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# Deciphering Morphological Variability: Addressing Taxonomic Ambiguities in Contemporary Species Delimitation (Hymenoptera, Figitidae)

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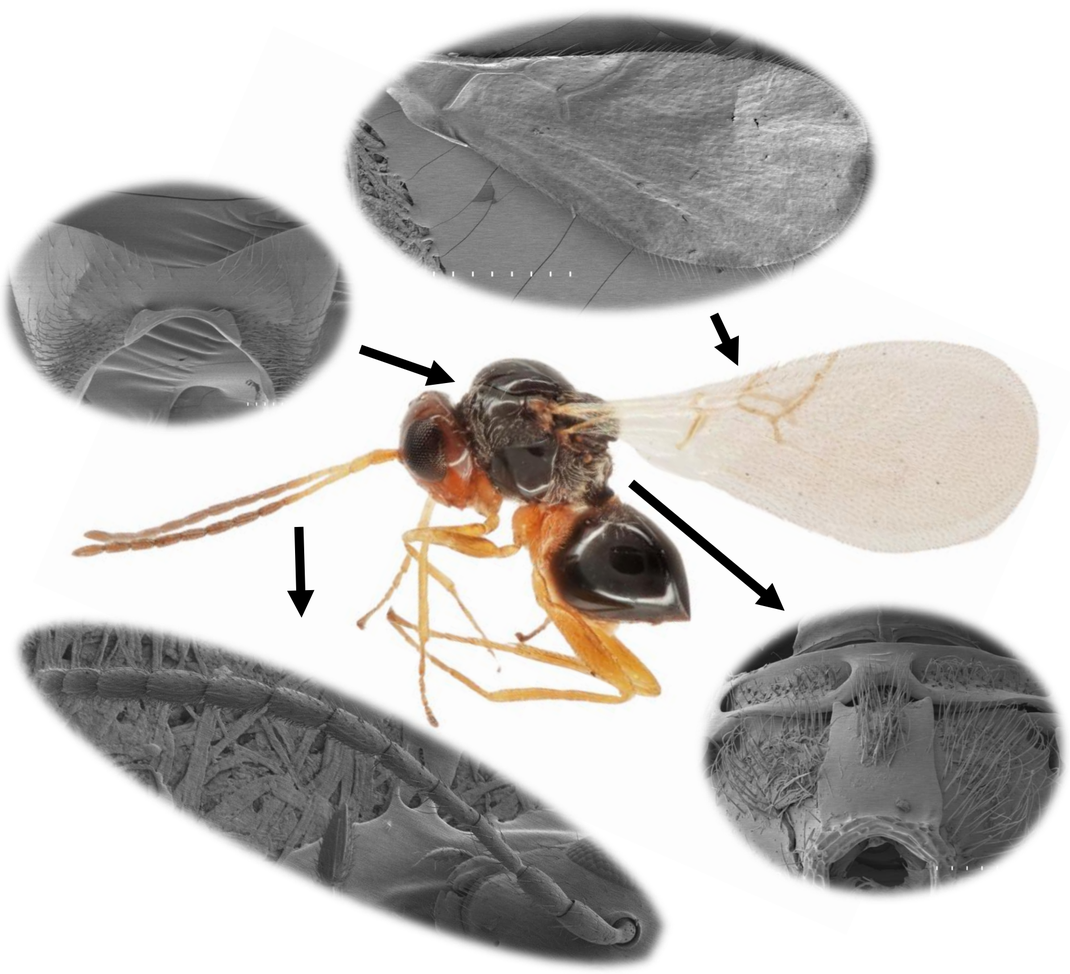
## Introduction

Species delimitation remains a major challenge in taxonomy, particularly in small organisms with high morphological similarity, such as hyperparasitoid wasps of the subfamily Charipinae. Traditional identification based only on morphological traits may be unreliable due to high intraspecific variability. Our case of study, subfamily Charipinae, are extremely small wasps with smooth and shiny body, with few distinguishing morphological traits, making species identification very difficult. Morphological features were analyzed according with the previous studies [1], key features include the presence or absence of pronotal (PN) and propodeal (PP) carinae, with the latter's shape being particularly important when present, as well as the shape and size of the radial cell (RC), the proportions between flagellomeres (FG) and the beginning of the rhinaria an club shaped (RN). Recently, species limits within this subfamily have been addressed making some taxonomic changes, in these previous study, interspecific distances ranged from 12.4% to 16.4% [2]. To continue addressing this issue, integrative taxonomy, combining morphological and molecular data, has been proposed as a more accurate approach for species identification. The main objective of this study is to check the validity of the morphological treats traditionally used to identify Charipinae species.



## Material and Methods

In this study, 80 specimens belonging to ten species from the genera *Alloxysta* and *Phaenoglyphis*, collected in Belgium, were analyzed to assess the reliability of traditional morphological characters used in Charipinae taxonomy. To achieve this, molecular analyses were conducted using three genetic markers: the mitochondrial cytochrome oxidase subunit I (COI) gene, the nuclear ITS2 region, and the ribosomal 16S RNA. Genetic distances, both intra- and interspecific, were calculated based on COI marker data, allowing for an evaluation of the genetic differentiation among the analyzed species. Additionally, phylogenetic analyses were performed to compare species delimitation based on morphological traits with that inferred from molecular data. The number of base substitutions per site from estimation of net average between groups of sequences are shown. Analyses were conducted using the Kimura 2-parameter model [3]. This analysis involved 50 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1876 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [4]. This approach enabled the assessment of congruence between traditional taxonomy and molecular evidence, providing valuable insights into intraspecific variability and the potential presence of cryptic species within Charipinae.

## Results

The previously established species boundaries based on morphological characters (Table 1) are confirmed. Genetic distances between species range from 0.10 to 0.33 (Table 3), while intraspecific variability varies from 0.03 to 0.0006 (Table 2). These results support the reliability of morphological traits for species delimitation within the studied group. Morphological features of the species studied are shown in Table 3 for comparative studies.

**Table 1.** Comparative table of diagnostic characteristics for the Charipinae species analyzed in this study. The table includes key morphological traits such as the presence/absence and shape of pronotal (PN) and propodeal carinae (PP), radial cell size and shape (RC), flagellomere proportions (FG), and the starting position of the rhinaria and club shape.

| Charipinae species | PN | PP | RC | RN | FG |
|---|---|---|---|---|---|
| ***A. brevis*** | - | X | closed | F4 | F1-F3 < F4 |
| ***A. castanea*** | X | X | parc. open | F3 | F2--F4 subequal |
| ***A. obscurata*** | X | - | parc. open | F4 | F1>F2, F2<F3, F3<F4 |
| ***A. pilipennis*** | X | X | closed | F3 | F2 – F4 subequal |
| ***A. ramulifera*** | X | X | closed | F4 | F1>F2=F3 |
| ***A. victrix*** | X | - | closed | F3 | F1>F2, F2—F4 |
| ***P. heterocera*** | X | X | closed | F3 | F1>F2<F3=F4 |
| ***P. longicornis*** | X | X | closed | F1 | F1>F2=F3<F4 |
| ***P. villosa*** | X | X | parc. open | F3 | F1>F2—F4 |
| ***P. xanthochroa*** | X | X | closed | F3 | F1>F2<F3=F4 |

**Table 2.** Estimates of Average Evolutionary Divergence over Sequence Pairs within Groups.

| | |
|---|---|
| *A. castanea* | 0,0325 |
| *A. victrix* | 0,0076 |
| *A. brevis* | 0,0071 |
| *P. villosa* | 0,009 |
| *A. pilipennis* | 0,0045 |
| *A. obscurata* | 0,0819 |
| *P. heterocera* | 0,0307 |
| *P. longicornis* | 0,0006 |
| *A. ramulifera* | 0,0007 |
| *P. xanthochroa* | 0,0065 |

## Discussion

Molecular analyses confirmed that species delimitation based on morphological traits is generally accurate, although some inconsistencies were identified and are currently being addressed. The integration of molecular data allowed for a refinement of species boundaries, leading to a more precise classification of Charipinae wasps. These findings underscore the importance of combining multiple lines of evidence in taxonomy, contributing to a deeper understanding of biodiversity and providing a robust framework for future ecological and evolutionary studies on these hyperparasitoid wasps.

Future research should focus on expanding molecular sampling to cover a wider geographic range and investigating possible cryptic species. Additionally, studying host-parasitoid relationships using molecular tools could improve our understanding of their ecological roles and evolutionary interactions.

**Table 3.** Estimates of Net Evolutionary Divergence between Groups of Sequences.

| | ***Acastanea*** | ***Avictrix*** | ***Abrevis*** | ***Pvillosa*** | ***Apilipennis*** | ***Aobscurata*** | ***Pheterocera*** | ***Plongicornis*** | ***Aramulifera*** | ***Pxanthochroa*** |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Acastanea*** | | | | | | | | | | |
| ***Avictrix*** | 0,1294 | | | | | | | | | |
| ***Abrevis*** | 0,0973 | 0,1274 | | | | | | | | |
| ***Pvillosa*** | 0,1566 | 0,1472 | 0,1554 | | | | | | | |
| ***Apilipennis*** | 0,0829 | 0,1506 | 0,1290 | 0,1808 | | | | | | |
| ***Aobscurata*** | 0,0807 | 0,0920 | 0,0940 | 0,1356 | 0,1166 | | | | | |
| ***Pheterocera*** | 0,1153 | 0,1512 | 0,1148 | 0,1311 | 0,1467 | 0,0915 | | | | |
| ***Plongicornis*** | 0,1232 | 0,1516 | 0,1184 | 0,1375 | 0,1433 | 0,0923 | 0,0217 | | | |
| ***Aramulifera*** | 0,1043 | 0,1468 | 0,1372 | 0,1941 | 0,1059 | 0,1079 | 0,1380 | 0,1383 | | |
| ***Pxanthochroa*** | 0,2409 | 0,3021 | 0,3300 | 0,2286 | 0,3041 | 0,2756 | 0,1950 | 0,1159 | 0,2914 | |

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