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SHORT COMMUNICATION

AN EXTREMELY LOW GENETIC DIVERGENCE ACROSS THE RANGE OF *EUSCORPIUS ITALICUS* (SCORPIONES, EUSCORPIIDAE)

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ABSTRACT. Little or no genetic divergence is detected using mitochondrial 16S rDNA sequence comparisons across the entire geographic range of the scorpion *Euscorpius italicus* (Herbst 1800) from Switzerland, Italy, Slovenia, Greece and Turkey. This is consistent with known absence of patterns of allozymes and morphological variation. *Euscorpius italicus* is found almost exclusively in human habitations. Its sister species, *E. naupliensis*, exhibits much higher genetic diversity within southern Greece. We suggest that the natural populations of the thermophilic *E. italicus* underwent a bottleneck during the glaciations, and that its modern range could be a result of dispersal with humans.

Keywords: Scorpions, genetic distance, DNA, 16S rRNA, biogeography

A large, conspicuous scorpion *Euscorpius italicus* (Herbst 1800) has been known to arachnologists for 200 years and to humankind for millennia. It is commonly found in many localities in Italy and Greece, being an especially common species in human habitations (Crucitti 1993; Braunwalder 2001). This species is found from the French Riviera to the northern and eastern shores of the Black Sea. *Euscorpius italicus* prefers a xeric microclimate (Birula 1917a, 1917b; Braunwalder 2001; Fet et al. 2001). In Italy, this species is locally very abundant and usually synanthropic. To the north of Italy it is limited by the southern Alpine valleys in Italy and Switzerland (Crucitti 1993; Braunwalder 2001); in Turkey and the Caucasus it also does not venture into high mountains (Birula 1917a, 1917b). The species' elevational preference seems to range from 0–500 m, while reported well-isolated ''island'' populations above 500 m could be attributed to recent human-mediated range expansion (Braunwalder & Tschudin 1997). Several subspecies were described in this species (Birula 1917a; Birula 1917b; Caporiacco 1950) but are currently not recognized (Kinzelbach 1975; Vachon 1981; Bonacina 1982; Fet & Sissom 2000)(Fig. 1). A detailed redescription and taxonomic history of *E. italicus* was recently published by Gantenbein et al. (2002), who also demonstrated the separate species status (well defined by both morphological and molecular criteria) for *E. naupliensis* (C. L. Koch 1837) from southern Greece, for many years considered a synonym of *E. italicus*.

In order to assess the species structure of *E. italicus*, we used comparative analyses of the mitochondrial 16S ribosomal RNA gene, a molecular marker that has been recently applied to resolve the species-level phylogeny of several species of *Euscorpius* (Gantenbein et al. 1999, 2000, 2001, 2002; Fet et al. 2002, 2003); for the detailed DNA analysis procedures and phylogenetic tree-building algorithms, see Gantenbein et al. (1999, 2000). Seven mtDNA sequences (ca. 400 base pairs each) were aligned using ClustalX 1.81 (Thompson et al. 1997). Two new DNA sequences, obtained for the present study, were deposited in GenBank (http://www.ncbi.nlm.nih.gov) under accession numbers: *Ei*TU1 (AY371536) Samugüney Village, Bulancak, Giresun, Turkey, 40°56′N, 38°15′E, 17 February 2003 (coll. A. Karataş), and *Ei*SM1 (AY371535) Silvi Marina, Abruzzo, Italy, 42°34′N, 14°05′E, 20 June 2000 (coll. F. Kovařík). Voucher specimens are deposited in the Unites States National Museum (USNM), Smithsonian Institution, Washington, DC, USA. Four additional DNA sequences of *E. italicus*

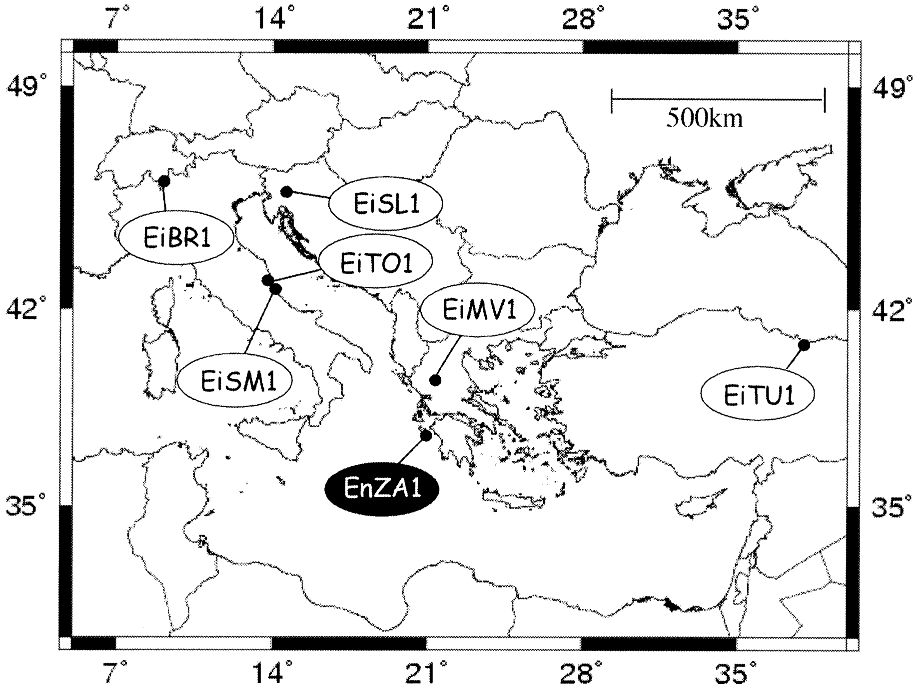

Figure 1.—Origin of population samples of *Euscorpius italicus* and *E. naupliensis* (outgroup; dark circle).

published earlier by our research group and its collaborators (Gantenbein et al. 1999, 2002) were extracted from the GenBank online database. Their abbreviations, accession numbers and geographic origin were: *Ei*BR (AJ389378), Brissago, Ticino, Switzerland, 46°07′N, 08°43′E, 25 May 1996 (coll. B. Gantenbein); *Ei*TO1 (AJ298067), Tortoreto, Abruzzo, Italy, 42°47′N, 13°55′E, 7 October 1997 (coll. M. Bellini); *Ei*MV1 (AJ506152), Metsovo, Epirus, Greece, 39°46′N, 21°10′E, 13 May 2001 (coll. V. Fet); *Ei*SL1 (AJ512752), Brje, Dobravlje, Aidovščina, Slovenia, 45°46′N, 13°50′E, 7 August 2000 (coll. B. Sket). As an outgroup, we used *E. naupliensis*: *En*ZA1 (AJ506153), Zakynthos Island, Greece, 37°46′N, 20°46′E, 20 August 1999 (coll. K. Palmer) (Gantenbein et al. 2002).

For estimation of within-species variation in species with moderate genetic variation, the application of networks and cladistic methods has been proposed to be the most efficient (Posada & Crandall 2001). Recently developed methods allow evaluation of the limits of parsimony (Templeton et al. 1992, 1995). Therefore, we calculated a statistical network that only connects haplotypes with a 95 % confidence limit using the program TCSalpha v1.01 (Clement et al. 2000). From the length of the DNA sequences we estimated the maximum number of steps that haplotypes can differ from each other for a 95% confidence limit. This statistical cladistic analysis revealed a very weak detectable geographic structure across the entire range of *E. italicus*. The statistical cladogram in Fig. 2 connects haplotypes of up to 7 mutation steps, whereas gaps are treated as the "fifth" base pair. The estimated level of divergence ranging from zero to four base pair substitutions (i.e. from 0–1.2 % uncorrected "p") is in a dramatic contrast with the elaborate, deep geographic structure detected using the same mitochondrial gene fragment in the congeneric species *E. germanus* (C.L. Koch 1837) and *E. alpha* Caporiacco 1950 (Gantenbein et al. 2000), *E. naupliensis* (C.L. Koch 1837) (Gantenbein et al. 2002), *E. tergestinus* (C.L. Koch 1837) (Fet et al. 2002), and *E. sicanus* (C.L. Koch 1837) (Fet et al. 2003); in each of the listed taxa, within-species divergences were up to 5%. The relatively poor genetic diversity of the mtDNA marker clearly supports the complete absence of nuclear variation at allozyme loci among Swiss populations of *E. italicus* (Gantenbein et al. 1998) compared with populations of other congeneric species (Gantenbein et al. 2001). The network (Fig. 2) also is consistent with the hypothesis of artificial transplantation, which is an important issue for phylogeographic studies on scorpions (Gantenbein & Largiadèr 2002). The Swiss haplotype (EiBR1) is identical with the Slovenian haplotype (EiSL1), which supports a very recent transplantation from the east into the region of

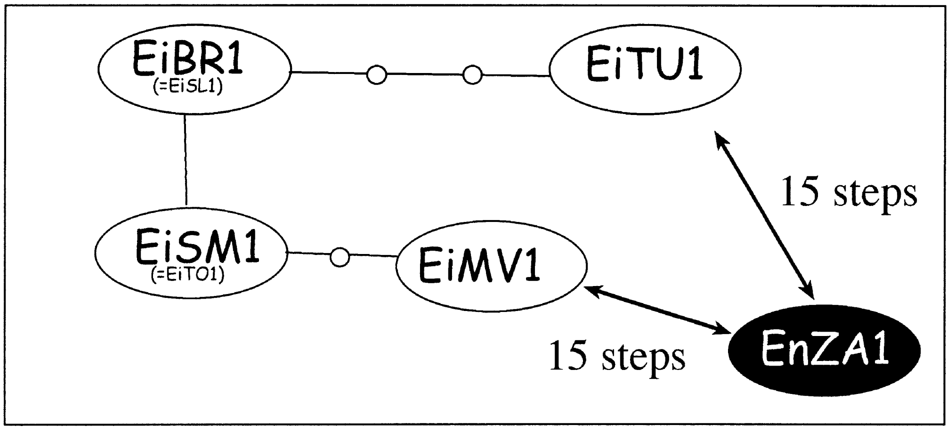

Figure 2.—Maximum Parsimony tree connecting isolated mtDNA haplotypes of a fragment of the 16S rRNA gene. Connections are exclusively drawn with a confidence limit of 95% (i.e., ≤ 7 steps) as calculated according to the method of Templeton et al. (1992). Large circles represent haplotypes; small circles represent hypothetical intermediate haplotypes that connect the haplotypes with each other. Network is not drawn proportionally to genetic distance. See text for the designation of haplotypes. Outgroup shown as a dark circle.

Northern Italy and Switzerland. On the other hand, the haplotypes from Italy (EiSM1/TO1) and from Greece (EiMV1) are each connected to each other with only a single mutational step.

Birula (1917a, 1917b) characterized in detail the geographic distribution of *E. italicus*, describing clearly two disjunct parts of the *E. italicus* range, ''western'' (Europe) and ''eastern'' (Anatolia and Caucasus). This idea still holds as the species has never been found in the eastern part of the Balkan Peninsula (Gantenbein et al. 2002). Birula (1917a, 1917b) considered the eastern part of the range (a narrow strip along the southern and eastern coasts of the Black Sea) reduced compared to the western, due either to southward increase of the Black Sea basin, or aridization of the climate in Anatolia. Morphologically, *E. italicus* from the ''western'' and ''eastern'' parts of the range are the same species (Gantenbein et al. 2002). Thus a strong case can be made for recent, even historical time, dispersal of *E. italicus* between the ''western'' and ''eastern'' portions of its range.

From the current network of sampled haplotypes one could speculate that Anatolia might have served as the refuge for *E. italicus* during the last Pleistocene glaciations. Evidence that Anatolia might have been an important refuge for plants and animals has been recently found for the gall wasp (*Andricus quercustozae*) by Rokas et al. (2003) who reported a higher within-population diversity in the Anatolian populations than in the European ones. We cannot infer any conclusions about genetic diversity of populations; for this, many more *E. italicus* populations and specimens per population need to be genotyped for the orthologous mtDNA fragment. The low genetic divergence between the haplotypes, however, supports a recent (postglacial) range expansion of this species. Similar low genetic diversity across a wide range was found in the congeneric species *E. flavicaudis*, which is not closely related to *E. italicus* (~10% sequence divergence between species; Gantenbein et al. 1999). In *E. flavicaudis*, the combined analysis of multilocus allozyme data and mtDNA sequence data also revealed a low diversity, which can be interpreted as the evidence of rapid range expansion, most likely by human transplantation (Gantenbein et al. 2001). *Euscorpius flavicaudis* is known to be an invasive species since it has been recently reported from places obviously outside its natural habitat, e.g., south of England (Benton 1991) and Uruguay (Toscano-Gadea 1998), where it manages to survive and reproduce. Hewitt (1996, 1999) lists several examples for rapid natural spreading of animals from glacial refuges into Europe, with dispersal rates of ~300 m per year and higher. Hewitt (1990) estimated that flightless grasshoppers like *Chorthippus parallelus* spread from southern Europe to England at a rate of about 300 m per year. In scorpions, however, much lower annual dispersal rates have been determined, which range between 1–30 m, males having a higher dispersal rate (Polis et al. 1985). If we assume as lower dispersal rates for scorpion species than for the flightless grasshoppers, we have to conclude that *E. italicus* and *E. flavicaudis* populations were both spread through human civilization. It is also very likely that these two species had two different glacial refuges: *E. italicus*, in Anatolia and *E. flavicaudis* probably in the south of Italy, which has been identified as a main refuge for many other species (Taberlet et al. 1998).

Unlike other species of *Euscorpius*, *E. italicus* was never reported from any of the Aegean islands, or from any Mediterranean islands such as Baleares, Sicily, Sardinia, Corsica or Malta; it has been only recorded from the offshore islands in the Adriatic Sea (Dalmatian coast of Croatia) and Ionian Sea (Corfu, Greece) (Gantenbein et al. 2002). At the present time, this species appears to be successfully dispersing with human assistance, since in parts of its range it is almost or exclusively synanthropic, being found only in human habitations or ruins but not in the wild (Crucitti 1993). Braunwalder (2001) documented that in only 33 records out of 1,031 records in southern Switzerland, *E. italicus* has been found in decidedly natural habitats. Another sign of its probable dispersal with humans is the fact that this species, like *E. flavicaudis*, establishes new reproducing populations, often remotely disjunct from its continuous range. As examples we can mention established populations in lower Don, Russia (Zykoff 1912); in Sion, Valais, Switzerland (Braunwalder 2001); in Ljubljana, Slovenia (Fet et al. 2001); and even in Yemen (Birula 1937) and Iraq (Fet & Kovařík 2003). Records from southwestern Romania (Mehadija, Oravitza; Birula 1917a, 1917b; confirmed by Vachon 1981) probably also refer to introduced populations. Single specimens of *E. italicus* have been found in many localities well outside the main range (Fet & Gruodis 1987; Fet & Sissom 2000; Gantenbein et al. 2002). Moreover, at least within Europe, the active transplantation of *Euscorpius* with the human peddlers of ''scorpion oil'' (an infusion of olive oil with live scorpions, allegedly of medicinal value) has been possible until recently (Komposch et al. 2001). This frequent anthropochory and synanthropy, absence from most islands, and high morphological and genetic similarity of the studied populations from Switzerland, Italy, Slovenia, Greece and Turkey, all suggest that the dispersal of *E. italicus* (likely from glacial refugia) might not be an ancient event. Further investigation of multiple populations could determine an exact refugial origin and possible ways of dispersal of *E. italicus*.

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