

Ph.D thesis

**Taxonomic and Phylogentic Research of
Palaeartic Charipinae Species
(Hymenoptera, Figitidae)**

Fülöp Dávid

Supervisor: Dr Melika George

Consultant: Dr. Péntzes Zsolt

HAS Biological Research Center, Institute of Genetics
University of Szeged, Faculty of Science and Informatics
Doctoral School of Biology

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Introduction

The subfamily Charipinae (Hymenoptera: Cynipoidea: Figitidae) is one of the many problematic groups of Hymenoptera. The taxon rank and name has been changed several times since its description. What is due to their simplified morphology and biology (koinobiont endohyperparasitoids). The subfamily used to be divided into three tribes, Protocharipini, Alloxystini and Charipini, monophyly of which is still under debate. One of the tribes, Protocharipini, is extinct. The members of the extant tribe Alloxystini are hyperparasitoids of aphids (Hemiptera: Aphididae) via Aphidiinae (Hymenoptera: Braconidae) and via Aphelinidae (Hymenoptera: Chalcidoidea), while the species of the extant Charipini are hyperparasitoids of psyllids (Hemiptera: Psyllidae) via Encyrtidae (Hymenoptera: Chalcidoidea).

From 288 described species of the subfamily, currently 175 are valid. The last comprehensive work was published in 1910. Since then only a few papers were published mainly dealt with the fauna of geographically restricted area, and two unrevised world catalogues were published, one of which gave a generic key.

Even the sister group relationship of the taxon is unclear. The data in the literature is controversial.

It is commonly accepted view that the identification problems are based on the small size of wasps, their

simplified morphology and the superficial species descriptions. Often species' descriptions are based on one specimen only and a few diagnostic characters, which very often are not enough for the precise species identification, more of that frequently different authors used different characters for species separation and it is impossible to determine if the variability of characters is intra- or interspecific.

For the species identification within the most species rich genus, *Alloxysta*, two different conceptions are known. According to some authors (e.g. Fergusson), only a few, very variable and generalist species exist whereas others (e.g. Evenhuis) suggest that the genus contains much more species which are less variable, hardly distinguishable and more specialized.

The solution of taxonomic problems within the members of the genus is in need because they are natural enemies of economically important aphid species and model species in ecological trophical webs research. Resolving the identification problem is essential for a correct interpretation of the results of the host-parasitoid community experiments and especially in the natural environment.

Objective of studies

Our main research focus was the establishing of the phylogenetic relationships within the genus *Alloxysta*. For its realisation, DNA based phylogenetic trees were constructed by using different methods and morphological characters were mapped onto them what is a widely used approach in phylogenetic and taxonomic studies. Morphometric characters used in the species descriptions were analysed by the ordination method.

Methods

Field data

The basic morphological investigations were carried out on specimens loaned from different museums whereas for more detailed studies we used freshly collected specimens by the help of sweeping and yellow pitfall trapping. The collected material after separation (~150 specimen) was stored in 96% ethyl-alcohol at -20°C until further analysis. More extensive stereomicroscope studies were carried out on the sequenced specimens with making slide micropreparations, following Prinsloo's method.

Morphometrics

The widely used metric characters for wing venation, width and length of antennomeres were investigated with PAST program using Principal Component Analysis (PCA).

Molecular phylogeny

We isolated and purified DNA from each specimen which we were able to distinguish „by eye”, tried to involve into this research as many specimens as was possible for us to identify to a particular morphotype („species”). The DNA was isolated from the metasoma by chelex method. We amplified 658 bp of cytochrom oxidase (COI) and 565 bp of nuclear 28S D2. The purified PCR products were sequenced from both direction.

The nuclear rDNA regions were aligned manually using secondary structures following the recommendations of literature. The COI alignment was carried out by using Promals. For basic descriptive statistic (e.g. base composition) and computing genetic distances (COI), DAMBE and MEGA5 programs were used.

The ambiguously aligned regions of D2 were excluded from the analysis, but in order to avoid losing of extra information, they were coded as binary data according to the simple gap coding method. We used parsimony, Bayesian, maximum-likelihood and distance based

approaches for analysing of our data set. The evolutionary models used in our phylogenetic reconstructions were selected with jModelTest and MrModeltest. The F81+G (loop) doublet (stem) was suggested for D2 and HKY+I (1. and 2. codon) GTR+G (3. codon) and gamma-distributed rates across sites and a proportion of invariant sites was suggested for COI. The partitioned analysis was carried out in a Bayesian framework, using MrBayes selected model type and default priors in the following data-partitioning schemes: COI by codon position, D2 stem, loop, gap.

Character mapping

The Hymenoptera Anatomy Ontology (HAO) nomenclature was used for the first time in Hungary to clarify the concepts we are referring to and make it easily comparable with other publications.

The characters taken from the literature (39) were mapped on the DNA based tree and estimated with bayesian partitioned analysis. We used a likelihood based approach of StochChar (Mesquite) program with Mk1 ("Markov k-state 1 parameter") model which is a k-state generalization of Jukes-Cantor model. The single parameter is the rate of change where any particular change is equally probable.

Results and discussion

Morphometrics

- Two specimen (with two different haplotypes) were found with asymmetric wing venation.
- In the PCA analysis the variance was very high (70-90%) along the first principal component axis. It should be noted that according the literature this axis is strongly influenced by the size because many characters display some degree of allometric growth.
- We were unable to separate groups correspond with the haplotypes neither on first and second nor second and third axes.
- Based on our analysis these metric characters may be unsuitable for subgeneric division of the genus.

Molecular phylogeny

- 53 specimens used for phylogenetic estimations were belonging to 41 COI and 17 28S D2 haplotypes.
- The COI sequences of the *Phaenoglyphis* specimens were 6 bp shorter than in *Alloxysta*
- The COI based mean intrageneric p distances were 0.105 in the genus *Alloxysta* and a bit higher 0.126 in the genus *Phaenoglyphis*. The distance between the two genera was 0.128. It should be noted that

the intergeneric distances between species could be lower than intrageneric distances.

- The phylogenetic trees reconstructed with different methods were congruent.

Character mapping

- 13 from the involved 39 morphological characters showed variation in our analysis:
 - relative position of anterior ocellus,
 - shape of head from anterior view,
 - Pedicel (female),
 - Transfacial line,
 - Hypostoma,
 - Pronotal carina,
 - Processes behind submedian pronotal depression,
 - Apex of scutellum,
 - Propodeal carina,
 - R1 vein,
 - length of Rs vein,
 - M, Cu1a and M+Cu1a veins,
 - Rs+M vein.
- Our results suggest that all of the variable characters tend to be homoplasious. For this reason taken alone they are not suitable to establish monophyletic groups and should be handled carefully in morphological based phylogenetical

studies. The species descriptions must take into account more anatomical structures.

- Combination of different morphological and metric characters can be used to separate species. The combination of different characters is suitable for subgeneric division as well. Our results support more Fergusson's species concept.

List of publications:

The present thesis is based on the following publications:

Paretas-Martínez, Jordi; Arnedo, Miquel A.; Melika, George; Selfa, Jesús; Seco-Fernández, Maria Victoria; **Fülöp, David**; Pujade-Villar, Juli (2007): Phylogeny of the parasitic wasp subfamily Charipinae (Hymenoptera, Cynipoidea, Figitidae). *Zoologica Scripta*, 36 (2): 153-172(20) IF: 2.364

Juli Pujade-Villar, Jordi Paretas-Martínez, Jesús Selfa, Maria Victoria Seco-Fernández, **David Fülöp**, George Melika (2007): *Phaenoglyphis villosa* (Hartig 1841) (Hymenoptera: Figitidae: Charipinae): a complex of species or a single but very variable species? *Annales de la Société Entomologique de France* 43 (2): 169-179. IF: 0.823

Fülöp D., Melika G., Bechtold M., Bozsó M. (2010): Checklist of charipines of Hungary (Hymenoptera, Figitidae: Charipinae). *Folia Entomologica Hungarica*, 71. IF: -

Pujade-Villar J., **Fülöp D.**, Paretas-Martínez J., Melika G. (2011): First record of *Phaenoglyphis villosa* (Hartig, 1841) from Korea (Hymenoptera: Cynipoidea: Figitidae: Charipinae). *ORSIS* 25: pp. 141-146. IF: -

Fülöp, D., Mikó, I., Seltmann, K., Péntes, Zs., Melika, G. (2013): *Alloxysta chinensis* a new Charipinae species from China (Hymenoptera, Figitidae). *Zootaxa* 3637 (3):394-400 IF: 0.927

Other publications:

Fülöp D. (2002): Habrocerinae and Tachyporinae (Coleoptera: Staphylinidae) from the Fertő-Hanság National Park – *In: Mahunka S. (eds.): The fauna of the Fertő - Hanság National Park, Hungary*, Hungarian Natural History Museum, Budapest, 413-415.

Fülöp D. (2005): Rove beetles (Coleoptera: Staphylinidae) of the Tisza valley – *In Gallé L. (eds.): Vegetation and Fauna of Tisza River Basin I, Tiscia Monograph Series*, Szeged, 95-147.

Melika, G., Choi, J-Y., Pujade-Villar, J. Péntes, Zs., **Fülöp, D.** (2007): A new species of cynipid inquiline of the genus *Ufo* Melika & Pujade-Villar, 2005 from Korea (Hymenoptera: Cynipidae: Synergini) – *The Journal of Asia Pacific Entomology* 10(3): 197-200.

Szél Gy., Retezár I., Bérces S., Szabó K., **Fülöp D.**, Péntes Zs (2007): Magyarország futrinkái [Ground beetles of Hungary]. – *In: Forró L. (eds): The Development of the Fauna of the Carpathian Basin*. Hungarian Natural History Museum, Budapest, pp. 81-106. [in Hungarian]

Bérces S., Szél Gy., Ködöböcz V., Kutasi Cs., Szabó K., **Fülöp D.**, Péntes Zs., Peregovits L. (2007): A magyar futrinka [The Hungarian ground beetle]. – *In: Forró L. (eds): The Development of the Fauna of the Carpathian Basin*. Hungarian Natural History Museum, Budapest, pp. 107-124. [in Hungarian]

Merkl O., Szabó K., **Fülöp D.**, Bozsó M., Máté A., Peregovits L., Soltész Z., Somogyi K., Péntes Zs. (2007): A pusztai gyalogcincér: taxonómiai helyzet, molekuláris genetikai és morfológiai vizsgálatok, elterjedés, életmód, természetvédelmi vonatkozások [Dorcadion cervae: taxonomical position, molecular genetic and morphometric studies, distribution, biology and references for conservation]. – In: *Forró L. (eds): The Development of the Fauna of the Carpathian Basin*. Hungarian Natural History Museum, Budapest, pp. 125-132. [in Hungarian]

Melika G., Péntes Zs., Mikó I., Bihari P., Ács Z., Somogyi K., Bozsóki Z., Szabó K., Bechtold M., Fári K., Fehér B., **Fülöp D.**, Csóka Gy. Stone, G. N. (2007): A Kárpát-medence tölgy gubacsdarázs faunájának kialakulása [The development of gall wasp fauna of the Carpathian basin] – In: *Forró L. (eds): The Development of the Fauna of the Carpathian Basin*. Hungarian Natural History Museum, Budapest, pp. 165-174. [in Hungarian]

G. Melika, M. Tavakoli, S.E. Sadeghi, Z. Péntes, **D. Fülöp**, M.A. Assareh, R. Atkinson, M. Bechtold, I. Mikó, M. R. Zargarán, D. Aligolizade, H. Barimani, F. Pirozi, R.J. Challis & G.N. Stone (2008): New Species of Oak Gallwaps from Iran (Hymenoptera: Cynipidae: Cynipini) – *Zootaxa* 1699:1-64. IF: 0.740

Fülöp D., Rudner J. (2008): Ground beetles (Coleoptera: Carabidae) of the Tisza Valley, Hungary. – In *Gallé L. (eds.): Vegetation and Fauna of Tisza River Basin II., Tiscia Monograph Series*, Szeged, 45-132

Zsolt Péntzes, George Melika, Zoltán Bozsóki, Péter Bihari, István Mikó, M Tavakoli, Juli Pujade-Villar, Balázs Fehér, **Dávid Fülöp**, Krisztián Szabó, Miklós Bozsó, Botond Sipos, Kálmán Somogyi, Graham N. Stone (2009): Systematic re-appraisal of the gall-usurping wasp genus *Synophrus* Hartig, 1843 (Hymenoptera: Cynipidae: Synergini) – *Systematic Entomology*, 34 (4): 688-711. IF: 2.467

Melika G., Pujade-Villar J., Stone G.N., **Fülöp D.**, Péntzes Z. (2009): New species of cynipid gallwasps of the genus *Plagiotrochus* Mayr, 1881 from Nepal and Jordan *Acta Zoologica Academiae Scientiarum Hungaricae* 55 (3) 263-274 IF: 0.514

Σ IF: 7,835