

Ph.D thesis

**Taxonomic and Phylogenetic Studies on
the Palaearctic *Saphonecrus* Dalla
Torre & Kieffer oak gall inquiline
species (Hymenoptera: Cynipidae,
Synergini)**

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Introduction

Cynipid galls represent a habitat and food resource for a relatively closed community of arthropods, in which parasitoid, hyperparasitoid and inquiline species may also be found beside the gall inducers. The least known and studied trophic level of the oak gall community is the inquilines. One of the underlying causes may be that the taxonomic identification of Synergini species is difficult. The tribe Synergini includes about 186 species (during our research other four newly described *Lithosaphonecrus* species were added), of which 48 is present in the Western Palaearctic. A number of important taxonomic and phylogenetic issues remain unresolved for cynipid inquilines, and in order to address them, molecular phylogenetic analyses may be needed besides the morphological re-appraisal of diagnostic species level characters. In case of cynipids, nuclear 28S D2 locus and mitochondrial cytochrome c oxidase I (coxI) gene sequences are frequently used.

The inquilines are now believed to represent polyphyletic (or paraphyletic) assemblage, in which species associated with rose cynipids are distinct from those associated with oaks. Molecular phylogenetic evidence suggests that the inquilines are not a monophyletic group, and instead comprise up to 3 distinct evolutionary lineages with independent origins within the Aylacini assemblage: a) rose-associates inquilines in *Synophromorpha* and *Periclistus*, b) oak-associated inquilines in *Ceroptres*, c) oak-associated inquilines in *Synergus* complex which consist of *Synophrus-Saphonecrus-Synergus* and the Afrotropical genus *Rhoophilus* attacking moth-induced galls. The recent phylogenetic reconstructions within the

Synergus complex of species support the monophyly of the large genus *Synergus* and the smaller *Synophrus*, while the monophyly of *Saphonecrus* was rejected. These results are also supported by the new molecular phylogeny which we propose in this thesis.

Objective of studies

Our main goals were to establish new phylogenetic relationships within the *Synergus* complex (extending with *Ufo*, *Lithosaphonecrus* and a „Saphonecrus-like” group), to establish their new taxonomy, and the description of a new genus, (*Lithosaphonecrus*), with the designation of the type-species and description of four new species. Other aims were to justify the necessity of the phylogenetic analysis of *Saphonecrus* genus and Saphonecrus-like groups and to outline the possible host plant and host gall associations of these group of species.

Methods

Field data

All the wasps in this study were laboratory reared from fresh galls collected in different localities in Russia, Japan, China and Taiwan during 2008–2012. Japanese galls were reared at the University of Edinburgh, UK; galls collected in Taiwan – at the National Chung Hsing University, Taichuing, Taiwan. Galls were placed in plastic containers at a room temperature and checked every day. Wasps that had emerged were aspirated and placed in 99% ethanol for further

laboratory processing. The galls were classified into the following categories based on their locations: bud galls (galls occur at the top bud or lateral buds), twig galls (galls on young twigs), catkin galls (galls on male inflorescences) and leaf galls (galls on veins of leaf or at the leaf base or petiole).

Molecular phylogeny

We chose 28S D2 and *coxI* markers because then we are able to incorporate and compare our results with others. Genomic DNA was extracted from legs from adult specimens, following the chelex extraction method.

A 619 bp aligned fragment of the mitochondrial *coxI* gene and 596 bp aligned fragment of 28S D2 region of nuclear rDNA were successfully amplified. Previously published sequences of other cynipid inquilines (*Saphonecrus*, *Synergus* and *Synophrus* species) were included in the final dataset altogether comprising sequences of 55 taxa.

Sequences were aligned using Muscle 3.6. Phylogenetic reconstruction was carried out in a Bayesian framework using MrBayes 3.2.1. In this analysis, codon positions of *coxI* and the entire 28S D2 were defined as separate partitions, resulting in four data partitions. For each partition the GTR+G evolutionary model was used

Morphology

The terminology used to describe gallwasp morphology follows other recent cynipid studies. Images of wasp anatomy were produced with a digital Nikon Coolpix 4500 camera attached to a Leica DMLB compound microscope, followed by processing in CombineZP (Alan Hadley) and Adobe Photoshop 6.0.

Results and discussion

The combined results of the morphological analysis and molecular phylogeny

- 50 specimens used for phylogenetic estimations were belonging to 32 COI and 23 28S D2 haplotypes.
- The obtained gene trees were congruent with each other.
- As a result of our work we could be delineated three main clades of inquilin species. Within these clades eight *Saphonecrus* subclades identified, each with posterior probabilities exceeding 0.68
- It would *be appropriate to reclassify* *Saphonecrus barbotini* and *S. gallaepomiformis* species into a newly established genus based on their biology, morphological characteristics and the results of phylogenetic studies. It is the sister group of *Synophrus*.
- The „conatus” lineage which includes *Saphonecrus connatus* also consist two Eastern Palaearctic species which are currently under description. These new specimens associated to Eastern Palaearctic *Callirhytis hakonensis* gall wasp species on *Quercus* (Q.) section *Quercus*. This fact is supporting suspected trans-Palaearctic distribution of "connatus" lineage. It is suspected as remains of an ancient separate lineage of the *Synergus* complex.
- Herein described new genus, *Lithosaphonecrus*, with four species (*L. formosanus*, *L. dakengi*, *L. huisuni* and *L. yunnani*) can be considered the first inquilin

species known to associate with gallwasps on *Lithocarpus* within Synergini tribus.

- We also identified another distinct clade whose members also associated the *Lithocarpus* species based on the results of our preliminary studies of undescribed *Saphonecrus* species from Eastern Palaearctic and Oriental Region. However, this is clearly distinct from the *Lithosaphonecrus* genus on the basis of their morphology and examined gene sequences.
- We relocated the previously classified *Ufo shirakashii* (Shinji) and *U. shirokashicola* (Shinji) species from *Ufo* genus to *Saphonecrus* genus as *Saphonecrus shirakashii* (Shinji) and *S. shirokashicola* (Shinji).
- There is no doubt that the current limits of the *Saphonecrus* genus must be changed, and a number of new monophyletic genera must be established.

Host gall associations

- We qualitatively analyzed host gall associations of examined *Saphonecrus* individuals and in the phylogenetic study previously separated clades.
- Our studies based on the observed distribution of four types of galls (bud-, leaf-, twig- and catkin gall) between *Saphonecrus* clades. The galls were in different positions on host plant or on leaves and had different surface patterns.
- The distribution of different gall types and gall positions support a number of cases on the one hand and the relationships between examined inquiline taxa and

the other hand the boundaries of distinct inquilin subgroups within lineages which designation based on morphological and molecular studies.

Host plant associations

- Our study suggests independent radiations of host plants and their associated inquilin *Saphonecrus* species. The most striking example is provided by the two *Saphonecrus* lineages present on *Lithocarpus*.
- The early split within the Fagaceae between *Quercus* and *Lithocarpus* or within *Quercus* is not reflected in the inquiline phylogeny.
- the section Cerris-specific, eastern taxon *Ufo* and the western “undulatus- haimi” clade seem to be associated with clades characteristic to the subgenus *Cyclobalanopsis* of *Quercus*.

List of publications:

The present thesis is based on the following publications:

Melika, G., Tang, C.-T., Yang, M.-M., Bihari, P., **Bozsó, M.**, & Péntzes, Zs. (2012): New species of cynipid inquilines of the genus *Ufo* Melika & Pujade-Villar, 2005 (Hymenoptera: Cynipidae: Synergini) *Zootaxa* 3478: 143–163.

Impact Factor: 0,97

Bozsó, M., Tang, C.-T., Péntzes, Zs., Yang, M.-M., Bihari, P., Pujade-Villar, J., Schwéger, S. and Melika, G. (2013): A new genus of cynipid inquiline, *Lithosaphonecrus* Tang, Melika & Bozsó (Hymenoptera: Cynipidae: Synergini), with description of four new species from Taiwan and China. *Insect Systematics & Evolution* doi 10.1163/1876312X-45032116.

Impact Factor: 1,11

Bozsó, M., Péntzes, Zs., Bihari, P., Schwéger, S., Tang, C.-T., Yang, M.-M., Pujade-Villar, J. and Melika, G. (2014): Molecular phylogeny of the inquiline cynipid wasp genus *Saphonecrus* Dalla Torre and Kieffer, 1910 (Hymenoptera: Cynipidae: Synergini). *Plant Protection Quarterly* 29(1), 26–31.