A preliminary molecular cladistic analysis of the dipteran family Sciaridae (Diptera: Sciaroidea)

Julia Seeber[1], Kai Heller[2], Wolfgang Arthofer[1] & Alexander Rief[1]

Institute of Ecology, University of Innsbruck

Abstract: Die Trauermücken (Insecta, Diptera, Sciaroidea: Sciaridae) sind eine weltweit verbreitete Familie mit ca. 1700 beschriebenen Arten. Da es nur wenige, morphologisch abgrenzende Merkmale gibt, ist die Klassifizierung schwierig und uneinheitlich. In der vorliegenden Studie wurde zum ersten Mal eine Klassifizierung anhand eines molekularen Gens, der cytochrome c oxidase subunit one (CO I), vorgenommen.

Key Words: Diptera, Sciaroidea, Sciaridae, Europe, Austria, Denmark, Germany, molecular phylogeny, CO I

[1] Institute of Ecology, University of Innsbruck, Technikerstrasse 25, 6020 Innsbruck, Austria, E-Mail: Julia.Seeber@uibk.ac.at

[2] Arthur-Zabel-Weg 25, 24226 Heikendorf, Germany, kaiheller@gmx.de

Introduction

Sciaridae (Diptera: Sciaroidea) are a dipteran family distributed worldwide and contain about 1700 named extant species. Until now, their classification in genera has been conducted solely based on morphological characteristics (in earlier times only wing venation, nowadays mainly genitalic characters). Sciaridae are extremely uniform and the few distinguishing characters used for generic classification appear to be very variable throughout the family. Enderlein (1911) and Frey (1942) were the first ones who arranged the genera of Sciaridae with respect to their supposed relationships. Two further attempts to figure out the generic phylogeny of Sciaridae have been conducted recently. Menzel & Mohrig (2000) presented a cladogram based upon manual character evaluation, whereas Vilkamaa & Hippa (2004) used a computer-aided parsimony analysis for the generic consensus tree. Both cladograms differ considerably from each other and no common agreement has been found so far concerning the intergeneric classification of Sciaridae. Thus it was interesting to evaluate the relationship of selected species on the basis of DNA similarity.

The application of molecular tools to assess phylogenetic relationships within families or clades has been adopted for a wide range of taxa, including various dipteran families (e.g. Drosophilidae, O'Grady & al. 2011; Calyptratae, Kutty & al. 2010). For the Sciaridae, no attempt to confirm or revise the existing classification via molecular analyses has been made until now. To get a first insight into the molecular cladistics of this widespread dipteran family we analysed DNA sequences of a mitochondrial gene (cytochrome c oxidase subunit one).

Material and methods

Taxon sampling

A total of 93 specimens belonging to 35 species and 10 genera were used in this study. Alpine species from "Hoher Burgstall" (Central Alps, Austria) were sampled via emergence traps throughout one growing season, the other species were hand-sampled in May 2010 in Northern Germany and Southern Denmark and in July/August 2010 in Carinthia (Austria). To avoid DNA degradation, salt water was used as primary collection fluid in the emergence traps. All specimens were stored in 70% EtOH at room temperature.

DNA extraction and sequencing

Genomic DNA was extracted from single fungus gnats using the peqGOLD Tissue DNA Mini Kit (peqlab), following manufacturer's instructions. Part of the mitochondrial cytochrome c oxidase subunit one gene (COI) was amplified using the universal invertebrate primers LCO1490 and HCO2198 described by Folmer & al. (1994). PCR was carried out in 10 µL reaction volumes containing 0.2 mM dNTPs (Genecraft), 1.0 µM of each primer, 1 × buffer (BioTherm), 3 mM $MgCl_2$, 5 µg of bovine serum albumin (BSA), 0.375 U *Taq* Polymerase (BioTherm), 2.825 µl of PCR-grade water and 1.5 µl of DNA extract. Thermal cycling comprised an initial denaturation at 94°C for 2 min, followed by 35 cycles at 94°C for 20 sec, 50°C for 30 sec, 72 °C for 45 sec and a final elongation at 72°C for 2 min. PCR products were purified using ExoSAP-IT (USB Corporation), subjected to sequencing PCR using Big-Dye Terminator mix (version 1.3, Applied Biosystems) and sequenced in forward and reverse directions. The sequences from up to three individuals per species were aligned with BioEdit (Version 7.0.9.0, Hall, 1999) and corrected manually. Sequences will be made available in GenBank.

Nomenclature

The applied nomenclature follows Fauna Europaea (Heller & Menzel 2011)

Phylogenetic analysis

In order to get a first overview of the molecular phylogenetic relationships, neighbouring trees of the corrected sequences were generated using ClustalX2 (Larkin & al. 2007).

Results

The intraspecific variation of all species was lower than the variation between species, indicating that COI is useful for species determination in Sciaridae. Only in the ubiquitous species *Corynoptera subparvula*, *Lycoriella castanescens* and *Epidapus absconditus* was an increased variation observed.

This arrangement of species, however, does not reflect the current generic arrangement of the species. Only the four *Cratyna* species, which all belong to the subgenus *Spathobdella*, clade together. Many species of the most species rich genus *Bradysia* in the upper part of figure 1 also seem to be closer related to each other than to other species, placed in other genera. They are currently positioned in the *fungicola*-group (*affinis*, *fungicola*, *pectoralis*), the *alpicola*-group (*alpicola*, *pauperata*), *nervosa*-group (*nervosa*, *placida*), *melanura*-group (*zetterstedti*), and the *praecox*-group (*praecox*). The remaining species of *Bradysia* seem to be neither closely related to each other nor to the formerly mentioned ones. They belong to the *rufescens*-group (*confinis*, *loriculata*), *angustipennis*-group (*flavipila*), *lobata*-group (*normalis*), *giraudii*-group (*lapponica*), *pallipes*-group (*heydemanni*) and the *hilaris*-group (*hilariformis*). The species group assignment follows Menzel & Mohrig (2000).

Lycoriella inflata appears to be more similar to the first complex of *Bradysia* species than to the other studied *Lycoriella*, *L. castanescens*, which may indicate that the current subgenus *Hemineurina* is not closely related to *Lycoriella* s. str. and may probably better be treated as a separate genus. The same applies to *Leptosciarella* s. str. and the subgenus *Hirtipennia*, whose representatives *L. (L.) fuscipalpa* and *L. (H.) hirtipennis*, are not especially similar concerning their COI sequences.

The genus *Corynoptera* s. l. with its studied representatives *C. subparvula* (*parvula*-group), *C. forcipata* (*forcipata*-group), *C. winnertzi* (*concinna*-group), *Claustropyga abblanda, Caustropyga refrigerata* and *Peyerimhoffia alpina* does not appear to be a natural group and the ongoing subdivision into several genera seems to be justified.

Lastly *Schwenckfeldina carbonaria*, which turned out to be a sister group to all other Sciaridae based upon morphological characters (Vilkamaa & Hippa 2004) is not at all isolated in our study but is most similar to *Corynoptera subparvula*, a much smaller and morphologically very different species.

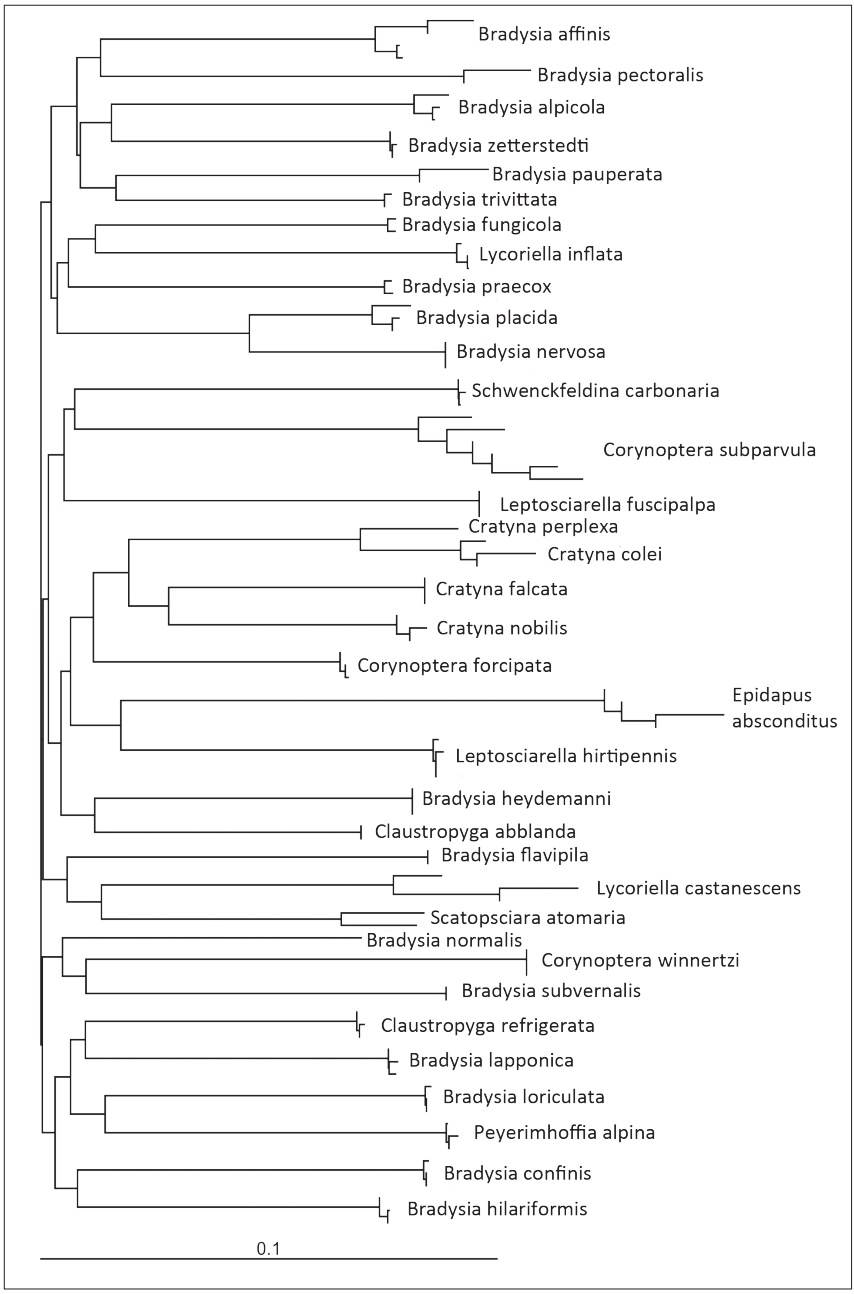

Fig. 1: Cladogram

Table 1: Sampled species used for DNA analysis with sampling localities.

Species	Sampling site
Bradysia affinis (Zetterstedt, 1838)	Austria, T, Burgstall
Bradysia alpicola (Winnertz, 1867)	Austria, T, Burgstall
Bradysia confinis (Winnertz, 1867)	Denmark, Als, Klingbjerg
Bradysia flavipila Tuomikoski, 1960	Austria, T, Burgstall
Bradysia fungicola (Winnertz, 1867)	Denmark, Als, Klingbjerg
Bradysia heydemanni (Lengersdorf, 1955)	Austria, K, Reißeck
Bradysia hilariformis Tuomikoski, 1960	Denmark, Sonderhav
Bradysia lapponica (Lengersdorf, 1926)	Austria, K, Bad Kleinkirchheim
Bradysia loriculata Mohrig, 1985	Austria, T, Burgstall
Bradysia nervosa (Meigen, 1818)	Denmark, Als, Klingbjerg
Bradysia normalis Frey, 1948	Austria, T, Burgstall
Bradysia pauperata (Winnertz, 1867)	Austria, T, Burgstall
Bradysia pectoralis (Staeger, 1840)	Denmark, Sonderhav
Bradysia placida (Winnertz, 1867)	Denmark, Sonderhav
Bradysia praecox (Meigen, 1818)	Austria, K, Bad Kleinkirchheim
Bradysia subvernalis Mohrig & Heller, 1992	Denmark, Als, Klingbjerg
Bradysia trivittata (Staeger, 1840)	Austria, T, Burgstall
Bradysia zetterstedti Mohrig & Menzel, 1993	Austria, T, Burgstall
Claustropyga abblanda (Freeman, 1983)	Denmark, Als, Klingbjerg
Claustropyga refrigerata (Lengersdorf, 1930)	Austria, T, Burgstall
Corynoptera forcipata (Winnertz, 1867)	Germany, SH, Fröruper Berge
Corynoptera subparvula Tuomikoski, 1960	Austria, T, Burgstall
Corynoptera winnertzi Mohrig, 1993	Denmark, Als, Klingbjerg
Cratyna (Spathobdella) colei (Freeman, 1990)	Austria, T, Burgstall
Cratyna (Spathobdella) falcata (Tuomikoski, 1960)	Austria, K, Bad Kleinkirchheim
Cratyna (Spathobdella) nobilis (Winnertz, 1867)	Austria, K, Bad Kleinkirchheim
Cratyna (Spathobdella) perplexa (Winnertz, 1867)	Austria, K, Bad Kleinkirchheim
Epidapus absconditus (Vimmer, 1926)	Austria, T, Burgstall
Leptosciarella (Hirtipennia) hirtipennis (Zetterstedt, 1838)	Denmark, Als, Klingbjerg
Leptosciarella (Leptosciarella) fuscipalpa (Mohrig & Mamaev, 1979)	Denmark, Als, Klingbjerg
Lycoriella (Hemineurina) inflata (Winnertz, 1867)	Austria, T, Burgstall
Lycoriella (Lycoriella) castanescens (Lengersdorf, 1940)	Austria, T, Burgstall
Peyerimhoffia alpina (Mohrig, 1978)	Austria, T, Burgstall
Scatopsciara (Scatopsciara) atomaria (Zetterstedt, 1851)	Austria, T, Burgstall
Schwenckfeldina carbonaria (Meigen, 1830)	Germany, SH, Fröruper Berge

Discussion

To our knowledge, we present the first approach of a molecular characterization of the family Sciaridae. We are aware that this preliminary study is far from being exhaustive. The species assortment is more or less random due to the available material. Some genera like *Sciara*, *Trichosia*, *Camptochaeta* and *Corynoptera* s. str. are still missing. The restriction to a single, maternally inherited gene may also introduce some bias to our results. Mitochondrial sequences are more variable in time than nuclear ones, so that higher level branching may not be reliably reflected in the resulting trees. Another critical point is, that in this study only a simple cluster analysis on similarity was performed and not a strict parsimony or maximum likelihood analysis with a defined outgroup. But even by a comparison of similarity it should be expected that closely related species should appear nearer to each other in the tree than to phylogenetically distant species as it was the case with *Spathobdella* in this study. By the analysis of more species, the

implementation of nuclear markers and the employment of more sophisticated statistical methods the confirmation of natural groups among Sciaridae will be possible. On the other hand the present study seems to indicate that some collective genera such as *Bradysia*, *Corynoptera* and *Lycoriella* do not constitute natural units in their currently accepted limits. Further studies, also including non-palaearctic representatives of other genera, may help to enlighten the phylogenetic history of Sciaridae.

References

Enderlein, G. (1911). Die phyletischen Beziehungen der Lycoriiden (Sciariden) zu den Fungivoriden (Mycetophiliden) und Itonididen (Cecidomyiiden) und ihre systematische Gliederung. – Archiv für Naturgeschichte **77**(1): 116-201.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. – Molecular Marine Biology and Biotechnology **3**: 294-299.

Frey, R. (1942). Entwurf einer neuen Klassifikation der Mückenfamilie Sciaridae (Lycoriidae). – Notulae Entomologicae **22**: 5-44.

Heller, K. & Menzel, F. (2011). Fauna Europaea: Sciaridae. – In: P.L.T. Beuk & T. Pape (eds): Fauna Europaea: Diptera: Nematocera. Fauna Europaea version 2.4. Available from www.faunaeur.org.

Kutty, S.N., Pape, T., Wiegmann, B.M. & Meier, R. (2010). Molecular phylogeny of the Calyptratae (Diptera: Cyclorrhapha) with an emphasis on the superfamily Oestroidea and the position of Mystacinobiidae and McAlpine's fly. – Systematic Entomology **35**(4): 614-635.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. & Higgins D.G. (2007). Clustal W and Clustal X version 2.0. – Bioinformatics 23: 2947-2948.

Menzel, F. & Mohrig, W. (2000). Revision der paläarktischen Trauermücken (Diptera: Sciaridae). – Studia dipterologica Suppl. **6** (1999): 1-761; AMPYX-Verlag, Halle (Saale).

O'Grady, P.M., Lapoint, R.T., Bonacum, J., Lasola, J., Owen, E., Wu, Y. & DeSalle, R. (2011). Phylogenetic and ecological relationships of the Hawaiian Drosophila inferred by mitochondrial DNA analysis. – Molecular Phylogenetics and Evolution **58**: 244-256.

Vilkamaa, P. & Hippa, H. (2004). The genus *Xenosciara* gen. n. and the phylogeny of the Sciaridae (Diptera). – Zootaxa 699: 1-24.