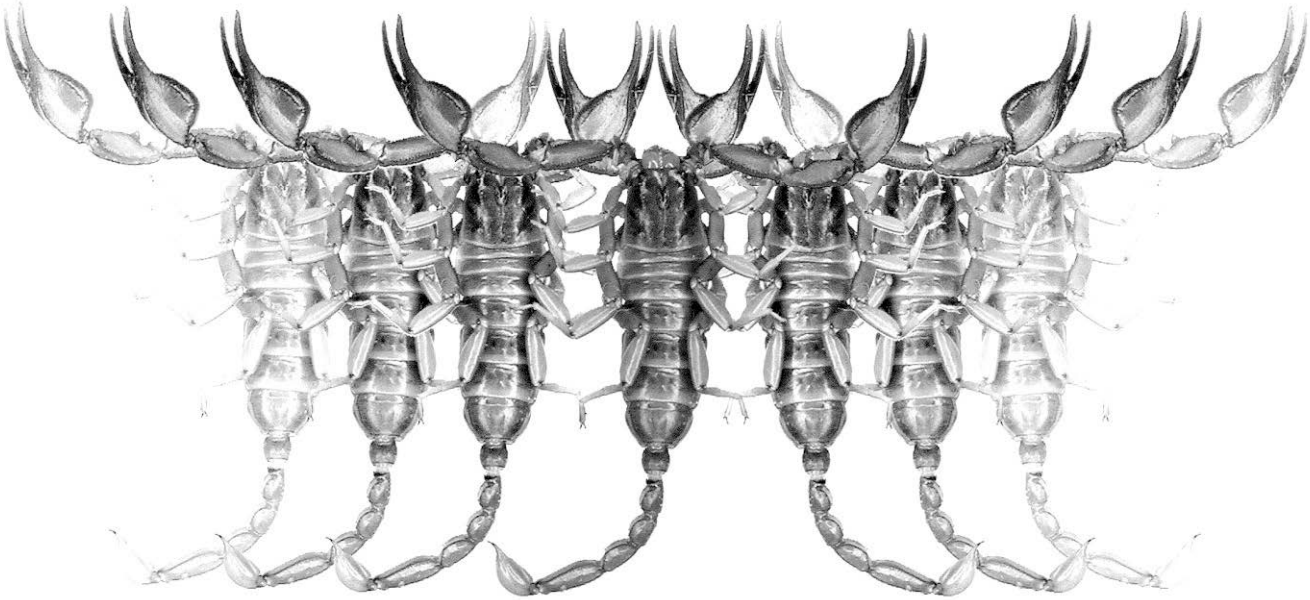
Euscorpius

Occasional Publications in Scorpiology



DNA markers confirm presence of *Euscorpius avcii* Tropea et al., 2012 (Scorpiones: Euscorpiidae) on Samos Island, Greece

Aristeidis Parmakelis, Panayiota Kotsakiozi, Gioele Tropea, Ersen Aydın Yağmur, Iasmi Stathi, Victor Fet & Michael E. Soleglad

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Derivatio Nominis

The name ***Euscorpius*** Thorell, 1876 refers to the most common genus of scorpions in the Mediterranean region and southern Europe (family Euscorpiidae).

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Summary

Euscorpius avcii Tropea et al., 2012 has been recently described from Dilek Peninsula in western Anatolia (Turkey, Aydın Province). The population from Samos Island in eastern part of the Aegean Sea is found to match closely the Anatolian *E. avcii*, making it a new, rare species for the Greek fauna, confirmed by two DNA markers as well as morphology. Samos also shares with western Anatolia two other local recently described scorpion species, *Iurus kinzelbachi* and *Neocalchas gruberi* (family Iuridae).

Introduction

Scorpions of the genus *Euscorpius* Thorell, 1876 (Scorpiones: Euscorpiidae) are very common in southern Europe and Anatolia. Ecologically diverse, they occupy a variety of habitats from xeric to mesic, from the Mediterranean shoreline to the high altitudes of the Alps and Balkans. The traditional taxonomy of this genus, based mainly on morphosculpture and coloration characters, was complicated and confusing.

Many authors reported *E. carpathicus* (L., 1767) or its subspecies from various localities in Greece including many islands (Kinzelbach, 1975) but without analyzing their actual specimens it is usually impossible to determine taxonomic affiliation. Some insights into morphology of island forms from Greece were offered by Fet (2000) who analyzed Balkan and Aegean collections from the National Museum of Natural History in Sofia, Bulgaria. Such studies are hindered by lack of material from many areas as well as existence of morphologically "cryptic" (i.e. exhibiting the same, "standard", largely invariable trichobothrial pattern) groups usually lumped under *E. carpathicus* (Fet & Soleglad, 2002; Kaltsas et al., 2008). Status of many populations reported in literature as "*E. carpathicus*" remains unclear, and has not been resolved on the basis of traditional morphology alone (Kaltsas et al., 2008; Vignoli & Salomone, 2008).

An ongoing investigation of many populations from Greece using multiple DNA markers (Parmakelis et al., in press) allowed revealing a diverse set of species, many of them undescribed. Here, we confirm that one of those populations present on Samos Island matches *Euscorpius avcii* Tropea et al., 2012, an oligotrichous form recently described from the neighboring mainland of Anatolia (Turkey).

Methods and Material

Abbreviations

NHMC, Natural History Museum, Iraklio, Crete, Greece; NHMW, Naturhistorisches Museum Wien, Vienna, Austria; ZMHB, Museum für Naturkunde der Humboldt-Universität zu Berlin, Berlin, Germany.

Material Studied

We studied 10 specimens (4 ♂, 6 ♀) from Samos Island, Greece, listed below. Two NHMC specimens were used for DNA analysis.

	Pectinal Teeth Number		Pedipalp Patella Trichobothria Number		
	males	females	ventral (*v*)	external	
				et	*em*
Dilek Peninsula, Turkey (modified after Tropea et al., 2012) (79 specimens)	7 (2) 8 (50) 9 (6) [n = 58]	6 (21) 7 (79) [n = 100]	6 (6), 7 (139) 8 (13) [n = 158]	5 (101) 6 (47) [n = 158]	4 (158) [n = 158]
Samos Island, Greece (10 specimens)	8 [n = 8]	6 (1) 7 (11) [n = 12]	6 (1) 7 (15) 8 (3) [n = 19]	4 (1) 5 (16) 6 (2) [n = 19]	3 (2) 4 (17) [n = 19]

Table 1: Morphological variation of *E. avcii* from Dilek and Samos.

1 ♀, Samos Island, Marathocampos, [1887], 37.7264°N, 26.69°E, leg. E. von Oerntzen (ZMB 15280); 3 ♂, 3 ♀, Samos Island, Valley of the Nightingales, road to Manolates, 30 May 1987, leg. E. Kritscher (NHMW 16.008/1-6); 1 ♀, Samos Island, S of Pyrgos, 37.7117°N, 26.8042°E, 2 June 1987, leg. E. Kritscher (NHMW 16.009); 1 ♂, Samos Island, Manolates, SA11, 55 m, 37.7978°N, 26.83°E, 30 August 2000, leg. I. Stathi (NHMC 2154) (DNA samples E01, E26); 1 juv. ♀, Samos Island, Pagondas to Spatharaoi, 300 m, 37.67°N, 26.832°E [for Pagondas], pinewood on limestone, 8 January 2007 (NHMC 7497).

Additional specimen from Turkey used for DNA analysis was from: 2 km S Güzelçamlı Village, 354 m, Kuşadası District, Aydın Province, 37.6895°N, 27. 2266°E, 13 June 2012, leg. E. Yağmur.

A distribution map of specimens from Samos and Turkey is presented in Fig. 1.

DNA analysis

All DNA work was performed in the University of Athens by P.K. and A.P. We compared DNA from one *Euscorpius* specimen originating from Manolates, Samos Island, Greece (a part of the large multi-marker study of *Euscorpius* populations across Greece) and two specimens from Güzelçamlı Village, Dilek, Turkey, supplied by E.Y.

Collected specimens were stored in absolute ethanol. DNA extraction was carried out following the protocol described in Parmakelis et al. (2005). Two mitochondrial genome regions (markers) targeted for amplification via the polymerase chain reaction (PCR) were large ribosomal subunit (LSU, *16S rDNA*) and the cytochrome oxidase subunit I (*COI*). For details on PCR primers and protocols, see Gantenbein et al. (1999) and Parmakelis et al. (2006). Automated sequencing of both strands of each purified amplicon was performed in a PE-ABI3740 automated sequencer (using Big-Dye terminator chemistry) using the same primers as in the PCR amplifications. DNA sequences produced for this study have been deposited in GenBank under the accession numbers: KC215588, KC215604: Samos Island, KF030936, KF030937: Turkey (*16S rDNA*) and KC21 5671, KC215688: Samos Island, KF030934, KF030935: Turkey (*COI*). Sequences were aligned with CodonCode Aligner v. 2.06 (Genecodes Corporation) using the Clustal algorithm. The gaps present in the alignment of *16S rDNA* were treated as missing. The genetic distances separating individual sequences were calculated using MEGA5 (Tamura et al., 2011). sequences of *E. avcii* from Dilek were used together with the *16S rDNA* and *COI* sequences reported in Parmakelis et al. (in press) in a Neighbor Joining tree reconstruction analysis (using MEGA5) in order to infer their relationships with other *Euscorpius* specimens.

Results

Morphology. General habitus and details of morphology of Samos specimens closely fit the detailed description of *E. avcii* Tropea et al., 2012 from Dilek peninsula, Turkey. Table 1 lists variable meristic characters scored from 10 Samos specimens, in comparison with 79 Dilek specimens. The same patterns of variation, well within a single species range, was observed in both populations.

Variation observed in 79 studied specimens (29 males, 50 females) of *Euscorpius avcii* in Dilek Peninsula, Turkey (after Tropea et al., 2012) was as follows: pectinal teeth in males predominantly numbered 8, pectinal teeth in females varied from 6 to 7, with 7 more common. Pedipalp patella ventral trichobothria numbered predominantly 7; in pedipalp patella external trichobothria, *et* varied from 5 to 6, with 5 more common; all other external series exhibited no variation (*est* = 4, *em* = 4, *esb*= 2, eb_a = 4, *eb* = 4).

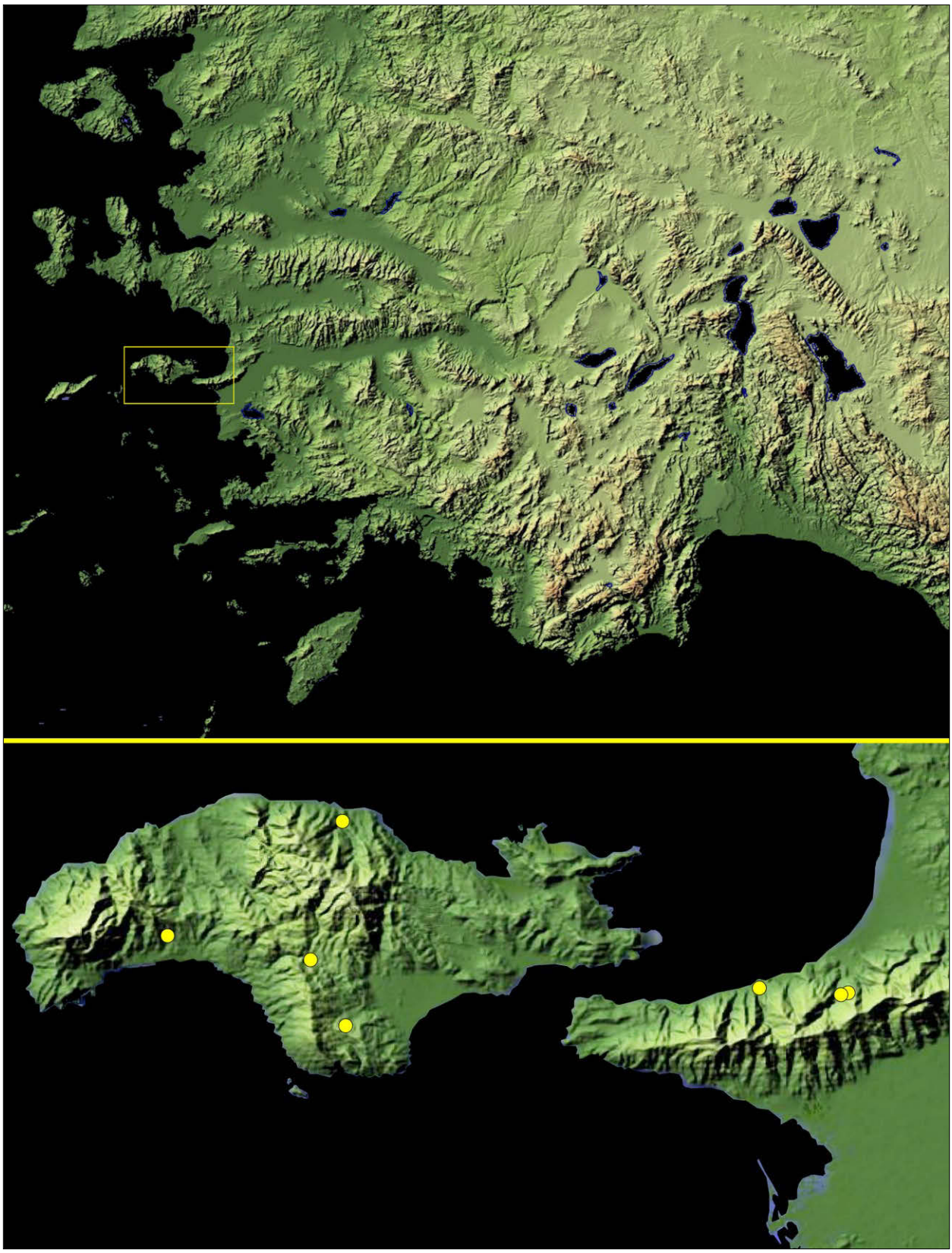

Figure 1: Distribution map of *E. avcii* in Samos Island (our data) and Dilek Peninsula (after Tropea et al., 2012, fig. 7).

Variation observed in 10 studied specimens (4 males, 6 females) from Samos closely followed Dilek patterns: pectinal teeth in males numbered 8; pectinal teeth in females predominantly 7. Pedipalp patella ventral trichobothria numbered predominantly 7; in pedipalp patella external trichobothria, *et* varied from 4 to 6, predominantly 5.

An interesting observation is a rare, asymmetric (3-4 and 4-3) reduction of *em* from 4 to 3 in two specimens, not found in Dilek; all other external series exhibited no variation (*est* = 4, *esb*=2, eb_a = 4, *eb* = 4). This trichobothrial series is fixed as *em*=3 in subgenus *Alpiscorpius*, but also independently exhibits a derived reduction of one accessory trichobothrium (from *em*=4 to *em*=3) in several lineages of subgenus *Euscorpius* s.str. in the Balkans and Greece (Fet, 2000; Fet & Soleglad 2002, 2007), most notably in *Euscorpius carpathicus* s.str. from Romania. Such minor reduction of trichobothrial numbers is common in island or isolated populations (Gantenbein et al., 2001; Soleglad & Fet, 2002).

Molecular Data. DNA sequences of *E. avcii* from Dilek and the sample from Samos clustered firmly together in the combined *16S rDNA* and *COI* phylogenetic tree. The sequence divergence between *E. avcii* from Turkey and Samos was 1.2%. For comparison, our data for the sequence divergence within *Euscorpius sicanus* (C. L. Koch, 1837), a species that is widespread within Greek mainland and islands, was 2.4% whereas within *E. avcii* (Turkish and Samos specimens considered together) it was only 0.8 %. The divergences were similar under both the Jukes-Cantor distance model and the Kimura K-2p model. We confidently confirm, therefore, that Dilek and Samos populations represent the same species.

Discussion

Euscorpius from Samos are very rare in collections. For a long time, the only specimen from Samos available in museums was a single female in Berlin (ZMHB 15280) collected near Marathocampos by Eberhardt von Oertzen (1856–1909) a prominent German naturalist who visited Greek islands in 1887 (Vrenozi & Dunlop, 2013). This specimen from Samos remained unpublished. The scorpion label bears no date but we know that von Oertzen collected ants from the same locality on 12 June 1887 (Forel, 1889: 255). His specimen of a recently described scorpion *Protoiurus rhodiensis* Soleglad et al., 2012 from Rhodes (ZMHB 8069) (fam. Iuridae) was collected in May 1887 (Kovařík et al., 2010).

At the same time, Werner (1934: 162) who collected on Samos in June 1932 and paid a special attention to scorpions, found *Mesobuthus gibbosus* (Buthidae; reported as *Buthus gibbosus*) and a rare *Iurus kinzelbachi* Kovařík et al., 2010 (Iuridae; reported as "*Jurus dufoureius"*) near Marathocampos but did not collect any *Euscorpius*.

Exactly 100 years after von Oertzen, in summer 1987, the Austrian zoologist Erich Kritscher (1927–2010) collected seven specimens from Samos (Kritscher, 1993: 384, reported as "*E. carpathicus carpathicus*"); his great scorpion collection from Greece is deposited in Vienna (NHMW). In addition to eight specimens of von Oertzen and Kritscher, we analyzed two new specimens collected by Greek zoologists in 2000 and 2007.

Vignoli & Salomone (2008: 199, fig. 10) mentioned an unnamed population from Samos, a small form with stocky pedipalps and pectinal teeth 7 in female, and 8 in male, which fits *E. avcii*. Their specimens had 5 *et* trichobothria, which fits *E. avcii* as well but they also had 5 trichobothria in the ventral series, which does not correspond to any known *Euscorpius* s.str. We suspect that their specimens belong to the subgenus *Alpiscorpius*, common in the mainland Anatolia.

Samos Island is located in the easternmost part of the Aegean Sea, and is separated by less than 2 km of seawater from the Dilek Peninsula. It has been connected to the Anatolian mainland by a landbridge in Pleistocene and earlier, and shares with the Anatolian mainland a number of endemic plant and animal species (see e.g. Sindaco et al., 2000). Among them, are at least two other interesting local scorpion species (family Iuridae), *Iurus kinzelbachi* (Kovařík et al., 2010; Soleglad et al., 2012) and *Neocalchas gruberi* (Fet et al., 2009) (first reported for Samos by Sissom, 1987, as "*Paraiurus nordmanni"*; see Fet et al., 2009; Yağmur et al., 2013).

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