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

V. Fet1, Ay. Karataş2, E. V. Fet1, and A. Karataş2

1 Department of Biological Sciences, Marshall University, Huntington, West Virginia 25755-2510, USA
2 Department of Zoology, Nigde University, Nigde, Turkey

Received November 28, 2002

Abstract—The first molecular data (16S rRNA gene sequences) on the Anatolian scorpion populations, tenta-

tively 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 Birula 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 Balk-

cans (Bonacina, 1980). E. ciliciensis has been either synonymized with E. mingrelicus or considered its sub-

species (Kinzelbach, 1975; Fet and Sissom, 2000). Fet (1986) studied type material of Birula from the Zoologi-

cal 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 ana-

lyzed 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: Ulukıslıa, Gümüş-Maden road, 37°28′ N, 34°37′ E, ca. 1750 m asl. No. 69,

May 23, 2001, ZDUN/S 2001/69 (coll. A. Karat;ş); 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 QiagenTM 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 (CGATTGAAACTCAGATCA; forward, 18-mer) and a scorpion-specific reverse primer (GTGCAAGAGTAGGCATAATCA, 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
A matrix of genetic distances (Kimura 2-parameter distances)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigde</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eskisehir</td>
<td>0.045</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. germanus</td>
<td>0.069</td>
<td>0.070</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. gamma</td>
<td>0.047</td>
<td>0.054</td>
<td>0.074</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. flavicaudis</td>
<td>0.107</td>
<td>0.109</td>
<td>0.115</td>
<td>0.094</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. italicus</td>
<td>0.068</td>
<td>0.065</td>
<td>0.100</td>
<td>0.061</td>
<td>0.097</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. carpathicus</td>
<td>0.065</td>
<td>0.069</td>
<td>0.091</td>
<td>0.061</td>
<td>0.094</td>
<td>0.047</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>E. candiota</td>
<td>0.093</td>
<td>0.096</td>
<td>0.114</td>
<td>0.078</td>
<td>0.122</td>
<td>0.065</td>
<td>0.059</td>
<td>0.000</td>
</tr>
</tbody>
</table>

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. min grelicus” 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 Eskağır 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.

The morphological investigation of the Nigde specimens showed that they could belong to the "E. min grelicus" 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 Eskisehir 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.
(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 candida." 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, Viperidae bulgaraghica). In the localities of Gümüs and Maden, Euscorpius were found under stones as well as in alpine meadows and pine forests (Pinus brutia, F. 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


