Research Article

Revising museum collections help to fill knowledge gaps in the Italian mammal fauna: the case of *Sorex araneus* and *Sorex antinorii* from South Tyrol

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Abstract

In Italy, after the elevation to species rank of the Valais shrew *Sorex antinorii*, all collection specimens from the museums previously attributed to the Eurasian shrew *Sorex araneus* were assigned to the former species. But no official verification of this “automatic” species attribution has ever been attempted. In our work we did the revision of 14 specimens of the *Sorex araneus* group from the Autonomous Province of Bolzano-South Tyrol in the north-east of Italy, by using molecular markers. The study was based on cytochrome b sequences, that allowed to assign unambiguously the analyzed specimens to one of the *Sorex* species. Among these South Tyrolean *Sorex* records we unexpectedly found only one specimen of *S. antinorii*, whereas the other 13 specimens could all be assigned to *S. araneus*. In this way, we were able to prove the occurrence of both *S. antinorii* and *S. araneus* for South Tyrol. Our work furthermore shows that *S. araneus* in Italy was never “gone” and it has to be added to the list of Italian mammals again. These findings thus stress the importance of a revision of museum collections by using modern technologies, to estimate the correct level of diversity of the small mammal fauna at regional and national level and, as in the case of *S. antinorii*, to better define its actual northern distribution limit in the Alps.

Introduction

The genus *Sorex* (Linnaeus) includes numerous species, whose status has not yet been sufficiently clarified (Spitzengerber, 1990). Therefore, information on the number of species in the literature varies considerably, ranging from 64 species reported in the first mammalian checklist of the world (Honacki et al., 1982) to 70 in Wilson and Reeder (1993) and 77 in Wilson and Reeder (2005), to the current 86 (Wilson and Reeder, 2005). In the course of time, many of its chromosomal races raised to species rank. In Italy, after the elevation to species rank of the Valais shrew *Sorex antinorii*, all collection specimens from the museums previously attributed to the Eurasian shrew *Sorex araneus* were assigned to the former species. But no official verification of this “automatic” species attribution has ever been attempted. In our work we did the revision of 14 specimens of the *Sorex araneus* group from the Autonomous Province of Bolzano-South Tyrol in the north-east of Italy, by using molecular markers. The study was based on cytochrome b sequences, that allowed to assign unambiguously the analyzed specimens to one of the *Sorex* species. Among these South Tyrolean *Sorex* records we unexpectedly found only one specimen of *S. antinorii*, whereas the other 13 specimens could all be assigned to *S. araneus*. In this way, we were able to prove the occurrence of both *S. antinorii* and *S. araneus* for South Tyrol. Our work furthermore shows that *S. araneus* in Italy was never “gone” and it has to be added to the list of Italian mammals again. These findings thus stress the importance of a revision of museum collections by using modern technologies, to estimate the correct level of diversity of the small mammal fauna at regional and national level and, as in the case of *S. antinorii*, to better define its actual northern distribution limit in the Alps.

Amori et al. (2008) reported five *Sorex*-species for Italy: *Sorex alpinus, S. antinorii, S. arunchi, S. minutas* and *S. samniticus*. Among these species, two can be assigned to the *S. araneus* group: the sub-endemic *S. antinorii* and the endemic *Sorex samniticus*. The latter is considered a phylogenetic basal species of the *S. araneus* group, characterized by not-rearranged fully acrocentric chromosomemes (Mackiewicz et al., 2017). In the latest checklist of Italian mammals (Loy et al., 2019), only four *Sorex*-species are recognized, as *Sorex arunchi*, described by Lapini and Testone (1998), was retracted after molecular genetic studies by Yannic et al. (2012) had shown its conspecificity with *S. antinorii*.

Although *S. araneus* s. str. is not listed in the current checklist of Italian mammals (Loy et al., 2019), there is some evidence of its presence, at least in some areas in the Northeast. In fact, preliminary analyses of the mandibles of *Sorex* sp. from the Italian Alps (Frituli-Venezia Giulia and Veneto regions), suggest the occurrence of populations with a closer morphology to *S. araneus* than to *S. antinorii* (Lapini and Cassol, 2017; Dorigo et al., 2016). Such observations make revision of collection material coupled with analysis of genetic data mandatory to support the actual occurrence of *S. araneus* s. str. in Italy (Loy et al., 2019).

Bertolino et al. (2015) already emphasized the importance of genetic studies to better understand the taxonomy and genetic diversity of Italian small mammals. Italy is an endemism-rich area for small mammals but for different reasons species richness and distribution, as well as possible contact zones between different species, are far from being fully described (Amori and Castiglia, 2018).

In some Italian museums, especially in the North, such as the Museum of Nature South Tyrol, there are still numerous specimens labeled as *S. araneus*. As a matter of fact, small mammal diversity in South Tyrol, a region in the far north of northeastern Italy, is largely neglected in scientific literature, although its peculiar geographical posi-
tion makes this region a putative contact zone between Italian endemic species and genetic lineages and their European relatives.

In this study, we employed molecular analyses to verify which of the species from the Sorex araneus group occur in the Autonomous Province of Bolzano - South Tyrol. Extracting DNA from preserved museum specimens, we compared South Tyrolean material with sequences available in GenBank of individuals of S. araneus and S. antinorii, collected in different sites of their current known European distribution range. In contrast to the hardly reliable discrimination of these species on the basis of preserved specimens’ morphology (skulls and bellow), molecular markers offer the opportunity to re-evaluate the species attribution, to define the distribution boundaries of S. antinorii and eventually confirm or reject the occurrence of S. araneus in northern Italy.

**Materials and methods**

We examined 14 specimens of Sorex sp. preserved in alcohol at the Museum of Nature South Tyrol (Bolzano), collected in different localities of the province (Fig. 1). Total genomic DNA was extracted by standard salting-out procedure (Aljanabi and Martinez, 1997) and a fragment of the mitochondrial cytochrome b gene was amplified for all the Sorex spp. specimens. Cytochrome b sequences were obtained using the primers L14734 (Ohdachi et al., 2001) and H15906 (Lebedev et al., 2007). PCR reactions were carried out in 25 µl reaction volume including 200 ng of each primer, 2.5 µl of 10× buffer, 2.5 µl MgCl2 50 mM, 0.2 mM dNTP, 2 U Taq polymerase (BioLine), and 50–500 ng of template DNA. Double stranded PCR products were purified using Sure Clean (BioLine) and prepared for automated sequencing using the same primers as used for the amplification. The products of amplification were sent for sequencing to an external service (Microsynth). Raw sequences where quality checked and trimmed using FinchTV v1.5 (Geospiza Inc.) and successively deposited on GenBank (Accession numbers from MW389518 to MW389531).

The obtained sequences (637 bp) of Sorex spp. from South Tyrol were aligned and compared respect to available sequences of both S. antinorii and S. araneus archived in GenBank. The alignment was performed by using the MUSCLE (Multiple Sequence Comparison by Log-Expectation) algorithm (Edgar, 2004), implemented in the software Seaview version 5.0.4 (Gouy et al., 2010). In order to properly assign the individuals to one of the two species of Sorex spp. putatively present in the area, our 14 sequences were aligned with a small set of cytochrome b from individuals from Switzerland and France (accession numbers in Fig. 2), i.e. the closest localities to South Tyrol available in GenBank. The aligned sequences were used to build a maximum likelihood phylogenetic tree using the software PhyML 3.0 (Guindon et al., 2010) and a Bayesian tree with MrBayes 3.2.4 (Ronquist and Huelsenbeck, 2003). Both the maximum likelihood (ML) and bayesian analyses (BA) were performed under the assumption of a HKY model with gamma rates. This model was chosen among 56 different evolutionary models using modelgenerator v 0.85 (Keane et al., 2006). The best fitting model was chosen by comparing the likelihood by Bayesian Information Criteria (BIC). The robustness of the ML tree was assessed using the Approximate Likelihood-Ratio (aLRT; Anisimova and Gascuel, 2006) implemented in PhyML. Bayesian inference was performed using two independent searches of one million of generations and sampling every 100 steps. After a burn-in of the first 25% genealogies, a 50%-majority rule consensus tree was built. Finally, the same dataset (n=39 after removing the two outgroup species) was used to obtain a parsimony network using the R package Pegas (Paradis, 2010).

Successively a larger dataset (n=277) gathered from GenBank was used to compare mtDNA diversity indexes for S. araneus in South Tyrol respect to other countries for which cytochrome b sequences were available. Genetic diversity indexes, i.e. the number of haplotype and haplotypic and nucleotide diversity, were estimated using the R package Pegas (Paradis, 2010).
Table 2 – Collection localities of the 14 examined specimens of Sorex spp., the name of the municipalities is given in German and Italian. All specimens are deposited in the Museum of Nature South Tyrol (Bolzano).

<table>
<thead>
<tr>
<th>Museum ID</th>
<th>Species</th>
<th>Collection year</th>
<th>Locality (municipality)</th>
<th>Altitude</th>
<th>Longitude</th>
<th>Latitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAM 159</td>
<td>S. araneus</td>
<td>1996</td>
<td>Eys (Laas/Lasa)</td>
<td>875</td>
<td>10.65490</td>
<td>46.62430</td>
</tr>
<tr>
<td>MAM 711</td>
<td>S. araneus</td>
<td>2007</td>
<td>Meranen (Mühlbach/Rio di Pusteria)</td>
<td>1510</td>
<td>11.65540</td>
<td>46.81870</td>
</tr>
<tr>
<td>MAM 915</td>
<td>S. araneus</td>
<td>2013</td>
<td>Laungalm (Unsere liebe Frau im Walde-St. Felix/Senale San Felice)</td>
<td>1780</td>
<td>11.09350</td>
<td>46.50700</td>
</tr>
<tr>
<td>MAM 919</td>
<td>S. araneus</td>
<td>2012</td>
<td>Weibenstein (Deutschnofen/Nova Ponente)</td>
<td>1500</td>
<td>11.41300</td>
<td>46.38800</td>
</tr>
<tr>
<td>MAM 1129</td>
<td>S. araneus</td>
<td>2014</td>
<td>Obere Gostalm (Moos in Passeier/Moso in Passiria)</td>
<td>1990</td>
<td>11.15870</td>
<td>46.88410</td>
</tr>
<tr>
<td>MAM 1232</td>
<td>S. araneus</td>
<td>2014</td>
<td>Traufsee (Sand in Taufers/Campo Tures)</td>
<td>2009</td>
<td>11.91410</td>
<td>46.92260</td>
</tr>
<tr>
<td>MAM 1253</td>
<td>S. araneus</td>
<td>2002</td>
<td>Monte Morel (Altrei/Anterivo)</td>
<td>1265</td>
<td>11.37854</td>
<td>46.28566</td>
</tr>
<tr>
<td>MAM 1404</td>
<td>S. araneus</td>
<td>2015</td>
<td>Hintereisen (Brenner/Brennero)</td>
<td>1456</td>
<td>11.32210</td>
<td>46.98600</td>
</tr>
<tr>
<td>MAM 1431</td>
<td>S. araneus</td>
<td>2015</td>
<td>Zanser Alm (Villnöß/Funes)</td>
<td>1950</td>
<td>11.77790</td>
<td>46.63600</td>
</tr>
<tr>
<td>MAM 1432</td>
<td>S. araneus</td>
<td>2015</td>
<td>Karthaus (Schlaus/Senaless)</td>
<td>1280</td>
<td>10.99110</td>
<td>46.70530</td>
</tr>
<tr>
<td>MAM 1440</td>
<td>S. araneus</td>
<td>2016</td>
<td>Streitmoos (Karneid/Cornedo all’Isarco)</td>
<td>1275</td>
<td>11.44330</td>
<td>46.46780</td>
</tr>
<tr>
<td>MAM 1665</td>
<td>S. araneus</td>
<td>2017</td>
<td>Simeleunüllig (Mölten/Meltina)</td>
<td>1136</td>
<td>11.23440</td>
<td>46.59900</td>
</tr>
<tr>
<td>MAM 1854</td>
<td>S. araneus</td>
<td>2019</td>
<td>Gurndalm (Aldein/Aldino)</td>
<td>2034</td>
<td>11.43569</td>
<td>46.35019</td>
</tr>
<tr>
<td>MAM 1132</td>
<td>S. antinorii</td>
<td>2014</td>
<td>Tramin (Tramin a.d. Weinstrasse/Termeno s.s.d.Vino)</td>
<td>870</td>
<td>11.22950</td>
<td>46.36050</td>
</tr>
</tbody>
</table>

Table 2 – Number of Sorex araneus individuals (n), number of haplotypes (H), haplotype diversity (Hd) and nucleotide diversity (π) and their variances.

<table>
<thead>
<tr>
<th>n</th>
<th>H</th>
<th>Hd</th>
<th>π</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>13</td>
<td>9</td>
<td>0.872±0.0079</td>
</tr>
<tr>
<td>Switzerland</td>
<td>9</td>
<td>6</td>
<td>0.834±0.0149</td>
</tr>
<tr>
<td>France</td>
<td>8</td>
<td>6</td>
<td>0.893±0.0104</td>
</tr>
<tr>
<td>Sweden</td>
<td>7</td>
<td>5</td>
<td>0.857±0.0161</td>
</tr>
<tr>
<td>Finland</td>
<td>13</td>
<td>10</td>
<td>0.628±0.0205</td>
</tr>
<tr>
<td>Hungary</td>
<td>17</td>
<td>10</td>
<td>0.919±0.0016</td>
</tr>
<tr>
<td>Poland</td>
<td>31</td>
<td>8</td>
<td>0.454±0.0124</td>
</tr>
<tr>
<td>Russia</td>
<td>138</td>
<td>56</td>
<td>0.862±0.0007</td>
</tr>
<tr>
<td>UK</td>
<td>54</td>
<td>26</td>
<td>0.816±0.0029</td>
</tr>
</tbody>
</table>

Results

After the alignment the cytochrome b sequences (n=41) includes 520 complete sites (no gaps, no ambiguities), 91 variable sites (17.5%) and 34 informative sites (6.5%). Both ML and BA phylogenetic analyses give a clear evidence of the presence of both S. araneus and S. antinorii in South Tyrol (Fig. 2 and Fig. S1 in supplementary information).

The two species are monophyletic and 13 specimens (nine haplotypes) can be attributed to S. araneus on the basis of their phylogenetic relationships (Fig. 2). Conversely, only one specimen can be assigned to S. antinorii (Fig. 2). The two species show an average uncorrected genetic distance equal to 0.015. According to the SP network the two species are monophyletic and 13 specimens (nine haplotypes) were assigned to S. antinorii and 4 specimens (3 haplotypes) to S. araneus.

The existence of hybrids between the two species is evident from the literature (Yannic et al., 2008; Basset et al., 2006; Balloux et al., 2000; Brünnen et al., 2002). Generally, the identified hybridization zones were very narrow (a few kilometers) and the number of admixed individuals was very low, suggesting strong reproductive isolation between S. antinorii and S. araneus, which proves the good species status of the latter. These findings further imply that the mitochondrial DNA attributable to S. araneus in South Tyrolean specimens is unlikely to be due to large introgression within S. antinorii. In any case, this aspect deserves further investigations by using other markers, such as microsatellite and SNPs, in order to exclude potential hybridization.

Discussion

In Italy, after the elevation to species rank of the Valais shrew S. antinorii, all specimens previously attributed to S. araneus were assigned to S. antinorii. However, some preliminary studies conducted by Lapini et al. (2001), Lapini and Cassol (2017) and Dorigo et al. (2016) on northern Italian Sorex specimens suggest that S. araneus was probably always present, at least in north-east Italy. Our data support this hypothesis and show how important a genetic revision of the species is for whole countries, but it is coupled with a lower level of nucleotide diversity. This could reflect a recent expansion following a bottleneck (i.e., during the last glaciation) or a relatively recent colonization event. However, a sampling bias cannot be excluded at this stage of the research and further analyses are required to assess the historical demography of this Italian population.

Another important and intriguing issue that needs to be carefully considered is the potential hybridization between S. antinorii and S. araneus. The existence of hybrids between the two species is evident from the literature (Yannic et al., 2008; Basset et al., 2006; Balloux et al., 2000; Brünnen et al., 2002). Generally, the identified hybridization zones were very narrow (a few kilometers) and the number of admixed individuals was very low, suggesting a strong reproductive isolation between S. antinorii and S. araneus, which proves the good species status of the latter. These findings further imply that the mitochondrial DNA attributable to S. araneus in South Tyrolean specimens is unlikely to be due to large introgression within S. antinorii. In any case, this aspect deserves further investigations by using other markers, such as microsatellite and SNPs, in order to exclude potential hybridization.
The importance of using museum collections to better describe past and current mammal biodiversity is recognized in the literature (Gippoliti et al., 2014), but in practice the reality is often different. Indeed, museum collections are rarely reanalyzed with modern technologies (i.e. molecular markers), due to lack of funding or technical difficulties. However, analysis of museum collections can improve our knowledge of mammal diversity and the combination of genetic and morphometric approaches also provides opportunities to fill knowledge gaps.®

References


Supplemental information

Additional Supplemental Information may be found in the online version of this article: Figure S1 Bayesian consensus tree.