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Conference Abstract

Is cytochrome c oxidase subunit I (COI) the right DNA barcoding marker for the *Chaetopteryx villosa* group?

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Abstract

Chaetopteryx villosa (Fabricius, 1798) is a caddisfly species distributed throughout Europe, except in the Balkan and Apennine Peninsula. However, phylogenetically close species belonging to the *C. villosa* group are widespread throughout entire Europe. Species of this group (*C. villosa*, *C. gessneri*, *C. fusca*, *C. sahlbergi*, *C. atlantica, C. bosniaca*, *C. vulture,* and *C. trinacriae*) have distinct distributions with some overlaps. Adult forms of these species are morphologically similar, whereas larval morphology is only known for some species. There are also indications of species hybridization (e.g., *C. villosa x fusca*). Presumably, the molecular approach for the species determination of this group would be highly beneficial. In the BOLD database, there are 154 specimens with *COI-5P* barcodes of *C. villosa* species. Out of the remaining species, *C. sahlbergi* has 27 specimens with a barcode, *C. fusca* 20, *C. gessneri* 5, *C. bosniaca* 5, and *C. atlantica* 1, whereas sequences from the species *C. vulture* and *C. trinacriae* are missing. Therefore, we tested the power of discrimination of the *COI-5P* marker in the *C. villosa* group, as the most common barcoding markers for species identification in animals.

Only sequences from public records originating from experienced research groups or taxonomists and containing a specimen photograph were taken as input. A total of 75

sequences from the BOLD database were obtained. Out of these sequences, 11 belonged to *C. fusca*, 5 to *C. gessneri*, 52 to *C. villosa*, 5 to *C. bosniaca,* and 2 to *C. sahlbergi*. For the generation of overview trees, *COI-5P* barcodes of *Rhyacophila fasciata* and *Rh. nubila* were used as outgroups. All sequences were trimmed at 5' and 3' ends, resulting in a final alignment length of 516 base pairs. Multiple sequence alignments and editing were done in the MEGA-X software. Analysis of nucleotide polymorphism was done in DNASP6 software. MEGA-X was used to calculate the pairwise distance and overall mean p-distance, and to construct the overview trees.

Analysis of DNA polymorphism revealed 14 haplotypes of *C. villosa*, 3 haplotypes of *C. fusca*, 2 haplotypes of *C. gessneri,* and one for species *C. bosniaca* and *C. sahlbergi*. There were no significant interspecific and intraspecific differences among haplotypes based on pairwise distances. The p-distance between one of the haplotypes of *C. fusca* and *C. villosa* was 0.000, whereas the p-distance among haplotypes of *C. villosa* varied from 0.001 to about 0.055. The mean overall p-distance among haplotypes of all species equaled 0.03. No species-specific clusters were observed when phylogenetic trees were constructed except for *C. gessneri*, regardless of the method used (i.e., NJ, UPGMA, ML, ME, or MP).

To minimize the possibility of species misidentification, we used only records submitted by NTNU-Norwegian University of Science and Technology (Norway), SNSB-Zoologische Staatssammlung Muenchen (Germany), Zoologisches Forschungsmuseum Alexander Koenig (Germany), University of Oulu, Zoological Museum (Finland), prof Hans Malicky and prof Mladen Kučinić. No records identified as hybrids were included in the analyses. With the exception of *C. gessneri*, COI-5P marker failed to separate the species of the *C. villosa* group. However, it is highly unlikely that poor species determination was the basis for such a result. To enable the comprehensive and unbiased evaluation of the relationships within this group, data coverage in BOLD database for most of the studied species should be enhanced, encompassing different geographical distribution of samples. Further studies are needed to detect the array of molecular markers suitable for the species delineation in a complex group such as *C. villosa*.

Keywords

DNA barcoding, Chaetopteryx villosa, Cytochrome c oxidase subunit I

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