

# 1 Appendix

Due to space constraints, the Appendix information is provided in a CD. It contains one folder for each chapter. The files contained in each chapter are briefly described in the following sections.

## 1.1 Chapter 2: Cuckoo wasps

- Table 1- Species: This table contains taxonomic information, classification, host species and the number of specimens used for each species (59 Chrysididae and 7 Crabronidae) for which CHC chromatograms were analyzed in this thesis.
- Table 2 - Specimens: This document contains three spreadsheet, each containing information for each specimen for which chromatograms were analyzed (species, gender, locality of collection).

## 1.2 Chapter 3: Phylogeny

- Figure S1. Gene models of all ten investigated nuclear protein-coding genes. Gene identifies refer to those given by Hartig et al. (2012) and the gene name in parenthesis designates the most similar homolog in *Drosophila melanogaster* Meigen 1830. Depicted are coding exons (blue bars) and introns (black bars) of each gene. Exon and intron boundaries were annotated by aligning transcripts of *Chrysis terminata* to the genomic nucleotide sequence of the corresponding gene of this species. Numbers specify the length (in nucleotides) of all sequenced exonic sections of a given gene (numbers in parentheses include also the length of the introns), using the *Chrysis terminata* nucleotide sequence as reference. An asterisk above an intron indicates that the intron was absent in some of the investigated species (indicated in Fig. 1).
- Figure S2. Phylogenetic relationships between and within major cuckoo wasp lineages (continued in Fig. S2) inferred in a Bayesian framework with the software MrBayes and applying the same supermatrix and the same substitution models as in the analysis with the software IQ-TREE (see Figs 1 and 2). Shown is the 50 % majority rule consensus tree. Posterior probability

values were inferred from 56,000 sampled trees and are indicated in the tree by colour codes (percent values were rounded to the first digit before the decimal point). *Cephalonomia tarsalis* (Bethyridae) and *Anteon* sp. (Dryinidae) served as outgroups for rooting of the tree.

- Figure S3. Continuation of Fig. S2.
- Table S1. Sample information (taxonomic information, locality of collection for all species used in the phylogenetic analyses).
- Table S2. Oligonucleotide primers used for the amplification of ten nuclear genes and COI in cuckoo wasp and selected outgroup species.
- Table S3. List of Genbank accession numbers.
- Table S4: GC content by gene in percent (%).
- File S1. Supermatrix with the multiple nucleotide sequence alignment (4,946 sites, 189 sequences referring to a total of 188 species) of the concatenated nucleotide sequences of ten nuclear-encoded genes and of one mitochondrial gene, all protein-coding.
- File S2. Table indicating gene boundaries in the supermatrix given in File S1.
- File S3. Supermatrix partition table in Nexus format.
- File S4. Compilation of host associations.
- File S5. Phylogenetic tree (phylogram) with bootstrap support values as depicted in Figs 1 and 2 in Newick format.
- File S6. Phylogenetic tree (phylogram) with posterior probability values as depicted in Figs S2 and S3 in Nexus format.

## 1.3 Chapter 4: Sexual dimorphism of CHC

- Sample information in Appendix of Chapter 2.
- Table CHC-Females: mean CHC composition for females of each species used in this thesis. CHC compounds are labeled by their class, retention index and their identification. Alkadienes remain unidentified, only separated by retention index. Values are relative amounts.
- Table CHC-Males: A table containing mean CHC composition for males of each species used in this thesis. Same as above.
- Table CHC-sex differences: 67 CHC compounds contribute to 75% of sex differences. These are indicated here. Order refers to the order of importance the CHC compound has in each species and ranges from 1 (the compound contributing to the most differences between sexes) to 16.

- Table Anosim Values: Result of the ANOSIM analysis for each species. P values have been also corrected by Bonferroni. N refers to the sample size.
- File Plot Anosim: graphs showing the results of ANOSIM analysis.
- Figure Species-specific: The first three compounds contributing to major differences between sexes in all species.

## 1.4 Chapter 5: Species specificity of CHC

- Sample information in Appendix of Chapter 2 and CHC profile composition in Appendix of Chapter 4.
- Table HedychrumLocalityCHCProfiles: Mean CHC profile composition for each species in each locality for the five species of *Hedychrum* used.
- Table Localities: information on the different localities used in the analysis by geographic ranges.
- Table IndVal Analysis. Results of the Indval Analysis conducted for the two sexes.
- Figures NDMS3dFem and NMDS3dMal. Tridimensional NMDS using all species in females and males respectively.
- Figures AnosimF and AnosimM: results of the Anosim analysis in females and males respectively.
- Figures ClusterAnalysis: results of different cluster analysis using different clustering methods, and different grouping options (see Methods of the chapter and explanation in the corresponding file).
- Figure Intraspecific-Variation: Bray-Curtis dissimilarities between specimens within species in females and males.
- Figures BoxplotsRelativeVariation: Boxplots showing intraspecific variation of CHC compounds separated by compound class in females and males.

## 1.5 Chapter 6: Effect of natural selection on CHC evolution

- Sample and species information in Appendix of Chapter 2.
- Table Phylogenetic Signal: Results of the Phylogenetic signal calculated for each CHC compound and for different metrics.
- Figure ChemicalDistances: Pairwise comparisons between all specimens of all species in females and in males. This includes more than 180000 comparisons.

## **1.6 Chapter 7: Evolutionary arms race between Hedychrum and their hosts**

- Hosts parasite relationships in File S4 of Chapter 3.
- Sample information in Appendix of Chapter 2.
- Table Provenance: Number of samples, provenance and year of collection for females and males of the species in this study.
- Table Primers: Characteristics of the primers used and number of nucleotides in the exonic regions of the nuclear genes used for the phylogenetic analyses.
- Table SubModels: Substitution models chosen by Modelfinder for the respective phylogenetic analysis in IQTree and MrBayes (in File Suppl)
- Table CHCFemandMales: Mean relative abundance  $\pm$  standard deviation for each peak or mixture of CHC compounds included in the NMDS of all species analyzed (females and males respectively).
- Table Methyl-BranchedCHC: Mean number of CHC and proportion of methyl-branched compounds, alkenes and alkanes in the different analyzed groups.
- File Supplementary. Intra- and interspecific variability of cuticular hydrocarbon profiles in female and male individuals of all host species using only five specimens in each species for the calculation.

## **1.7 Chapter 8: A protocol for analyzing CHC profiles**

- File Visualization. Graphic visualization of the same identifications in each chromatograms.

## **1.8 Chapter 9: Tool for the rapid identification of methyl-branched compounds**

- R Script. File containing the two functions explained in this chapter. List of ions that need to be provided.