

**A NEW SPECIES OF SCORPION FROM AUSTRIA,
ITALY, SLOVENIA AND CROATIA: *EUSCORPIUS
GAMMA* CAPORIACCO, 1950, STAT. NOV.
(SCORPIONES: EUSCORPIIDAE)**

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Abstract

SCHERABON B., GANTENBEIN B., FET V., BARKER M., KUNTNER M., KROPF C., HUBER D.: A new species of scorpion from Austria, Italy, Slovenia and Croatia: *Euscorpius gamma* Caporiacco, 1950, stat. nov. (Scorpiones: Euscorpiidae). In GAJDOŠ P., PEKÁR S. (eds): Proceedings of the 18th European Colloquium of Arachnology, Stará Lesná, 1999. Ekológia (Bratislava), Vol. 19, Supplement 3/2000, p. 253-262.

Scherabon (1987) recorded for the Austrian fauna two separate forms under *Euscorpius germanus* (C. L. KOCH), a so-called typical (T-Form) from Tyrol and Carinthia, and a "Karawanken-Form" (K-Form) limited to southern Carinthia. New morphological data on animals collected in Carinthia, Italy and Slovenia, as well as genetic analyses (allozymes and mitochondrial DNA), show that the so-called "K-Form" is in fact a separate species belonging to the *Euscorpius mingrelicus* complex, a phylogenetic lineage distinct from *E. germanus* (C. L. KOCH). The correct name for this species is *E. gamma* CAPORIACCO, 1950, stat. nov. (= *E. germanus histrorum* CAPORIACCO, 1950). It was described from northeastern Italy and the adjacent territory of Slovenia as a subspecies of *E. germanus*; we fix a lectotype of this species from the Risano (now Rizana) River in northern Istria, Slovenia. *E. gamma* is also found in Croatia. A number of other forms of the "*E. mingrelicus* complex" are found in the Balkans and Anatolia; their status is still unclear. We also fix a neotype of *Euscorpius mingrelicus* (KESSLER, 1874) from Batumi, Georgia (Caucasus).

Introduction

Several species of the scorpion genus *Euscorpius* (Euscorpiidae) are common in southern Europe. However, the number of species in *Euscorpius* and their phylogenetic relationships are not clear. This study concerns one of the least known lineages which we prefer to call a “*Euscorpius mingrelicus* complex”. BONACINA (1980) clearly demonstrated that this group of taxa, which he treated as “subspecies” of *E. mingrelicus* (KESSLER, 1874), was separate from *E. germanus* (C. L. Koch, 1837) and its “subspecies”. *Euscorpius germanus* was described from “southern Tyrol (now Trentino – Alto Adige in Italy) and northern Italy”; see FET, BRAUNWALDER (1997) for the detailed taxonomic history. On the other hand, *E. mingrelicus* was described from Georgia (Caucasus). These taxa have been analysed by many authors (BIRULA, 1900, 1917; CAPRA, 1939; CAPORACCIO, 1950; KINZELBACH, 1975; BONACINA, 1980; SCHERABON, 1987; FET, 1993; LACROIX, 1995). The status of several “subspecies” of *E. mingrelicus* inhabiting a vast area between Italy and the Caucasus remains unclear (CRUCITI, 1993).

Materials and methods

Euscorpius material

Original data obtained by SCHERABON (1987) on the morphology of Austrian “*E. germanus*” (s.l.) were based on 400 specimens partly deposited in the Naturhistorisches Museum Wien, Vienna, Austria (NMW). Data on *E. mingrelicus* from FET (1993) (685 specimens from Caucasus) were used here for comparison. A large series of *E. mingrelicus* from Georgia, collected by V.F. in 1985, is deposited in the Zoological Museum of the Moscow State University, Moscow, Russia, with some specimens also in NMW. For additional morphological comparisons, we (V.F.) studied collections from Austria, Italy and Slovenia deposited in NMW, Museo Zoologico “La Specola”, University of Florence, Florence, Italy (MZUF), and University of Ljubljana, Ljubljana, Slovenia. Detailed morphological and locality data will be published elsewhere, and are available from V. F. Abbreviations used are: Tv, trichobothrial series (usually 5-6) on the ventral aspect of the patella (formerly treated as “tibia”) of the pedipalp; et, terminal cluster (usually 4-5) of the trichobothrial series on the external aspect of the patella (tibia); est and dsb, external subterminal and dorsal suprabasal trichobothria on the fixed finger of the pedipalp.

Live specimens used for allozyme and DNA analysis were collected by authors (B.S., B.G., M.K., C.K. and D.H.) in Austria (Carinthia), Italy (Alto Adige) and Slovenia (near Nova Gorica), and used for the allozyme (B.G.) or DNA (B.G., V.F., M.B.) analysis. The sampling sites are shown in Fig. 1. As an outgroup we selected the West Mediterranean species *Euscorpius flavicaudis* (DEGEER, 1778) (Lauris, France) collected by A. Scholl. Sampling areas were in the size range 300-400 m². In general, only a few animals were taken from any one site because small sample sizes (n < 10) are already expected to result in relatively good estimates of gene frequencies at allozyme loci. This statement is likely to be true due to a low genetic variability detected within *E. germanus* populations in an earlier allozyme analysis (GANTENBEIN et al. 1998).

Molecular analysis

Comparative analyses of the mitochondrial 16S ribosomal RNA and allozymes has been recently used for resolving species-level phylogeny of *Euscorpius* (GANTENBEIN et al., 1999); this work should be consulted for detailed techniques and protocols. For the detailed allozyme

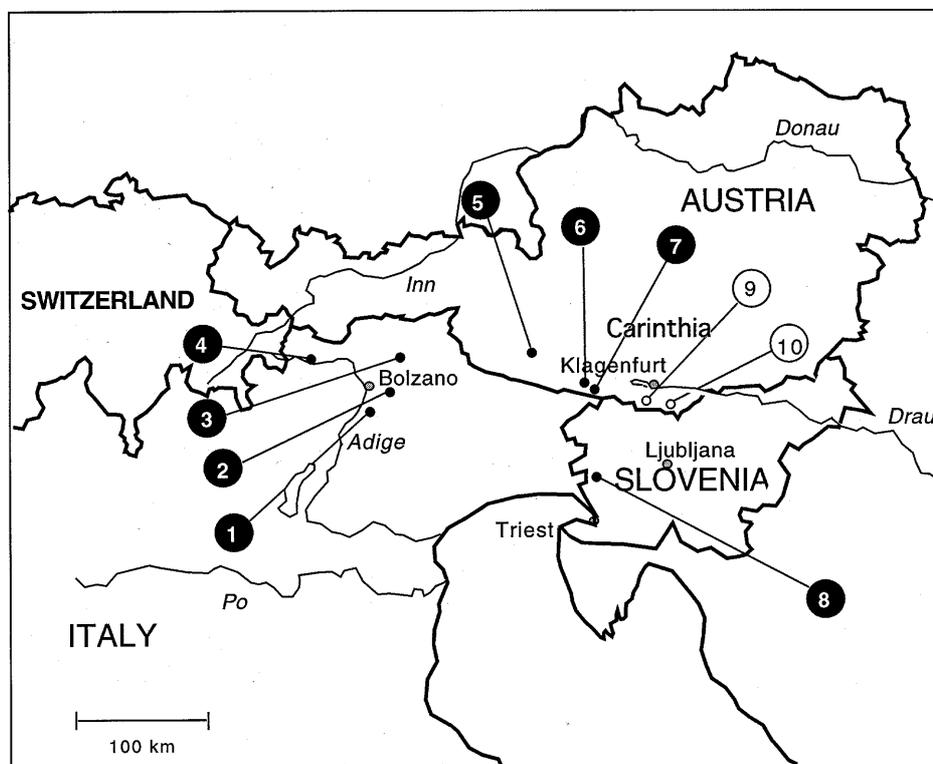


Fig. 1. Sampling sites of analysed *E. germanus* populations using allozymes and/or mtDNA sequences. Black circles: "T-Form" (= *E. germanus*): 1 Bremer (Eggental); 2 Völs; 3 Brixen; 4 Schluderns; 5 Oberdrauburg; 6 Dobrebratsch; 7 Schütt; 8 Crnice (near Nova Gorica). White circles: "K-Form" (= *E. gamma*): 9 Koschuta; 10 Trögerer-Klamm.

and DNA analysis procedures, see GANTENBEIN et al. (1998, 1999). We scored the same allozyme set (18 loci) as described in these studies. Alleles at loci were labelled according to the relative mobility of the most common allele in the *E. flavicaudis* population from Lauris. For DNA analysis, total DNA was extracted from fresh or preserved (95% ethanol) muscle tissue using a standard extraction method. An approximate 400 bp fragment of the mitochondrial (mt) 16S rRNA gene was amplified by the polymerase chain reaction (PCR) using the primers 16Sbr, or LR-J-12887 (SIMON et al., 1994; CGATTTGAACTCAGATCA; forward, 18-mer) and a scorpion-specific reverse primer (GTGCAAAGGTAGCATAATCA, 20-mer). A total of six mtDNA sequences were used for the analysis. For phylogenetic tree-building algorithms, see GANTENBEIN et al. (1999).

Results and discussion

Morphology

BIRULA (1900: 19) was the first to give a clear distinction between *E. germanus* from Tyrol and *E. mingrelicus* from the Caucasus, i.e. between the nominotypical populations of both species. He listed several diagnostic features of *E. mingrelicus* including: intensive dark pigmentation; larger size (average ca. 38 mm while in *E. germanus*, ca. 28 mm); swollen and rounded chela manus (as opposed to more flattened in *E. germanus*); developed metasomal carinae II-IV (carinae absent in *E. germanus*), and Tv=6 (as opposed to Tv=5 in *E. germanus*). Further studies of populations from the Alps, Balkans, and Anatolia, identified usually as *E. germanus*, revealed a significant range of variation, and to a large extent blurred the picture clearly perceived by BIRULA (1900, 1917). *E. mingrelicus* was for a long time treated as a subspecies of *E. germanus* (e.g. CAPORIAMCO, 1950; KINZELBACH, 1975). However, BONACINA (1980) again demonstrated the existence of two separate species using new characters: trichobothria of the fixed finger of the pedipalp, and the shape of the pectinal sensilla as revealed by the SEM.

As demonstrated by SCHERABON (1987) through a detailed morphological analysis, the Austrian fauna included what was then considered two separate forms of *Euscorpius germanus*: a so-called typical (T-Form) in Nordtirol, Osttirol and Carinthia, and a separate "Karawanken-Form" (K-Form) limited to the southern Carinthia. Our comparison of the "K-Form" with the type specimens of *Euscorpius germanus gamma* CAPORIAMCO, 1950 (Slovenia, Italy) and *E. germanus histrorum* CAPORIAMCO, 1950 (Croatia, Slovenia) demonstrates that these specimens are morphologically identical. Both Caporiacco's forms were considered subspecies of *E. mingrelicus* by BONACINA (1980). They have been distinguished by the Tv number as being 6 in *E. mingrelicus gamma* (= *E. m. caprai*) and 5 in *E. m. histrorum* (= *E. m. boninoi*) (BONACINA, 1980: 75). However, statistical analysis of the "K-Form" shows that Tv numbers vary between 5 and 6. The degree of expression of metasomal carinae also varies in studied specimens which was noticed already by CAPORIAMCO (1950). All these animals clearly belong not to *E. germanus* but to what could be called a "*E. mingrelicus* complex" as indicated by the proximity of the trichobothria *est* and *dsb* on the fixed finger of the pedipalp, and metasomal carination (see BONACINA, 1980: 75; SCHERABON, 1987, Abb. 6 and 23). However, an exhaustive statistical study of 685 specimens of *E. mingrelicus* from the Caucasus (FET, 1993) demonstrated that the absolutely dominant trichobothrial values of this population are Tv=6 (90.6%) (83.2% of specimens had Tv=6-6). Conversely, 162 specimens of the "K-Form" from the Karawanken Alps (SCHERABON, 1987) had Tv=5 (68.5%) or 6 (29.6%); only 13% had Tv=6-6. Additional characters that distinguish the nominotypical Georgian population of *E. mingrelicus* from the Austrian/Balkan populations, are higher number of trichobothria in group *et* (5 in 91.9%); larger size (average 38 mm in Georgia, 32 mm in the Karawanken) and darker pigmentation of Georgian specimens (BONACINA, 1980; SCHERABON, 1987; FET, 1993).

Table 1. Allele frequencies at 18 loci and sample sizes of analysed *E. germanus* and *E. gamma* populations.

	Population	Schluderns	Brixen	Brenner	Vois	Oberdrauburg	Dobratsch	Schütt	Crnice	Koschuta	Trögerner Klamm	<i>E. flavicaudis</i> Lauris
	Sample size	(4)	(4)	(7)	(10)	(4)	(4)	(4)	(3)	(6)	(4)	(11)
Locus	Allele											
Aat-1	100				0.05	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	96				0.95							
Aat-2	88	1.00	1.00	1.00								
	113								1.00	1.00	1.00	
	107	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
Alpdh	100	1.00		0.21	0.4					1.00	1.00	1.00
	95		1.00	0.79	0.6	1.00	1.00	1.00	1.00			
Ark	104								1.00			
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00				1.00
	93									1.00	1.00	
Ddh	101	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	100											1.00
Gapdh	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Gtdh	100									1.00	1.00	1.00
	95											
Hk	90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
	107									1.00	1.00	
Idh-1	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			1.00
	100											0.96
	95											0.04
Idh-2	94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	100									1.00	1.00	
Mdh-1	87	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00
Mdh-2	89								1.00			
Mpi	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	135	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
	118								1.00			
Pep	110									1.00	1.00	
	100											1.00
	100											0.83
	107					0.25	0.12	0.25	1.00			
	104	1.00	1.00	1.00	1.00	0.75	0.88	0.75				
	98									0.83	0.13	
	94											0.17
6-Pgd	78									0.17	0.87	
	100											0.68
	98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	93									1.00	1.00	
Pgi	88											0.32
	100											1.00
	92	1.00	1.00	1.00	1.00	1.00	0.88	1.00	1.00			
	86						0.12					
Pgm	80									1.00	1.00	
	100											1.00
	98		0.12		0.1	0.25		0.37	0.33	1.00	1.00	
	94		0.12	0.14	0.2	0.51						
	91	1.00	0.76	0.86	0.7	0.12	0.75	0.12	0.67			
Pk	80					0.12	0.25	0.51				
	101									1.00	1.00	
	100											1.00
	98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			

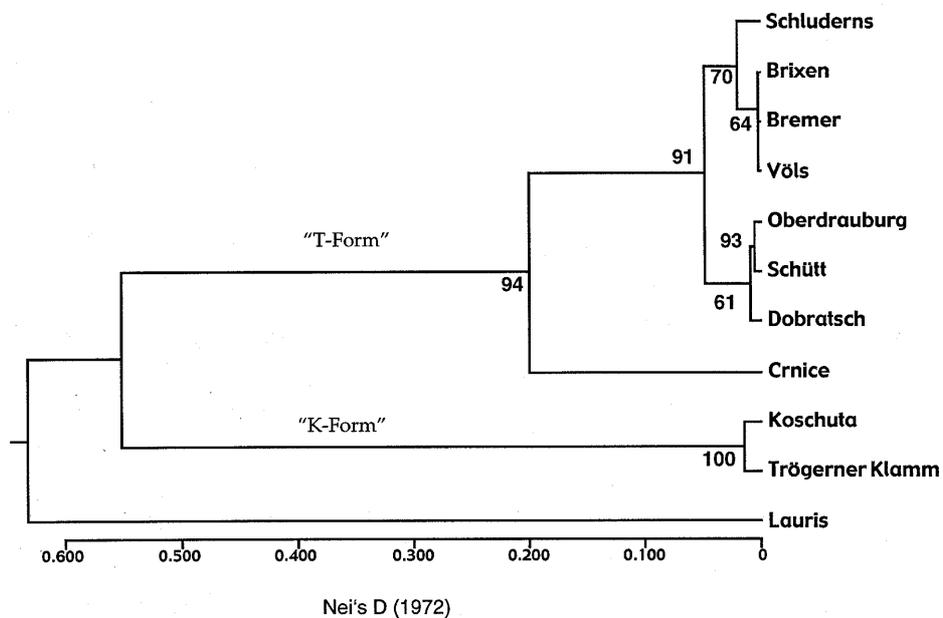


Fig. 2. Phenogram (Nei's D/UPGMA) based on allozyme data (18 loci) separating the "T-Form" (= *E. germanus*) from the "K-Form" (= *E. gamma*) by a high genetic distance. Numbers at the node represent bootstrap values (% over 1000 pseudoreplicates). Only high values (>70%) are given.

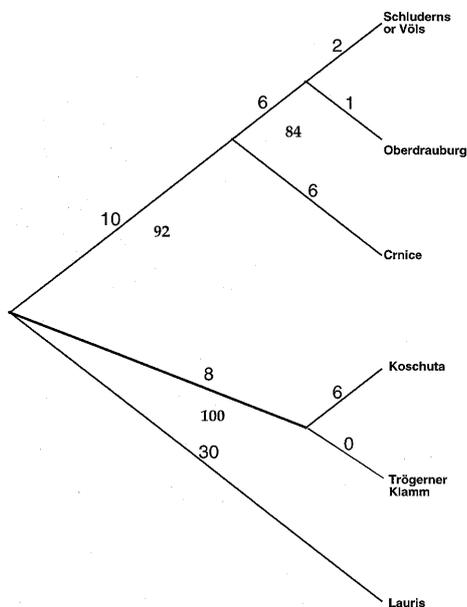


Fig. 3. Phylogeny based on 16S mtDNA data separating the "T-Form" (= *E. germanus*) from the "K-Form" (= *E. gamma*). The single most parsimonious tree found (69 steps, CI 0.899) using the Exhaustive Search under the MP criterion of PAUP. Numbers refer to the assumed lengths of branches. Bold numbers refer to bootstrap values (% over 1000 pseudoreplicates).

Allozyme data

The allozyme data revealed a low genetic variability within population samples and confirm the data observed for *E. germanus* (“T-Form”) in GANTENBEIN et al. (1998). The samples belonging to the “K-Form” were fixed for private alleles at 9 out of 18 gene loci if they were compared to the “T-Form” population samples (Table 1). Due to this high observed genetic differentiation two clearly distinct clusters were revealed in the calculated UPGMA phenogram (Fig. 2). The nodes of these two clusters were highly supported in bootstrap analysis (>94%). The derived phenogram (Fig. 2) resulted in a strikingly identical tree topology to the 16S mtDNA phylogeny (Fig. 3).

DNA data

For seven nucleotide sequences of the mtDNA 16S gene, the PAUP Exhaustive Search using the maximum parsimony (MP) criterion, yielded a single MP tree with a length of 69 steps (Fig. 3). (Sequences of Völs and Schluderns were identical). A high tree stability was indicated by the consistency index (CI=0.899). The five ingroup *Euscorpis* specimens split clearly into two highly supported clades (92% and 100% bootstrap support), exactly repeating the allozyme UPGMA phenogram (Fig. 2). These two clades correspond exactly to the “T-Form” (one specimens from Oberdrauburg, Austria; two from Schluderns and Völs, Italy; and one from Crnice, Slovenia) and “K-Form” (two specimens from Koschuta and Trögerner Klamm, Austria) as defined by SCHERABON (1987).

DNA distance data (Table 2) also confirm the existence of two clades. Within-clade distance varied from 0.8% to 3.5% in the “T-Form” clade, and was 1.6% in the “K-Form” clade. At the same time, the distance between “T-Form” and “K-Form” clades varied from 5.6% to 7.5%, which fits well into the distance between congeneric morphospecies in *Euscorpis* (GANTENBEIN et al., 1999). Distance between both forms and the outgroup (*E. flavicaudis*) was from 9.8% to 11.5%.

Type material

CAPORACCO (1950) did not designate holotypes of his taxa, therefore we designate here lectotypes for *E.g. gamma* and *E.g. historum* from the existing syntype material (MZUF).

Euscorpis germanus gamma CAPORACCO, 1950. Lectotype (designated here): female (MZUF 5581), mouth of the Rizana (Risano) River, north Istria, Slovenia. Paralectotypes (11 specimens): Slovenia (mouth of Rizana River, Kamno, Vrsno, Krn, Trenta valley, and Panovizza, now Panovec), Italy (Cave di Predil), and unspecified localities in Soca (Isonzo) River valley in Slovenia and Italy.

E. germanus historum Caporiacco, 1950. Lectotype (designated here): male (MZUF 5583), Risnjak Mts., Croatia. Paralectotypes (4 specimens): Croatia (Monte Maggiore, now Mt. Ucka, Istria) and Slovenia (Mt. Re, or Nanos; 1 juvenile MZUF 5583). Current depository of paralectotypes for both species (other than MZUF) is not clear but the original Caporiacco’s material came from museums in Trieste and Genova.

T a b l e 2. Pairwise distances between taxa: mtDNA data (below diagonal: absolute distances; above diagonal: mean distances).

		1	2	3	4	5	6
1	Oberdrauburg	–	0.008	0.035	0.064	0.075	0.109
2	Völs (=Schluderns)	3	–	0.035	0.058	0.075	0.103
3	Crnice	13	13	–	0.056	0.067	0.114
4	Trögerner Klamm	24	22	21	–	0.016	0.098
5	Koschuta	28	28	25	6	–	0.115
6	Lauris	41	39	43	37	43	–

We also designate here a neotype of *E. mingrelicus* (KESSLER, 1874) from Batumi, Georgia (Caucasus) which has not been fixed by FET (1993). This is a female specimen deposited in the Natural History Museum Vienna, Austria (NMW 14644). It belongs to the large series studied by FET (1993: 3) and has $Tv=6-6$ and $et=5-5$. Elsewhere, we also plan to designate a neotype of *E. germanus* (C. L. KOCH, 1837) from northern Italy (GANTENBEIN et al., in prep.).

Taxonomy

Two independent systems of molecular markers (allozymes and 16S mtDNA) confirm morphological analysis of SCHERABON (1987) and reveal an identical pattern: a deep phylogenetic divergence between the Karawanken population of *Euscorpius* (“K-Form” of SCHERABON, 1987) (Koschuta and Trögerner Klamm), as opposed to the cluster of “typical” *E. germanus* populations from western Carinthia, northern Italy and western Slovenia. Morphology of the “K-Form” is identical to that of BONACINA’s (1980) *E. mingrelicus gamma* (= *E. m. caprai*) and *E. m. histrorum* (= *E. m. boninoi*) from Italy, Slovenia and Croatia. At the same time, the “K-Form” differs from the nominotypical *E. mingrelicus* (Kessler, 1874) from Georgia. A number of other forms exist in the Balkans and Anatolia which are not identical with either “K-Form” or the Georgian *E. mingrelicus*. We suggest therefore to treat the Alpine-north Balkan form as a separate species, *Euscorpius gamma* Caporiacco, 1950, stat. nov. According to the principle of the first reviser (IZCN Article 24), priority of this name was fixed by VALLE et al. (1971: 96) who were first to synonymize Caporiacco’s *E. germanus gamma* and *E.g. histrorum*, selecting *E.g. gamma* as a senior synonym. Both Caporiacco’s subspecies were described on the same date and in the same work (CAPORIAMCO, 1950).

Key

The species *Euscorpius gamma* is included in the existing key of the genus as follows (modified from CAPORIAMCO, 1950; BONACINA, 1980; GANTENBEIN et al., 1999).

1. Four trichobothria ventrally on the chela manus 2
- Six or more trichobothria ventrally on the chela manus 5

2. Usually 7 or more trichobothria on the ventral aspect of the pedipalp patella, and 23-29 on its external aspect (group *em* usually has 4 trichobothria) *Euscorpius carpathicus* (L., 1767) (s.l.)
 — Usually 5-6 trichobothria on the ventral aspect of the pedipalp patella, and 20-22 trichobothria on its external aspect (group *em* usually has 3 trichobothria) **3**
3. Trichobothria *est* and *dsb* on the fixed finger of the pedipalp are removed from each other so that ratio *et-est/est-dsb* is approximately 1.0. Metasomal segments lack carinae *Euscorpius germanus* (C. L. KOCH, 1837) (s.l.)
 — Trichobothria *est* and *dsb* on the fixed finger of the pedipalp adjacent to each other; ratio *et-est/est-dsb* varies usually from 1.5 to 2.0. Metasomal segments with traces of dorso-lateral carinae **4**
4. Almost always 6 trichobothria on the ventral aspect and 5 trichobothria in the group *et* on the external aspect of the pedipalp patella; darker coloration; larger size (average 38 mm) *Euscorpius mingrelicus* (KESSLER, 1874)
 — Usually 5 or, more rarely, 6 trichobothria on the ventral aspect and 4 trichobothria in the group *et* on the external aspect of the pedipalp patella; lighter coloration; smaller size (average 32 mm) *Euscorpius gamma* CAPORACCO, 1950
5. Six trichobothria ventrally on the chela manus. Usually 10 to 13 trichobothria on the ventral aspect of the pedipalp patella, and 26-29 on its external aspect *Euscorpius flavicaudis* (DEGEER, 1778)
 — Eight to 11 trichobothria ventrally on the chela manus. Usually 11 to 13 trichobothria on the ventral aspect of the pedipalp patella, and 26 to 45 on its external aspect (*em* usually with 5 trichobothria; an additional group *esba* with 4 to 11 trichobothria is usually present) *Euscorpius italicus* (HERBST, 1800)

It is important to indicate that *E. germanus* and *E. gamma* are sympatric in the Isonzo (Soca) River valley in Slovenia and Italy, and in part of western Slovenia. In Carinthia, two species are allopatric, and a small gap between their ranges is defined by the geographic gap between the Carnian Alps (*E. germanus*) and the Karawanken Alps (*E. gamma*). It remains to be seen whether the range of *E. gamma* extends further south and east to the Balkan peninsula. A number of forms clearly belonging to the “*E. mingrelicus* complex” has been recorded from Bosnia-Herzegovina, Serbia, Albania, Montenegro, Macedonia, Greece and Turkey (CAPORACCO, 1950; BONACINA, 1980; FET, 1986, 1993; LACROIX, 1995) which might not be conspecific with either *E. gamma* or *E. mingrelicus*. Due to often unclear morphological characters in *Euscorpius*, molecular analyses could play a decisive role in delineation of these taxa in the future studies.

DNA sequence availability

All sequences were deposited in the EMBL Nucleotide Sequence Database with the following accession numbers: *E. germanus*: AJ389380, AJ249552, AJ249553; *E. gamma*: AJ249554, AJ249555; *E. flavicaudis*: AJ389381.

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