Molecular phylogeny of Trichoptera

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A robust phylogeny of Trichoptera and its related sister order Lepidoptera is required to answer questions about the evolution of pheromone communication systems and other traits. Regions of 18S and 28S were sequenced to construct a molecular phylogeny of Trichoptera. The suborder Annulipalpia sensu stricto was shown as a monophyletic taxon. Due to a polytomy, monophyly of both Integripalpia and Spicipalpia remains questionable.

Keywords: 18S, 28S, caddisflies, phylogeny, Trichoptera

Despite its obvious importance in Lepidoptera evolution, little is known about the evolution of sex pheromone systems within Trichoptera. Trichoptera and primitive Lepidoptera share the same type of pheromone components and the same pheromone-producing gland (Löfstedt & Kozlov, 1997). These types of components are considered as a possible autapomorphy of the super order Amphiesmenoptera (Löfstedt & Kozlov, 1997). To evaluate the suggestion put forward by Löfstedt & Kozlov, a robust phylogeny of Trichoptera and its related sister order Lepidoptera is needed.

The order Trichoptera (caddisflies) is the sister order of the Lepidoptera (caddisflies and moths). It is generally accepted that both orders are monophyletic (Morse, 1997). Together, the orders constitute the super order Amphiesmenoptera. Distinction of the trichopteran suborders is based on the ways of cocoon preparation in the larval stages. The three major suborders are Annulipalpia sensu stricto (the netspinners and retreat makers), Integripalpia (the tube case makers) and Spicipalpia (the closed cocoon makers). The suborder Spicipalpia is controversial; although most taxonomists consider Spicipalpia as a separated suborder, some taxonomists place spicipalpian families within the suborder Annulipalpia (see Morse, 1997). In this study, the suborder that is indicated as Annulipalpia sensu stricto does not include spicipalpian families. Modern consensus, mainly based on morphological data, generally affirms the monophyly of Integripalpia and of Annulipalpia sensu stricto, but the monophyly and relationships of Spicipalpia and its families remains inconclusive (Morse, 1997). In this study, a molecular phylogeny was constructed based on DNA sequences of 18S and 28S regions.

MATERIAL AND METHODS

Collection and rearing of the insects

Fifteen trichopteran species and four lepidopteran species were used in the data analysis. The Trichoptera species were collected as adult, larvae or pupae in Skåne, south Sweden, during the period 1996-1998 by V. Ivanov. The adults were kept in 70% alcohol or stored in a -20°C freezer immediately after collection. Larvae and pupae were reared in the laboratory and put in alcohol or in the freezer as soon as they became adult.

The primitive Eriocrania semipurpurella (Lepidoptera: Eriocraniidae) was trapped with sticky traps in 1997 by G. Svensson and stored in a climate room at 4°C. The sequences of Ascalapha odorata (18S: U65140; 28S: U65200), Galleria mellonella (18S: U65138; 28S: U65198) and Papilio troilus (18S: U65139; 28S: U65199) were downloaded from the National Center for Biotechnology Information (Leipe & Soussov, 1995).

DNA extraction, amplification and sequencing

The DNA of the other species was extracted following QIAamp Tissue Protocol described in QIAamp Blood and Tissue Handbook, Qiagen tissue Kit NR. 29304 (1997). One part of 18S
rRNA and one part of 28S rRNA were amplified and sequenced. The primers were designed based on known sequences. The 18S primer pair gave fragments of 258 bp, whereas 28S gave 228 bp fragments. Polymerase Chain Reaction (PCR) conditions were optimised for each primer pair and each species with the help of the modified Taguchi methods described by Cobb & Clarkson (1994). After amplification, the products were purified in Millipore tubes. Subsequently, the fragments were labelled by using the ABI Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). The products were purified following the protocol Ethanol/Sodium Acetate Precipitation Procedure of Applied Biosystems (1997, pp. 18-19; only half of the advised amounts were used (S. Gröndahl, personal communication)). Both strands of 1-3 individuals were sequenced on an Applied Biosystems Prism 310 Genetic Analyser (Perkin Elmer).

Data analysis
The sequence data were aligned in Clustal X (Thompson et al., 1997) using the default settings. A molecular phylogenetic tree was produced in version 3.1.1 for Macintosh of the computer program PAUP (Swofford, 1991) by using the options maximum parsimony (MP) and the bootstrap method (100 replicates) with heuristic search using stepwise addition and nearest-neighbor interchanges branch-swapping algorithm. The four Lepidoptera species were used as outgroup.

RESULTS AND DISCUSSION
After alignment, the sequences had a total length of 486 characters of which 170 were variable and 67 of these were parsimony-informative. The phylogeny (Fig. 1) showed the suborder

![Diagram](attachment:image.png)

**Figure 1.** The maximum parsimony phylogenetic tree based on 18S and 28S sequences as obtained with the bootstrap method (100 replicates) with heuristic search using stepwise addition and nearest-neighbor interchanges branch-swapping algorithm. The four Lepidoptera-species (L) were used as outgroup. Species with (I) belong to the suborder Integripalpia, (S) species belong to the suborder Spicipalpia and the as (A) indicated species belong to the suborder Annulipalpia *sensu stricto*. Above the branches, the bootstrap values are shown. The tree length was 290 and the CI 0.700.
Annulipalpia sensu stricto as a monophyletic taxon, which corresponded with preliminary analyses by Kjer et al. (1999).

Monophyly of both Integripalpia and Spicipalpia could not be demonstrated due to a polytomy. Within Integripalpia, molecular data support Limnephilidae (represented by *Glyphotaelius pellucides* and *Potamophylax latipennis*). Preliminary molecular data of Kjer et al. (1999) suggest monophyly of Integripalpia. Earlier studies of Ivanov (1993; 1997) and R.W. Holzenthal, K.M. Kjer & S.J. Weller (pers. comm. in: Morse, 1997; hereafter cited as: Holzenthal et al., 1997) suggest that the suborder Spicipalpia is not a monophyletic taxon.

Based on morphological data, Ivanov (1993, 1997) concludes that the sister families Rhyacophilidae and Hydrobiosidae arose at the base of Annulipalpia sensu stricto and that only Glossosomatidae and Hydroptilidae are allied with Integripalpia. However, preliminary molecular data of Holzenthal et al. (1997) suggest that Spicipalpia and Integripalpia form an internal clade that includes a paraphyletic Spicipalpia. In the study presented, *Rhyacophila nubila* (Rhyacophilidae; Spicipalpia) was placed in the polytomy formed by Integripalpia and Spicipalpia. This did not correspond with the phylogeny of Ivanov (1993, 1997).

The phylogeny obtained is not resolved due to the very conservative 18S and 28S fragments. Sequencing of more DNA fragments, like mtDNA, is necessary to be able to produce a robust phylogeny of the sister orders Trichoptera and Lepidoptera.

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**REFERENCES**


