

***Euscorpius balearicus* Caporiacco, 1950, stat. nov. (Scorpiones:Euscorpiidae): molecular (allozymes and mtDNA) and morphological evidence for an endemic Balearic Islands species**

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Abstract

The geographic variation of the circum-Mediterranean scorpion species *Euscorpius carpathicus* (L.) was traditionally analysed using morphological characters such as trichobothrial patterns, which resulted in the recognition of 23 subspecies; however, the biological reality of these subspecies remains unclear. Here, we focus on populations from the western Mediterranean and provide new molecular evidence that those from the island of Mallorca (Balearic Islands, Spain) represent a highly divergent lineage separate from *E. carpathicus* from the mainland of France (Vaucluse) and Italy (Liguria and Piemonte). This divergence is evidenced by morphological analysis. Moreover, allozyme and mtDNA divergences (about 10%) agree with our hypothesis that the Balearic island populations became isolated from the mainland about 5 Ma BP since the refilling of the Mediterranean Basin and have to be considered autochthonous. This hypothesis is additionally supported by the comparison of the genetic differentiation between artificially transplanted island populations and mainland populations in the congeneric species *E. flavicaudis* (de Geer). The phylogenetic species concept (PSC) is applied to elevate the subspecies *E. carpathicus balearicus* Caporiacco, 1950 to species rank. A lectotype is designated for this species.

Key words: scorpions, island populations, endemic species, allozymes, 16S mtDNA sequences, trichobothria, phylogeny

Introduction

Traditionally, the species of the genus *Euscorpius* Thorell, 1876 (Scorpiones: Euscorpiidae) were distinguished by using the patterns of trichobothria of the pedipalp. Currently, nine valid species exist in the genus *Euscorpius*: *E. alpha* Caporiacco, 1950; *E. beroni* Fet, 2000; *E. carpathicus* (Linnaeus, 1767); *E. gamma* Caporiacco, 1950; *E. germanus* (C. L. Koch, 1837); *E. mingrelicus* (Kessler, 1874), *E. flavicaudis* (de Geer, 1778); *E. italicus* (Herbst, 1800); and *E. tergestinus* (C. L. Koch, 1837) (Fet 2000, Fet & Sissom 2000, Gantenbein et al. 2000a, Scherabon et al. 2000). The taxonomic sta-

tus and phylogenetic relationships among these taxa were confusing when based on the traditional morphological characters. However, recent phylogenetic studies using nuclear and mitochondrial markers (Gantenbein et al. 1999, 2000b; Scherabon et al. 2000; Huber et al. 2001) suggested an unexpected (from a morphological point of view) evolutionary history for six of the above-mentioned species. Unfortunately, samples from *E. beroni*, *E. mingrelicus* and *E. tergestinus* were not yet available in these analyses and would be urgently needed in future studies.

Among the *Euscorpius* species, *E. carpathicus* is the most diverse, with 23 formally valid subspecies (Capori-

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acco 1950, Fet & Sissom 2000). Some of the subspecies refer to island populations (e.g., *E. c. balearicus* di Caporiacco, 1950: Balearic Islands; *E. c. candiota* Birula, 1903: Crete); in these cases their geographic ranges are very clearly defined. However, other subspecies refer to mainland populations; their geographic ranges are very confusing, and the biological validity of the taxa remains questionable; it is unclear if some subspecies are sympatric and if hybridisation occurs (Fet & Braunwalder 2000). Clinal variation in number of trichobothria on the ventral side of the pedipalp patella has been used to derive phylogenetic relationships among different populations (Vachon 1962, Curcic 1972, Valle 1975). However, morphological patterns are not necessarily correlated (Hillis 1987), and there have been no objective criteria to distinguish whether polytrichy or oligotrichy is pleiomorphic (Gantenbein et al. 1999).

Here we focus on the species' island populations on Mallorca (= *E. c. balearicus*, Balearic Islands) and compare their genetic and morphological differentiation with western Mediterranean populations from the mainlands of France and Italy. We analysed the genetic variation in nuclear markers (18 allozyme loci) and mitochondrial (mt) markers (~350 bp 16S mtDNA sequences) as in previous studies (Gantenbein et al. 1998, 1999). Along with the molecular analyses we provide a morphological study of the Balearic populations.

In order to evaluate the divergence status of the Balearic populations we compare the genetic differentiation between Balearic and mainland populations in *E. carpathicus* with the mainland-to-island differentiation in the congeneric species *E. flavicaudis*. Since it is known that scorpions can be easily transplanted artificially (Vachon 1981, Goyffon 1992, Kritscher 1992), and the *E. flavicaudis* population on Corsica is most probably not native (= allochthonous), we make predictions about the status of the *E. carpathicus* populations on the Balearic Islands by comparing the genetic divergences. Furthermore, we evaluate the inter-island morphological differentiation by comparing populations from the island of Mallorca with populations from the Cabrera Islands (Pons & Rambla 1993).

Material and methods

Specimens used for molecular analyses

The western Mediterranean sampling sites are shown in Fig. 1. Samples are numbered as in Table 1. Austrian samples are not shown here but were illustrated in Fig. 1 of Huber et al. (2001). From the island of Mallorca (Balearic Islands, Spain) we collected samples from Calvia and Banyalbufar. For comparison of 'eastern' Mediterranean populations we also included two population samples from the island of Crete (Greece), from

Vai (coll. I. Stathi) and Kallikratis (coll. A. Widmer) (not shown in Fig. 1). The corresponding EMBL sequence accession numbers are also listed in Table 1.

Specimens used for morphological analysis

In addition to the lectotype specimen (see below), the following 89 specimens were used for morphological analysis:

Spain: Balearic Islands (circa 1974–1996; courtesy of Dr. G. X. Pons):

Cabrera Archipelago: Cap Llebeig, 1 adult female; Cova de na Boixa, 1 subadult and 1 juvenile female; Cova des Burri, 1 subadult male; Cova des Cap Ventos, 1 adult female; Es Burri, 1 juvenile; Estell des Coll,

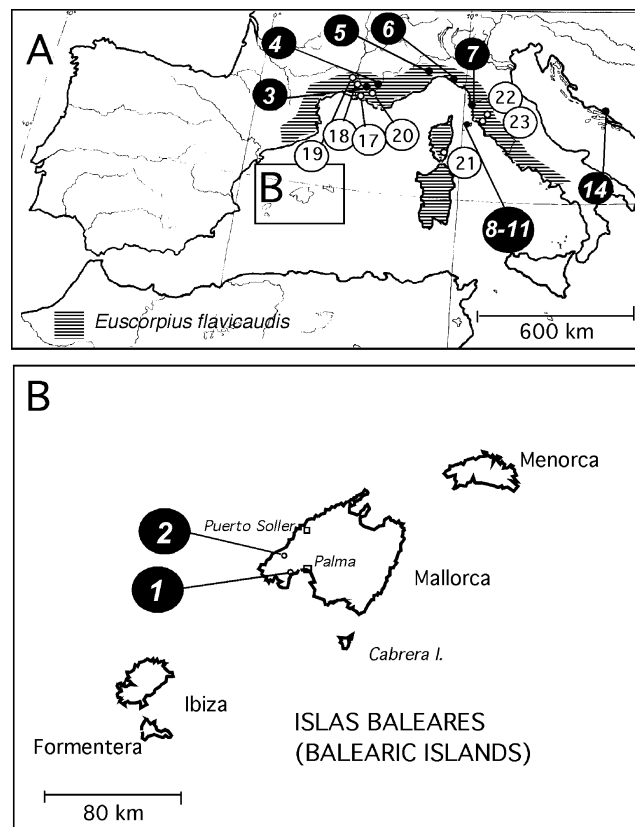


Figure 1. Map of the western Mediterranean showing sampling sites of *Euscorpium* populations. *E. balearicus* stat. nov.: B1 Banyalbufar, B2 Calvia (Spain, Balearic Islands), *E. carpathicus*: 3 Mathis, 4 Peyruis (both France, Vaucluse), 5 La Morra (Italy, Piemonte), 6 Vernazza (Italy, Liguria), 7 Castellina (Italy, Tuscany), 8 Valdana, 9 Procchio, 10 Lacona, 11 Monte Perone (all Italy, Island of Elba), 14 Mala Duba (Croatia), *E. flavicaudis*: 17 Marignane (France, near Marseille), 18 Lauris, 19 Ardèche, 20 Balazuc (all France, Vaucluse), 21 Bocca dell'Oro (France, Island of Corsica), 22 Casino di Terra, 23 Riparbella (both Italy, Tuscany) (not shown are 12 Krems, 13 Hochosterwitz (both Austria) and 15 Kallikratis, 16 Vai (both Greece, island of Crete). Shaded area represents geographic range of *E. flavicaudis* according to Kinzelbach (1975).

Table 1. Taxa, populations, and details on samples for the biochemical analyses. Asterisks mark island populations. References are 1 Gantenbein et al. (1999), 2 Huber et al. (2001), 3 Gantenbein et al. (2000a).

Taxon	Pop. No.	Population	Country, region	Sample size (n) Allozymes	mtDNA	Haplotype	EMBL accession number	Reference	coll.
<i>E. balearicus</i> Caporiacco stat. nov <i>E. carpathicus</i> (L.)	1	Calvia	Spain, Mallorca*	12	2	EbCA1-2	AJ309209, AJ309210	this work	A. Scholl
	2	Banyalbufar	Spain, Mallorca*	11	1	EbBA1	AJ309208	this work	A. Scholl
	3	Mathis	France, Alpes Maritimes	27	3	EcMA1	AJ389376	1	A. Scholl
	4	Peyruis	France, Alpes Maritimes	13	1	EcMA1	ditto	1, 2	A. Scholl
	5	La Morra	Italy, Piemonte	12	2	EclM1	AJ389377	2	B. & I. Gantenbein
	6	Vernazza	Italy, Liguria	12	1	EcVE1	AJ298062	–	A. Scholl
	7	Castellina	Italy, Tuscany	3	–	–	–	–	A. Scholl
	8	Valdana	Italy, Elba*	2	–	–	–	–	A. Scholl
	9	Procchio	Italy, Elba*	15	2	EcPR1	AJ309211	this work	A. Scholl
	10	Lacona	Italy, Elba*	12	–	–	–	–	A. Scholl
	11	Monte Perone	Italy, Elba*	3	–	–	–	–	A. Scholl
	12	Hochosterwitz	Austria, Carinthia	2	2	EcHO1	AJ298065	2	D. Huber
	13	Krems	Austria, E Tyrolia	3	2	EcHO1	ditto	2	D. Huber
	14	Mala Duba	Croatia	3	2	EcMD1-2	AJ298063, AJ298064	2	A. Widmer
15	Kallikratis	Greece, Crete*	2	2	EcKA1-2	AJ309213, AJ309214	this work	A. Widmer	
16	Vai	Greece, Crete*	5	2	EcVA1	AJ309212	this work	I. Stathi	
<i>E. flavicaudis</i> (de Geer)	17	Lauris	France, Alpes Maritimes	49	1	EFLA1	AJ389381	1, 2, 3	A. Scholl
	18	Marignane	France, Alpes Maritimes	18	–	–	–	–	M. Lörtscher
	19	Ardèche	France, Alpes Maritimes	17	–	–	–	–	J. Rüetschi
	20	Balazuc	France, Alpes Maritimes	31	–	–	–	–	A. Scholl
	21	Bocca dell'Oro	France, Corsica*	5	1	ER11	AJ309215	this work	B. & I. Gantenbein
	22	Casino di Terra	Italy, Tuscany	3	–	–	–	–	A. Scholl
	23	Riparbella	Italy, Tuscany	9	1	ER11	AJ309215	this work	A. Scholl

1 subadult and 1 juvenile female; Estell de s'Esclata-sang, 1 juvenile; Estell de Fora, 1 juvenile; Estell de sa Teula, 1 juvenile male; Estell Xapat de Llevant, 1 adult male and 1 juvenile; Estell Xapat de Ponent, 1 subadult male and 1 juvenile; Ets Malgrats, 1 subadult and 1 juvenile male; Illa de ses Rates, 2 juvenile females and 1 subadult male; Illa des Conills, 1 juvenile; Illa de sa Torre, 1 adult female and 1 adult and 2 subadult males; L'Esponja, 1 juvenile; L'olla - Clot des Guix, 1 adult female; Monument als Francesos, 2 adult and 1 juvenile female, 2 subadult males and 1 juvenile; Na Foradada, 1 subadult female and 1 juvenile; Na Redona, 1 adult juvenile male and 2 juveniles; Penyal Blanc, 2 adult females and 1 adult male.

Mallorca: Banyalbufar, 3 adult and 2 subadult females, 1 subadult male, and 2 juveniles; Biniaraix, 1 adult female and 1 subadult male; Binibona, 1 adult female; Cala Sanutges, 1 adult male; Calvia, 1 subadult female and 1 subadult male; Cap Blanc, 1 adult male; Comuna de Biniamar, 1 adult female; Comuna de Bunyola, 1 juvenile female; Comuna de Caimari, 1 adult female, 1 juvenile male and 1 juvenile; Cuber, 1 subadult male; Font des Guix, 1 subadult female; Font des Noguer, 1 juvenile; Illa de Formentor, 1 adult female; Manut, 1 adult female; Mortix, 1 adult male; Puerto Alcudia, 1 adult and 1 subadult female and 1 juvenile male; Puig de sa Font, 1 subadult male; Puig de Santuiri, 3 adult females; Puigpunyent, 1 adult female; Sa Torre Nova, 1 adult female; Serra de Tramuntana, 1 adult female and 1 subadult male; Son Moragues, 1 adult and 1 juvenile female and 1 subadult male; Son Pocos, 1 juvenile male.

Menorca: Cap de s'Indio, 1 subadult male and 1 juvenile; Illa den Colom, 1 adult female, 1 subadult male and 1 juvenile; Monte Toro, 1 juvenile.

Laboratory methods

All specimens were killed by deep-freezing and stored at -80°C 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. 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* (Lauris, Vacluse). Due to high resolving power, differences in electromorph mobility could be traced up to 1 mm.

Genomic DNA was extracted from fresh or preserved (ethanol 94–98%) muscle tissue (usually pedipalp or metasoma) using a standard phenol/chloroform and precipitation method. We amplified a fragment (~450 bp) of the 16S mitochondrial (mt) rRNA, using the same primers and PCR conditions as in except that MgCl_2 concentration was increased to 2.75 mM to achieve a higher stability of the PCR, and that PCR products were

purified with the QiagenTM quick purification kit that allows easy one-step concentrating of the product.

Cycle sequencing reactions this time were performed with Pharmacia BiotechTM AmershamTM Sequencing Kit for LI-CORTM. We followed strictly the instructions of the manufacturer and sequenced ~350 bp using the same primer and cycling profile as in Gantenbein et al. (1999). Fragments were resolved on the automated sequencer (LI-COR model 4200), and all sequences were checked manually for sequencing errors.

Morphological analyses

For morphological analysis and redescription, we used standard techniques as given in Sissom (1990). Trichobothrial patterns, important in *Euscorpium* classification, were interpreted according to the conventions in Vachon (1981). All measurements are given in mm. Statistical data presented in the 'Taxonomy' section is broken down as follows: minimum – maximum (mean) (\pm standard deviation) [number of samples]: {corrected minimum – corrected maximum (mean \pm standard deviation)} \rightarrow coefficient of variability (standard deviation / mean).

Statistical and phylogenetic analyses

Allele frequencies at allozyme loci were calculated using Genepop 3.1d (Raymond & Rousset 1995). Exact tests for Hardy-Weinberg equilibrium (HWE) at loci were calculated for each population using the algorithm implemented in Genepop. Level of significance for multiple independent tests was adjusted using the Bonferroni procedure (Rice 1989). Furthermore, within-population genetic variability estimates, i.e. the mean number of alleles per locus, the percentage polymorphism (0.95 criterion), and the observed and expected heterozygosity were calculated using Biosys-2 (Swofford & Selander 1989). The distribution of allozyme diversity was quantified as *F*-statistics (Weir & Cockerham 1984) using the analysis of molecular variance model (AMOVA; Excoffier et al. 1992) in Arlequin 2.000 (Schneider et al. 2000) among *E. carpathicus* and *E. flavicaudis* samples. For phylogenetic analyses of allozymes we calculated the chord distance (Cavalli-Sforza & Edwards 1967) and used these distances for construction of a phenogram by the Neighbour-Joining algorithm (NJ) (Saitou & Nei 1987). Confidence in tree topology was assessed by re-sampling 1,000 pseudo-replicates (Felsenstein 1985). All phylogenetic analyses of allozyme data were calculated with PHYLIP 3.57c (Felsenstein 1995).

25 mtDNA sequences representing different haplotypes were aligned using Clustal X (Higgins et al. 1991) and by eye. New haplotypes are listed in Table 1, along with sequences from earlier studies that were retrieved

from the EMBL database. All ambiguities and gaps were omitted as described in Swofford et al. (1996), resulting in 347 characters remaining. Identical haplotypes (Table 1) were not considered in further analyses. In order to select the most appropriate DNA model of nucleotide substitution we calculated hierarchic likelihood ratio test statistics using the program Modeltest 3.06 (Posada & Crandall 1998) which is implemented in PAUP* 4.0b8 (Swofford 1998) and tests 56 different substitution models based on a NJ tree using Jukes-Cantor (1969) distances. Details about likelihood ratio tests are given in Huelsenbeck & Crandall (1997) and in Huelsenbeck & Rannala (1997). The HKY85 + Γ model (Hasegawa et al. 1985) was selected. The 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). In a further step, the molecular clock hypothesis (i.e., equal rates across all sequences) was tested using the χ^2 approximated likelihood ratio test statistics with OTUs -2 degrees of freedom ($df = 15$ minus $2 = 13$) which was not rejected with a P -value of 0.98. Therefore, we explored the tree space by 100 heuristic tree searches and by randomising the order of the sequence input using the clock enforcement option in PAUP*. For Maximum Parsimony (MP) analysis we omitted all ambiguities but included five gaps (ending up with 352 bp in the alignment). The latter were treated as a 'fifth' base since we are convinced that this information ought to be considered in cladistical analyses especially for ribosomal RNA sequences (McGuire et al. 2001). Transitions (ti) were down-weighted relative to transversions (tv)

according to the ML-estimated ti/tv ratio, which was about 3:1 in favour of ti (see 'Results'). Tree search was performed by the branch-and-bound algorithm. Tree stability of best trees was evaluated by calculating the consistency index excluding uninformative sites (CIu), and the retention index (RI) (Kitching et al. 1998).

In all phylogenetic trees *Euscorpius (Tetratrichobothrius) flavicaudis* was used as an outgroup; its outgroup position to the subgenus *Euscorpius (Euscorpius)* has been demonstrated in the recent molecular study of Gantenbein et al. (1999).

DNA sequence availability. All sequences have been deposited in the EMBL nucleotide sequence database (<http://www.ebi.ac.uk>) (accession numbers in Table 1). The sequence alignment file ALIGN_000241 in nexus format can be obtained from the EMBL alignments database.

Results

Allozyme variation

The allele frequencies of all included *Euscorpius* populations are given in Appendices 1 and 2. Generally, a population is fixed for an allele at many loci. The two samples of *E. balearicus*, stat. nov. from the Island of Mallorca have private (= unique) alleles at six out of 18 gene loci (*Aat-1*, *Mdh-1*, *Mdh-2*, *Mpi*, *6-Pgd*, *Pep*, Appendix 1) which isolates these populations clearly from all *E. carpathicus* samples. However, the other included island populations, from Elba and Crete, are also distin-

Table 2. Pairwise Cavalli-Sforza (1967) chord distances (above diagonal), and pairwise F_{ST} values (Weir & Cockerham 1984) (below diagonal) between *E. balearicus*, stat. nov. (lines 1, 2) and *E. carpathicus* samples. Asterisks are given for F_{ST} values that are not significant at the $P = 0.05$ level.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 Calvia	–	0.02	0.38	0.41	0.37	0.43	0.41	0.36	0.41	0.39	0.39	0.46	0.43	0.51	0.40	0.60
2 Banyalbufar	0.16	–	0.35	0.39	0.37	0.43	0.42	0.35	0.40	0.36	0.36	0.45	0.42	0.51	0.42	0.62
3 Mathis	0.87	0.85	–	0.04	0.26	0.15	0.24	0.18	0.23	0.19	0.19	0.13	0.12	0.27	0.36	0.49
4 Peyruis	0.90	0.87	0.27	–	0.30	0.20	0.26	0.23	0.28	0.23	0.23	0.16	0.15	0.29	0.37	0.48
5 La Morra	0.91	0.89	0.86	0.90	–	0.22	0.20	0.28	0.33	0.38	0.38	0.28	0.25	0.26	0.42	0.54
6 Vernazza	0.84	0.82	0.68	0.71	0.74	–	0.19	0.20	0.26	0.31	0.31	0.20	0.18	0.26	0.44	0.52
7 Castellina	0.89*	0.85	0.78	0.83	0.77	0.62*	–	0.27	0.32	0.36	0.36	0.33	0.30	0.52	0.41	0.48
8 Valdana	0.92	0.88	0.84	0.88	0.92	0.70	0.84	–	0.05	0.10	0.10	0.26	0.24	0.51	0.38	0.52
9 Procchio	0.95	0.94	0.86	0.93	0.96	0.84	0.98	1.00	–	0.05	0.05	0.31	0.29	0.61	0.38	0.52
10 Lacona	0.95	0.92	0.82	0.91	0.96	0.84	0.97	1.00	1.00	–	0.00	0.37	0.34	0.72	0.38	0.52
11 Monte Perone	0.91	0.87	0.78	0.86	0.94	0.76	0.90	1.00	1.00	–	–	0.37	0.34	0.72	0.38	0.52
12 Hochosterwitz	0.91	0.87	0.69	0.75	0.91	0.62*	0.83*	0.97	0.99	0.99	0.97*	–	0.01	0.38	0.43	0.51
13 Krems	0.90	0.86	0.65	0.71	0.89	0.60	0.81*	0.89	0.97	0.97	0.92*	-0.19*	–	0.34	0.39	0.51
14 Mala Duba	0.92	0.89	0.82	0.86	0.91	0.72*	0.87	0.95	0.99	0.99	0.96	0.90*	0.84*	–	0.46	0.60
15 Kallikratis	0.88	0.84	0.84	0.87	0.92	0.78*	0.80	0.89	0.98	0.97	0.91*	0.86	0.83	0.88	–	0.21
16 Vai	0.92	0.90	0.89	0.91	0.93	0.84	0.88	0.92	0.98	0.97	0.93*	0.91	0.89	0.92	0.72	–

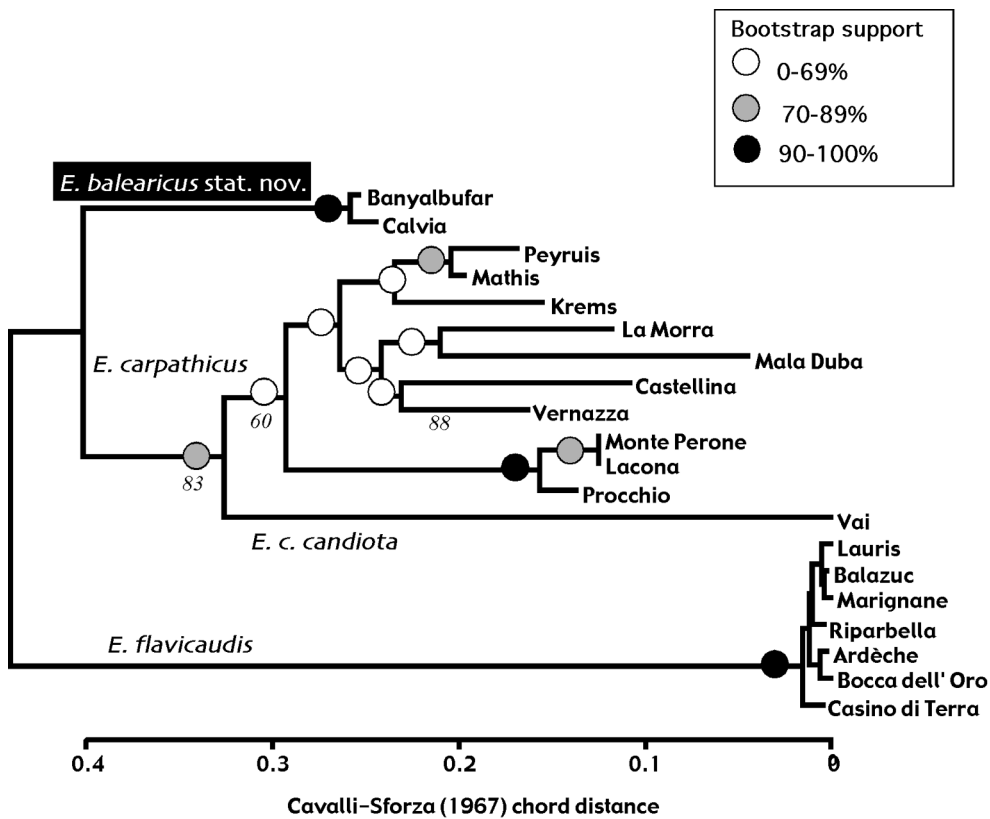


Figure 2. Neighbour Joining (NJ) tree of *Euscorpis balearicus* stat. nov. and *E. carpathicus* samples ($n \geq 3$) using chord distance as an input matrix. Distances are based on 18 allozyme loci. Circle colour at nodes refers to bootstrap support calculated over 1,000 pseudo-replicates (inlet). Numbers at nodes refer to bootstrap values. The tree was rooted using *E. flavicaudis*.

guished by private alleles: one locus (*Gapdh*) for the three samples from Elba, and five to six (*Idh-1*, *Idh-2*, *Gtdh*, *Mdh-2*, *Pep*, *6-Pgd*) loci for the two samples from Crete (Vai, Kallikratis). Genetic differentiation among *E. carpathicus* is also considerable, e.g., the southern Croatian sample (Mala Duba) was fixed for private alleles at three loci. Beyond this, the mainland samples vary mainly in gene frequencies but are not fixed for private alleles.

8 out of 52 exact tests for Hardy-Weinberg equilibrium deviate significantly at the $P = 0.05$ level. However, there are no deviations observed after Bonferroni-correction ($P_{adjusted} = 0.00096$, $k = 52$). The mean heterozygosity is low for both observed and expected heterozygosity (0.03 ± 0.02 and 0.05 ± 0.03 , respectively). The mean proportion of polymorphism is 11%, which corresponds to about 2 out of 18 loci being polymorphic within all population samples. These genetic variability estimates are in agreement with previous studies on *Euscorpis* based on much smaller data sets (Gantenbein et al. 1998, 1999).

Pairwise F_{ST} values between *E. balearicus*, stat. nov. and *E. carpathicus* populations range from zero to the maximum value, and therefore show a population structure significantly differentiated into small metapopulations (Table 2). The majority of pairwise F_{ST} values are

significant at the 0.05 level (Table 2). F_{ST} over all 16 *E. carpathicus* populations is estimated to 0.87 ($P < 0.0000$). The overall F_{ST} value among mainland populations remains high if the island populations from Mallorca, Elba and Crete are excluded ($F_{ST} = 0.77$, $P < 0.0000$). Such high F_{ST} values can be explained by the fixation of alternative alleles at the polymorphic loci between many samples (Appendix). Pairwise genetic distances ranged from 0 to 0.60 (Table 2), which confirms a high genetic differentiation of all samples. F_{ST} among all seven *E. flavicaudis* populations was considerably lower but was still significant ($F_{ST} = 0.13$, $P < 0.0000$). If the two populations of *E. balearicus* stat. nov. (Calvia and Banyalbufar) are compared with the *E. carpathicus* populations they are always separated by high values of F_{ST} and genetic distance (0.82–0.92 and 0.38–0.60, respectively).

A phylogram based on the genetic variation of 18 allozyme loci is presented in Fig. 2. The estimated phylogeny confirms *E. balearicus* stat. nov. (Banyalbufar and Calvia) always as a highly separated split-off from all other *E. carpathicus* populations (bootstrap support 83%). Within the *E. carpathicus* clade the island population from Crete (Vai) is also clearly isolated from all other included *E. carpathicus* samples. The phylogenetic relations among the remaining populations within the *E. carpathicus* clade are less clear. The island popula-

tions from Elba (Procchio, Lacona, Monte Perone) are genetically moderately separated from the mainland samples from France (Mathis, Peyruis) and Italy (La Morra, Vernazza, Castellina). The genetic differentiation within *E. flavicaudis* is very low compared with *E. balearicus* and *E. carpathicus* samples. This is true even for the island population from Corsica (Bocca dell'Oro).

mtDNA analysis

To estimate pairwise distances between 15 haplotypes (Table 3), we used two distance measures: uncorrected "p" and Maximum Likelihood (ML) distances using the HKY85 + Γ substitution model with ML-estimated parameters (base frequencies: $\pi_A = 0.37$, $\pi_C = 0.11$, $\pi_G = 0.11$, and $\pi_T = 0.41$; $\alpha = 0.18$; transition (ti) / transversion (tv) ratio = 3.29 ($\kappa = 9.38$). The tree shape parameter (of the gamma distribution was estimated to 0.18, which indicates strong heterogeneity of mutation rates across sites. These distances among *Euscorpius* haplotypes range from 0 to 0.10 and 0.14, respectively. The haplotypes from Mallorca (Calvia and Banyalbufar) are isolated from all other included *E. carpathicus* sequences by average distances of about 8% (uncorrected) and 10% (HKY85 + Γ), respectively. The estimated phylogenies based on Maximum Likelihood (ML) and weighted Maximum Parsimony (MP) revealed similar tree topologies (Fig 3A and B). The heuristic tree search using ML revealed a single tree island (tree score $-\ln L$ of 972.5). The branch-and-bound search revealed eight equally parsimonious trees with 109 steps. High tree stability of the eight cladograms is indicated by relatively high tree scores

(CIu = 0.78, RI = 0.87). In both phylogenies the three *E. balearicus* stat. nov. haplotypes are clearly separated from the *E. carpathicus* clade, by high bootstrap values at the nodes (Fig. 3). The three included haplotypes of *E. carpathicus candiota* Birula, 1903 from Crete are also separated from the remaining *E. carpathicus* sequences. Within the latter clade the phylogenetic relations are less clear.

Taxonomy

L. di Caporiacco (1950) gave a very brief description of his subspecies from the Balearic Islands, which is not sufficient at the modern level of scorpion taxonomy. No more detailed description was given by any of the few authors who have mentioned the Balearic subspecies since (Vachon & Jaques 1977, Lacroix 1991, Dupré 1997). Kinzelbach (1975) considered the Balearic populations as belonging to „*Euscorpius mesotrichus* Hadzi, 1929“. This name is a junior homonym and is not available (Fet & Sissom 2000). The identity of Kinzelbach's species is currently under study by the present authors. Below, we provide a morphological redescription of the Balearic taxon, which is hereby formally assigned species status (see molecular analyses for justification). A female lectotype is designated.

***Euscorpius balearicus* Caporiacco, 1950, stat. nov.**

Euscorpius carpathicus balearicus Caporiacco, 1950: 187, 227; Vachon & Jaques (1977: 431); Bartolozzi et al. (1988: 295); Lacroix (1991: 19); Dupré (1997: 15); Fet & Sissom (2000: 361–362).

Table 3. Distance matrix of the sequence divergence (uncorrected *p*) (upper right) and of HKY85 + Γ (lower left) calculated from pairwise comparisons of 16S mtDNA sequences in *Euscorpius*. Distances in boldface are given for comparisons between *E. balearicus*, stat. nov. and other sequences. See Table 1 for abbreviations of haplotypes. Gaps were not considered.

	<i>EbBA1</i>	<i>EbCA1</i>	<i>EbCA2</i>	<i>EcMA1</i>	<i>EcLM1</i>	<i>EcVE1</i>	<i>EcPR1</i>	<i>EcHO1</i>	<i>EcMD1</i>	<i>EcMD2</i>	<i>EcVA1</i>	<i>EcKA1</i>	<i>EcKA2</i>	<i>EfRI1</i>	<i>Efla1</i>
<i>EbBA1</i>	–	0.01	0.01	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.10	0.10
<i>EbCA1</i>	0.01	–	0.00	0.08	0.08	0.07	0.08	0.08	0.07	0.08	0.07	0.08	0.08	0.10	0.10
<i>EbCA2</i>	0.01	0.00	–	0.08	0.08	0.07	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.10	0.10
<i>EcMA1</i>	0.15	0.15	0.14	–	0.02	0.02	0.02	0.04	0.03	0.03	0.07	0.07	0.07	0.12	0.12
<i>EcLM1</i>	0.15	0.14	0.15	0.03	–	0.03	0.03	0.04	0.03	0.03	0.06	0.07	0.07	0.12	0.12
<i>EcVE1</i>	0.14	0.13	0.14	0.03	0.04	–	0.03	0.03	0.03	0.03	0.06	0.07	0.07	0.11	0.11
<i>EcPR1</i>	0.15	0.14	0.15	0.02	0.03	0.03	–	0.03	0.03	0.03	0.06	0.07	0.07	0.11	0.11
<i>EcHO1</i>	0.15	0.14	0.15	0.05	0.05	0.04	0.03	–	0.02	0.02	0.05	0.06	0.06	0.10	0.10
<i>EcMD1</i>	0.14	0.13	0.14	0.04	0.04	0.03	0.04	0.02	–	0.00	0.05	0.05	0.06	0.11	0.11
<i>EcMD2</i>	0.14	0.14	0.14	0.04	0.04	0.03	0.04	0.02	0.00	–	0.05	0.06	0.06	0.11	0.11
<i>EcVA1</i>	0.14	0.14	0.14	0.11	0.10	0.10	0.10	0.08	0.08	0.08	–	0.05	0.04	0.12	0.11
<i>EcKA1</i>	0.15	0.14	0.15	0.12	0.10	0.11	0.11	0.09	0.08	0.09	0.07	–	0.01	0.11	0.12
<i>EcKA2</i>	0.14	0.13	0.14	0.12	0.10	0.11	0.10	0.08	0.09	0.09	0.06	0.01	–	0.11	0.11
<i>EfRI1</i>	0.23	0.23	0.23	0.25	0.24	0.24	0.23	0.19	0.22	0.22	0.27	0.23	0.22	–	0.00
<i>Efla1</i>	0.23	0.23	0.23	0.25	0.24	0.24	0.23	0.19	0.22	0.22	0.26	0.24	0.23	0.00	–

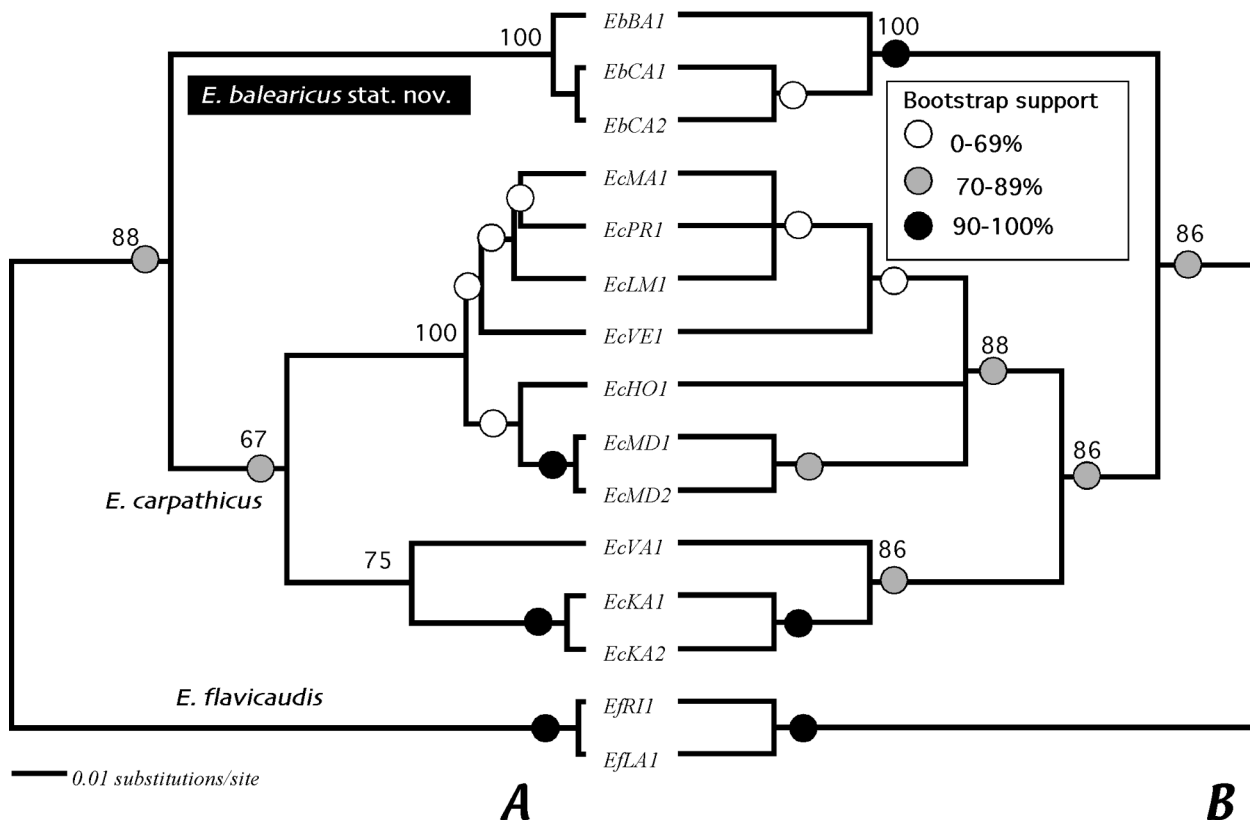


Figure 3. Phylogenies of *Euscorpis balearicus* stat. nov. and *E. carpathicus* 16S mtDNA sequences (347 bp) including *E. flavicaudis* as out-group. For explanation of haplotype abbreviations see Table 1. **A**) Maximum Likelihood (ML) tree ($-\ln L = 972.50$) using the HKY85 + (model with the ML-estimated parameters, $\pi_A = 0.37$, $\pi_C = 0.11$, $\pi_G = 0.11$, and $\pi_T = 0.41$, $\alpha = 0.18$, transition (ti) / transversion (tv) ratio = 3.23 ($\kappa = 9.38$). **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 109 steps (Clu = 0.78 and RI = 0.87). Numbers at nodes are bootstrap values in percent over 1,000 pseudo-replicates. Circle colours at the nodes refer to three classes of bootstrap support (inlet).

Type. Lectotype (designated here): subadult female, MZUF 5976 (Museo Zoologico "La Specola" dell'Università de Firenze, Florence, Italy), Puerto Soller, Mallorca (Majorca), Balearic Islands, Spain, collector unknown.

Four labels were contained in the lectotype vial: (1) Syntype (printed), (2) 5976 (handwritten, pencil), (3) *E. carpathicus* (L.) Puerto Soler (Datgean) Mayorca (hand written, ink), and (4) *Euscorpis carpathicus balearicus* Puerto Soller (Maiorca) del Musei Genova (hand written, very faint ink).

The lectotype is designated here for the purposes of nomenclatural stability as we consider this taxon valid at the species level. This designation is done in compliance with Article 74.7 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999). It is the only specimen now available from the original syntype series from Puerto Soller which, according to Caporiacco (1950: 187, 227), included three females and was deposited in MZUF.

Diagnosis. Small to medium in size, the largest mature female examined was 37 mm in length, the largest male 34 mm. Coloration is light brownish-tan with little contrasting patterns. Metasoma reduced proportionally, pedipalps unusually large (see morphometric ratio comparisons below). Metasomal carinae essentially obsolete on segments I–IV except for weakly granulated dorsal carinae. Pedipalp patellar external trichobothria numbers are: $eb = 4$, $eb_a = 4$, $esb = 2$, $em = 4$, $est = 4$, and $et = 6-10$; ventral aspect of patella 9–14. The number of trichobothria occurring in the et and ventral series are among the largest found in the genus as a whole. Pectinal tooth counts: female 6–8, male 7–9.

Female (lectotype) (Measurements from lectotype and other specimens in Table 4).

Colouration. Basic colour light brownish-tan, with some orange overtones. Carapace slightly darker than mesosoma, slight fuscous patterns visible on lateral aspects. Inner dorsal and ventral carinae of pedipalp femur

Table 4. Morphometrics (mm) of *Euscorpium balearicus* Caporiacco, stat. nov.

	Female lectotype: Mallorca, Puerto Soller	Female: Mallorca, Serra de Tramuntana	Male: Mallorca, Mortix	Male: Cabrera, Illa de sa Torre
Total length	27.05	36.80	33.50	28.45
Carapace length	3.80	6.20	5.15	4.45
Mesosoma length	12.25	13.10	12.55	9.95
Metasoma length	8.10	13.00	11.30	9.90
Metasomal segment I				
length	1.20	1.80	1.50	1.35
width	1.20	1.75	1.50	1.45
Metasomal segment II				
length	1.30	2.10	1.80	1.55
width	1.10	1.50	1.35	1.25
Metasomal segment III				
length	1.40	2.20	2.00	1.75
width	1.00	1.45	1.30	1.20
Metasomal segment IV				
length	1.70	2.60	2.35	2.00
width	0.95	1.35	1.25	1.15
Metasomal segment V				
length	2.50	4.30	3.65	3.25
width	0.90	1.40	1.30	1.10
Telson length	2.90	4.50	4.50	4.15
Vesicle length	1.90	3.15	3.45	3.15
width	0.95	1.50	1.70	1.45
depth	0.80	1.55	1.80	1.45
Aculeus length	1.00	1.35	1.05	1.00
Pedipalp length	13.65	23.40	18.95	15.60
Femur length	3.35	5.85	4.70	3.80
width	1.25	2.00	1.70	1.40
Patella length	3.45	5.60	4.55	3.75
width	1.45	2.20	1.90	1.55
Chela length	6.85	11.95	9.70	8.05
Palm length	3.40	5.95	4.80	4.15
Palm width	1.85	3.45	3.00	2.35
Palm depth	2.10	4.00	3.40	2.70
Movable finger length	4.00	7.10	6.10	4.80
Pectinal teeth	7–7	7–7	8–8	7–7
Pectinal middle lamellae	4–4	5–4+	5+–4+	3–4

and patella reddish-brown; digital carina of chela darker than palm; finger condyles, base of movable finger and finger denticles reddish. Eyes and tubercles dark brown to black, leg articulation spots reddish.

Carapace. Generally smooth and shiny at 10×; ocular lateral carinae area slightly granulose at 20×. Anterior edge essentially straight exhibiting subtle wide concave depression from lateral eyes. Two pairs of lateral eyes present, anterior largest; median eyes and tubercle small with following length and width formulas: $152/380$

(anterior edge to median tubercle center|carapace length), and $52/325$ (width of median tubercle|width of carapace at that point).

Mesosoma. Terga essentially smooth on entire surface at 10×; carinae absent on tergite VII. Sternites smooth and shiny, carinae absent on sternite V. Stigmata small, slit-like to sub-oval.

Metasoma. Generally short compared to mesosoma and pedipalp. Carinae: Segments I–IV: dorsal lateral, lateral, and inferior lateral and median essentially obso-

lete; dorsal weakly granulate, not exhibiting elongate posterior spine. Segment V: dorsal lateral rounded and weakly granulate, lateral obsolete, inferior lateral and median weakly granulate. Intercarinal area essentially smooth.

Telson. Elongate and smooth, aculeus with medium curve. 5–7 pairs of long setae on ventral aspect of vesicle.

Pectines. Length|width formula of pecten 314|147 (length taken at anterior lamellae|width at widest point including teeth). 7-7 pectinal teeth and 4-4 middle lamellae. Fulcra present, the most distal essentially obsolete. Sensory areas well developed, covering one half to two thirds of tooth surface. Small, clear setae scattered on anterior lamellae. Pectinal basal plate with slight, wide anterior indentation, length|width formula 367|168.

Genital operculum. Plates separated but connected with membrane for most of length.

Sternum. Pentagonal, wider than long, length|width formula 272|304.

Chelicerae. Movable finger: ventral distal denticle extends well beyond dorsal denticle counterpart; two subdistal denticles on dorsal edge; ventral edge lacking dentition and serrulae, but with heavy brush-like setae on distal half of edge. Fixed finger: standard with four denticles, the basal two conjoined on common base.

Pedipalps. Large compared to metasoma. Femur: dorsal internal and external, and ventral internal carinae crenulate to serrulate; ventral external rounded and granulose; ventral and external surfaces with irregular granulation, dorsal surface smooth, and internal surface with 7+ large granules. Patella: dorsal and ventral internal carinae crenulate to serrulate, dorsal and ventral external granulate, and external median rounded and irregularly granulate. Dorsal and ventral surfaces smooth and dorsal patellar spur (DPS) well developed and pointed, ventral patellar spur (VPS) very weak, represented as small granule. Chela carinae: digital strong, exhibiting slight granulation proximally; subdigital in relief, represented by 1–2 granules; dorsal secondary essentially obsolete, slightly indicated on extreme proximal aspect; dorsal marginal rounded, continuous and granulose; dorsal internal very rounded and granulose; ventroexternal strong extending to external condyle of finger, external to trichobothrium Et_i and granulose on proximal one-half; ventromedian very flat, essentially obsolete; ventrointerior rounded and granulose; and external secondary rounded and irregularly granulose. Chelal finger dentition: median denticle row straight; 6/7 inner denticles, 7/7 outer denticles, and 4/5 inner accessory denticles for fixed and movable fingers, respectively. Trichobothria patterns: type C, neobothriotaxic (major additive) on patella. Femur: trichobothrium d positioned proximal in relation to i . Patella: ventral series number 13/12 and external series number $eb = 4/4$, $eb_a = 4/4$, $esb = 2/2$, $em = 4/4$, $est = 4/4$, and $et = 8/8$. Chela: Ventral

series number 4/4, V_4 on external surface, removed from ventroexternal carina.

Legs. Two pairs of pedal spurs present, tarsal spines absent, unguis medium length with average curve. Tarsus III: ventral median spinule row formed by 6 elongated spinules; one offset pair of ventral distal spinules; 2–3 pairs of flanking setae on ventral aspect. Basitarsus I–IV: four proventral spinules on leg I, and two on leg II.

Male. Similar to female, although smaller in overall size, comparative carapace lengths from sexually mature specimens: females: 5.05–6.2 (5.37; $n = 8$), males: 4.4–5.15 (4.67; $n = 3$). Bases of chelal fingers of sexually mature males equipped with conspicuous proximal scalloping on the denticle edges, whereas in the female these edges essentially straight. Telson vesicle of sexually mature males swollen and larger in all dimensions than vesicle of mature females. Morphometric ratio comparisons involving carapace length and width and depth of the telson vesicle exhibit well over 30% difference between mean values from eight females and three males:

Carapace length/telson width: difference in mean values = 33.5%

Females

3.59–4.39 (3.919) (± 0.262) [008]: {3.66–4.18} $\rightarrow 0.067$

Males

2.71–3.07 (2.936) (± 0.197) [003]: {2.74–3.13} $\rightarrow 0.067$

Carapace length/telson depth: difference in mean values = 37.3%

Females

3.70–4.35 (3.997) (± 0.210) [008]: {3.79–4.21} $\rightarrow 0.052$

Males

2.80–3.07 (2.910) (± 0.141) [003]: {2.77–3.05} $\rightarrow 0.048$

Genital papillae present in male, extending from genital operculum posterior edge. Pectines more developed in the male, in size and in number of teeth:

Pectinal tooth counts:

Males

7–9 (7.357) (± 0.520) [056]: {6.837–7.877} $\rightarrow 0.071$

Females

6–8 (6.494) (± 0.527) [081]: {5.966–7.021} $\rightarrow 0.081$

Difference between male and female pectinal tooth counts moderate, of roughly one tooth, a mean difference of 13.3%.

Variation within species. We examined 89 additional specimens from the Balearic Islands Mallorca (43 specimens), Menorca (6 specimens), and the Cabrera Archipelago (40 specimens). Only the patellar external et series and ventral aspect exhibited variability in trichobothria numbers as follows:

Patella, *et* series:

Mallorca

6–10 (8.220) (± 0.832) [059]: {7.388–9.052} -> 0.101

Menorca

7–8 (7.417) (± 0.515) [012]: {6.902–7.932} -> 0.069

Cabrera

6–7 (6.750) (± 0.441) [028]: {6.309–7.191} -> 0.065

Cabrera Islets

6–7 (6.543) (± 0.504) [046]: {6.040–7.047} -> 0.077

Non Mallorca

6–8 (6.733) (± 0.562) [086]: {6.171–7.295} -> 0.083*E. balearicus*6–10 (7.338) (± 1.002) [145]: {6.336–8.339} -> 0.136

Patella, ventral aspect:

Mallorca

9–14 (12.129) (± 0.992) [070]: {11.137–13.120} -> 0.082

Menorca

10–11 (10.167) (± 0.389) [012]: {9.777–10.556} -> 0.038

Cabrera

9–11 (10.031) (± 0.474) [032]: {9.557–10.505} -> 0.047

Cabrera Islets

9–11 (10.064) (± 0.567) [047]: {9.496–10.631} -> 0.056

Non Mallorca

9–11 (10.066) (± 0.512) [091]: {9.554–10.578} -> 0.051*E. balearicus*9–14 (10.963) (± 1.274) [161]: {9.689–12.237} -> 0.116

There is a significant drop in the number of accessory trichobothria in specimens from the islands of Menorca, Cabrera and islets surrounding Cabrera proper compared with those found on the larger island of Mallorca. For example, the difference in mean values of the *et* series between Mallorca and the Cabrera Islets is 25.6%, roughly a 1.7 drop in trichobothria number. For ventral trichobothria counts, the mean value difference is 20.5%, a drop of more than 2 trichobothria. Figure 4 illustrates typical trichobothria patterns of the external aspect of the patella for *E. balearicus*. Note in particular the relatively large number of trichobothria in the *et* series and ventral aspect of the patella. Also important is the pattern and number of trichobothria for the other five external series, which is constant across all specimens examined.

Comparison with *Euscorpius carpathicus* (L.). The 89 *E. balearicus* specimens discussed above were compared to 53 specimens of *E. carpathicus*, including 29 specimens from France (Mathis and Peyruis), and 24 specimens from Italy (Vernazza, 15 specimens; La Morra, 2 specimens; Procchio (Elba Island), 7 specimens). Significant morphological differences between the two species were identified involving numbers of accessory trichobothria, pectinal tooth counts, morphometric ratios based on metasoma and pedipalp measurements, and carinal granulation of the metasoma.

Trichobothria. *E. balearicus* exhibited higher numbers than *E. carpathicus* in both variable trichobothria series, as follows:

Patella, *et* series:*E. balearicus*6–10 (7.338) (± 1.002) [145]: {6.336–8.339} -> 0.136*E. carpathicus*5–8 (6.291) (± 0.567) [055]: {5.724–6.858} -> 0.090

Patella, ventral surface:

*E. balearicus*9–14 (10.963) (± 1.274) [161]: {9.689–12.237} -> 0.116*E. carpathicus*8–10 (8.714) (± 0.563) [056]: {8.151–9.277} -> 0.065

For the *et* series, the difference in mean values is 16.64% roughly one trichobothrium. For the ventral series, the value difference is 25.8%, slightly more than two trichobothria.

Pectinal tooth counts. *E. carpathicus* has higher pectinal tooth counts than *E. balearicus* for both males and females, exhibiting over 12% difference in mean values:

E. balearicus

Males

7–9 (7.357) (± 0.520) [056]: {6.837–7.877} -> 0.071

Females

6–8 (6.494) (± 0.527) [081]: {5.966–7.021} -> 0.081*E. carpathicus*

Males

8–10 (8.432) (± 0.545) [044]: {7.886–8.977} -> 0.065

Females

6–9 (7.322) (± 0.571) [059]: {6.751–7.893} -> 0.078

The difference in mean values is 14.61% (roughly one pectinal tooth) in males and 12.75% (slightly less than one pectinal tooth) in females.

Morphometric ratios. The pedipalp development of *E. balearicus* is quite exceptional, especially when compared to its somewhat reduced metasoma. Following are morphometric ratio comparisons involving the chelal movable finger and fifth metasomal segment lengths, and the total lengths of the pedipalp (sum of lengths for segments femur, patella and chela) and metasoma (sum of lengths for segments I–V). Eight adult females and three sexually mature males were used in these calculations as compared to a set of six adult females and eight males from French and Italian populations of *E. carpathicus*.

Movable finger length/metasoma segment V length:

Females: 22.9% mean difference

*E. balearicus*1.56–1.67 (1.608) (± 0.039) [008]: {1.57–1.65} -> 0.024*E. carpathicus*1.26–1.37 (1.309) (± 0.042) [006]: {1.27–1.35} -> 0.032

Males: 34.6% mean difference

E. balearicus

1.48–1.67 (1.544) (± 0.110) [003]: {1.43–1.65} -> 0.071

E. carpathicus

1.10–1.19 (1.147) (± 0.030) [008]: {1.12–1.18} -> 0.026

Pedipalp length/metasoma length:

Females: 21.9% mean difference

E. balearicus

1.69–1.81 (1.752) (± 0.053) [008]: {1.70–1.81} -> 0.030

E. carpathicus

1.37–1.47 (1.437) (± 0.033) [006]: {1.40–1.47} -> 0.023

Males: 25.3% mean difference

E. balearicus

1.50–1.68 (1.585) (± 0.090) [003]: {1.50–1.67} -> 0.057

E. carpathicus

1.25–1.29 (1.265) (± 0.014) [008]: {1.25–1.28} -> 0.011

The two morphometric ratios as applied to both female and male samples exhibited 22–35% mean value differences in all four comparison sets. The pedipalp in general is longer in *E. balearicus* relative to its reduced metasoma than in *E. carpathicus*.

Metasomal carinae development. This is more pronounced in *E. carpathicus* than in *E. balearicus* whose carinae are essentially obsolete on segments I–IV except for weakly developed dorsal carinae. Table 5 contrasts the carinal development of all five metasomal segments of *E. balearicus* with two populations of *E. carpathicus*. In particular, note the development of dorsal lateral and inferior lateral carinae in *E. carpathicus* for segments I–IV, and the difference in granulation on the inferior carinae of segment V (also see Fig. 5). Also noteworthy is the presence of a smooth, single inferior median carina on segment IV of *E. carpathicus*, which is obsolete in

E. balearicus. In Fig. 5 note the subtle irregular granulation of the inferior median carina of segment V in *E. balearicus*, both male and female, as compared to male and female *E. carpathicus*. Also, the inferior lateral carinae in *E. balearicus* are barely distinguishable as compared to the crenulate carinae exhibited by *E. carpathicus*. Fig. 5 also illustrates the stark difference in pigmentation between the two species: Italian specimens of *E. carpathicus* (Fig. 5C, D) are much darker, a dark maroon to black, in contrast to *E. balearicus* (Fig. 5A, B), which are a light brownish-tan with little contrasting pigmentation.

Discussion

Genetic differentiation between island and mainland populations with respect to time

Two independent classes of inherited markers, allozymes (nuclear marker) and mtDNA sequences (a maternally inherited marker with a few exceptions (Gyllenstein et al. 1991, Wallis 1999) provide evidence that the island populations on Mallorca are highly divergent from the other included mainland and other island populations of *E. carpathicus* (Appendix 1 in electr. suppl., Tables 2–3, Figs 2–3). This divergence is confirmed by the morphological analysis (especially the morphometric ratios of the pedipalp, and colouration).

The strong genetic divergence of the island populations is evidenced by the occurrence of private alleles at six out of 18 allozyme loci (Table 1), and by a mtDNA genetic distance of ~14% (Table 3). Assuming a molecular clock rate of about 1–2% sequence divergence per Ma (Brown et al. 1979, Fleischer et al. 1998, Knowlton & Weight 1998, Gantenbein et al. 2001) we end up with

Table 5. Metasomal carinae comparisons of *E. balearicus* Caporiacco, stat. nov. and *E. carpathicus* (L.) (two populations, France and Italy).

	<i>E. balearicus</i>	<i>E. carpathicus</i> (France)	<i>E. carpathicus</i> (Italy)
SEGMENTS I–IV			
Dorsal	Weak, slightly granulose	Medium, granulose	Medium, granulose
Dorsal lateral	Obsolete	Weak, granulose anterior half, I–III	Weak, granulose anterior half, I–IV
Lateral	Obsolete	Obsolete	Obsolete
Inferior lateral	Barely visible, rounded and smooth	Distinct, smooth, III–IV	Distinct, roughly granulose, III–IV
Inferior median	Obsolete	Smooth, IV	Smooth, IV
SEGMENT V			
Dorsal lateral	Weakly granulose	Granulose	Granulose
Lateral	Obsolete	Obsolete	Obsolete
Inferior lateral & median	Weak, irregularly granulose	Distinct, crenulate to serrulate	Distinct, crenulate to serrulate

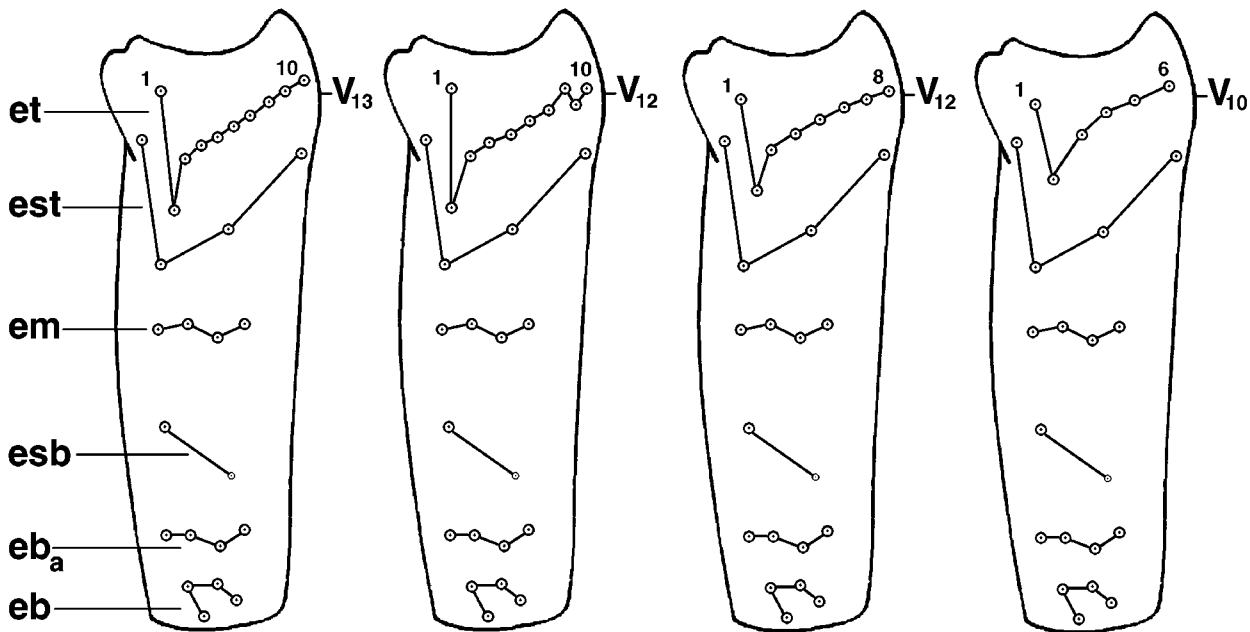


Figure 4. Idealized trichobothrial pattern of external aspect of pedipalp patella of *Euscorpium balearicus* stat. nov., showing typical distribution of the *et* series. Position and number of the terminal ventral trichobothrium is also shown. *et* = external terminal, *est* = external subterminal, *em* = external median, *esb* = external suprabaasal, *eb_a* = external basal-a, and *eb* = external basal; *V* = ventral.

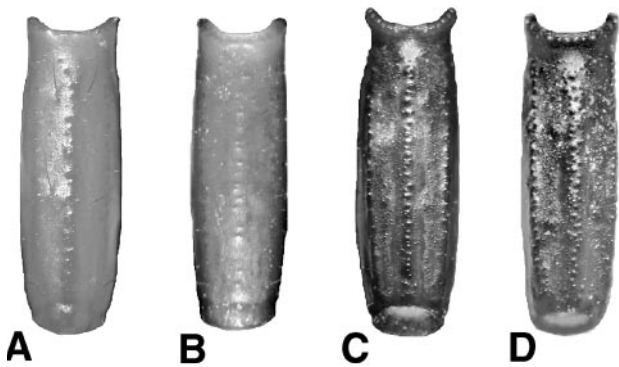


Figure 5. Metasomal segment V, ventral view, showing inferior lateral and median carinae. A, B. *Euscorpium balearicus* Caporiacco, stat. nov.; C, D. *Euscorpium carpathicus* (L.). A. male, Cabrera, Estell Xapat de Llevant; B. female, Mallorca, Serra de Tramuntana; C. male, Vernazza, Italy; D. female, Vernazza, Italy.

a divergence time of about 5 Ma BP. This time frame would be expected from geological data for the natural colonisation of these islands assuming existing land bridges between the Balearic islands and the Iberian mainland 5.6 Ma BP as a consequence of the desiccation of the Mediterranean basin (Hsü 1972, Hsü et al. 1977). However, 5.2 Ma BP the Mediterranean was refilled within a short time (~100 yrs), and populations have been kept isolated from the mainland populations ever since. Although this hypothesis of colonisation might be

simplistic and evidence for reticulate biogeographical patterns has recently been shown in beetles (Palmer & Cambefort 2000), it can explain our observed genetic divergence. This pattern of colonisation is also supported if the genetic differentiation between island and mainland populations in *E. flavicaudis* is considered. The island population from Corsica (Bocca dell’Oro) is genetically very similar to populations from the adjacent coast of South France (Fig. 2, Appendix 2). Therefore, we interpret the island population of Corsica not as autochthonous (= native) as has been suggested (Vachon 1983, Goyffon 1992). Because we did not find a similarly low genetic divergence between the populations of the Balearic Islands and the mainland populations of *E. carpathicus* we assume natural colonisation for the former. Finally, similarly high genetic differentiation was reported between congeneric *Euscorpium* species using the same marker set (Scherabon et al. 2000, Gantenbein et al. 2000a). For example, the genetic divergence between the species *E. italicus* and *E. carpathicus* was recently estimated as 8% (Gantenbein et al. 1999), and between the two Alpine sister species *E. alpha* and *E. germanus* as 7% (Gantenbein et al. 2000a). It should be mentioned that samples of *E. carpathicus fanzagoi* (Simon 1879) (Fet & Sissom 2000), which occurs on the Iberian Peninsula (Pyrenees), could not be obtained for this study. However, because of morphological differences we doubt that this subspecies is genetically similar to the populations on the Balearic Islands.

A new endemic species for the Balearic Archipelago

The Balearic Islands represent the most isolated archipelago in the western Mediterranean region, and therefore it is not surprising that a remarkable endemic fauna has been reported: a recent review listed 568 presumed endemic species, whereas from 230 species no taxonomic or distributional uncertainties exist (Palmer et al. 1999). The Balearic Islands consist of two island groups, i.e. the Gymnestic Islands (Mallorca, Menorca, Cabrera) and the Pityusic Islands (Eivissa, Formentera). Each of these island groups harbours slightly different species sets, or certain species can be absent from one of them (Palmer et al. 1999). However, although we have no genetic data on *Euscorpium* populations from the island of Menorca or Cabrera we believe that the inter-island genetic differentiation is very low as was revealed in a recent allozyme and mtDNA study on the fruit fly *Drosophila subobscura* Collin, 1936 (Castro et al. 1999). A somewhat higher genetic differentiation is expected between populations of the Gymnestic and the Pityusic islands (Palmer et al. 1999) and needs to be tested for the scorpion populations in future studies.

Because of the aforementioned genetic divergence of the Balearic population relative to the other included *E. carpathicus* samples and because of the high degree of endemism on the Balearic Islands we here elevate the former subspecies *E. carpathicus balearicus* di Caporiacco, 1950 to the species rank. As the biological species concept (BSC) (Mayr 1942) cannot be used for island populations, we follow the phylogenetic species concept (PSC) (Cracraft 1989). We justify the elevation to species with our genetic and morphological data, which implies a highly divergent island population as the most likely hypothesis. A similar strategy was recently applied to the island population of scorpions from Cyprus described as *Mesobuthus cyprius* Gantenbein & Kropf, 2000 (Buthidae; Gantenbein et al. 2000b). However, reliable morphological differentiation in this cryptic buthid species could only be confirmed by hemispermaphore analysis, although this species is genetically highly differentiated from mainland populations. Two similar cases of cryptic speciation in the presence of highly divergent phylogenetic lineages at the species level were recently studied in Appalachian *Nesticus* cave spiders (Hedin 1997) and in Californian trapdoor spiders (Bond et al. 2001). In such cases traditional species concepts fail. However, in the case of *E. balearicus* stat. nov. considerable morphological differentiation in size and body coloration is obvious and seems to reflect a highly divergent evolutionary lineage.

Recently, we demonstrated (Gantenbein et al. 1999) that the phylogeny of *Euscorpium* could include vicariant events similar to those 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-Trans-Mediterranean (ATM) lineages. Oosterbroek & Arntzen (1992) indicated that Iberian/Italian elements are older than the ATM lineages; the phylogeny presented here seems to reflect the ancient age of an isolated Iberian lineage, as opposed to *E. carpathicus*.

Genetic differentiation of the included island populations of Crete and Elba

From our genetic data it is possible that other lineages deserving species status might exist within *E. carpathicus*. The island populations from Crete are clearly separated from the other included populations (from France, Italy, Austria and Croatia), being fixed for private alleles at five and six loci, respectively, out of 18 scored allozyme loci (Appendix 1). This nuclear genetic differentiation together with a mtDNA divergence of ~8% between Crete and mainland sequences (Table 3) lie within expectations if these populations are considered autochthonous (Hsü et al. 1977). The Crete population was described as a subspecies *E. carpathicus candiota* (Birula, 1903). So far, phylogenetic analyses have not clearly confirmed the isolated position of this population (bootstrap support 40–70% in Figs 2–3; Fet 1986, Fet & Sissom 1990). New samples are needed in order to evaluate the evolutionary history of *E. carpathicus* in the eastern Mediterranean area.

The island populations of Elba are genetically only moderately differentiated (one to two loci different out of 18 loci) from the adjacent coast of Tuscany (Castellina). 18,000 yrs ago the Mediterranean sea level was about 120 m lower than it is today (Fairbanks 1989), due to the Pleistocene glaciations. Since water depth between Elba and the mainland is less than 120 m, the last connection between these two land masses is recorded for the Würm glaciation. The island of Elba has been isolated since then. Therefore, a moderate divergence between island and mainland populations is expected for this case where populations have probably naturally colonised the island by land bridges. However, we cannot fully exclude that this moderate level of genetic differentiation of the Elba populations to the adjacent mainland can be interpreted by random genetic drift alone, and in this case colonisation might have occurred even less than 18,000 yrs ago.

Genetic population structure of *Euscorpium*

Because scorpions are sit-and-wait predators and generally known to have a low dispersal rate (Polis et al. 1985) it is not surprising that members of the genus *Euscorpium* are highly structured genetically as indicated

by high F_{ST} estimates even among mainland populations (Table 2, Appendices 1–2). This finding parallels the genetic population structures of other scorpions such as *Paruroctonus mesaensis* Stahnke, 1957 (Vaejovidae; Yamashita & Polis 1995; Yamashita & Fet 2001) and in *Mesobuthus* Vachon, 1950 (Buthidae; Gantenbein et al. 2000b), that showed restricted gene flow among metapopulations. These estimates of genetic differentiation are even higher than those measured in salamanders and gophers with F_{ST} 's of 0.53 and 0.23, respectively (Hewitt & Butlin 1997). In this context the genetic differentiation within *E. flavicaudis* is surprising, because populations separated by rather large geographic distances are genetically very similar (Appendix 2). Only the population of Casino di Terra was slightly differentiated from all other *E. flavicaudis* populations. The reason for this low differentiation can be either a low mutation rate or efficient DNA repair system, or this species has expanded its range in a very short time (~5,000 yrs) with the help of human activities. The fact that on the island of Corsica an identical mtDNA haplotype was collected as found in Tuscany supports the latter hypothesis. However, it is not yet possible to make predictions about the direction of artificial colonisation, because one would need more specimens from several localities. It is also likely that on Corsica both haplotypes (*Ef*LA1 and *Ef*RI1) can be found (2 bp difference) due to multiple transplantations from France and Italy.

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Appendix 2. Allele frequencies at 18 allozyme loci of seven *E. flavicaudis* populations.

Sample number	<i>E. flavicaudis</i>												
	Locus	Allele	17	18	19	20	21	22	23				
ALPDH	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
ARK	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
DDH	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
GAPDH	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
AAT-1	88	0.06	0.06	0.06	0.11	0.25	0.33	0.33	0.06				
AAT-1	100	0.94	0.94	0.94	0.89	0.75	0.67	0.67	0.94				
AAT-2	88	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02				
AAT-2	100	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98				
GTDH	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
HK	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
IDH-1	95	0.08	0.36	0.06	0.19	0.19	0.19	0.19	0.11				
IDH-1	100	0.92	0.64	0.94	0.81	0.81	0.81	0.81	0.89				
IDH-2	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
MDH-1	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
MDH-2	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
MPI	100	0.99	1.00	0.88	1.00	1.00	1.00	1.00	1.00				
MPI	101	0.01	0.01	0.12	0.02	0.02	0.02	0.02	0.11				
PEP	130	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13				
PEP	94	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87				
6-PGD	700	0.34	0.33	0.12	0.19	1.00	1.00	1.00	0.89				
6-PGD	88	0.66	0.67	0.88	0.81	1.00	1.00	1.00	1.00				
PGI	100	1.00	0.97	0.68	0.97	0.60	1.00	1.00	0.94				
PGI	102	0.03	0.03	0.32	0.03	0.40	0.03	0.03	0.06				
PGM	94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
PGM	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
PK	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				