial).

pectinal tooth counts in *E. naupliensis* is also reflected by its slightly smaller overall size as compared to *E. italicus* (roughly 28% smaller based on comparative sizes of 20 carapace lengths per species, male and female). Following are statistical data for the pectinal tooth counts:

Male: 5.8% mean value difference

naupliensis
8-11 (9.313) (±0.656) [067]: {8.657- 9.970} z 0.070
italicus
8-12 (9.855) (±0.546) [117]: {9.309-10.400} z 0.055

Female: 10.7% mean value difference

naupliensis
6-9 (7.415) (±0.631) [118]: {6.784-8.047} z 0.087
italicus
7-9 (8.209) (±0.458) [139]: {7.751-8.667} z 0.056

Carination and Coloration

Comparison of relative development of the metasomal and pedipalpal carinae uncovered some subtle differences. Probably the most significant difference is the weak to total obsolescence of the inferior median carina on segments I-IV found on *E. naupliensis*. On some males, there was a trace of this carina on segment IV. In contrast, *E. italicus* in general has a weak partially granulated carina on segment IV and usually traces of a smooth carina on segment III. Both species exhibited granulated dorsal carinae on segments I-IV to one degree or another, *E. italicus* showing the most granulation. *E. naupliensis* does appear to be slightly lighter in overall coloration, especially when compared to specimens of Caporiacco's subspecies *E. i. etruriae*, which is quite dark.

Molecular Analyses

Allozymes

The estimated allele frequencies of the nine ingroup populations of *E. italicus* and *E. naupliensis* reveal that the two samples from Zakynthos and Peloponnese are fixed for private alleles (i.e., alleles that are not found in other European samples of *E. italicus* nor in any of the "*E. carpathicus*" complex samples; see Appendix 1; Gantenbein *et al.*, 2001) at eight out of 18 total scored loci (Appendix 1). The observed and the estimated expected heterozygosities are about zero (Appendix 1), thus the within-population variability can be neglected but could be a fact of low sample size. However, since similar heterozygosities have been observed in other *Euscorpius* species with enormously low variability we consider the allele frequencies as relatively good estimates.

The constructed neighbor-joining phenogram reveals a clear and high divergence clustering of two lineages, which are supported with high bootstrap values (Fig. 27). The level of divergence between these two clades is similar to the level between congeneric

Table IV

Distance matrix of the sequence divergence for uncorrected *p* (lower left) and for HKY85 + Γ (upper right) calculated from pairwise comparisons of 16S mtDNA sequences. Distances in boldface are given for comparisons between *Euscorpius italicus* and *E. naupliensis*. See Table 1 for abbreviations of haplotypes. Gaps were not considered.

	*Ef*LA1	*Ei*BR1	*Ei*MV1	*Ei*SL1	*Ei*TO1	*En*IT1	*En*IT2	*En*ZA1
*Ef*LA1	-	0.148	0.151	0.141	0.123	0.133	0.139	0.149
*Ei*BR1	0.108	-	0.037	0.022	0.019	**0.065**	**0.069**	**0.069**
*Ei*MV1	0.112	0.033	-	0.040	0.016	**0.068**	**0.072**	**0.072**
*Ei*SL1	0.105	0.020	0.036	-	0.022	**0.068**	**0.073**	**0.073**
*Ei*TO1	0.095	0.017	0.015	0.020	-	**0.047**	**0.051**	**0.051**
*En*IT1	0.102	**0.054**	**0.057**	**0.057**	**0.042**	-	0.006	0.035
*En*IT2	0.105	**0.057**	**0.060**	**0.060**	**0.045**	0.006	-	0.039
*En*ZA1	0.112	**0.057**	**0.060**	**0.060**	**0.045**	0.032	0.035	-

species, i.e. between *E. alpha* and *E. germanus* (Gantenbein *et al.*, 2000) but it is even higher than the level observed between *E. italicus* and *E. tergestinus* (the latter reported as *E. carpathicus*) in Gantenbein *et al.* (1999).

DNA

The sequence divergences between the eight distinct haplotypes are given in Table IV. The divergence between *E. italicus* and *E. naupliensis* is about 6% (7% corrected for multiple hits), which is comparable to divergences reported between congeneric species, i.e. *E. alpha* and *E. germanus* (Gantenbein *et al.,* 2000). The genetic divergence of about 3% within the species *E. naupliensis* is considerable but these populations are also geographically well-isolated. The phylogenetic analysis of the fragment of the 16S rRNA gene reveals a clear separation of the studied *E. italicus* samples into two groups, and the resulting tree topologies of Maximum Likelihood (ML) and Maximum Parsimony (MP) are in congruence with the topology of the allozyme tree (Fig. 27). The branch-and-bound search for the most likely tree found a single tree with the given the substitution model (Fig 28). In MP, there are 16 parsimony-informative characters, the exhaustive tree search finds a single tree with 69 mutation steps; its retention index is 0.94 and consistency index is 0.89, indicating a low level of homoplasy. The clades are supported by rather high bootstrap values.

Discussion

Clearly, *E. italicus* and *E. naupliensis* are two closely related species considered within the overall context of the genus *Euscorpius*. Caporiacco (1950) was correct when he observed this close relationship in morphology, consequently defining *E. i. zakynthi* as a subspecies of *E. italicus*. Although Caporiacco's brief discussion of the reduced patellar external trichobothria did not deal with specific series or the identification of individual trichobothria, he did isolate the most important character separating the two species, as discussed in detail above. Vachon (1981) and Lacroix (1991), using the current trichobothrial nomenclature defined for *Euscorpius* by Vachon (1974, 1975), clarified this reduction in the patellar external accessory trichobothria by identifying, discussing and illustrating the apparent loss of the unique esb_a series (found exclusively on *E. italicus*).

E. italicus and *E. naupliensis* share many important trichobothria based characters which differentiate them, in part, from the other species of *Euscorpius*, and place them both in the subgenus *Polytrichobothrius* Birula, 1917. An important shared character, unmatched by any other *Euscorpius* species, is the variable neobothriotaxy found on the ventral aspect of the chela. *Euscorpius (Tetratrichobothrius) flavicaudis* is the only other *Euscorpius* species that exhibits neobothriotaxy on this segment surface, but in a fixed and limited manner: only two accessory trichobothria are present, one each found on the ventral and external surfaces of the palm, respectively. In contrast, variable numbers of accessory trichobothria are found on both the ventral and external surface of *Euscorpius italicus* and *E. naupliensis*, and in many cases, one or more accessory trichobothria are even found on the ventroexternal carina of the chelal palm which separates the two surfaces. Statistically, the patterns and overall numbers of accessory trichobothria of the chela are essentially the same between these two species (i.e., mean value difference less than 2%) further endorsing the hypothesis that they indeed are based on the same derivation. The unique *em*=5 trichobothria series found on the patella extern is another important character shared by these two species, only matched consistently by *E. flavicaudis*. In *E. hadzii* Caporiacco, *em* series ranges from four to five but is predominantly four (58%) (Fet & Soleglad, 2002). The $eb_a = 6$ series, where *E. italicus* shows compliance for well over 80% of samples, is also shared by *E. naupliensis*, although the latter does show the loss of one these accessory trichobothrium in some populations. *E. flavicaudis* also exhibits $eb_a = 6$. In *E. hadzii*, eb_a series is variable from six to eight, but predominantly seven (59%) (Fet & Soleglad, 2002). *E. italicus* and *E. naupliensis* share similar numbers and distributions of the highly variable patella *et* and ventral (*v*) series, among the highest found in the genus. However, these series are clearly important within the species level only and therefore do not necessarily

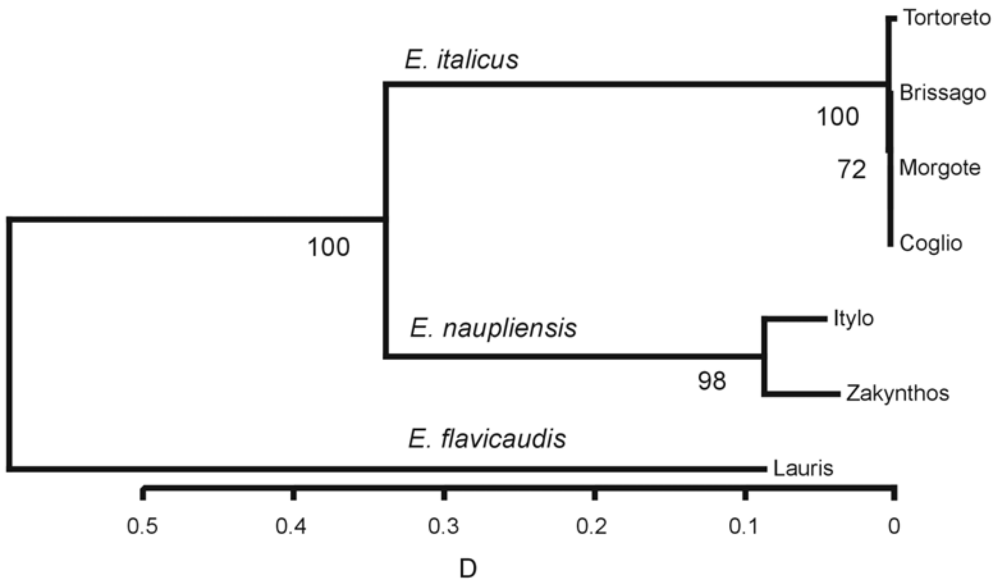


Fig. 27. Neighbor Joining (NJ) tree of *Euscorpius italicus* and *E. naupliensis* based on allozyme data (n $ 2) using Cavalli-Sforza & Edwards (1967) chord distance as an input matrix. Distances are based on 18 allozyme loci. Numbers at nodes refer to bootstrap values. The tree was rooted using *E. flavicaudis*.

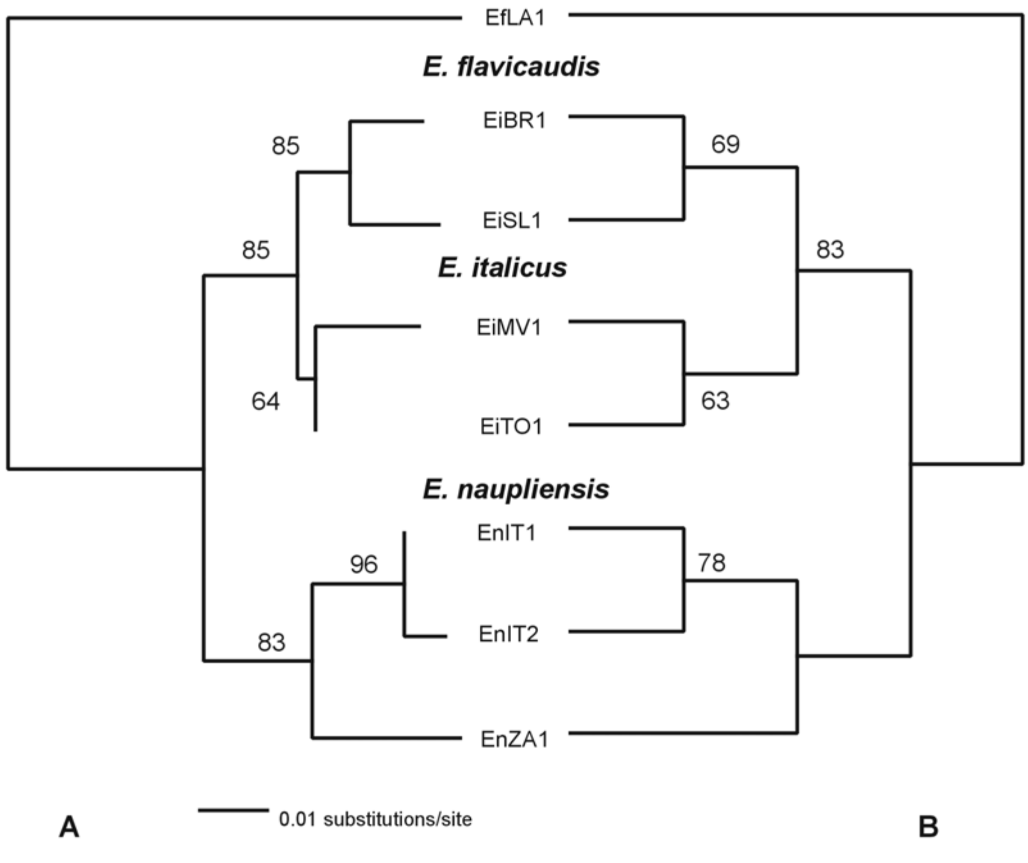


Fig. 28. Phylogenies of *Euscorpius italicus* and *E. naupliensis* based on 16S mtDNA sequences (348 bp), including *E. flavicaudis* as an outgroup. For explanation of haplotype abbreviations see the Material & Methods section. (**A**) Maximum Likelihood (ML) tree (-lnL = 784.35) using the HKY85 + Γ model with the ML-estimated parameters, $\pi_A = 0.34$, $\pi_C = 0.13$, $\pi_G = 0.12$, $\alpha = 0.37$, transition (ti) / transversion (tv) ratio = 1.60 ($\kappa = 4.275$). (**B**) Strict consensus tree of weighted Maximum Parsimony analysis (weighting ti three times over tv) including 352 bp, gaps = "fifth" base. The branch-and-bound tree search revealed eight equally parsimonious trees with 69 steps (CIu = 0.94 and RI = 0.89). Numbers at nodes are bootstrap values in percent over 1,000 pseudoreplicates.

reflect phylogenetic information above this level. For example, the somewhat diverse assemblage of *E. flavicaudis*, *E. hadzii*, and *E. balearicus* also have high trichobothria numbers in their *et* and *v* series similar to the ranges seen in *E. italicus* and *E. naupliensis*.

E. naupliensis and *E. italicus* seem to be "good" biological species according both to the gene trees and the clear morphological evidence. It is also important to notice that *E. italicus* and *E. naupliensis* show a close relationship to the "*E. carpathicus* complex" (Gantenbein *et al.*, 1999). Because of concordant phylogenetic patterns, however, it will be crucial for future studies to conduct an overall genetic analysis including more populations of *E. italicus*. Noteworthy, the genetic variability within population at nuclear markers is almost zero (Appendix 1).

The genetic divergence within the species *E. naupliensis*, i.e. between the sequences from the Peloponnesus and the island of Zakynthos are about 3%, which corresponds to about 3Myrs divergence time using the recently calibrated buthid molecular clock (Gantenbein & Largiadèr, 2002). This dating suggests a split which again predates the last Pleistocene glaciations, which is clearly the case for the split between *E. naupliensis* and *E. italicus* with a genetic divergence of about 7%. According to Stathi & Mylonas (2001, Fig. 1) the island of Zakynthos was isolated from the mainland of Greece after the Messinian salinity crisis (5.2 Myrs ago) (Hsü *et al.*, 1977) and remained isolated since then. If this divergence is the result from the 5Myr separation we would have to assume that the "clock" ticks slower in *Euscorpius* than in the buthid *Mesobuthus*. However, since our analysed fragment is rather short it might be just a "noise" and the observed divergence can be attributed to this main geological event. The split between *E. italicus* and *E. naupliensis* dates further back and could be connected with the Alpine orogenesis.

The newly confirmed species *Euscorpius naupliensis* is clearly limited to Peloponnese and nearby islands. Endemic species are common in Peloponnese; see e.g. Thaler & Knoflach (1998) for spiders; Brown (1977) and Leestmans & Arheiliger (1988) for butterflies; Carpaneto (1986) for beetles; Barbieri *et al.* (2000) for freshwater fish; Tan & Iatrou (2001) for vascular plants. In reptiles, *Lacerta graeca* and *Podacris peloponnesiaca* are endemics of Peloponnese, while *Anguis cephallonicus* and *Algyroides moreoticus* are found in Peloponnese, Zakynthos and the nearby Kefallonia (Meliadou *et al.*, 1999, Fig. 1). The peninsula's long-term effective separation from the mainland has led to endemic speciation.

A still unresolved issue is the origin of a clearly disjunct range of *E. italicus*, which is not found in the eastern part of Greece. Birula (1917a, 1917b) characterized in detail the geographic distribution of *E. italicus*, describing clearly two disjunct parts of *E. italicus* range, "western" and "eastern"; this notion still holds as the species has not been found in eastern part of the Balkan Peninsula. Birula (1917a, 1917b) considered the eastern part of the range (a narrow strip along the southern and eastern coasts of the Black Sea) reduced comparing to the western, and suggested two possible hypotheses for such disjunction: either southward increase of the Black Sea basin, or aridization of climate in Anatolia. Morphologically, *E. italicus* from the "western" and "eastern" parts of the range are the same species. Further genetic data (now lacking from the Turkey and Caucasus) will show whether a considerable divergence is hidden behind this morphological uniformity (in which case one could interpret the disjunct range as the result of reduction). On the contrary, if "western" and "eastern" populations will prove genetically very similar, a strong case can be made for recent, even historical time, dispersal of *E. italicus*.

Remarkably, *E. italicus* was never reported from any of the Aegean islands, from where extensive samples of other *Euscorpius* species (belonging to the "*E. carpathicus*" complex) are available (Kinzelbach, 1975; Fet, 2000; Stathi & Mylonas, 2001). Neither was this species found on any Mediterranean islands such as Baleares, Sicily, Sardinia, Corsica, and Malta; it has been, however, recorded from the offshore islands in the Adriatic Sea (Dalmatian coast of Croatia) and Ionian Sea (Corfu).

At the present time, this species is successfully dispersing with humans, and in parts of its range is almost or exclusively synanthropic being found only in human habitations or ruins but not in the wild (Crucitti, 1993). Braunwalder (2001) accounted only for 33 out of 1,031 records in southern Switzerland, in which *E. italicus* has been found in decidedly natural habitats. Dispersing with humans, this species often establishes new reproducing populations, often remotely disjunct from its continuous range. As examples we can mention established populations in lower Don, Russia (Zykoff, 1912; Fet, 1989); in Sion, Valais, Switzerland (Braunwalder, 2001); in Ljubljana, Slovenia (Hadñi, 1943; Fet *et al.*, 2001); and even in Yemen (Birula, 1937) and Iraq (Fet & KovaÍík, in press). Records from southwestern Romania (Mehadija, Oravitza; Birula, 1917a, 1917b; confirmed by Vachon, 1981) probably also refer to introduced populations. Single specimens of *E. italicus* often have been found in many localities well outside the main range, e.g. in Austria (NMW collection), inland France (Vachon, 1983), Germany (Kinzelbach, 1975), and Lithuania (Fet & Gruodis, 1987). Introduction by humans might be true for the French record from Marseilles (type of *Scorpius provincialis* C. L. Koch), where *E. italicus* was never found again (Vachon, 1983; Lacroix, 1991). This frequent anthropochory, absence on the most islands, and high genetic similarity of the studied populations from Italy, Switzerland, and Greece all suggest that the dispersal of *E. italicus* (likely from some refugia) might not be an ancient event.

Acknowledgements

Special thanks are to W. David Sissom for his advice on the analysis of the hemispermatophore structure, and to Sarah Whitman for her enormous efforts to make the rich type collections of the Museo Zoologico "La Specola" (Florence, Italy) available for our study. We are grateful to Kevan Palmer (UK), Boris Sket (Slovenia), and Marco Bellini (Italy) for collecting live specimens for molecular analyses. We thank Adolf Scholl, Carlo R. Largiadèr and W. Ian Towler for their enthusiastic cooperation in the molecular phylogenetic research of *Euscorpius*. We also are grateful to all those who over the years donated and loaned material and rare literature, and facilitated study of *Euscorpius* in many European museums, including (but not limited to) Janet Beccaloni, Alberto Bonacina, Matt Braunwalder, Boñidar ,ur...if, Hieronymus Dastych, Jason Dunlop, Gérard Dupré, Jürgen Gruber, Paul Hillyard, Dietmar Huber, Ragnar Kinzelbach, František KovaÍík, Matjañ Kuntner, Jean-Bernard Lacroix, Kirill Mikhailov, Vladimir Ovtsharenko, Boris Sket, Marco Valle, Valerio Vignoli, and Mark Volkovich. B.G. was supported by a fellowship of the Swiss National Science Foundation (SNF) for young scientists. E.V.F. was supported by the Department of Biological Sciences, Marshall University.

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Appendix 1

Allele frequencies at 18 scored allozyme loci of the studied populations of *Euscorpius italicus* and *E. naupliensis*. Also given are the observed (H_O) and expected (H_E) heterozygosity estimates (Nei, 1978).

Locus		*E. italicus*				*E. naupliensis*		*E. flavicaudis*
	allele (N)	Tortoreto (4)	Vico Morgote (10)	Brissago (10)	Coglio (10)	Zakynthos (2)	Itylo (11)	Lauris (49)
ALPDH	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ARK	104	1.00	1.00	1.00	1.00			
	100					**1.00**	**1.00**	1.00
DDH	102					**1.00**	**1.00**	
	101	1.00	1.00	1.00	1.00			
	100							1.00
GAPDH	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AAT-1	100						**1.00**	1.00
	90	1.00	1.00	1.00	1.00			
	88					**1.00**		
AAT-2	117	1.00	1.00	1.00	1.00	1.00	1.00	
	100							0.98
	88							0.02
GTDH	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
HK	100							1.00
	93	1.00	1.00	1.00	1.00			
	88					**1.00**	**1.00**	
IDH-1	100							0.92
	95							0.08
	90	1.00	1.00	1.00	1.00			
	84					**1.00**	**1.00**	
IDH-2	100							1.00
	93	1.00	1.00	1.00	1.00	1.00	1.00	
MDH-1	109					**1.00**	**1.00**	
	100							1.00
	89	1.00	1.00	1.00	1.00			
MDH-2	100							1.00
	89	1.00	1.00	1.00	1.00	1.00	1.00	
MPI	101	1.00	1.00	1.00	1.00	1.00	1.00	
	100							1.00
PEP	104	1.00	1.00	1.00	1.00	1.00	1.00	0.01
	100							0.086
	94							0.13
6-PGD	100							0.66
	98					**1.00**	**1.00**	
	88							0.34
	87	1.00	1.00	1.00	1.00			
PGI	102	0.13						
	100	0.88	1.00	1.00	1.00			1.00
	95					**1.00**	**1.00**	
PGM	100							1.00
	85						**0.23**	
	80	1.00	1.00	1.00	1.00	1.00	0.77	
PK	103	1.00	1.00	1.00	1.00	1.00	1.00	
	100							1.00
	H_O (SE)	0.01(0.06)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.015 (0.06)	0.048 (0.12)
	H_E (SE)	0.01(0.06)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.020 (0.09)	0.049 (0.12)