Molecular phylogeny of panorpodid scorpionflies: An enigmatic, species-poor family of Mecoptera (Insecta)

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Abstract

The Panorpodidae are one of the species-poor taxa in Mecoptera, but explanation of the puzzling vicariant distribution of the two subordinated genera, \textit{Brachypanorpa} in the USA and \textit{Panorpodes} in East Asia and western North America, is a rewarding task. This first phylogenetic analysis of the Panorpodidae is based on 3661 aligned nucleotide positions and contains all described taxa of Panorpodidae, with the exception of two \textit{Panorpodes} species inhabiting Asia. Five genes, three mitochondrial and two nuclear, were used in separate as well as in a combined analysis. Bayesian inference and parsimony analysis both led to congruent trees, but the different gene partitions were incongruent in that \textit{Panorpodes} resulted as either paraphyletic or monophyletic. Based on our molecular data, the genus assignment of \textit{Panorpodes colei} Byers, 2005 is probably correct despite the species’ isolated occurrence in western North America. Based on morphological and molecular data, we recommend further research on a new \textit{Brachypanorpa} population, which would probably lead to the description of a new species.

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Introduction

The scorpionfly family Panorpodidae consists of the genera \textit{Panorpodes} MacLachlan, 1875 and \textit{Brachypanorpa} Carpenter, 1931, with a total of only 10 described species. Thus, Panorpodidae is rather species-poor compared to the closely related Panorpidae with about 380 species (Penny 1997). \textit{Brachypanorpa} comprises five known species (Carpenter 1931). These occur in the southwestern Appalachian Mountains (\textit{B. carolinensis}, \textit{B. jeffersoni}) and the western United States (\textit{B. montana}, \textit{B. oregonensis}, \textit{B. sacajawea}), respectively. Due to this disjunct distribution, the extant populations are thought to be relicts of an ancestral clade that was more widespread in North America during the early Tertiary (Byers 1997). Recent events like Pleistocene glaciation might explain the present relict distribution (Grimaldi and Engel 2005). The second genus, \textit{Panorpodes}, consisted of three species from Japan (\textit{P. maculata}, \textit{P. paradoxa}, \textit{P. pulchra}) and one species from Korea (\textit{P. komaensis}) until Byers (2005) rather surprisingly described a fifth species, \textit{Panorpodes colei}, from the Sierra Nevada, USA. It has remained unclear whether the sister group of Panorpodidae is Bittacidae, as Whiting (2002) suggested based on molecular data, or Panorpidae, as Willmann (1989) assumed on the basis of morphological characters.
The biology of the Panorpodidae is still not well known, in spite of existing information about some Brachypanorpa species (Carpenter 1953a, b; Byers 1997; Sauer et al. unpublished). The fossil record of Panorpodidae is rather poor; two specimens in Baltic amber have been described as Panorpa species (Carpenter 1954). So far no efforts have been made to elucidate the phylogeny of Panorpodidae. The only suggestion on relatedness within this group that has been made is that B. carolinensis and B. jeffersoni may form sister species, although this was not explicitly stated in the relevant article of Byers (1976). Specimens of Brachypanorpa and Panorpodes have solely been used as outgroup taxa in a phylogenetic study of the genus Panorpa (Misof et al. 2000), and as representatives of Panorpodidae in a study on mecopteran phylogeny (Whiting 2002).

In the present paper, we investigate the relationships within Panorpodidae, using fragments of three mitochondrial genes, cytochrome c oxidase subunits I and II (COI and COII) and 16S rRNA, as well as two nuclear genes, 28S rRNA and elongation factor 1z (EF-1z), in a Bayesian and a parsimony analysis. These genes have been used successfully in previous phylogenetic studies on Mecoptera (Misof et al. 2000; Whiting 2002). The present set of taxa consists of specimens from all described Brachypanorpa species and three Panorpodes species, including the recently described P. colei. Additionally, a morphologically exceptional specimen (Brachypanorpa sp.) from a geographically isolated Brachypanorpa population occurring in northern California that is generally similar to B. oregonensis (N.D. Penny, personal communication, 2005) was included in the analysis.

Material and methods

Taxon sampling and outgroup selection

We were able to obtain specimens of all described Brachypanorpa species and three of the five described Panorpodes species, either by direct collecting (B. carolinensis and B. jeffersoni) or from museum loans. Bittacus sp. was used as an outgroup, and three species of Panorpa were also included. The specimens used were stored in pure ethanol, with the exception of B. sacajawea which was pinned and dried. The species list and additional information on the specimens are given in Table 1.

DNA extraction

DNA samples were obtained by extracting DNA from leg musculature of ethanol-preserved specimens or from legs and antennae of dry-pinned material. The remains of the specimens used are stored at the Institute of Evolutionary Biology and Ecology of the University of Bonn. DNA was extracted using the NucleoSpin® tissue kit (Machery-Nagel), following the manufacturer's standard protocol.

PCR amplification

PCR followed the protocol described for 12S rRNA by Misof et al. (2000). As the yield of DNA from pinned and old specimens was very low, reamplification of the PCR products was necessary to gain sufficient amounts of DNA for sequencing. Due to poor DNA preservation in some of the specimens, not all DNA fragments could be sequenced for all species. Table 2 gives an overview of the primers used and indicates specimens for which not all gene fragments could be sequenced successfully.

DNA sequencing

PCR products were cleaned with the NucleoSpin® PCR kit (Machery-Nagel). Cycle sequencing of the PCR products was conducted using the ABI big dye terminator kits V1.5 and V2.0. An ABI 377 sequencer was used for sequencing both strands of DNA of all specimens used.

DNA alignment

DNA sequences of the protein-coding genes COI, COII and EF-1z were aligned with ClustalX (Thompson et al. 1997) using the default parameters. Controlling of the alignment by eye, and translation into amino acids with BioEdit v5.0.9 (Hall 1997) led to no ambiguities. Ribosomal RNA fragments were also prealigned with ClustalX in a first step; afterwards, highly variable regions that were ambiguously aligned were excluded in BioEdit.

Phylogenetic reconstruction

The dataset was analyzed in three separate ways: nuclear gene sequences, mitochondrial gene sequences and a combined dataset.

The best model of nucleotide substitution for the combined dataset under the Akaike Information Criterion (AIC; Akaike 1974) was calculated with Modeltest 3.7 (Posada and Crandall 1998) prior to Bayesian analysis with MrBayes 3.0b4 (Huelsenbeck 2000). The general time-reversible model of DNA substitution with gamma-distributed rate variation across sites and some invariable sites (GTR + I + γ) (Yang 1994) was selected as the best-fitting model for the mitochondrial and the combined datasets. For the dataset containing only nuclear genes, the GTR + γ model was selected as best-fitting. Bayesian analysis was conducted using four Markov Chain Monte Carlo (MCMC) samplers, three hot and one cold, and a random starting tree. We used 1,000,000 generations and sampled trees every 100 generations. The default model settings for the priors
were applied. Trees received before reaching stable ln likelihood sums were discarded as burn-in.

Prior to the parsimony analysis, we checked the three protein-coding genes for saturation in the third-codon position. As the plot of genetic distances in codon positions one and two versus position three showed no clear signs of saturation (data not shown), we decided to use all codon positions in our analyses.

Parsimony analyses were performed in PAUP* 4.0b10 (Swofford 2003), using heuristic searches with tree-bisection-reconnection (TBR) branch swapping and 1000 random-addition sequence replicates with 10 trees held at each step. Bootstrap support values were calculated from 10,000 replicates.

Results

Alignment

After the exclusion of about 200 ambiguously aligned positions mainly in the C-region of domain I of the 28S
rRNA, the resulting alignment was 3661 positions long. Average sequence lengths of the individual loci are given in Table 2. The alignment is available from the authors upon request.

**Dataset of nuclear genes**

For the nuclear dataset, consisting of 28S rRNA and EF-1α gene fragments, the Bayesian and parsimony searches yielded the same tree (Fig. 1A). Both Panorpidae and Panorpodidae form well-supported clades, the respective posterior probability/bootstrap values being 100/100%. Both Panorpodes (89/99%) and Brachypanorpa (84/99%) are monophyletic. Within Brachypanorpa, the support for individual nodes is relatively weak. Only the sister-taxon relationships B. jeffersoni–B. carolinensis (77/86%) and B. oregonensis–B. sp. (79/99%) receive substantial support.

**Dataset of mitochondrial genes**

With the mitochondrial dataset, the two methods of calculation again resulted in identical trees (Fig. 1B). In contrast to the Panorpodidae (98/100%), the Panorpidae clade (<50/79%) is not well supported, but the main difference to the nuclear dataset is that *P. colei* is placed at the base of Panorpodidae instead of as a member of a monophyletic genus Panorpodes, thus turning Panorpodes into a paraphyletic group (76/100%). Within Brachypanorpa, support for the sister-taxon relationships B. jeffersoni–B. carolinensis (100/100%) and B. oregonensis–B. sp. (100/100%) is strong again; B. sacajawea is placed at the most basal split. The position of *B. montana* receives only weak support (76/61%). Nevertheless, there is good support for the monophyly of a group containing *B. sacajawea, B. montana, B. carolinensis* and *B. jeffersoni* (96/100%), with *B. carolinensis* and *B. jeffersoni* being the more derived species.

**Combined dataset**

In the combined dataset, the species of the genus Panorpa (100/100%) and the family Panorpodidae (100/100%) both form monophyletic clades (Fig. 2). Similar to the nuclear-gene dataset, Panorpodes forms a monophyletic group, but the support is lower in this analysis (<50/93%). The monophyly of Brachypanorpa shows 100% bootstrap support and a posterior probability value of 100. Within the genus Brachypanorpa, B. oregonensis, and B. sp. form a monophyletic group (100/100%), the sister taxon of the remaining Brachypanorpa species. The remaining species also form a monophyletic group (98/100%), with *B. sacajawea* placed at the stem (82/84%), and *B. carolinensis* and *B. jeffersoni* as more derived sister species (100/100%).

**Discussion**

We present the first molecular systematic study of scorpionfly species in Panorpodidae, using Bayesian and parsimony analyses on mitochondrial as well as nuclear DNA sequences. Parsimony and Bayesian reconstruction resulted in almost identical trees. Nuclear and mitochondrial genes also led to a nearly consistent tree, but were not congruent on the essential placement of *P. colei*, a taxon that has been geographically isolated.
from its closest relatives for up to 35 million years if we assume the Beringian Bridge I to be the relevant connection between the American and Asian taxa (Sanmartin et al. 2001).

Consistent with Willmann (1989), the Panorpodidae form a well-supported monophyletic clade, as do the Panorpidae. In contrast to the genus Brachypanorpa, which we confirm to be monophyletic, the molecular analysis failed to reveal with certainty whether Panorpodes is the monophyletic sister-taxon of Brachypanorpa, as the nuclear genes (Fig. 1A) and the combined dataset (Fig. 2) suggest, or whether P. colei and the remaining Panorpodidae share a common ancestor, which would turn Panorpodes into a paraphyletic group (Fig. 1B). This second topology is not completely devious, as Willmann (1989) already presumed Panorpodes to be a paraphylum, because no autapomorphic morphological characters are described. But then we would expect P. colei as the sister taxon to Brachypanorpa, as the two co-occur in western North America. Since this is not the case, we think that probably the mitochondrial dataset is misleading in depicting Panorpodes as a paraphyletic group. The geographical separation of P. colei corresponding with a presumably long time of genetic isolation might serve as an explanation for our methods’ failure to clearly identify the correct phylogenetic placement of P. colei. Therefore, our conclusion that this species was probably correctly assigned to the genus Panorpodes by Byers (2005) remains to be confirmed by more detailed studies.

The genus Brachypanorpa, on the other hand, is well-supported as a monophyletic group, irrespective of the used dataset and analysis. This was to be expected, as the shortened rostrum and the female’s reduced wings must be accepted to be derived features characterizing the genus Brachypanorpa (Carpenter 1931). Within Brachypanorpa we were able to identify two well-supported sister groups (B. oregonensis–B. sp., B. carolinensis–B. jeffersoni), and to demonstrate that B. sacajawaea also belongs to the second group.
Brachypanorpa montana also seems to belong to this second group, but due to the age (~46 years) and inappropriate storage of the studied material, we were unable to sequence most DNA/RNA fragments of the available B. montana specimen; thus, it was impossible to identify the position of the species with certainty. The phylogenetic relationships of the Brachypanorpa species reflect their geographical distribution (Fig. 2) only partially, as the Appalachian species B. carolinensis and B. jeffersoni form a monophyletic group together with B. sacajawea and B. montana, occurring in the western USA. Brachypanorpa oregonensis and B. sp. are also distributed in the western USA (Oregon, California, Idaho, Utah; Carpenter 1953a, b). We hypothesize that most likely Pleistocene glaciation events have led to this now-scattered distribution of the Brachypanorpa species, as Grimaldi and Engel (2005) have already suggested.

The specimen of Brachypanorpa sp. we obtained from N. Penny not only differs from B. oregonensis and all other known Brachypanorpa species morphologically, by its dark brownish wing pattern, but also shows distinct genetic differences compared to its sister species, B. oregonensis. More genetic data on the gene flow between the two populations would probably lead to results justifying the description of Brachypanorpa sp. as a new species. Although we found remarkable genetic differences between Bittacus and all included Panorpidae and Panorpodidae species, our analysis design
does not allow us to conclude that Panorpidae and Panorpodidae are sister taxa, as Willmann (1989) had already proposed on the basis of morphological characters. Nevertheless, we are skeptical about Whiting’s (2002) hypothesis that the Bittacidae form the sister taxon of Panorpodidae, which was based on a combined analysis with low support values (Bootstrap < 50%, Bremer support 2), while none of the four individual genes Whiting used confirmed this relationship by itself.

In summary, we were able to resolve large parts of the genetic relationships within Panorpodidae, facilitating more detailed future research on the evolutionary history within this group. Additional DNA sequences of B. montana and the addition of the two unsequenced Panorpodes species from Japan and Korea should lead to an even more complete resolution of panorpodid phylogeny.

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