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FIRST DNA PHYLOGENY OF *EUSCORPIUS* THORELL, 1876 (SCORPIONES, EUSCORPIIDAE) AND ITS BEARING ON TAXONOMY AND BIOGEOGRAPHY OF THIS GENUS

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ABSTRACT.– A new phylogeny for the four species of the scorpion genus *Euscorpius* Thorell, 1876 : (((*E. carpathicus*, *E. italicus*), *E. germanus*), *E. flavicaudis*) is proposed based on a comparative analysis of mitochondrial DNA and nuclear gene variation (allozymes). The study revealed that the traditional subgenus *Euscorpius* s.str. (*E. carpathicus* + *E. germanus*) is polyphyletic. A new subgenus is introduced, *Alpiscorpius* subg. n., with the type species *Euscorpius germanus* (C.L. Koch, 1837). Phylogenetic and biogeographic implications are discussed.

KEY–WORDS.– *Euscorpius*, Mitochondrial 16S rRNA, Allozymes, Phylogeny, Mediterranean, Trichobothria

RESUME.– Une nouvelle phylogénie fondée sur l'analyse comparative de l'ADN mitochondrial et de la variabilité génétique nucléaire (allozymes) est proposée pour les quatre espèces de scorpions du genre *Euscorpius* Thorell, 1876 : (((*E.carpathicus*, *E. italicus*), *E. germanus*), *E. flavicaudis*). L'étude démontre que le traditionnel sous–genre *Euscorpius* s. str. (*E.carpathicus* + *E. germanus*) est polyphylétique. Un nouveau sous–genre est proposé, *Alpiscorpius* subg. n., avec comme espèce type *Euscorpius germanus* (C.L. Koch, 1837). Sont discutées les implications phylogénétiques et biogéographiques.

MOTS–CLES.– *Euscorpius*, 16S rARN mitochondrial, Allozymes, Phylogénie, Méditerranéen, Trichobothries

INTRODUCTION

Several species of the scorpion genus *Euscorpius* are common in the circum–Mediterranean region and in southern Europe. Traditionally included in the family Chactidae, this genus was recently placed in a separate family, Euscorpiidae (STOCKWELL, 1992). True Chactidae are currently limited to the South American fauna. Although taxonomy of this genus has been studied since the 1830s, the number of species in *Euscorpius* and their phylogenetic relationships are not clear. There are currently five « good » species, and 43 (!) formally valid subspecies in *Euscorpius* (FET & SISSOM, in press).

One of the most clear (and commonly not disputed) taxonomic arrangements within *Euscorpius* was its subgeneric division introduced by the Russian scorpiologist Alexei BIRULA (1917). He used, in fact, the single diagnostic character – number of trichobothria (mechanoreceptory bristles) on the ventral aspect of the manus of pedipalp

chela (series *Vm*) – to distinguish three subgenera: *Euscorpius* s.str., with three trichobothria; *Tetratrichobothrius* Birula, 1917 – with four; and *Polytrichobothrius* Birula, 1917 – with six to nine trichobothria (BIRULA, 1917; VACHON, 1963). In modern terminology, more trichobothria are included in this series, and Birula's diagnostic character should be read as four *Vm* trichobothria in *Euscorpius* s.str., six in *Tetratrichobothrius*, and eight to 11, for *Polytrichobothrius* (Table III). Both new subgenera proposed by Birula were monotypic, based on *Euscorpius flavicaudis* (DeGeer, 1778) (type species of *Tetratrichobothrius*) and *E. italicus* (Herbst, 1800) (type species of *Polytrichobothrius*) (see e.g. VACHON, 1975; LACROIX, 1991 a,b). The nominotypical subgenus, *Euscorpius* Thorell, 1876, included the type species of the genus, *E. carpathicus* (L., 1767) and, « by default », several other taxa, number and scope of which has been under scrutiny for the last 80 years (see e.g.: HADZI, 1931; DI CAPORIACCO, 1950; CURCIC, 1972; KINZELBACH, 1975; VACHON, 1975, 1981; VACHON & JAQUES, 1977; BONACINA, 1980; FET, 1986, 1993).

Comparative analyses of mitochondrial ribosomal RNA sequences (12S and 16S) are now routinely used in molecular systematics for resolving species–level phylogenies of such different animal groups as ungulate mammals (MIYAMOTO *et al.*, 1989), fruit flies (RUTTKAY *et al.*, 1992), and horseshoe crabs (AVISE *et al.*, 1994). Due to their lack of recombination, mitochondrial genes (« haplotypes ») are used to construct phylogenies which are compared against the geographic background (« phylogeography »). Nucleotide distances serve as an estimate of species divergence, while more sophisticated cladistic algorithms (e.g. SWOFFORD, 1993) are used to construct phylogenetic trees. We report here the first molecular data, based on the mtDNA sequences from four species belonging to all three known subgenera of *Euscorpius*. Allozyme data of population samples are used to underlay and compare the obtained DNA species tree topology. We believe that these data shed new light on the long–discussed phylogenetic relationships within the genus *Euscorpius*, for the first time reaching to the directly inheritable characters: protein and DNA sequences.

METHODS AND MATERIALS

***Euscorpius* material.** The original DNA sequence and allozyme data were obtained from four species of European scorpions belonging to the genus *Euscorpius* (Scorpiones, Euscorpiidae). Live specimens of *E. carpathicus*, *E. germanus*, *E. italicus* and *E. flavicaudis* from Switzerland, Italy and France were collected by B.G and A.S. and stored at –80° C prior used in gel electrophoresis or DNA extraction. Sampling sites and specimens used for the DNA and allozyme study are given in Table I.

Outgroup species. As outgroups for the phylogenetic analyses of DNA sequences, we selected three North American scorpion species (Tab. I) : *Vaejovis spinigerus* (Wood, 1863) (fam. Vaejovidae), collected by Dr. Joseph Bigelow in Yuma, Arizona, USA; and *Hadrurus concolor* Stahnke, 1969 (fam. Iuridae)[1] and *Centruroides exilicauda* (Wood, 1863) (fam. Buthidae), collected by V.F. at Bahia de los Angeles, Baja California Norte, Mexico. Such choice of outgroups was dictated by the fact that families of Vaejovidae and Iuridae are among the family–level taxa considered to be close to Euscorpiidae (so–called « chactoid » group of families; SISSOM, 1990). On the other hand, family Buthidae represents as remote as possible among extant scorpions outgroup to Euscorpiidae (SISSOM, 1990).

[1] This species was described and always listed as *H. concolorous*; however, the correct Latin adjective is « concolor », while « concolorous » should be regarded as an incorrect original spelling according to the International Code of Zoological Nomenclature.

The closest outgroup taxon to *Euscorpius* should in fact also be sought in North America (Mexico), namely the genus *Megacormus*, which is currently placed in Euscorpiidae (STOCKWELL, 1992; FET & SISSOM, in press) and is remarkably close to *Euscorpius* (SOLEGLAD, 1976); however, specimens of *Megacormus* were not available for the DNA study.

DNA techniques. Total DNA was extracted from fresh or preserved muscle tissue using a standard chloroform–phenol/ethanol extraction method. Our previous experience shows that scorpion tissue preserved in 95 % ethanol (but not 70 %) yields a good quality, nondegraded DNA (FET & VEZZETTI, 1994). A fragment of the mitochondrial 16S rRNA gene was amplified using the following PCR primers: the forward primer is a scorpion–specific version of the « universal » primer 16Sbr, or LR–J–12887 (SIMON *et al.*, 1994) while the reverse primer has a scorpion–specific sequence designed by one of the authors (V.F.). These primers have sequences CGATTTGAACTCAGATCA (forward, 18–mer) and GTGCAAAGGTAGCATAATCA (reverse, 20–mer) which correspond to the positions 12,867–12,887 and 13,218–13,310 in the *Drosophila yakuba* mitochondrial genome (CLARY & WOLSTENHOLME, 1985). Extracted DNA was amplified by the polymerase chain reaction (PCR) using a Perkin Elmer 2400 PCR Thermocycler (USA team) or a PCT–100–machine (MJ Research, USA) (Swiss team). PCR was carried out in 50 μl volumes with the following conditions: 1.5 mM $MgCl_2$; annealing temperature 48° C; 40 cycles; using the *Taq* Polymerase kit by a Perkin Elmer (USA team) or QIAGEN™ (Swiss team). The resulting PCR product was verified on 1.5% agarose electrophoretic gel and purified using Ultrafree MC 30,000 cellulose filters (Millipore, Inc.) (USA team) or the QIAquick PCR Purification Kit (QIAGEN™) . (Swiss team). Cycle sequencing of the purified PCR product was performed independently in two facilities by the USA and Swiss teams. For the USA team, it was carried out by the sequencing service at the Molecular Genetics Facility, University of Georgia (Athens, Georgia, USA). The Swiss team performed cycle sequencing at the University of Berne using the forward primer (18–mer, fluorescent dye end–labelled, IRD800; LI–COR) and the SEQUITHERM™ LONG–READ™ CYCLE SEQUENCING KIT. The cycle sequencing reaction were carried out according to the recommendations of the manufacturers using 200–400 ng of DNA per sequence and applying 35 cycles consisting of the following temperature profile: 20 s at 94° C, 15 s at 52° C, and 60 s at 63° C. The sequence reaction products were then resolved on an automated DNA sequencer (USA team: ABI model 9600; Swiss team: LI–COR model 4200). Sequences of 385 base pairs (bp) were obtained for six *Euscorpius* specimens (for *E. carpathicus* and *E. germanus* we had more than one population analysed) and three outgroups.

A total of nine DNA sequences were used for the analysis, these sequences were aligned by CLUSTALV (HIGGINS *et al.*, 1991) and by eye. For further analyses we excluded all sites that could not unambiguously determined for all species (369 characters remained). The Kimura distance (KIMURA, 1980) was calculated using PHYLIP/DNADIST (Phylogeny Inference Package, Version 3.57c, FELSENSTEIN, 1995) setting the transition/transversion ratio to 2 and including deletions/insertions. Using these distances as a matrix, a tree was created by the neighbor–joining method (N–J) (SAITOU & NEI, 1987) using PHYLIP/NEIGHBOR. N–J allows for unequal rates of molecular changes among branches in contrast to UPGMA (SWOFFORD *et al.*, 1996; AVISE, 1994). Bootstrap values were obtained by 1,000 pseudoreplicates of DNA sites (PHYLIP/SEQBOOT). Resulting trees were analysed using the PHYLIP/CONSENSE program using *C. exilicauda* as the outgroup. A maximum likelihood analysis was done using PHYLIP/DNAML which uses the algorithm described by FELSENSTEIN (1981). *C. exilicauda* was used as outgroup. Maximum Parsimony analysis (MP) was carried out using the tree–building algorithm available in the Phylogenetic Analysis Using Parsimony (PAUP) 3.1.1 computer program (SWOFFORD, 1993). Bootstrap resampling statistics of PAUP (1,000 replicates) with branch–and–bound search method was used to confirm the tree topology. Since the number of taxa used for total analysis was not high,

we were able to perform the exhaustive search option in PAUP. Insertions were treated as a « fifth » base. As a measure for tree stability of the MP analysis the consistency index (KLUGE & FARRIS, 1969) was calculated with PAUP.

Allozyme analysis. Horizontal starch gel electrophoresis of allozymes was carried out according to the protocols of earlier studies (SCHOLL *et al.*, 1978; GANTENBEIN *et al.*, 1998). Tissue of the pedipalp was homogenized in ten volumes of buffer (Tris–HCl, 0.1 M pH 8.0), homogenates were then centrifuged for 10 min (13,000 rpm). 25 μl of supernatant fractions were applied to starch gels. We scored 18 gene loci on three buffer systems: N–(3–aminopropyl)–morpholine–citrate (AC, pH 6.2, modified from CLAYTON & TRETIAK, 1972), Tris–citrate (TC, pH 7.3, AYALA *et al.*,1972) and Tris–borate–EDTA (TBE, pH 9.3, modified from AYALA *et al.*,1972). The loci scored were: ALPDH (alanopine dehydrogenase; EC 1.5.1.17), ARK (arginine kinase; EC 2.7.3.3), AAT–1 and AAT–2 (aspartate aminotransferase; EC 2.6.1.1), DDH (dihydrolipoamide oxidase; EC 1.8.1.4), GAPDH (glyceraldehyde–3–phophate dehydrogenase; EC 1.2.1.12), GPI (glucose–6–phosphate isomerase; EC 5.3.1.9), GTDH (glutamate dehydrogenase; EC 1.4.1.2), HK (hexokinase; EC 2.7.1.1), IDH–1 and IDH–2 (isocitrate dehydrogenase; EC 1.1.1.42), MDH–1 and MDH–2 (malate dehydrogenase; EC 1.1.1.37), MPI (mannose–6–phophate isomerase; EC 5.3.1.8), PEP (peptidase; EC 3.4.–.–), PGM (phosphoglucomutase; EC 5.4.2.2), 6–PGD (6–phosphogluconate dehydrogenase; EC 1.1.1.44), and PK (pyruvate kinase; EC 2.7.1.40).

We refer to the observed electromorphs as alleles which are identified by their electrophoretic mobility (in mm) relative to the most common mobility in the *E. flavicaudis* population from Lauris, France (assigned mobility=100).

Nei's genetic distance (NEI, 1972) was calculated from pairwise comparisons of populations (PHYLIP/GENDIST). Nei's distance measure is expected to rise linearly with time since separation of gene pools (NEI, 1987). Using these distances as a matrix, a tree was created by the unweighted pair group method using arithmetic averiges (UPGMA) and by the neighbor–joining method (N–J). Bootstrap values were obtained by 1,000 pseudo replicates of allele frequencies (PHYLIP/SEQBOOT). Resulting UPGMA trees were analysed using the PHYLIP/CONSENSE program.

RESULTS

DNA data. The DNA sequences of the mtDNA 16S gene are listed in the Appendix I and a distance matrix including six *Euscorpius* specimens and three outgroup scorpion taxa is presented in Table 2. Genetic distance between the *Euscorpius* species varied from 0.02 to 0.13 while that of *Euscorpius* versus two outgroup « chactoid » taxa was 0.21 to 0.44.

The Exhaustive Search using the maximum parsimony (MP) criterion yielded a single MP tree with a length of 299 steps (Fig. 1A). A high tree stability was indicated by a consistency index (CI) of 0.836. The topology of this tree was strikingly congruent to the N–J tree based on Kimura distances (Fig. 1B). In the two trees of figure 1, all *Euscorpius* sequences formed a distinct cluster supported by high bootstrap values (98%). The Iuridae and Vaejovidae sequences grouped together outside the *Euscorpius* clade (bootstrap values >82%) which confirmed their presumed status as true outgroup species.

Within the *Euscorpius* clade, the two *E. germanus* and the two *E. carpathicus* sequences clustered together, respectively. High bootstrap values were observed for the node of the *E. carpathicus* clade in both analyses (> 99%). The *E. germanus* group was supported with a high bootstrap value in the N–J tree (86%), however, a slightly lower bootstrap value was observed for this node (60%) in the single most parsimonious tree.

In both approaches *E. italicus* sequence clustered closely to the *E. carpathicus* clade with high support from bootstrap analysis (> 75%).

Finally, *E. flavicaudis* was found to be an outgroup to the other *Euscorpius* taxa (bootstrap values >70%). The maximum likelihood analysis (tree not shown) revealed an identical tree topology. Ln likelihood was −1688.78 and 379 trees were examined. Confidence limits of branch lengths did not suggest for alternative tree arrangements and crude likelihood ratio tests suggested that all branches were significant ($P < 0.05$).

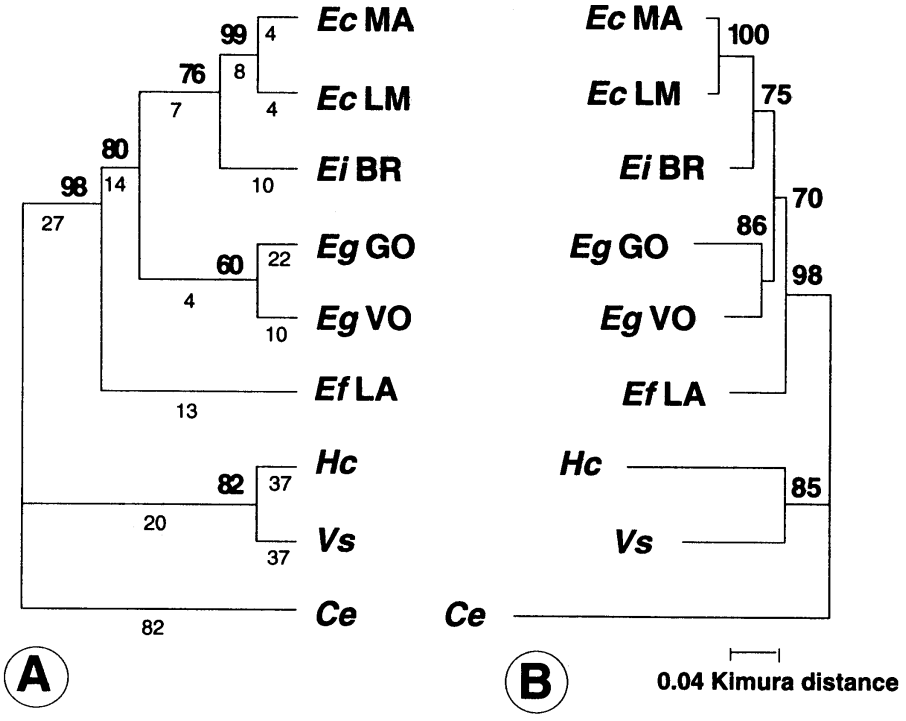

Fig. 1A. The single most parsimonious tree found (299 steps, CI 0.83) using the Exhaustive Search under the MP criterion of PAUP (SWOFFORD, 1993). Insertions were treated as a « fifth » base; numbers refer to the assumed lengths of branches. Numbers in bold refer to bootstrap values obtained from the majority-rule consensus tree out of 1,000 pseudoreplicates using the branch-and-bound search for best trees. 1B. Neighbor-joining (N-J) phenogram based on the Kimura distance (KIMURA, 1980). Numbers in bold refer to bootstrap values out of 1,000 pseudoreplicates). In both trees *C. exilicauda* (*Ce*) was preset as outgroup. Abbreviations are given in Table I.

Allozyme data. The observed allele frequencies are given in Appendix II. Populations were fixed at most gene loci. The *E. germanus* populations were fixed for different alleles at eight out of 18 gene loci. Nei's genetic distances (D) between pairs of populations ranged from 0.35 to 1.78 (Table II).

The derived UPGMA phenogram resulted in the same tree topology as the unrooted N-J tree (Fig. 2A, B). The two *E. carpathicus* populations clustered at a relatively low level with the *E. italicus* population. The two populations of *E. germanus*

were separated at about the same genetic distance level as compared to the level at which *E. italicus* clustered with the *E. carpathicus* clade (Fig. 3A). The population of *E. flavicaudis* was highly genetically differentiated from the remaining *Euscorpius* populations. It clustered, however, with the *E. germanus* clade in the UPGMA tree but this node was weakly supported by a relatively low bootstrap value (56%). All other nodes were strongly supported (> 88%).

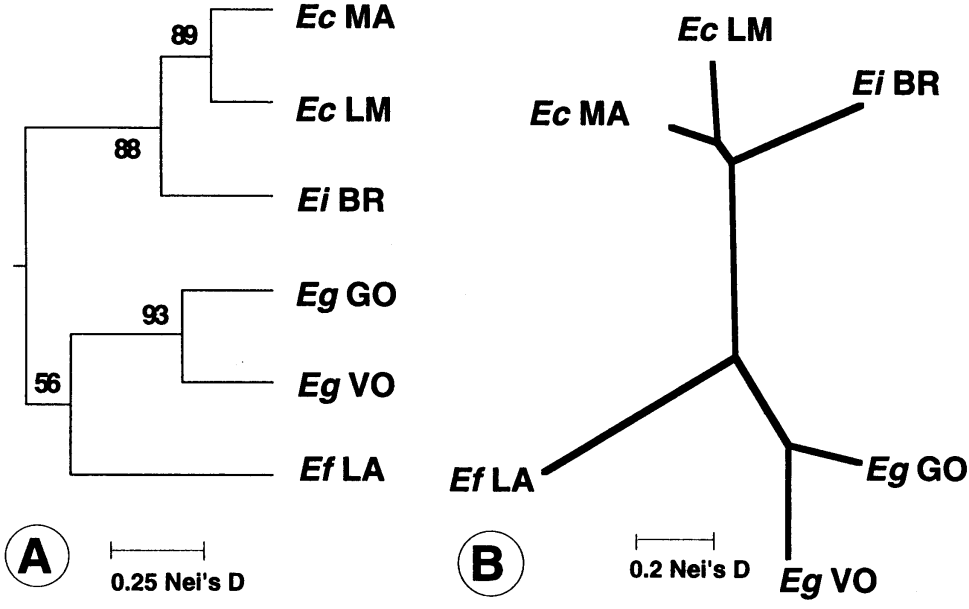

Fig. 2A. UPGMA phenogram; and 2B. Unrooted neighbor–joining network of *Euscorpius* populations using Nei's genetic distance (NEI, 1972) as a matrix. Abbreviations are given in Table I. Numbers at the nodes refer to bootstrap values out of 1,000 pseudoreplicates.

DISCUSSION

(a) Nuclear allozyme data versus mtDNA haplotype data

The two independent systems of molecular markers (allozymes and 16S mtDNA sequences) revealed an almost identical phylogenetic pattern applying methods based on models of evolutionary change (pairwise distance methods/maximum likelihood) and the maximum parsimony criterion. The only discrepancy concerned the position of *E. flavicaudis* in the UPGMA tree based on allozyme data (Fig. 2A). In contrast to the mtDNA gene trees (Fig. 1), *E. flavicaudis* clustered with the *E. germanus* group (Fig. 2A) and did not form an outgroup to the remaining *Euscorpius* species. However, this node was only weakly supported by a low bootstrap value. Whether this inconsistency reflects an almost simultaneous divergence of three major *Euscorpius* lineages (namely *E. carpathicus/E. italicus, E. germanus* and *E. flavicaudis*) should be assessed using additional phylogenetic analysis.

(b) Polyphyly of the subgenus *Euscorpius* s.str.

The first and unexpected observation in our phylogeny is that the traditional nominotypical subgenus *Euscorpius* s.str. is found to be polyphyletic. The goal of modern phylogenetic taxonomy is to avoid existence of polyphyletic genus-group taxa. Our topology splits the subgenus *Euscorpius*, as it is commonly accepted, into two clades. The first one includes the type species of the genus, *Euscorpius* (*Euscorpius*) *carpathicus*, and forms a sister group to the subgenus *Polytrichobothrius*. Another clade is represented in our study by two populations of *Euscorpius* (*Euscorpius*) *germanus* from northern Italy and Switzerland.

We propose to solve this taxonomic problem by restricting the nominotypical subgenus *Euscorpius* s. str. and by establishing a **new subgenus** based on *Euscorpius germanus* as a type species. This new subgenus would embrace studied populations of *E. germanus* and might in future include other closely related subspecies and species, such as *E. mingrelicus* (Kessler, 1876) recently reestablished by BONACINA (1980).

We preserve two monotypic Birula's subgenera in their entirety. The monotypic subgenus *Tetratrichobothrius*, which is based on *E. flavicaudis*, forms an outgroup to all other three subgenera in DNA analysis. On the other hand, the monotypic subgenus *Polytrichobothrius*, which is based on *E. italicus*, is a terminal taxon which forms a sister group of the subgenus *Euscorpius* s.str (Figs. 1–2).

(c) Proposed taxonomic changes

The taxonomic changes we propose are to introduce a new subgenus within *Euscorpius* Thorell, 1876, as follows:

Alpiscorpius Gantenbein *et al.*, **subg. nov.**, type species *Scorpius germanus* C. L. Koch, 1837, now *Euscorpius germanus* (C. L. Koch, 1837). Etymology: name derived from the Alpine mountain belt of Europe where the type species is found.

Diagnosis of *Alpiscorpius* , subg. nov.

Morphological characters. Ventral aspect of the pedipalp patella (*Tv* series) usually with 5 to 6 trichobothria. Metasomal carinae reduced to obsolete. Sensilla of pectinal organs elongated (BONACINA, 1980), in the type species reaching 20 micrometers in length (FET & BROWNELL, 1998).

Molecular characters. According to allozyme analysis and to the mitochondrial 16S rRNA nucleotide sequence of their type species, *Alpiscorpius* **subg. nov.** forms a sister group to the clade which includes type species of the subgenera *Euscorpius* Thorell, 1876 and *Polytrichobothrius* Birula, 1917.

Taxa included. The type species, *Euscorpius germanus* (C. L. Koch, 1837), was described from « southern Tirol (i.e. modern Trentino – Alto Adige in Italy) and northern Italy »; see FET & BRAUNWALDER (1997) for the detailed taxonomic history. This species traditionally included several subspecific taxa with very complicated and unclear relationships (DI CAPORIACCO, 1950). BONACINA (1980) limited *E. germanus* (C. L. Koch) to the Alpine regions of Italy (from Piemonte in the West to Goriziano in the East), Switzerland, and Austria, plus some Balkan populations; and reestablished as a « good » species *E. mingrelicus* (Kessler, 1876) (the Eastern Mediterranean area) (BONACINA, 1980; FET, 1993). We include only type species, *E. germanus* in the new subgenus *Alpiscorpius*. Position of *E. mingrelicus* remains to be determined.

Amended scope of the nominotypical subgenus. The type species of the genus *Euscorpius* Thorell, 1876 is *E. carpathicus* (Linnaeus, 1767) which is the only species

we currently include in the subgenus *Euscorpius* s. str. The species *E. carpathicus* includes a great number of subspecific taxa (DI CAPORIACCO, 1950; VACHON & JAQUES, 1977; FET & SISSOM, in press), many of them originally described and often treated as species, especially from geographic isolates such as the Baleares, Sardinia, Sicily, Crete, Crimea etc. (e.g. BIRULA, 1917; KINZELBACH, 1975; VACHON, 1975, 1981; FET, 1986, 1997). KINZELBACH (1975) suggested that many Balkan and Aegean populations of *E. carpathicus* are comprised by two sibling species, and/or their hybrids. It is beyond the scope of this paper to analyze phylogeny of the « *E. carpathicus* » complex but it is possible that in contains many « good » species.

(d) Trichobothrial patterns and position of the subgenus *Polytrichobothrius* Birula, 1917

Before the advent of chaetotaxy (trichobothrial pattern) analysis, taxonomy of *Euscorpius* was extremely confusing and was based mainly on morphosculpture and coloration characters. Already KRAEPELIN (1899) and BIRULA (1917) started to use large and conspicuous trichobothria of *Euscorpius* series *Vm* and *Tv* as taxonomic characters. This character set was further studied by HADZI (1931 etc.) and DI CAPORIACCO (1950) who used overall trichobothrial counts on different aspectes of pedipalp chela and patella [commonly called « tibia »; see SISSOM (1990) for correct terminology]. Later, VACHON (1963, 1975, 1981) demonstrated the value of trichobothrial charactes in *Euscorpius* in more detail, considering separate « series » of trichobothria and changes within those. It is also possible that overly detailed attention given to the trichobothrial patterns has obscured search for other diagnostic characters.

HADZI (1931) regarded « polytrichy », or presence of multiple trichobothria (versus their smaller number designated as « mesotrichy » or « oligotrichy ») in *Euscorpius* as a primitive character. This suggestion was accepted by DI CAPORIACCO (1950) and CURCIC (1972). Obviously, as pointed in FET (1986), there are at least two choices: either gain or loss of trichobothrial number; without strong support from other data sets, there is no particular reason to choose between one or another. Moreover, most authors addressed polytrichy « in general », as an overall trend, and not necessarily addressed the exact trichobothrial series (*Tv*) used by Birula to diagnose subgenera.

Depending on this treatment of polytrichy as primitive or derived condition, the subgenus *Polytirichobothrius* (orignally erected for *E. italicus*) was considered either an « ancestral » group (i.e. outgroup) to two other subgenera (HADZI, 1931) or a derived group compared to *Euscorpius* s.str. (KINZELBACH, 1975). The last author (KINZELBACH, 1975, Abb. 19) suggested the following topology for *Euscorpius* species (based solely on trichobothrial data) : ((*E. germanus*, *E. carpathicus*), (*E. italicus*, *E. flavicaudis*)). KINZELBACH (1975) was the only author who abolished the subgenus *Tetratrichobothrius* and placed *E. flavicaudis* in subgenus *Polytrichobothrius*. He also considered *E. germanus* an « ancestral » species of all other *Euscorpius* and suggested a « theory of mixed characters » for a hybridogenic origin of species he included in *Euscorpius* s.str. Our DNA and allozyme data do not confirm any of hybridogenic suggestions.

Our phylogeny which is based on DNA and allozyme data, strongly suggests quite a different topology: (((*E. carpathicus*, *E. italicus*), *E. germanus*), *E. flavicaudis*) (Figs. 1–2). Here, the subgenus *Tetratrichobothrius* forms an outgroup to all other taxa, while *Polytrichobothrius* is a sister group to *E. carpathicus* (L.). Thus *E.* (*Polytrichobothrius*) *italicus* (Herbst) becomes in fact one of the most derived taxa, therefore the notion of « ancestral polytrichy » for the genus *Euscorpius* does not apply. Also, in recent years, more variations in trichobothrial number and patterns have been discovered in *Euscorpius* species which show considerably wider limits of variation within each

species than was thought before (Table III). For example, there are oligotrichous populations of both *E. flavicaudis* (BONACINA & RIVELLINI, 1986) and *E. italicus* (VACHON, 1981; LACROIX, 1991b). With inclusion of some polytrichous populations of *E. carpathicus* (HADZI, 1931; DI CAPORIACCO, 1950; VACHON & JAQUES, 1977) the number of *Tv* trichobothria seems essentially overlapping between *E. flavicaudis* (10–13), *E. carpathicus* (7–12) and *E. italicus* (11–13). In fact, the only « polytrichous » trichobothrial series truly gained in *E. italicus*, besides the *Vm* series, is in the *esba* series on the external aspect of pedipalp patella (Table 3). This series varies widely in number from 4 to 11 trichobothria in most of the studied populations from Italy to Caucasus, but is absent in some Peloponnesos (Greece) populations (VACHON, 1981, fig. 13). Therefore, the only major trichobothrial character distinguishing Peloponnesos populations of *E. italicus* from *E. carpathicus* is the original Birula's character: the number of *Vm* trichobothria.

KINZELBACH (1975) considered low number of *Vm* trichobothria a plesiomorphic character. We agree with this statement; it is true for other genera of Euscorpiidae (SOLEGLAD, 1976) and for most other represetatives of « chactoid » scorpion families (SISSOM, 1990). According to our phylogeny, subgenera *Euscorpius* and *Alpiscorpius* preserve this plesiomorphic number. Therefore, gain of the accessory *Vm* trichobothria in *Tetratrichobothrius* (one) and *Polytrichobothrius* (three to six) could be regarded as two independent events rather than a synapomorphic gain (KINZELBACH, 1975) or a trend in « gradual loss » of the original high number (HADZI, 1931). It is thus interesting that our molecular-data phylogeny accommodates two conflicting trends for trichobothrial number change in different clades: gain in *Vm* and *esba/esb* series in *Polytrichobothrius*, and loss in the *Tv* series in *Alpiscorpius*.

(e) Phylogenetic implications for biogeography

Historical biogeography of *Euscorpius* recently has been included in a comprehensive cladistic analysis of the Mediterranean biota (OOSTERBROEK & ARNTZEN, 1992). However, the primary data on scorpions in this study have been taken from KINZELBACH (1975) who proposed a phylogeny based solely on trichobothrial data.

The branching pattern in our phylogeny (Figs. 1–2) implies a different sequence of speciation events. The first evolutionary split is between the *Tetratrichobothrius* clade (which leads to the modern *E. flavicaudis*) and other groups. *E. flavicaudis* is the only species of *Euscorpius* with a rather narrow range, currently an endemic of the Western Mediterranean area. It is presently found only in the eastern Spain (with the Baleares), southern France (with Corsica), Italy, and coastal North Africa. It is also the only species of *Euscorpius* which is not found in the Balkan Peninsula. This peculiar biogeographic pattern was detected by OOSTERBROEK & ARNTZEN (1992, fig. 12) for several other animal groups, and interpreted as the most ancient split between Iberian/Italian lineages versus younger, Asia Minor – Transmediterranean (ATM) lineages. Such vicariance was observed in six other animal groups, including newts, some butterflies and crane flies by OOSTERBROEK & ARNTZEN (1992) who indicated that Iberian/Italian elements are older than the ATM lineages. We might suggest that, as in the listed groups, ancestors of *Euscorpius* could be once (at the beginning of the Tertiary) limited to the western part of the Mediterranean region.

OOSTERBROEK & ARNTZEN (1992) suggested that major events of *Euscorpius* evolution probably happened from Early to Middle Miocene (20–10 MYBP). Our phylogeny, since it contains the single distinct Iberian/Italian clade (subgenus *Tetratrichobothrius*), indicates possible existence of even deeper split between the major *Euscorpius* lineages. Similar Miocene events (early evolution in the Western

Mediterranean and further dispersal eastward) are suggested for newts, and are confirmed by both fossil and molecular data.

Further interpreting our phylogeny, we should notice the split between the endemic Alpine clade (*Alpiscorpius*) and the major ATM lineage (*Euscorpius* + *Polytrichobothrius*). This leads to the assumption that ecological differentiation of the ancestors of modern *E. germanus* as orophylic and mesophylic animals (as opposed to xerophylic *E. carpathicus* and especially *E. italicus*) could have been an ancient event. In other words, modern forms inhabiting the Alpine zone of Europe are not necessarily a result of speciation due to the recent (Pleistocene) glacial events (KLICKA & ZINK, 1997) but could well have been evolving in this area since the beginning of the Alpine orogenesis. This time scale can explain high genetic divergence we observe in the *Alpiscorpius* clade, especially when contrasted with the (*Euscorpius* + *Polytrichobothrius*) clade.

According to our phylogeny, *Polytrichobothrius* is the most derived taxon of all four subgenera. Recent discovery of an oligotrichous population of *E. italicus* (with a typical *esba* series completely missing) from the Peloponnesos (Greece) (VACHON, 1981; LACROIX, 1991b) could indicate that ancestral forms of *E. italicus* have evolved in the Balkan Peninsula from where they later spread westward (as far as Italy and Switzerland) and eastward (Turkey to Caucasus). Similar biogeographic scenario could be true for another « ATM lineage » species, *E. carpathicus* with its current center of diversity in the Balkan Peninsula (KINZELBACH, 1975).

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REFERENCES

AVISE, J. C., 1994. Molecular markers, natural history and evolution. New York, Chapman & Hall.

AVISE, J., W. S. NELSON & H. SUGITA, 1994. A speciational history of « living fossils » : molecular evolutionary patterns in horseshoe crabs. Evolution, 48 (6) : 1986–2001.

AYALA, F. J., POWELL, J. R., TRACEY, M. L., MOURÃO, C. A. & S. PEREZ-SALAS, 1972. Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics, 70 : 113–139.

BIRULA, A. A. (Byalynitsky-Birula, A.A.), 1917. Arachnoidea Arthrogastra Caucasica. Pars I. Scorpiones. Mémoires du Musée du Caucase, Tiflis: Imprimerie de la Chancellerie du Comité pour la Transcaucasie, A (5), 253 pp. (in Russian). English translation: 1964. Arthrogastric Arachnids of Caucasia. 1. Scorpions. Jerusalem: Israel Program for Scientific Translations, 170 pp.

BONACINA, A., 1980. Sistematica specifica e sottospecifica del complesso « *Euscorpius germanus* » (Scorpiones, Chactidae). Riv. Mus. civ. Sci. nat. « E. Caffi », 2 : 47–100.

BONACINA, A., 1982. Note preliminari sulla sistematica sottospecifica di *Euscorpius italicus* (Herbst) (Scorpiones, Chactidae). Riv. Mus. civ. Sci. nat. « E. Caffi », 4 : 3–16.

BONACINA, A. & G. RIVELLINI, 1986. *Euscorpius flavicaudus cereris*: nuova sottospecie dell'Italia meridionale (Scorpiones, Chactidae). Riv. Mus. civ. Sci. nat. « E. Caffi », 10 : 73–78.

CAPORIACCO, L. di., 1950. Le specie e sottospecie del genre « *Euscorpius* » viventi in Italia ed in alcune zone confinanti. Atti Accad. naz. Lincei Memorie (ser. 8), 2 : 159–230.

CLARY, D. O. & D.R. WOLSTENHOLME, 1985. The mitochondrial DNA molecule of *Drosophila yakuba* : Nucleotide sequence, gene organization and genetic code. J. Mol. Evol., 22 : 252–271.

CLAYTON, J. W. & D.N. TRETIAK, 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Can., 29 : 1169–1172.

CRUCITTI, P., 1993. Distribution and diversity of Italian scorpions. Redia, 76 (2) : 281–300.

CURCIC, B. P. M., 1972. Considerations upon the geographic distribution and origin of some populations in the genus *Euscorpius* Thorell (Chactidae, Scorpiones). Rapp. Comm. int. Mer Medit., 21 (3) : 83–88.

FELSENSTEIN, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol., 17 : 368–376.

FELSENSTEIN, J., 1995. PHYLIP (Phylogeny Inference Package), Version 3.57c. Seattle, University of Washington.

FET, V., 1986. Notes on some *Euscorpius* (Scorpiones: Chactidae) from Greece and Turkey. Riv. Mus. civ. Sci. nat. « E. Caffi », 9 : 3–11.

FET, V., 1993. Notes on *Euscorpius mingrelicus* (Kessler, 1874) from the Caucasus. Riv. Mus. civ. Sci. nat. « E. Caffi », 16 : 1–8.

FET, V., 1997. A note on *Euscorpius carpathicus* (Scorpiones: Chactidae) from the Crimea. J. Arachnol., 25 (1) : 106–108.

FET, V. & M. E. BRAUNWALDER, 1997. On the true authorship and taxonomic history of *Euscorpius germanus* (C. L. Koch, 1837) (*nec* C. L. Koch, 1836; *nec* Schaeffer, 1766) (Scorpiones: Chactidae). Bull. Br. Arachnol. Soc., 10 (8) : 308–310.

FET, V. & P.H. BROWNELL, 1998. Morphological variation in the pectinal sensory organ in scorpions. Abstracts/XIV International Congress of Arachnology/22nd Annual Meeting of the American Arachnological Society (Chicago) : 16.

FET, V. & D. V. RECHKIN, 1990. Scorpion trichobothriotaxy: a principal component analysis. Riv. Mus. civ. Sci. nat. « E. Caffi », 14 : 191–206.

FET, V. & W. D. SISSOM (in press). Family Euscorpiidae. *In*: Fet, V. *et al.* (eds.), Catalog of the Scorpions of the World (1758–1998). New York Entomological Society, New York.

FET, V. & R. M. VEZZETTI, 1994. PCR amplification of mitochondrial DNA from preserved scorpions. Ancient DNA Newsletter, 2 (1) : 14.

GANTENBEIN, B., L. BÜCHI, M. E. BRAUNWALDER & A. SCHOLL, 1998. The genetic population structure of *Euscorpius germanus* (C. L. Koch) (Scorpiones: Chactidae) in Switzerland. *In*: Selden, P. A. (ed.), Proceedings of the 17th European Colloquium of Arachnology, Edinburgh 1997 : 33–40.

HADZI, J., 1931. Der Artbildungsprozess in der Gattung *Euscorpius* Thorell. Arch. Zool. Ital., 16 (1–2) : 356–362 (IX Congrès International de Zoologie).

HIGGINS, D. G., A. J. BLEASBY & R. FUCHS, 1991. CLUSTAL V: improved software for multiple sequence alignment. Cabios, 8 : 189–191.

KIMURA, M., 1980. A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol., 16 : 111–120.

KINZELBACH, R., 1975. Die Skorpione der Agäis. Beiträge zur Systematik, Phylogenie und Biogeographie. Zool. Jb. (Syst.), 102 (1) : 12–50.

KLICKA, J. & R.M. ZINK, 1997. The importance of recent ice ages in speciation : A failed paradigm. Science, 277 : 1666–1669.

KLUGE, A. G. & J. S. FARRIS, 1969. Quantitative phyletics and the evolution of anurans. Syst. Zool., 18 : 1–32.

KRAEPELIN, K., 1899. Scorpiones und Pedipalpi. *In* F. Dahl (ed.), Das Tierreich. Herausgegeben von der Deutschen Zoologischen Gesellschaft. Berlin: R. Friedländer und Sohn Verlag, 8 (Arachnoidea): 1–265.

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

LACROIX, J.-B., 1991b. Faune de France (Arachnida: Scorpionida). 6e note. 2A. Sub-genus (*Polytrichobothrius*) Birula, 1917. Arachnides, 10 : 14–25.

MIYAMOTO, M. M., S. M. TANHAUSER & P. J. LAIPIS, 1989. Systematic relationships in the artiodactyl tribe Bovini (family Bovidae) as determined from mitochondrial DNA sequences. Syst. Zool., 38 (4) : 342–349.

NEI, M., 1972. Genetic distance between populations. Am. Nat., 106 : 283–292.

NEI, M., 1987. Molecular Evolutionary Genetics. Columbia Univ. Press, New York.

NEI, M., J. C. STEPHENS, & N. SAITOU, 1985. Methods for computing the standard error of branching points in an evolutionary tree and their application to molecular data from humans and apes. Mol. Biol. Evol., 2 : 66–85.

OOSTERBROEK, P. & J. W. ARNTZEN, 1992. Area-cladograms of Circum-Mediterranean taxa in relation to Mediterranean palaeogeography. J. Biogeogr., 19 : 3–20.

RUTTKAY, H., M. SOLIGNAC & D. SPERLICH, 1992. Nuclear and mitochondrial ribosomal RNA variability in the *obscura* group of *Drosophila*. Genetica, 85 : 143–179.

SAITOU, N. & M. NEI, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4 : 406–425.

SCHOLL A., B. CORZILLIUS & W. VILLWOCK, 1978. Beitrag zur Verwandtschaftsanalyse altweltlicher Zahnkarpfen der Tribus Aphaniini (Pisces, Cyprinodontidae) mit Hilfe elektrophoretischer Untersuchungsmethoden. Z. zool. Syst. Evolut.-forsch., 6 : 116–132.

SIMON, C., F. FRATI, A. BECKENBACH, B. CRESPI, H. LIU & P. FLOOK, 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Amer., 87 (6) : 651–701.

SISSOM, W. D., 1990. Systematics, biogeography and paleontology. *In* G. A. Polis (ed.), Biology of Scorpions, pp. 64–160. Stanford, California: Stanford University Press.

SOLEGLAD, M. E., 1976. A revision of the scorpion subfamily Megacorminae (Scorpionida : Chactidae). Wasmann J. Biol., 34 (2) : 251–303.

STOCKWELL, S. A., 1992. Systematic observations on North American Scorpionida with a key and checklist of the families and genera. J. Med. Entomol., 29 (3) : 407–422.

SWOFFORD, D. L., 1993. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1.1. Laboratory of Molecular Systematics, Smithsonian Institution, Washington, DC.

SWOFFORD. D. L., G. J. OLSEN, P. J. WADDELL & D. M. HILLIS, 1996. Phylogenetic Inference. *In:* Hillis, D. M., C. Moritz & B. K. Mable (eds.), Molecular Systematics (2nd edition). Sinauer, Sunderland, MA.

VACHON, M., 1963. Remarques sur l'utilisation, en systématique, des soies sensorielles (trichobothries) chez les Scorpions du genre *Euscorpius* Thorell (Chactidae). Bull. Mus. natn. Hist. nat. Paris (2) 34 : 347–354.

VACHON, M. 1975. Recherches sur les Scorpions appartenant ou déposés au Muséum d'Histoire naturalle de Genève. I. Contribution à une meilleure connaissance des espèces et des sous–espèces de Scorpions du genre « *Euscorpius* » Thorell, 1876 (Fam. des Chactidae). Rev. Suisse Zool., 82 (3) : 629–645.

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

VACHON, M., 1981. Remarques sur la classification sous–spécifiques des espèces appartenant au genre *Euscorpius* Thorell, 1876 (Scorpionida, Chactidae). C. R. VIème Coll. Arachnol. express. française, Modena–Pisa 1981, Atti Soc. Tosc. Sci. Nat., Mem., ser. B, 88 (suppl.) : 193–203.

VACHON, M. & M. JAQUES, 1977. Recherches sur les Scorpions appartenant ou déposés au Muséum d'Histoire naturelle de Genève. 2. Contribution à la connaissance de l'ancienne espèce *Scorpius banaticus* C. L. Koch 1841, actuellement considerée comme synonyme de *Euscorpius carpathicus* (Linné 1767) (Fa. des Chactidae). Rev. Suisse Zool., 84 (2) : 409–436.

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Table I. Sampling sites and specimens used for DNA and allozyme analysis.

Species	Sampling site	Country	Specimens analyzed DNA	Specimens analyzed Allozymes	Abbreviation in analysis
E. carpathicus	Mathis	France, Vaucluse	1	27	*Ec* MA
	La Morra	Italy, Piemonte	1	12	*Ec* LM
E. italicus	Brissago	Switzerland, Ticino	1	10	*Ei* BR
E. germanus	Gondo	Switzerland, Valais	1	9	*Eg* GO
	Völs	Italy, Trentino	1	10	*Eg* VO
E. flavicaudis	Lauris	France, Vaucluse	1	33	*Ef* LA
Outgroup species					
V. spinigerus	Yuma	USA, Arizona	1	-	*Vs*
H. concolor	Bahia de Los Angeles	Mexico, Baja California	1	-	*Hc*
C. exilicauda	Bahia de Los Angeles	Mexico, Baja California	1	-	*Ce*

Table II. Distance matrix of calculated Nei's distance (NEI, 1972) (upper right) and of Kimura's distance (KIMURA, 1980) (lower left). The DNA study included three additional outgroups for comparison.

	Ec MA	*Ec* LM	*Ei* BR	*Eg* GO	*Eg* VO	*Ef* LA	*Hc*	*Vs*	*Ce*
***Ec* MA**	-	0.35	0.70	1.08	1.37	1.70	-	-	-
***Ec* LM**	0.02	-	0.79	1.33	1.38	1.78	-	-	-
***Ei* BR**	0.06	0.06	-	1.49	1.64	1.48	-	-	-
***Eg* GO**	0.14	0.14	0.12	-	0.52	1.20	-	-	-
***Eg* VO**	0.10	0.10	0.09	0.10	-	1.15	-	-	-
***Ef* LA**	0.13	0.13	0.10	0.13	0.10	-	-	-	-
Hc	0.31	0.30	0.29	0.32	0.30	0.27	-	-	-
Vs	0.25	0.23	0.23	0.26	0.22	0.25	0.24	-	-
Ce	0.40	0.41	0.38	0.43	0.41	0.38	0.46	0.46	-

Table III. Trichobothrial patterns in *Euscorpius*. Abbreviations : *Vm*: ventral series on the manus. *Tv*: ventral (inferior) series on the patella (= « tibia »). Groups of trichobothria in the external (posterior) series on the patella (= « tibia »): *et* – terminal, *est* – subterminal, *em* – median, *esba* – suprabasal « a », *esb* – suprabasal, *eba* – basal « a », *eb* – basal. Data compiled from : BONACINA, 1980, 1982; BONACINA & RIVELLINI, 1986; DI CAPORIACCO, 1950; FET, 1986, 1993, 1997, unpublished data; FET & RECHKIN, 1990; LACROIX, 1991a,b; SOLEGLAD, 1976; VACHON, 1963, 1975, 1978, 1981; VACHON & JAQUES, 1977.

TAXA	*V m*	*T v*	*et*	*est*	*e m*	*esba*	*esb*	*eba*	*eb*
"chactoid" type, e.g. *Vaejovis*	4	2	4	1	2	-	2	-	5
Megacorminae	4	6-12	3-4	4	3-5	-	2	3-4	3
Euscorpius flavicaudis (DeGeer, 1778)	6	10-13	6-8, rarely 9	4	4-5	-	2	6	5
E. italicus (Herbst, 1800)	8 to 11	11-13 rarely 10, (14-18?)	5-9	4	5, rarely 4, 6	4-11 (0 in Peloponnesos)	2 (+2)	4-8	3-4
E. carpathicus (Linnaeus, 1767)	4	7-12, rarely 6, 13-14	5-8, rarely 3-4, 9	4, rarely 3, 5	4 (3 in *E.c. banaticus*)	-	2	4-6	4-5
E. mingrelicus (Kessler, 1874)	4	6-7, rarely 5	4-6	4	3	-	2	4	4
E. germanus (C. L. Koch, 1837)	4	5-6	4	4	3	-	2	3-4	4

Appendix I : DNA sequences of the 16S rRNA gene used for this study. Abbreviations are given in Table I.

***Ec* MA**
TGGCACAGACTTCCTTAATTTTATTATTGCATGAAAAAGGTTTCTTAATCCAACATCGAG
GTCACAAACTTTCTT?ACGATGAGAACTCTTTAAGAAAATAATGCTGTTATCCCTATAGT
AACTTATTCTTTTTTAAAAATTTTTTGATTTTTCAAGAATTATATTCACATTATTAAAATAA
AATTTTATTTATCTACTGCCCCAGTAAAATAATATTTAATTTATGTTGATATTAAATTATAA
TTATAAAGTTTAATAGGGTCTTTTTGTCTTTAACGTTAATTTTAGCTTTTTTACTAAAATAT
AAAATTTGAAATATAGTAATAAGACATGAATATTTAGTTAAACCATTCATTCCAGTCCTA
AATTAAAAGACTA

***Ec* LM**
GACAGACTTCCTTAGTTTTATTATTGCATGAAAAAGGTTTCTTAATCCAACATCGAGGTC
ACAAACTTTCTTGATGATAAGAACTCTTTAAGAAAATAATGCTGTTATCCCTATAGTAAC
TTATTCTTTTTTAGAAATTTTTTGATTTTTCAAGAATTATATTCACATTATTAAAATAAAATT
TTATTTATCTACTGCCCCAGTAAAATAATTTTTAGTTTATGTTGATATTAAATTATAATTAT
AAAGTTTAATAGGGTCTTTTTGTCTTTAATGTTAATTTTAGCTTTTTTACTAAAATATAAAA
TTTGAAATATAGTAATAAGACATGAATATTTAGTCAAACCATTCATTCCAGTCCTAAATTA
AAAGACTA

***Ei* BR**
CGACAGCTCCTTAATTTTATTATTGCATGATAAAGGTATCTTAATCCAACATCGAGGTCA
CAAACTTTCTTGATGATAAGAACTCTTTAAGAAAATTATGCTGTTATCCCTATAGTAACTT
ATTCCTTTTTAAAAATTTTTTGATTTTTCAAGAATTGTATTCATATTATAAAAATAAAATTT
TTATTTATTTACTGCCCCAGTAAAATAATTTTTAATTTATTCTGATATTAAATTATAATTAT
AAAGCTTAATAGGGTCTTCTTGTCTTTAATGTTAATTTTAGCTTTTTTACTAAAATATAAA
ATTTGAAGTATAATAATAAGACATGATTAATTAGTTAAACCATTCATTCCAGTCCTAAATT
AAAAGACTA

***Eg* GO**
GCTTCCTTATTTTATTATTGCATGAAATAGGTATCTTAATCCAACATCGAGGTCACAAAC
TTTCTTGATGATAAGAACTCTTTAAGAAAATTATGCTGTTATCCCTATAGTAACTTGTTCT
TTTTTAAAAACTTTTTGGTTTTTCAAGAGTTATCCTCACATTATTAAAATAAAATTTTATTT
ATTTACTGCCCCAGTAAAATACTTCGTGGTTTATATTGATGTTAAATTAGAATTATAAAG
CTTAATAGGGTCTTCTTGTCTTTAAAATAAATTTTAGCCTTTTTACTAA?CTGTAAAATTT
GAAGTATAATAATAGGACAGGATTATTTGGTTAAACCATTCATTCCAGCCCTCAACTAC
AGC

***Eg* VO**
TCGAACAGACTTCCTTATTTTATTATTGCATGAAATAGGTATCTTAATCCAACATCGAGG
TCACAAACTTTCTTGATGATAAGAACTCTTGAAGAAAATTATGCTGTTATCCCTATAGTA
ACTTATTCTTTTTTAAAAATTTTTTGATTGTTCAAGAGTTATTCCCACGTTATTGAAATAAA
ATTTTATTTATTTACTGCCCCAGTAGAATAATTTTTAGTTTATGTTGATATTAAATTGAGA
TTATAAAGCTTAATAGGGTCTTCTTGTCTTTAAAATGGATTTTAGCTTTTTTACTAAAATA
TAAAATTTGAAGTATAATAATAAGACATGATTATTTAGTTAAACCATTCATTCCAGTCCTA
AATTACAAGACTA

***Ef* LA**
TCGAACAGACTCCCTTATTTTATTATTGCATGAAATAGGGCTCTTAATCCAACATCGAG
GTCACAAACTTTCTTGATGATAAGGACTCTTAAAGAAAATTATGCTGTTATCCCTACAGT
AACTTGTTCCTTCTTAAGAAATTTTTGATTTTTCAAGAGTTGTACTCACATTATATAAATA
AAATTTTATTTATTTACTGCCCCAGTAAAATAATTTTTTAACTATGTTGATGTTAGATTTTT
AATTATAAAGCTTGATAGGGTCTTCTTGTCTTTAATAAGAATTTTAGCTTTTTTACTAAAA

TATAAAATTTGAAGTATAATAATAAGACAGGAGTACTCAGTTAAACCATTCATTCCAGTC
CTAAATTACAAGACTA

Hc

TCGAACAGACTTCCTTAGTAAGCTATTGCGCCAACTAGGACTTTTAATCCAACATCGAG
GTCACAAAAATTTTTGATGATAAGAACTCTTAAAAAAAAATTATGCTGTTATCCCTACAGT
AACTTATTCCTTATTAAAAACTCCTCGATTTTACAAGATTAGAAATCACAATATAAAAATG
ATATTAACTCATTAACTGCCCCAGTTAAACAATTTCACCCTTGATTTGAAAACAAAAGAT
TAATTGTAAAGCTTGATAGGGTCTTCTCGTCCCTAAACAAAATTTTAGCTTTTTTACTAA
ATGATAAAATTCAAAATATTTAAATAAGACAGAAATATTCAGTAAAACCCTTCATTCCAG
TCCCAAATTAAGAGACTA

Vs

TCGAACAGACTTCCTTATTTCATTATTGCACGAATTAGGAATTTTAATCCAACATCGAGG
TCACAAACTTTTTTGATGATAAGAACTCTCTAAAAAAAATTATGCTGTTATCCCTACAGTA
ATTAATTTTTTAATTAAAAACTTTTTGATTTTTCAAGATTTATTTATCATAATTTAAAAATAAA
AAATTTTTATCTGCTGCCCCAGCAAAACAATTTTAAGTTTAGTTTGAAAATAAACTCTGG
ATTGTTAAACTTGATAGGGTCTTCTCGTCCCTATTAGAAATTTTAGCTTTTTTACTAAATT
ATTAAATTCAAAATATTATTATGAGACAAAAATATTTAGTCAAGCCCTTCATTCCAGTCCT
TAATTAGAAGACAA

Ce

TCGAACAGACTCCTTTCATTCCTCTTGCGGAATGAAGGAAATTTAATCCAACATCGAGG
TCGCAAACATACTTGTCGATTTGAGCTTTCGAAGTATATTACGCTGTTATCCCTAAAGTA
ACTTATTTAAACTTCAAAAATTTTGGGTATTAAGATAATGTTATCCTCATGCTCTAAAAGT
GTTTTTCTTTCTACCGCCCCAGTAAAACATATTTTTAATTTATTAAATTATTTTATGTAAAG
CTTTATAGGGTCTTCTTGTCTAAAAGAAACATTTTAGCCTTTTTACTAAAAAGTAAAATTC
AAAAGAAAAAGTTAAGAAAGAAACTCTTCAGTTTATCCCTTCATTCCAGTCTTAAATTAC
AAGACTA

Appendix II : Allele frequencies at 18 gene loci and sample sizes of analyzed *Euscorpius* populations.

Species		*E. flavicaudis*	*E. germanus*		*E. italicus*	*E. carpathicus*	
Sampling site		Lauris	Gondo	Völs	Brissago	Mathis	La Morra
(Sample size)		(33)	(9)	(10)	(10)	(27)	(12)
Locus	Allele						
AAT-1	123					1.00	
	107						1.00
	100	1.00					
	96		1.00	0.05			
	90				1.00		
	78			0.95			
AAT-2	117				1.00	0.94	1.00
	113		1.00				
	110					0.06	
	107			1.00			
	100	0.97					
	88	0.03					
ALPDH	105						0.04
	100	1.00		0.40	1.00	1.00	0.96
	95		1.00	0.60			
ARK	100	1.00	1.00	1.00		1.00	0.96
	93				1.00		0.04
DDH	102		1.00	1.00	1.00	0.04	
	101					0.96	1.00
	100	1.00					
GAPDH	100	1.00	1.00	1.00	1.00	1.00	1.00
GTDH	100	1.00			1.00	1.00	1.00
	90		1.00	1.00			
HK	100	1.00	1.00	1.00			
	93				1.00	1.00	1.00
IDH-1	100	0.91				0.04	
	95	0.09	1.00	1.00			
	93					0.26	
	90				1.00	0.70	1.00
IDH-2	100	1.00					
	93		1.00		1.00	1.00	
	87			1.00			1.00
MDH-1	100	1.00		1.00		0.04	
	89		1.00		1.00	0.96	1.00
MDH-2	100	1.00	1.00	1.00			
	89				1.00	0.96	1.00
	77					0.04	
MPI	135			1.00			
	107		0.83				
	104						1.00
	101				1.00		
	100	1.00	0.17				
	94					1.00	
PEP	107					1.00	0.33
	104			1.00	1.00		
	100	0.87					
	98		1.00				0.67
	94	0.13					
6-PGD	100	0.65					
	97		1.00	1.00		1.00	1.00
	88	0.35					
	87				1.00		
PGI	100	1.00			1.00		
	97					0.20	1.00
	93		1.00	1.00		0.80	
PGM	100	1.00					
	98		0.11	0.10			
	94		0.06	0.20			
	93					0.96	
	91		0.83	0.70			
	89					0.04	1.00
	80				1.00		
PK	103				1.00	1.00	1.00
	100	1.00	1.00				
	98			1.00			