Resurrection of Crypturgus subcribrosus Eggers 1933 stat. n., and its close phylogenetic relationship to Nearctic Crypturgus (Coleoptera, Scolytinae)

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Abstract

Crypturgus subcribrosus Eggers is removed from synonymy under C. cinereus (Herbst) and reinstated as a valid species based on evidence from DNA sequence data and morphological features. Phylogenetic analyses of Elongation Factor 1α and Cytochrome Oxidase I sequences in conjunction with morphological characters revealed a sister relationship between C. subcribrosus and two Nearctic species of Crypturgus, with C. cinereus unrelated to any of these taxa. Type material of C. cinereus has been located and lectotype with paralectotypes are designated. Amended diagnoses that include DNA barcodes are presented for C. subcribrosus and C. cinereus together with an identification key to the Fenno-scandian species of Crypturgus.

Key words: Cytochrome Oxidase I, Coleoptera, Crypturgus, Curculionidae, DNA barcodes, Elongation Factor 1α, phylogeny, Scolytinae

Introduction

Species of the bark beetle genus Crypturgus Erichson breed in conifers throughout the Holarctic region. Fifteen species are currently recognised, but a long and troubled history of disputed synonymies (e.g. Wood & Bright, 1992; Pfeffer, 1995) indicates that this number is perhaps rather approximate. Many Crypturgus species are difficult to distinguish morphologically and ecologically from their closest relatives, lacking readily observed diagnostic characters (Fig. 1). These problems are further exaggerated by their small adult body size that ranges between 0.9 to 1.5 mm in length and the weakly sclerotised body which often results in deformation of pinned museum specimens.

Most taxonomic problems in northern Europe and Scandinavia surround the species validity of C. subcribrosus Eggers, a current synonym (Schedl, 1946) of C. cinereus (Herbst). Although recent classifications and some faunal reports agree on the synonymous status (Pfeffer, 1995; Mandelshtam & Popovichev, 2000; Bright & Skidmore, 2002), several other entomological sources present C. subcribrosus as a valid species (e.g. Hansen, 1956; Lekander et al. 1977; Silfverberg 1992; Lundberg 1995; Voolma et al., 2000; Ehnström & Axelsson, 2002; Knížek 2004). This taxon was originally described as different from C. cinereus by the longer tuft of hairlike setae on the female elytral declivity, and by the less deeply impressed strial punctures and finely shagrinarte cuticular surface of the interstriae (Eggers, 1933; see also Fig. 1). However, these characters vary within species and are difficult to transform into diagnostic characters. Morphological characters alone are therefore not always sufficient for species delimitation.

To test the taxonomic status of C. subcribrosus, we sequenced DNA from one mitochondrial and one nuclear gene fragment and compared these data to adult morphology. We furthermore analysed several other
crypturgine taxa to provide comparative genetic data from morphologically well defined species. One particularly interesting comparison was made for another species pair that is associated with conifers in the same boreal region – between the closely related *C. pusillus* (Gyllenhal) and *C. hispidulus* Thomson. As many as ten synonyms have been published for the first species, mostly for taxa south of the European Alps (Wood & Bright, 1992). The north-Palaearctic *C. hispidulus* has never been placed in synonymy, despite its close morphological similarity to *C. pusillus*. These taxa can be distinguished in most cases by differences in the strial punctures and interstrial setae of the elytra (e.g. Hansen, 1956; Pfeffer, 1995).

With an updated identification key to the Scandinavian species, including diagnostic DNA barcodes, these beetles will hopefully in future provide a more readily accessible model system for studying variation in their unique behavioural ecology. Their fascinating life style involves among other things an inquiline behaviour where colonising pairs enter their host via the entrance holes of larger species (often *Ips*, *Dryocoetes* or *Tomicus* spp, see e.g. Schwarz, 1894; Ehnström & Axelsson, 2002). More rarely will large aggregations of colonial pairs form during high colonisation densities (Kirkendall, 1983; B. Jordal, pers. obs) as in some other crypturgine genera (Jordal, 2006). The frequent occurrence of *Crypturgus* in boreal conifers makes them an interesting and promising model system in the study of reproductive behaviour.

**FIGURE 1.** Dorsal view *Crypturgus cinereus* (A) and *C. subcribrosus* (B).
Methods

Type material of *C. cinereus* and *C. subcribrosus* was examined in the collections of Museum fur Naturkunde der Humboldt Universität, Berlin (MNH), Museum Zoologicum Universitatus, Helsinki (MZU), Naturhistorisches Museum, Vienna (NHMW), and United States National Museum, Washington D.C. (USNM). Additional specimens used in the phylogenetic analyses (Table 1) were identified using the original description of Eggers (1933) and the identification keys made by Schedl (1946), Hansen (1956), Wood (1982) and Pfeffer (1995).

**TABLE 1.** Locality data and GenBank accession numbers for the specimens included in the phylogenetic study. The first author collected the samples unless otherwise noted. Vouchers are in the first author’s collection unless otherwise stated.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Voucher label</th>
<th>Locality</th>
<th>COI accession number</th>
<th>EF-1a accession number</th>
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<td>A44affGC</td>
<td>Canary Islands: Fuerteventura 2002, ex Euphorbia regis-jubae</td>
<td>AY514942</td>
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<td>Canary Islands: Lanzarote 2002, ex Euphorbia balsamifera</td>
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<td><em>Cisurgus occidentalis 04</em></td>
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<td>Morocco: N Agadir, 2002, ex Euphorbia echinus</td>
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<td>AY500978</td>
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<tr>
<td><em>Cisurgus wollastoni EH</em></td>
<td>Ci09wolEH</td>
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<td>AY500981</td>
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<tr>
<td><em>Cisurgus wollastoni LP</em></td>
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<td>AY500982</td>
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<tr>
<td><em>Crypturgus alutaceus</em></td>
<td>CryAlu1 (Cognato lab)</td>
<td>USA (A. Cognato, coll.)</td>
<td>EU011815</td>
<td>EU011827</td>
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<td><em>Crypturgus borealis</em></td>
<td>SCR01/ Cr1 (Farrell lab)</td>
<td>Canada: Kanaskana NP 1999 (M. Reid, coll.)</td>
<td>AF187130</td>
<td>AF186680</td>
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<td>CgCry01_0</td>
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<td>Cr03med_I</td>
<td>Italy: Bufalars, Scanzana (S. Gallo), 1992</td>
<td>EU011814</td>
<td>-</td>
</tr>
<tr>
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<td>Sweden: Jämtland, Svenstavik, 2005, ex Picea abies</td>
<td>EU011812</td>
<td>EU011825</td>
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</table>

...to be continued.
Partial gene fragments of Cytochrome Oxidase I and Elongation Factor 1-α were PCR amplified and directly sequenced with the primer pairs S1718 and A2411 (COI) and S149 and A1043 (EF-1α) (see e.g. Jordal & Hewitt, 2004). After trimming the flanking regions for ambiguities and missing data, the data matrix contained 640 characters of COI (214 parsimony informative) and 925 aligned characters of EF-1α (171 parsimony informative). ClustalX (Thompson et al. 1997) was used to align a maximum of 79 EF-1α intron nucleotides under various alignment parameters. The slight variation between alignments had no influence on the topology. DNA sequences are available from GenBank under the accession numbers listed in Table 1.

Unweighted parsimony analyses were performed on each data set separately and in combination using Paup* (Swofford, 1999). Node support was estimated from 200 bootstrap replicates using 20 random additions per heuristic search. The data were also analysed in a Bayesian framework, using a mixed model of evolution in MrBayes 3.1.1 (Huelsenbeck & Ronquist, 2004). Partitions included COI, EF-1α and morphological characters, and each of the two molecular partitions was fitted to a HKY+Γ model of evolution as determined by ModelTest (FindModel web server at Los Alamos National Laboratory). Parameter settings for the Markov Chain Monte Carlo run included 1000000 generations; samples were collected every 1000 generation, with the first 300 samples discarded (stationarity in likelihood values reached before 27000 generations). Two parallel runs were performed on the data, each run consisting of one heated and three cold sampling chains. Trees were rooted with two species of Aphanarthrum (Table 1), the sister group of Crypturgus plus Cisurgus (see Jordal & Hewitt, 2004).

Results

Phylogenetic analyses. Maximum parsimony and Bayesian analyses of the combined molecular and morphological data resulted in identical topologies with well-resolved and well-supported species groups and higher clades (Fig. 2). C. subcribrosus was nested within the Nearctic clade as the sister group to C. borealis Swaine. Although this relationship was only moderately supported by bootstrapping or posterior probability, the clade consisting of these two species and C. alutaceus Schwarz received maximum support. The same relationship was also found in the separate analyses of morphological data and each of the molecular data partitions. C. subcribrosus was furthermore distinctly divergent from C. cinereus and differed by as many as 19.4 % of the COI sites and 6.2 % of the EF-1α sites (uncorrected p-distance). C. cinereus grouped with maximum support with C. mediterraneus Eichhoff in the separate analyses of the COI and morphology data, and in the combined parsimony and Bayesian analyses. Inclusion of missing C. mediterraneus data from the EF-1α partition had therefore no influence on the topological placement of this taxon.

Crypturgus hispidulus was clearly distinct from its putative sister taxon C. pusillus and differed by 12.4 %
of the COI sites and 3.9 % of the EF-1α sites. These two taxa grouped with moderate to strong support as the sister lineage to *Cisurgus*. Separate analyses of the EF-1α data resulted in an identical topology to the one that resulted from the combined data. The COI data were also largely congruent with the combined data and differed only by a paraphyletic distribution of *C. hispidulus* and *C. pusillus* with respect to *Cisurgus*. However, this clade was not supported by any bootstrap support (<50%).

**FIGURE 2.** Phylogenetic tree resulting from the parsimony analysis of the combined molecular and morphological data (L=985, CI=0.61, RI=0.81, gaps treated as 5th character). This topology was identical to the Bayesian topology. Bootstrap support (maximum parsimony) is given above nodes and Bayesian posterior probabilities below. The phylogenetic tree is rooted with *Aphanarhtrum armatum* and *A. affine*.

**Morphological characters.** Optimisation of morphological characters (Tables 2 and 3) on the combined data topology revealed relatively low levels of homoplasy (CI=0.73, RI=0.93). Among the characters most relevant to define the closest relative of *C. subcribrosus*, character 4 (elytral interstriae) supported the sister relationship to *C. borealis*. The weakly elevated interstriae in these two taxa differ from all other by its dull appearance due to very fine micro-chagrination (Fig. 1b). The morphologically similar *C. cinereus* and *C. mediterraneus* differ further from those taxa by a much more sharply elevated and shiny interstriae that lacks any trace of shagrination (Fig. 1a). Character 5 (female declivity) has traditionally supported a closer relationship between *C. subcribrosus* and *C. cinereus*, but the long setae on the female declivity have, according to the new phylogenetic hypothesis, evolved independently in these taxa. The Nearctic taxa and *C. subcribrosus* are further distinguished from other *Crypturgus* with reticulate pronotum by having sparse, obscure punctures (see Fig. 1b).

A new morphological character included in this study consists of patches of closely set micro-sensillae on
the lower part of the female declivity (Fig. 3). The size of the patches occurs as two small patches in *C. cinereus*, *C. mediterraneus* and *C. alutaceus*, and usually as two larger (albeit highly variable) patches in *C. borealis*. This character is apparently secondarily lost in *C. subcribrosus* according to the new phylogenetic hypothesis.

**TABLE 2.** List of morphological characters included in the combined phylogenetic analyses.

<table>
<thead>
<tr>
<th>Taxon / character</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>0</td>
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<td>0</td>
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<td>A. armatum</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
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<td>1</td>
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<tr>
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<td>1</td>
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<td>1</td>
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<td>CI (avg. 0.73)</td>
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<tr>
<td>RI (avg. 0.93)</td>
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<td>1.0</td>
<td>0.86</td>
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<td>0.86</td>
<td>0.86</td>
</tr>
</tbody>
</table>

**TABLE 3.** Character matrix for morphological characters listed in Table 2. All intraspecific samples were coded with the same character state as confirmed by morphological examination.

Discussion

DNA sequence data from two independent genetic markers revealed a clear genetic distinction between *C. cinereus* and *C. subcribrosus*. Not only were these taxa separated by more than 19% of the COI nucleotide sites, but were also more diverged than other sibling species in this genus (Fig. 2). In fact, these taxa were not sister lineages in any of the phylogenetic reconstructions, providing strong evidence for an independent origin of each taxon. *Crypturgus subcribrosus* is therefore reinstated as a valid species name (see below).
The sister relationship of *C. subcribrosus* to the Nearctic *C. borealis* may seem surprising given the historical synonymy of this taxon under *C. cinereus*. However, *C. subcribrosus* shares with *C. borealis* several morphological character states to the exclusion of *C. cinereus* and *C. mediterraneus* (characters 3 and 4). This was further supported in the separate parsimony analysis of the morphological characters.

The independent phylogenetic position of *C. subcribrosus* and *C. cinereus* may also provide hints about their geographical origin. A nested position of *C. subcribrosus* within the Nearctic clade provides some evidence for an easterly Palaearctic or Nearctic origin of this taxon. *C. alutaceus* is mainly restricted to the southeastern parts of the US, while *C. borealis* has a broader distribution over the remaining parts of USA and Canada (Wood, 1982). Of course, current geographical ranges cannot be taken as direct evidence for historical distributions, but merely indicate more or less plausible scenarios. The same applies to the close relationship between *C. cinereus* and *C. mediterraneus* that may indicate a more southerly Palaearctic origin, e.g. the Mediterranean area, for the ancestor of *C. cinereus*. More samples of close relatives, in particular the Mediterranean *C. numidicus* Ferrari and the Asian *C. tuberosus* Niisima, are needed to evaluate these hypotheses in a statistical framework. However, an allopatric speciation hypothesis seems more likely given that these species have very similar ecologies and overlap completely in host plant preference that would otherwise permit gene flow between populations and thus limit the possibility for sympatric divergence (e.g. Arnegard & Kondrashov, 2004; Jordal et al. 2006).

**Reinstatement of Crypturgus subcribrosus**

*Crypturgus subcribrosus* Eggers 1933
(Fig. 1b, 3a–b).

*Crypturgus subcribrosus* Eggers 1933: Entomologische Blätter für Biologie und Systematik der Käfer 29, p.5. Holotype in NHMW, examined. **New status.**

**Diagnosis.** Length 1.0–1.3 mm, mature colour bronze yellow or brown, rarely dark brown. Surface of the pronotum reticulate, dull, with sparse and shallow, obscure punctures. Striae of elytra weakly impressed, interstriae gently rounded, micro-shagrinate, dull. Female declivity usually with a long tuft of yellow hair-like setae (sometimes abraded in older specimens), never with two patches of micro-sensillae within the tuft. COI barcodes deposited in GenBank under accessions EU011808- EU011811.

**Remarks.** This species is very similar to *C. cinereus* but can usually be distinguished by the less elevated interstriae of the elytra which has a micro-shagrinare texture, by the strongly reticulated pronotum that appears more finely shagrinated with fewer and obscure punctures, and that the female elytral declivity lack patches with micro-sensillae. In his original description of *C. subcribrosus*, Eggers (1933) also noted the gently rounded interstriae and 'skin-like' appearance of the cuticle: "Zwischenräume auch ganz flach, fein hautartig gerunzelt". Exceptional specimens do overlap in this character, however, and longer series from each locality is sometimes needed to obtain correct identification based on morphology only. These problems are well illustrated by the fact that Eggers also mixed the two species in his own collection in NHMW (Knizek, pers. obs).

*Crypturgus cinereus* (Herbst 1793)
(Fig. 1a, 3c–d)

Diagnosis. Length 1.1–1.5 mm, mature colour brown to black. Surface of pronotum reticulate, subshining, with fine but distinct and densely set punctures. Striae of elytra deeply impressed, interstriae elevated, rugose, without reticulation or shagrination, shiny. Female declivity with a short tuft of yellow hair-like setae (sometimes abraded in older specimens) and with a pair of small patches with micro-sensillae in the middle of the tuft. COI barcodes deposited in GenBank under accessions EU011803- EU011806.

Remarks. The type material of *C. cinereus* was previously not located and assumed lost (Wood & Bright, 1992; Pfeffer, 1995). However, C. Holzschuh discovered the original syntype series by Herbst in the Humboldt University collection in 1996. We have examined the six syntypes from which Holzschuh designated a female lectotype and five paralectotypes (3 males, 2 females) and concur with his designation. However, Holzschuh (pers. comm.) never published his designation of the lectotype and is therefore not a valid designation. We therefore designate the female specimen labelled as lectotypus by Holzschuh as the lectotype of *C. cinereus*.

The type specimen of *B. minutus* Duftschmid is the only one that has not been located in this or any previous work on this genus. However, there is no indication from the original description that this taxon is anything else than a typical *C. cinereus* specimen. The type specimens of *C. corsicus* Eggers in USNM and NHMW differ slightly and specimens in the USNM collection are intermediate between *C. cinereus* and *C. subcribrosus*. However, without further genetic data, it seems prudent to keep this taxon as a synonym of *C. cinereus*.

Key to Fennoscandian species of Crypturgus

1. Dorsal side of pronotum reticulate, dull; female declivity with a tuft of hair-like setae .................... (2)
   - Dorsal side of pronotum shiny; male and female declivity with individually placed setae ................ (3)

2. Strial punctures deep, interstriae sharply elevated and shiny. Dorsal side of pronotum with distinct punctures. Small patches with micro-sensillae present on female declivity .................. *Crypturgus cinereus*
   - Strial punctures shallow, interstriae gently rounded, micro-shagrinate and dull. Dorsal side of pronotum with sparse, obscure punctures. Patches with micro-sensillae on elytral declivity absent in both sexes ......
       .......................... ........................................................................................................... *Crypturgus subcribrosus*

3. Elytral disc and sides with erect, long setae .......................................................... *Crypturgus hispidulus*
   - Elytral disc glabrous, with rather few, fine setae close to the elytral margins .................................. *Crypturgus pusillus*
Acknowledgement

We wish to thank C. Holzschuh for locating the type series of *C. cinereus*. This paper was supported by the Museum of Natural History and Archaeology, Trondheim, by grant 170565/V40 from the Norwegian Research Council and by a grant from The Royal Norwegian Society of Sciences and Letters to BHJ, and grant MZE-0002070201 from the Ministry of Agriculture of the Czech Republic to MK.

References


