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Contrasting mitochondrial DNA diversity estimates in Austrian *Scutovertex minutus* and *S. sculptus* (Acari, Oribatida, Brachypylina, Scutoverticidae)

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ABSTRACT

Important insights into the evolutionary and demographic history of species can be obtained from inter-specific comparisons of patterns and from determining the degree of genetic diversity. Analysis of sequences of the mitochondrial cytochrome oxidase I (COI) gene revealed remarkable differences in mtDNA diversity estimates and the distribution of mitochondrial lineages between the two closely related oribatid mite species *Scutovertex sculptus* and *S. minutus* in Austria. Divergence time estimates revealed an age of approximately 48–74 Myr for the split between the two *Scutovertex* species and age estimates of about 2–3 and 8–12 Myr for the most recent common ancestors of Austrian *S. minutus* and *S. sculptus*, respectively. Genetic diversity was considerably lower in *S. minutus* than in *S. sculptus*. A clear geographic sub-division into samples originating from north and south of the Central Alps became evident in *S. minutus*, whereas no phylogeographic structure was found in *S. sculptus*. Together with a high genetic diversity this is suggestive of a panmictic population and exceptional dispersal ability, most probably facilitated by phoresy on birds. The lack of sub-structure with regard to habitat types in *S. sculptus* suggests that this species can cope with a wide range of environmental conditions.

Introduction

Patterns and degree of intraspecific genetic diversity can provide important information for deducing the evolutionary history of species and are assumed to reflect population size, ecology, and the species' ability to adapt to different environments. Several biological and demographic factors such as population structure, population bottlenecks, natural selection, life cycle, and reproductive mode affect genetic diversity (Caballero and Hill 1992; Charlesworth et al. 1993; O'Brien 1994; Hedrick 2005; Subramanian 2009).

The oribatid mite family Scutoverticidae is assigned to a subgroup of the Circumdehiscentiae ("Higher Oribatida") at the base of the Poronota and is characterized by wrinkled nymphs and adults lacking pteromorphae (Grandjean 1953). To date, the family comprises eight genera with more than 60 species worldwide, whereby *Scutovertex* is the most speciose. The two most common members of this genus are *Scutovertex sculptus* and *S. minutus* with a widespread Palaearctic distribution. These species have probably been confused in the past, but recent re-descriptions should prevent misidentification (Schäffer and Krisper 2007; Pfingstl et al. 2008). Both species have similar body size and an approximately six month-long generation time (Grafeneder personal communication). Furthermore, similar to *S. sculptus*, *S. minutus* seems to be panphytophagous (Smrž 2006). Their preferred habitats are sun-exposed mosses and lichens, implying extreme living conditions for both mites. Thus, it is important for these species to be able to tolerate rapid environmental changes such as desiccation, inundation, and temperature fluctuation (Smrž 1992). Their adaptation to such extreme environmental conditions allows them to play a major role as pioneer organisms. For example, *S. sculptus* has been recorded on a variety of dumps (Skubała 1999, 2004), on saline soils and in salt marshes (Seniczak et al. 1985; Schuster 1959) or other dry habitats (Schatz 1995; Migliorini and Bernini 1999). *S. minutus* is commonly found on rocks and artificial habitats such as roofs (Smrž 1992), walls (Steiner 1995a) or roadside slopes (Eitminaviciute 2006), and sometimes on tree trunks (Pschorn-Walcher and Gunhold 1957). It has been also reported to be a pioneer species in a chalk quarry in England (Parr 1978). Moreover, there is evidence that scutoverticid mites, and in particular the species included in the present study, might be important bioindicators, e.g. of air pollution (Weigmann and Kratz 1987; Steiner 1995a, 1995b).

The present study set out to achieve two goals. First, it aimed to test for potential differences in the patterns of genetic diversity in *S. minutus* and *S. sculptus* in Austria. Given their high similarity

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in most life history traits we hypothesized that, although both species occur in different types of habitat, they would show similar patterns and degrees of genetic variation, and a clear phylogeographic structure, assuming that they are small moss-dwellers with low dispersal ability. Second, this study investigated the possibility of intraspecific sub-structuring reflecting a potential specialization to different habitat types populated in *S. sculptus*. We hypothesized that *S. sculptus* populations living in mosses on rocks are distinct from those inhabiting mosses on saline soils due to a potential adaptation to the markedly different environmental conditions in these habitats. To test these hypotheses we analysed sequences of the mitochondrial cytochrome oxidase I (COI) gene, which has been widely used for resolving phylogenetic and evolutionary questions in mites (Salomone et al. 2002; Heethoff et al. 2007; Ros and Breeuwer 2007; Marangi et al. 2009).

Material and methods

Sampling and DNA extraction

In this study we investigated 48 specimens of *S. sculptus* and 35 of *S. minutus*, collected from a total of 18 localities in Austria between 2005 and 2008 (Fig. 1 and Table 1). Specimens were extracted from mosses and lichens with Berlese–Tullgren funnels and preserved in absolute ethanol. Species identification was done using the criteria defined in Schäffer and Krisper (2007), and Pfingstl et al. (2008). Total genomic DNA was extracted from single individuals with a modified CTAB (hexadecyltriethylammonium bromide) method (Boyce et al. 1989) (for details see Appendix). After DNA extraction, the sclerotized body remnants were mounted on permanent slides and used for sex determination.

PCR and DNA sequencing

The mitochondrial COI gene was amplified using the primers COI_Fsy (5′-GNTCAACAAWTCATWAAG-3′) and COI_Rsy (5′-TAAACTTCNGGYTGNCCAAAAAATCA-3′) for COI-region 1 (modified after Heethoff et al. 2007), Mite COI-2F and Mite COI-2R (Otto and Wilson 2001) for COI-region 2. Polymerase chain reaction (PCR), purification of PCR products, and sequencing followed the protocol described in Schäffer et al. (2008). Sequences are available from GenBank under the accession numbers listed in Table 1.

Data analysis

Sequence verification was performed by comparisons with known mite COI sequences in GenBank. Sequences were aligned by eye in MEGA 3.1 (Kumar et al. 2004) and number of haplotypes, haplotype (H_d) and nucleotide diversity (π), as well as rates of synonymous (K_S) and non-synonymous (K_A) substitutions were calculated in DnaSP v4.50.3 (Rozas et al. 2003). To assess their statistical significance, inter-specific differences in H_d and π were tested against a null distribution obtained by randomizing sequences between species (1000 permutations) and recalculating these indices (Muñoz-Fuentes et al. 2005). To test for selection based on the K_A/K_S ratio, we applied the Kumar method (Nei and Kumar 2000; Kumar et al. 2001) as implemented in MEGA, which assesses the statistical significance by means of 1000 bootstrap replicates.

Phylogenetic inference was based on maximum parsimony (MP) and Bayesian inference (BI), conducted in PAUP* 4.02a (Swofford 2002) and MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003), respectively. Preliminary phylogenetic studies (Schäffer et al. unpublished) showed that *S. pannonicus* – another species of the genus *Scutovertex* – is closely related to the two studied species and therefore served as the outgroup. Heuristic tree search under MP criteria applied random addition of taxa and TBR branch swapping (1000 replicates). Statistical support for the resulting topologies was assessed by bootstrapping (1000 pseudo-replicates). For Bayesian inference of phylogeny the data were partitioned by codon position with the GTR+I+G model (Rodríguez et al. 1990) applied to each partition. Posterior probabilities were obtained from a Metropolis-coupled Markov chain Monte Carlo simulation (2 independent runs; 8 chains with 7 million generations each; chain temperature: 0.2; trees sampled every 100 generations). Chain stationarity and run parameter convergence were checked using Tracer v1.4 (Rambaut and Drummond 2007; available at http://beast.bio.ed.ac.uk/Tracer/). The first 10% of all sampled trees were discarded as burn-in before calculating a 50% majority rule consensus tree from the remaining trees.

To test for rate constancy among all taxa, and hence, to justify the use of a molecular-clock model for estimating divergence times, we performed a Bayesian relative rates test according to the method described by Wilcox et al. (2004). The posterior

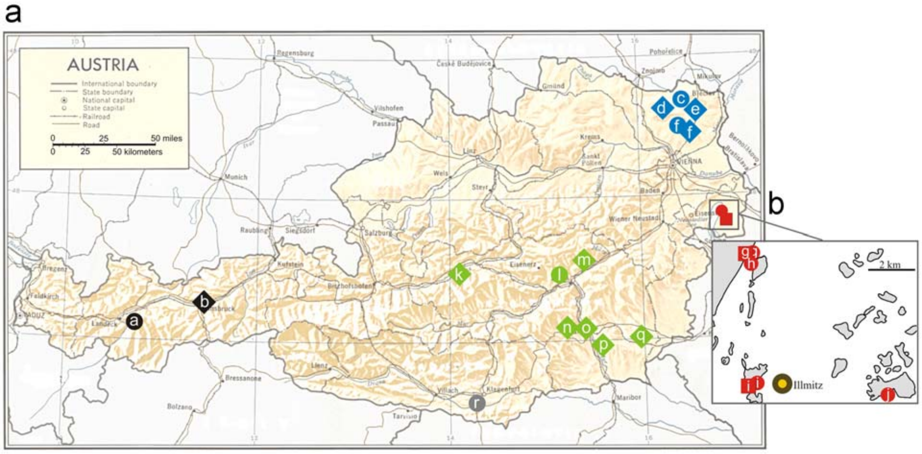

Fig. 1. (a) Map of Austria (modified from www.lib.utexas.edu/maps/europe/austria.gif) with sampling sites. (b) Lakes of the "Seewinkel" in the surroundings of Lake Neusiedl (Burgenland). ● =*S. sculptus*, ♦ =*S. minutus*, ■ =*S. pannonicus*. Number code of sampling sites is the same as in Table 1. Each Austrian province is marked by one color.