

The identity of the Finnish *Osmoderma* (Coleoptera: Scarabaeidae, Cetoniinae) population established by COI sequencing

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The hermit beetle *Osmoderma eremita* (Coleoptera: Scarabaeidae) is a flagship species for invertebrate conservation efforts by the European Union. This taxon has recently been revealed as a species complex likely encompassing five cryptic species. The northernmost population of *Osmoderma* is found on the island of Ruissalo in Turku, Finland. This population has been protected as species *O. eremita*, but its true species affinity has never been established. To resolve its identity, we sequenced the mitochondrial COI gene from seven specimens sampled in Ruissalo. Based on a phylogenetic hypothesis generated from the sequences combined with previously published data, the Finnish hermit beetle was identified as *Osmoderma barnabita*. Information regarding the ecology and life cycle of *O. eremita* should then not uncritically be assumed to apply to the Finnish population. Rather, the Finnish population should be treated as a separate entity in conservation and management of European *Osmoderma*.

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1. Introduction

Efficient conservation efforts will always rely on accurate information on the taxonomy and ecological traits of the species to be conserved. Without proper data, the focus of conservation management can be directed towards the wrong aspects of the target population and its environment (Murray *et al.* 2008, Rutishauser *et al.* 2012).

Small subpopulations can offer insights into the evolutionary trajectories of species, and are therefore considered to be valuable objects in conservation genetics (Moritz 1994, 1995, Phillimore & Owens 2006). However, without proper recognition of species boundaries, it will also be impossible to define the size and true threat status of any population. Thus, the up-to-date protection of species is based on knowledge of species

taxonomy, of the limits of its populations, and how protected populations are developing in relation to other populations.

Insect taxa may conceal a significant amount of cryptic species – i.e. species with similar morphology but differing in important hidden traits, frequently of genetic character (Collins & Paske-witz 1996, Williams *et al.* 2006, Bickford *et al.* 2007, Waters *et al.* 2011). Molecular techniques offer valid tools to detect such cases. In particular, sequences of mitochondrial DNA have been used to resolve even challenging species complexes (e.g. Moritz *et al.* 1987, Sperling & Harrison 1994, Hebert *et al.* 2003a, Trizzino *et al.* 2012). Importantly, the resolution of exact species boundaries and the definition of actual populations will allow us to gain more precise information of ecological characteristics and distribution patterns of target species, subsequently leading to more efficient conservation management. In the context of conservation of threatened species, DNA-techniques come with an added benefit, since instead of entire individuals, only small anatomical fragments can be used in research (Svensson *et al.* 2009, Oleksa *et al.* 2012). This eliminates the need for removing whole individuals from small and vulnerable populations.

The hermit beetle *Osmoderma eremita* Scopoli, 1763 is a saproxylic species inhabiting hollow trees (Ranius & Nilsson 1997). This taxon is currently a flagship for invertebrate conservation within the European Union. As it inhabits an environment in rapid decline across Europe, old deciduous forests (Anonymous 1992, Luce 1996), it is currently listed in species Annexes II and IV of the Habitats directive, and has thereby been granted the status of priority species in all member countries (European Commission 2007). In the regional assessment of Europe by IUCN, *Osmoderma eremita* is listed as Near Threatened (Nieto *et al.* 2010), while the status in Finland is Vulnerable (Rassi *et al.* 2010).

Taxonomically, the genus *Osmoderma* has undergone great changes during the 21st century. In the 1990s, Tausin (1994a, b), and later Sparacio (2000) and Gusakov (2002) discovered high morphological diversity both among and within the *Osmoderma* species. Nonetheless, separate

taxonomical names for individual morphs were not generally acknowledged until Audisio *et al.* (2007, 2009) applied sequencing techniques to identify two clades and likely five species in the area of Europe. The West European clade includes *Osmoderma eremita* Scopoli, 1763, *Osmoderma cristinae* Sparacio, 1994, and *Osmoderma italicum* Sparacio, 2000, while the East European clade includes *Osmoderma barnabita* Motschulsky, 1845, and *Osmoderma lassallei* Baraud & Tausin, 1991.

The classification introduced by Audisio *et al.* (2007, 2009) was based on a total of only twenty-six sequenced individuals. The easternmost specimens were collected from Northern Greece, Croatia, Germany, Slovenia, Slovakia and South-west Sweden (Audisio *et al.* 2009). Although Svensson *et al.* (2009) conducted a complementary study of the taxonomy of Swedish and Polish populations, there is still a lack of data on the exact identity of *Osmoderma* species in many parts of Eastern Europe.

From an ecological perspective, *Osmoderma eremita* has been well studied (Hedin 2003, Ranius *et al.* 2005, DuBois 2009), whereas its ecological differences compared to the eastern clade species are still unknown. As the eastern and western clades have likely separated several million years ago (Audisio *et al.* 2009), some features in their ecology and physiology have probably diverged. Thus, identifying the species affinity of individual populations remains a priority, and the ecology of the eastern clade needs to be established.

In Finland, a single population of *Osmoderma* occurs on the island of Ruissalo (Landvik 2000). This population was discovered in the 19th century (Rassi *et al.* 1986), and the closest European populations are located on the eastern coast of Sweden and southern border of Estonia (Antons-son *et al.* 2003, Ranius *et al.* 2005). The population has been identified as the species *Osmoderma eremita* (Rassi *et al.* 1986, 1991, 2001, 2010), but the data of Audisio *et al.* (2007, 2009) suggest that it might belong to the eastern clade. In this study, we employ sequences of the mitochondrial COI gene to resolve the species identity of the hermit beetle population in Finland.

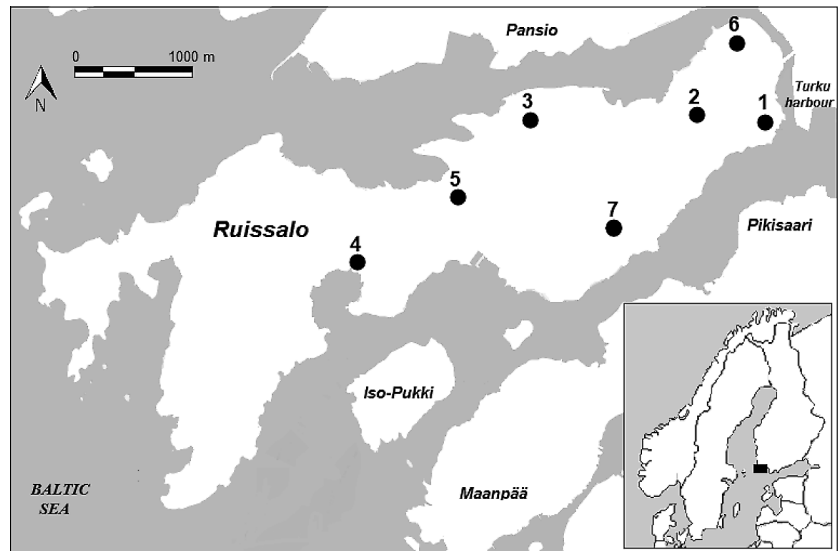


Fig. 1. Collecting sites (black dots) during field period 15.VII.–3.VIII. 2010 of the *Osmoderma* specimens sequenced from the island of Ruissalo, south western Finland. For sampling information of the localities, see Table 1.

2. Material and methods

In the case of the *Osmoderma* complex, the mitochondrial gene cytochrome oxidase subunit I (COI) offers a reliable marker for species-level identification, since it serves to distinguish between species the status of which has been confirmed on the basis of other types of data: the same separation between species of *Osmoderma* complex is supported by differences in morphology, geographical range and mtDNA COI sequences (Baraud & Tauzin 1991, Tauzin 1994a, b, Sparacio 2000, Audisio *et al.* 2007, 2009).

To obtain sequence material of the Finnish population, the authors were given permission to sample eight specimens from the main tree stands

within Ruissalo (Fig. 1). Individuals of *Osmoderma* (five males, three females) were collected by using pheromone traps (trap design modified from Svensson *et al.* 2009, M. Landvik unpubl.). Females were collected from the eastern part of Ruissalo, whereas male specimens were collected from protected forests and roadside trees across Ruissalo (Table 1). Seven sequences were subsequently successfully obtained, as one sample was contaminated. Museum samples were excluded from the study because they lack information on exact collection sites.

Genomic DNA was extracted from one leg of each individual using QIAgen's DNEasy Tissue Extraction kit following the manufacturer's protocol. Approximately 1,400 base pairs of the COI

Table 1. Details of Finnish *Osmoderma* specimens included in molecular analyses. All individuals were collected by M. Landvik from Ruissalo island, Turku. Sequences of mtCOI genes were deposited in GenBank (accession numbers KC476172–KC476178). The numbers in ID codes correspond to those in the map in Fig. 1. The coordinates are given in decimal degrees-system.

ID code	Accession numbers	Gender	Locality	Collecting date	Latitude (N)	Longitude (E)
FIN1	KC476172	female	Tammerterho	15.VII.2010	60.436098	22.201974
FIN2	KC476173	male	Ekars	19.VII.2010	60.436627	22.190312
FIN3	KC476174	male	Kauppila	21.VII.2010	60.436209	22.168682
FIN4	KC476175	male	Krottila	27.VII.2010	60.426303	22.140755
FIN5	KC476176	male	Choraesus	28.VII.2010	60.426303	22.140755
FIN6	KC476177	female	Marjaniemi	29.VII.2010	60.442073	22.200644
FIN7	KC476178	male	Kansanpuisto	3.VIII.2010	60.429258	22.173816

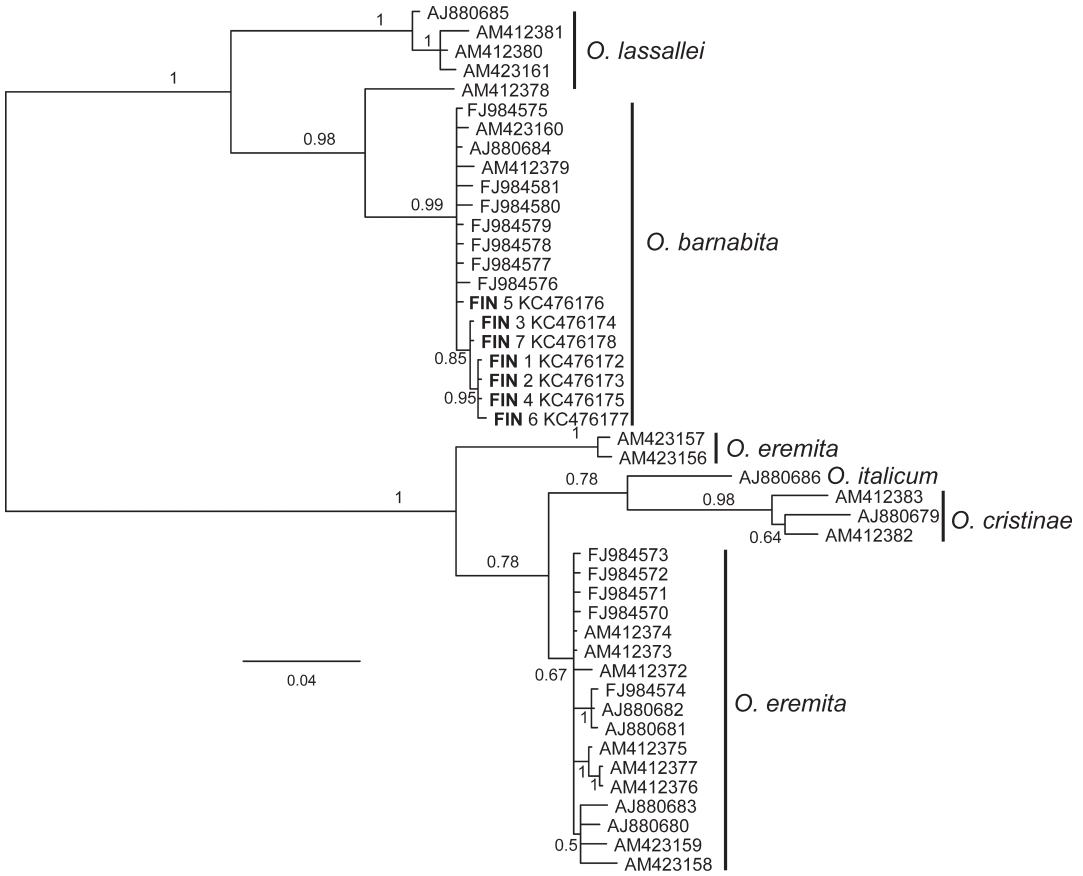


Fig. 2. An unrooted topology based on the COI sequences of 45 *Osmoderma* individuals analyzed using Bayesian inference. Here, sequences of the Finnish individuals; FIN1–FIN7, have been combined with data from Audisio *et al.* (2009) and Svensson *et al.* (2009). Haplotypes are labelled by GenBank accession numbers. Numbers above nodes refer to posterior probabilities of the nodes, and the scale bar shows the inferred number of substitutions per site.

gene were sequenced from the seven individuals using the primer pairs LCO1490 (5' GGT CAA CAAATC ATA AAG ATA TTG G 3')/HCO2198 (5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3') and Jerry (5' CAA CAY TTA TTT TGA TTT TTT GG 3')/Pat (5' ATC CAT TAC ATA TAA TCT GCC ATA 3') (Folmer *et al.* 1994). PCR protocols followed Wahlberg and Wheat (2008).

To infer the position of the Finnish *Osmoderma* population among European clades, the sequences generated were combined with previously published sequences of Central European and Mediterranean specimens (Audisio *et al.* 2009, Svensson *et al.* 2009) as extracted from GenBank. We note that our intention is not to in-

fer the phylogenetic relationships of the different species, but to investigate the identity of the Finnish specimens. The total dataset included COI sequences from 45 individuals for an aligned dataset of 1551 base pairs. Not all individuals yielded full sequences, resulting in some missing data. An optimal model to estimate phylogenies was chosen using jModelTest (Posada 2008) based on the AIC. This criterion identified the GTR+G model as the most optimal fit to the data. The data were analyzed using MrBayes 3.1 (Ronquist & Huelsenbeck 2003) under the GTR+G model and run twice independently for 10 million generations, with one cold and three heated chains, and sampling every 1,000 generations. Convergence of the two independent runs

Table 2. Estimates of evolutionary divergence between and within taxa in the *Osmoderma* species complex. Data is based on mitochondrial DNA COI gene sequences from the present study, and from Audisio *et al.* (2009) and Svensson *et al.* (2009). Shown is the number of base substitutions per site from averaging over all sequence pairs between groups.

	<i>O.eremita</i>	<i>O.italicum</i>	<i>O.cristinae</i>	<i>O.lassallei</i>	<i>O. barnabita</i> **	<i>O. barnabita</i>
<i>O. eremita</i>	0.012					
<i>O. italicum</i>	0.038	n.a.				
<i>O. cristinae</i>	0.049	0.044	0.016*			
<i>O. lassallei</i>	0.101	0.098	0.100	0.006*		
<i>O. barnabita</i> **	0.106	0.098	0.087	0.062	0.001*	
<i>O. barnabita</i>	0.115	0.115	0.105	0.068	0.006	0.008*

* = Intraspecific divergence.

** = Finnish specimens.

n.a. = Not available as only one individual sequenced.

was checked in Tracer 1.5 (Drummond & Rambaut 2007) and the first 2,500 sampled generations were deleted as burn-in.

Estimates of average evolutionary distances over sequence pairs between and within groups were calculated in MEGA5 (Tamura *et al.* 2011) using the maximum composite likelihood model (the most complex model available in MEGA5). The rate variation among sites was modelled with a gamma distribution (shape parameter = 1). The analysis involved 45 nucleotide sequences, including all codon positions. Any ambiguous positions were removed for each sequence pair.

3. Results

Finnish *Osmoderma* specimens were characterised by COI haplotypes closely related to those of *O. barnabita* and distant from those of *O. eremita* (Fig. 2). Among the Finnish *Osmoderma* individuals we detected two haplotypes of COI, none of which has yet been reported from any area outside of Finland. A failed PCR amplification with the LCO/HCO primer pair resulted in missing data, leaving the position of specimen FIN5 unresolved among the other *O. barnabita* sequences (Fig. 2). However, the specimen FIN5 does not differ from the other Finnish COI sequences in the second half of the gene.

Interspecific and intraspecific genetic distances among *Osmoderma* clades suggest long-term separation between *O. barnabita* and *O. eremita* populations, likely reflecting species-

level differentiation (Table 2). Intraspecific variation within the Finnish population proved rather restricted (two haplotypes differing at only one site in the COI), probably reflecting the small size of this isolated population, but also the low number of sequenced specimens. Our estimates of average genetic distances are slightly lower than those reported by Audisio *et al.* (2009), although this is most likely due to the larger number of *O. barnabita* individuals included in our study. Otherwise the results are similar to Audisio *et al.* (2009) and Svensson *et al.* (2009).

4. Discussion and conclusions

The haplotype data presented in this study identify the Finnish population of *Osmoderma* as unequivocally belonging to *Osmoderma barnabita*. This resolves the longstanding debate of the actual identity of the species (Alexander *et al.* 2010, Sverdrup-Thygeson *et al.* 2010), as originally spurred by the findings of Audisio *et al.* (2007). It also demonstrates that *O. barnabita* is widely dispersed in east Europe from Greece to Finland, supporting the hypothesis advanced by Audisio *et al.* (2007).

A re-evaluation of the protection of the *Osmoderma*-species complex was called for by Audisio *et al.* (2007), and has since been implemented by the IUCN in collaboration with the European Union (Nieto & Alexander 2010). With respect to the conservation of the Finnish population of *O. barnabita*, the current results offer a

clear-cut message: while populations of *Osmoderma* have been extensively studied in Sweden (Ranius & Nilsson 1997, Ranius 2000, 2001, 2002, 2006, 2007, Hedin 2003, Ranius et al. 2009), at a distance of less than 300 kilometres from the Finnish range, these populations represent a different species – the western clade *O. eremita*. Hence, information on the ecology and life cycle derived from the Swedish population should not uncritically be assumed to apply to the Finnish population – nor should findings from French (DuBois 2009, DuBois et al. 2010) or Italian (Chiari et al. 2012a, b) populations of *O. eremita*. The nearest populations conspecific with the Finnish *O. barnabita* likely occur in the Baltic and in the surroundings of St. Petersburg in Russia (Ranius et al. 2005). These latter populations have been much less studied. Hence, we believe that the Finnish population should be treated as a separate entity in species conservation, and that joint research projects on the ecology and life history of *O. barnabita* should urgently be implemented in collaboration with Baltic and Russian ecologists. Given the clear distinction of clades (Fig. 2), the current phylogram suggests that the identity of *Osmoderma* populations in areas around Finland may be easily established based on DNA barcodes using small fragments of live individuals.

Small and genetically divergent subpopulations have been identified as valuable targets of conservation (Moritz 1994, Taberlet & Bouvet 1994). Although isolated populations are often under threat for anthropogenic reasons, they also broaden the gene pool of a species and play a significant role in the process of speciation (Frankham et al. 2009). To what extent the Finnish population of *O. barnabita* genetically differs from populations of the same species in Croatia, Germany, Greece, Poland and Slovakia is yet to be resolved. The haplotypes detected in the Finnish population have not been found elsewhere in Europe, but this may reflect the small sample size of both the European and the Finnish material. Therefore, it is yet to be established whether haplotypes found in other parts of Europe actually occur in Finland and vice versa. In order to gain a wider perspective, a larger number of specimens from across Europe should be examined by DNA sequencing.

What the Finnish species should actually be called is at present a moot point. In this study, we have used the name *Osmoderma barnabita* Motschulsky, 1845 to designate the eastern clade occurring in Finland. At present, the official species name is *Osmoderma coriarius* (De Geer 1774) (Schoolmeesters 2011), but in practice, the name *O. barnabita* appears more widely established. The conflict of names results from Gusakov's (2002) suggestion to reintroduce the name *Gymnodus coriarius* (De Geer, 1774) for the eastern species of hermit beetles. Even though the type specimen and the syntype behind De Geer's description are currently unavailable for analysis, the original description includes the collecting locality and an illustration (De Geer 1774, Bergsten, J., Natural History Museum of Sweden, pers. comm.). Hence, we know for a fact that the material used by De Geer in originally describing *Scarabeus coriarius* De Geer, 1774 originated from Lövstabruk, Sweden and thus most likely represented the western clade of *Osmoderma* i.e. *Osmoderma eremita sensu stricto* (De Geer 1774, Audisio et al. 2007, Bergsten, J. NHMS, pers. comm.). As the true rationale of scientific binomials is to allow researchers around the globe to refer to the same taxon with a single, unequivocal name, we therefore suggest that the resurrected name *Osmoderma barnabita* is used in reference to Finnish hermit beetle species.

Overall, the results of our study show the power of molecular techniques – and the importance of critically examining the status of even well-known species. If the flagship species of European insects hides multiple cryptic species, then what about the less-studied members of our insect fauna – no less important nor less endangered?

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