Entomological Review, Vol. 83, Suppl. 2, 2003, pp. S249–S252. From Zoologicheskii Zhurnal, Vol. 82, no. 12, 2003.

First Data on the Molecular Phylogeny of *Euscorpius* (Scorpions: Euscorpiidae) from Turkey

V. Fet[1], Ay. Karataş[2], E. V. Fet[1], and A. Karataş[2]

[1] *Department of Biological Sciences, Marshall University, Huntington, West Virginia 25755-2510, USA*

[2] *Department of Zoology, Nigde University, Nigde, Turkey*

Received November 28, 20023

Abstract—The first molecular data (16S rRNA gene sequences) on the Anatolian scorpion populations, tentatively identified as *Euscorpius ciliciensis* Birula, are presented. Phylogenetic analysis shows their affinities to the European species *E. gamma* and *E. germanus*, but not to the "*E. carpathicus*" species complex.

The species composition and taxonomic rank of most Turkish *Euscorpius* Thorell are unclear (Fet and Braunwalder, 2000). An enigmatic species, *E. ciliciensis* Birul 1898, was described from the Cilician Taurus Mountains of Turkey, a record high altitude (2400 m asl) for the genus *Euscorpius*. Kessler (1874) described *E. mingrelicus* from Georgia, and this species has been subsequently reported in Turkey and even in the Balkans (Bonacina, 1980). *E. ciliciensis* has been either synonymized with *E. mingrelicus* or considered its subspecies (Kinzelbach, 1975; Fet and Sissom, 2000). Fet (1986) studied type material of Birula from the Zoological Institute, St. Petersburg, and confirmed that it belongs to the "*E. mingrelicus*" complex but was not sure as of its status. Fet (1993) analyzed in detail the morphology of *E. mingrelicus* from Georgia and Russia (Krasnodar Region) but not from Turkey. Fet and Sissom (2000) listed a number of Balkan and Anatolian subspecies under *E. mingrelicus*. Scherabon *et al.* (2000) demonstrated a distinct species, *E. gamma*, from Europe (Austria, Italy, Slovenia, and Croatia) belonging to the "*E. mingrelicus*" complex; therefore, the scope of *E. mingrelicus* was reduced. The members of this species complex from Turkey have not been studied in detail, and the morphological characters existing for the species delineation are not sufficient. We analyzed the mtDNA (the 16S rRNA gene) from a specimen collected close to the type locality and also from another population in Anatolia. These are the first DNA data on Euscorpius from Turkey.

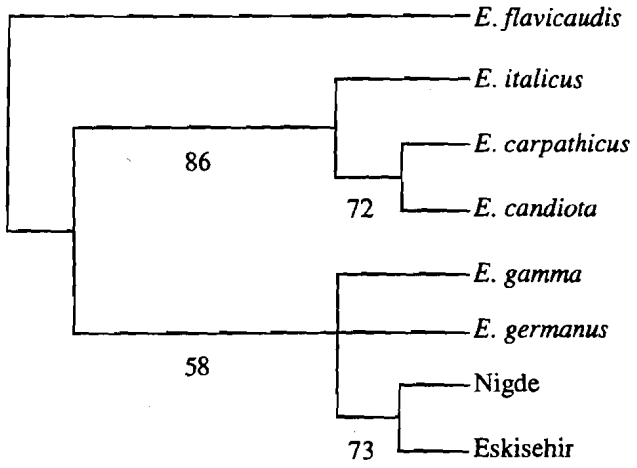
MATERIAL AND METHODS

Material. The 2 adult females of *Euscorpius* used in this study were collected with the following label data: 1 female, Turkey; Nigde: Ulukisla, Gümüş-Maden road, 37°28′ N, 34°37′ E, ca. 1750 m asl. No. 69, May 23, 2001, ZDUN/S 2001/69 (coll. A. Karataş); 1 female, Turkey, Eskişehir: Alpu, Otluk Village: 39°46′ N, 30°57′ E, ca. 1200 m asl., April 6, 2001 (coll. F. Caliskan), ZDUN/S 2001/41. The scorpions were preserved in 96% ethanol and sent for DNA analysis to Marshall University, West Virginia, USA.

DNA analysis. The comparative analysis of the mitochondrial 16S ribosomal RNA gene has been recently used for resolving the species-level phylogeny of *Euscorpius* (Gantenbein *et al.*, 1999, 2000, 2001; Scherabon *et al.* 2000); for the detailed DNA analysis procedures and phylogenetic tree-building algorithms, see Gantenbein *et al.* (1999, 2000). Total DNA was extracted from fresh or preserved (95% ethanol) muscle tissue (a leg) using a Qiagen™ DNeasy extraction kit. 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 (CGATTTGAACTCAGATCA; forward, 18-mer) and a scorpion-specific reverse primer (GTGCAAAG-GTAGCATAATCA, 20-mer). These primers corresponded to the positions 11.173–11.190 and 11.625–11.606 in the *Limulus polyphemus* mitochondrial genome (Lavrov *et al.*, 2000). The resulting PCR product was verified on 1% agarose electrophoretic gel and purified using Ultrafree MC 30000 cellulose filters (Millipore, Inc.). Automated Sanger dideoxy sequencing of the double-stranded PCR product was performed at the Molecular Genetics Instrumentation Facility, University of Georgia (Athens, GA), on the ABI 9600 Sequencer (US team).

Phylogenetic analysis. 8 mtDNA sequences representing different haplotypes were aligned using Clustal X 1.81 (Thompson *et al.*, 1997). Two new DNA sequences were deposited to the GenBank (http://www.ncbi.nlm.nih.gov) under the accession numbers AY152394 (EciGU1) and AY152395 (EciES1). Six DNA sequences published earlier by our research group and its collaborators (Fet *et al.*, 2002; Gantenbein *et al.*, 1999, 2000, 2001; Scherabon *et al.*, 2000; Huber *et al.*, 2001) were extracted from the GenBank online database. The corresponding taxa, their

[1] This article was submitted by the author in English.



Maximum Parsimony (MP) consensus phylogeny for the studied species and populations of *Euscorpius*. The numbers represent bootstrap values.

geographic origin, abbreviations, and accession numbers were: *E. flavicaudis* (DeGeer, 1778): Lauris, Vaucluse, France, EfLA (AJ389381); *E. italicus* (Herbst 1800): Tortoreto, Abruzzo, Italy, EiTO1 (AJ298067); *E. carpathicus* (Linnaeus 1767), Baile Herculane, Romania, EcRO1 (AY172337); *E. "carpathicus canuiota"* Birula, 1903: Kallikratis, Crete, Greece, EcKA1 (AJ309213); *E. germanus* (C.L. Koch 1837): Oberdrauburg, Carinthia, Austria, EgOB (AJ249553); and *E. gamma* Caporiacco, 1950: Troegerner-Klamm, Carinthia, Austria, EgaTR (AJ249555). As an outgroup, we used *E. flavicaudis*. The software package PAUP* Version 4.0b10 (Swofford, 1998) was used for the sequence analysis to perform the genetic distance calculation, Maximum Parsimony (MP), and Neighbor-Joining (NJ) algorithms. The statistical support of the inner clades of the phylogenetic tree was determined by bootstrapping (1000 replicates).

RESULTS

In the maximum parsimony analysis, of the dataset of 380 total characters, 28 were parsimony-informative. The exhaustive search under PAUP* (explored under weighting of transitions : transversions : gaps as 2 : 1 : 0, 2 : 1 : 4, 3 : 1 : 0, 3 : 1 : 1, 3 : 1 : 4, 5 : 1 : 0, and 5 : 1 : 4) found a single shortest MP tree, 109 steps long, CI = 0.82, RI = 0.53 under weighting 3 : 1 : 0. Under the six neighbor joining distance models studied (absolute, Kimura, Felsenstein, Jukes–Cantor, HKY85, and Tamura–Nei), the resulting phylogeny was identical to the MP tree. The MP bootstrap consensus tree is presented in figure.

The highest statistical support among the studied material was for the clade of *E. italicus* + "*E. carpathicus* complex" (86% in both MP and NJ). Another supported monophyletic lineage includes two "*E. carpathicus* complex" taxa: *E. carpathicus* from Romania and *E. "carpathicus candiota"* from Greece (support 72% in MP and 63% in NJ).

In both the MP and NJ analyses, the sister group to the Anatolian populations was the Alpine species *E. germanus*, while *E. gamma* formed a sister group to the clade of (*E. germanus* + Anatolian populations). However, the bootstrap test only supported the polytomy of these three clades (58% in MP and 68% in NJ). The two Anatolian populations formed a single clade with high statistical support (bootstrap 73%) only in the MP analysis.

Distance data (Table 1) are presented for the Kimura distance model.

DISCUSSION

The morphological investigation of the Nigde specimens showed that they could belong to the *"E. mingrelicus"* species complex judging from the smooth surface of the metasomal segments, the distance between the trichobothria on the fixed pedipalp finger, and the low number of external median trichobothria (*em* = 3) on the pedipalp patella. At the same time, the Eskişehir specimens had *em* = 3 but developed metasomal carinae with denticles, which is a feature present usually in the "*E. carpathicus*" complex (Fet and Soleglad, 2002). Moreover, *E. carpathicus* (L.) usually has *em* = 3 as do certain unclear forms from the Balkans

A matrix of genetic distances (Kimura 2-parameter distances)

	1	2	3	4	5	6	7	8
1 Nigde	–							
2 Eskisehir	0.045	–						
3 *E. germanus*	0.069	0.070	–					
4 *E. gamma*	0.047	0.054	0.074	–				
5 *E. flavicaudis*	0.107	0.109	0.115	0.094	–			
6 *E. italicus*	0.068	0.065	0.100	0.061	0.097	–		
7 *E. carpathicus*	0.065	0.069	0.091	0.061	0.094	0.047	–	
8 *E. candiota*	0.093	0.096	0.114	0.078	0.122	0.065	0.059	–

(e.g., "*E. germanus croaticus* Caporiacco" from Croatia and an unnamed form from the Rodope Mts., Bulgaria) (Fet, 1993, 2000; Gantenbein *et al.*, 2000). Some of these forms can also have reduced metasomal carination. Therefore, the existing morphological characters were not clearly diagnostic for the Turkish populations.

Our preliminary DNA data analysis clearly suggests that the taxa closest to the analyzed Anatolian populations are the European species *E. gamma* Caporiacco, 1950 and *E. germanus* (C. L. Koch 1837). On the contrary, a very separate lineage is strongly supported for the "*E. carpathicus*" complex species with *E. italicus* as an outgroup. This complex was represented in our study by *E. carpathicus* and *E. "carpathicus candiota."* The first species was defined and diagnosed recently on its morphological basis by Fet and Soleglad (2002) and on its molecular basis by Fet *et al.* (2002); the status of the Crete population remains unclear. Our molecular data strongly suggest that the Anatolian populations are not closely related to the "*E. carpathicus*" complex, and, therefore, the morphological similarity (*em* = 3, metasomal sculpture) could be homoplasious.

Recently, Scherabon *et al.* (2000) provided a justification of the species level for *E. gamma*, which was originally described as a subspecies of *E. germanus* and later included under *E. mingrelicus* (Fet and Sissom, 2000). Our current DNA data are obtained from the most western (*E. gamma*) and eastern (Turkey) populations (putative species) that could belong to this complex (Bonacina, 1980; Fet, 1993, 2000).

The exact taxonomic identity of the populations studied so far remains unclear. However, since one of them was collected at the type locality of *E. ciliciensis* (Karagöl, Bolkar Dagh, which is ca. 10 km to both the villages of Gümüş and Maden), it seems reasonable to suggest that this name is applicable. The Taurus mountain range is one of the most zoogeographic barriers in Anatolia and a place of high endemism for plants and animals (e.g. *Rana holtzi, Vipera bulgardaghica*). In the localities of Gümüs and Maden, *Euscorpius* were found under stones as well as in alpine meadows and pine forests (*Pinus brutia, P. nigra*). The second locality, Alpu (Eskişehir), has a mixture of dry grasslands and pine forests and higher summer temperatures than the Taurus locality.

No other molecular data on the "*E. mingrelicus*" complex are available. It remains to be seen whether the morphological and molecular divergence between *E. ciliciensis* and *E. mingrelicus* is sufficient to recognize two (or more) species. The recorded genetic distance between the two Anatolian populations is 4.5% (Table 1). Divergence at this level is commonly found within a single species of *Euscorpius* for the same 16S gene (Fet *et al.*, 2002; Gantenbein *et al.*, 1999, 2000, 2001; Scherabon *et al.*, 2000). Note, however, that the distance between the European *E. gamma* and the Anatolian populations is also low (4.7–5.4%), lower than that between the alpine sister species *E. germanus* and *E. alpha* (over 6%; Gantenbein *et al.*, 2000). Further investigation of the multiple populations from Turkey, the Caucasus, and the Balkans is necessary to resolve the composition of the *"E. mingrelicus"* complex and its relationship with the other European species of this diverse genus.

ACKNOWLEDGMENTS

We thank Michael Soleglad and Benjamin Gantenbein for their valuable insights, help, and enthusiasm in the study of *Euscorpius*.

REFERENCES

1. Birula, A.A., Ein Beitrag zur Kenntniss der Skorpionenfauna Kleinasiens. Horae Societatis Entomologicae Rossicae, *Preprint*, October 1898; collated issue: 1901, vol. 33, nos. 1–2, pp. 132–140.
2. Byalynitskii-Birulya, A.A., Arachnoidea Arthrogastra Caucasica. Pars I. Scorpions. Zapiski Kavkazskogo Muzeya (Mémoires du Musée du Caucase), Tiflis: Imprimerie de la Chancellerie du Comité pour la Transcaucasie, A(5), p. 253 (in Russian; published August 1917). English translation: Byalynitskii-Birulya, A.A., 1964. Arthrogastric Arachnids of Caucasia. 1. Scorpions. Jerusalem: Israel Program for Scientific Translations, 1917, 170 pp.
3. Fet, V., Notes on Some *Euscorpius* (Scorpiones: Chactidae) from Greece and Turkey, *Riv. Mus. Civ. Sci. Natur.* "Enrico Caffi" (Bergamo), 1986, vol. 9 (1985), pp. 3–11.
4. Fet, V., Notes on *Euscorpius* mingrelicus (Kessler, 1874) from the Caucasus, *Riv. Mus. Civ. Sci. Natur.*, "Enrico Caffi" (Bergamo), 1993, vol. 16, pp. 1–8.
5. Fet, V., Scorpions (Arachnida, Scorpiones) from the Balkan Peninsula in the Collections of the National Museum of Natural History, Sofia, *Hist. Natur. Bulgarica*, 2000, vol. 11, pp. 47–60.
6. Fet, V. and Braunwalder, M.E., The Scorpions (Arachnida, Scorpiones) of the Aegean Area: Current Problems in Taxonomy and Biogeography, *Belg. J. Zool.*, 2000, vol. 130 (suppl. 1), pp. 17–22.
7. Fet, V. and Sissom, W.D., Family Euscorpiidae, 2000, pp. 355–381. Fet, V., Sissom, W.D., Lowe, G., and Braunwalder, M.E., *Catalog of the Scorpions of the World* (1758–1998), New York: New York Entomological Society, 2000, pp. 1–690.
8. Fet, V., Gantenbein, B., Fet, E.V., and Popa, V., *Euscorpius carpathicus* (Linnaeus, 1767) from Romania (Scorpiones: Euscorpiidae): Mitochondrial DNA data, *Biogeographica* (Paris), 2002, vol. 78, no. 4, pp. 141–147.
9. Fet, V. and Soleglad, M.E., Morphology Analysis Supports Presence of More Than One Species in the *"Euscorpius carpathicus"* Complex (Scorpiones: Euscorpiidae), *Euscorpius*, 2002, no. 3, pp. 1–51.
10. Gantenbein, B., Fet, V., Barker, M., and Scholl, A., Nuclear and Mitochondrial Markers Reveal the Existence of Two Parapatric Scorpion Species in the Alps: *Euscorpius germanus* (C.L. Koch 1837) and *E. alpha* Caporiacco, 1950, stat. nov. (Scorpiones, Euscorpiidae), *Rev. Suisse Zool.*, 2000, vol. 107, no. 4, pp. 843–869.

11. Gantenbein, B., Fet, V., Largiadér, C.R., and Scholl, A., First DNA Phylogeny of *Euscorpius* Thorell, 1876 (Scorpiones: Euscorpiidae) and Its Bearing on Taxonomy and Biogeography of This Genus, *Biogeographica* (Paris), 1999, vol. 75, no. 2, pp. 49–65.

12. Gantenbein, B., Soleglad, M.E., and Fet, V., *Euscorpius balearicus* Caporiacco, 1950, stat. nov. (Scorpiones: Euscorpiidae): Molecular (Allozymes and mtDNA) and Morphological Evidence for an Endemic Balearic Islands Species, *Organisms, Diversity, and Evolution*, 2001, vol. 1, no. 4, pp. 301–320.

13. Kessler, K.F., On Russian Scorpions, *Trudy Russkago Entomologicheskago Obshchestva v S.-Peterburge* (Transactions of the Russian Entomological Society in St. Petersburg), 1874, vol. 8, no. 1, pp. 3–27 (in Russian).

14. Kinzelbach, R., Die Skorpione der Ägäis. Beiträge zur Systematik, Phylogenie und Biogeographie, *Zool. Jahrb., Abt. Syst.*, 1975, vol. 102, no. 1, pp. 12–50.

15. Lavrov, D.V., Boore, J.L., and Brown, W.M., The Complete Mitochondrial DNA Sequence of the Horseshoe Crab *Limulus polyphemus*, *Mol. Biol. Evol.*, 2000, vol. 17, pp. 813–824.

16. Scherabon, B., Gantenbein, B., Fet, V., Barker, M., Kuntner, M., Kropf, C., and Huber, D., A New Species of Scorpion from Austria, Italy, Slovenia, and Croatia: *Euscorpius gamma* Caporiacco, 1950, stat. nov. (Scorpiones: Euscorpiidae) Gajdoḍ, P. and Pekár, S., Eds., *Proc. 18th European Coll. Arachnol., Stará Lesná, 1999, Ekologia* (Bratislava), 2000, vol. 19, suppl. 3, pp. 253–262.

17. Swofford, D.L., PAUP* Phylogenetic Analysis Using Parsimony (*and other methods). Version 4. Sunderland: Sinauer Associates, 1998.

18. Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G., The Clustal X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by Quality Analysis Tools, *Nucl. Acids Res.*, 1997, vol. 24, pp. 4876–4882.