German Barcode of Life reveals unexpected diversity of Ceraphronoidea (Hymenoptera)

Marina Moser[‡,§,|], Cristina Vasilița[‡,§,¶], Maura Haas-Renninger[‡,|], Ecaterina Pirvu[#], Michael Haas[‡,|], Lars Krogmann[‡,§,|]

‡ State Museum of Natural History Stuttgart, Stuttgart, Germany
§ Biological Systematics (190w), Institute of Biology, University of Hohenheim, Stuttgart, Germany
| Center of Excellence for Biodiversity and Integrative Taxonomy (KomBioTa), Stuttgart, Germany
¶ Karlsruhe Institute of Technology, Eggenstein-Leopoldshafen, Germany
# Alexandru Ioan Cuza University, Iași, Romania

Abstract

Insect populations still experience marked declines globally, contributing to the ongoing biodiversity crisis. Counteracting these declines requires sound taxonomic and ecological knowledge on all levels of biodiversity, from genes to species to ecosystems. The superfamily Ceraphronoidea (Hymenoptera) has remained relatively obscure due to complex challenges in exploring its diversity and ecological roles. Despite their ecological importance as parasitoids or hyperparasitoids, these wasps are under-represented in scientific exploration and conservation. In a case study within the German Barcode of Life (GBOL) Dark Taxa project, we aim to bridge this knowledge gap through a comprehensive taxonomic investigation covering 2,136 specimens of Ceraphronoidea across 18 locations in the putatively well-studied State of Baden-Württemberg (south-western Germany). Our study identifies a surprising species richness of at least 193 conjectural species, based on COI-barcoding clusters, extrapolates key species richness estimators for the German ceraphronoid fauna and records a species new to the German fauna: *Creator spissicornis* (Hellén, 1966). By setting a foundational benchmark for Ceraphronoidea biodiversity, our research advocates for the inclusion of dark taxa in

broader insect biodiversity assessments, contributing meaningfully to the discourse on conservation priorities and strategies.

Keywords

dark taxa, biodiversity, Hill numbers, species richness estimators, Germany, GBOL

Introduction

Biodiversity is experiencing exceptionally rapid losses with current extinction rates at least 100 times higher than the background extinction rate (Pimm et al. 2006, Ceballos et al. 2015, Cowie et al. 2022). The extent to which insects have been affected was publicised in the "Krefeld study" in 2017, which reported a decline of 76% in the biomass of flying insects in protected areas throughout Germany over the course of 27 years (Hallmann et al. 2017). For insects, the declines can be ascribed to a combination of four major drivers: (1) habitat loss and conversion for intensive land use; (2) pollution, particularly by pesticides and fertilisers; (3) biological factors such as pathogens and introduced species; and (4) climate change (Sánchez-Bayo and Wyckhuys 2019). The knowledge necessary for understanding and counteracting these drivers to conserve insect biodiversity comes from taxonomic and ecological research. Researchers still have to describe, explore and understand the majority of biodiversity and its dynamics, from genetics to species and the ecosystem level (Kim and Byrne 2006, Shaw et al. 2025).

Insects make up over half of all described species and provide ecosystem services in all four categories, i.e. provisioning, regulating, cultural and supporting services (Ceballos et al. 2015), which makes them critical for ecosystem stability and human survival (Kim and Byrne 2006). At the same time, the majority of insect species are regularly neglected in conservation studies and policies (Shaw and Hochberg 2001, Ceballos et al. 2015, Habel et al. 2019). Amongst insects, parasitoid wasps are particularly species-rich with 115,000 described and up to 630,000 estimated species worldwide (Heraty 2017). Their parasitoid lifestyle is characterised by larval stages feeding on a host organism and eventually killing it to complete their development. Parasitoidism makes these wasps a key component of resilient ecosystems and balanced biological control programmes on account of their ability to effectively regulate the populations of their hosts (Godfray 1994).

Despite their ecological and economic importance, the taxonomy and biology of parasitoid wasps remain insufficiently understood. Very little is known about the life histories of most parasitoid Hymenoptera, including host ranges (Whitfield 1998, Moser et al. 2024), habitat requirements (Shaw 2006), phenology (Haas-Renninger et al. 2024), distribution patterns (Quicke 2012) and evolutionary history (Peters et al. 2017, Blaimer et al. 2023). In addition to their extraordinary species diversity, parasitoid wasps have been particularly hard to study using traditional morphological approaches. Many groups contain a plethora of morphologically highly similar taxa, which genetic markers recognise as cryptic species complexes rather than individual species (e.g. Smith et al.

(2008), Chesters et al. (2012), Derocles et al. (2015), Gebiola et al. (2016)). At the other end of the spectrum, the significant morphological disparity of males and conspecific females has made their association, based on morphology alone, extremely difficult (Gokhman 2018).

Molecular methods add a significant source of information to integrative taxonomic research. DNA barcoding has become a standard method in characterising and identifying animal species and has significantly furthered taxonomy (Hebert et al. 2003). Recent technical (e.g. Srivathsan et al. (2021)) and conceptual (e.g. Hartop et al. (2022)) optimisations of this method facilitate the barcoding of species-rich samples, thus forming the basis for large-scale biodiversity research (Meier et al. 2025). Additionally, this method is particularly beneficial in dark taxa, i.e. taxa in which the majority of species richness is still undescribed and taxonomic expertise and resources are highly insufficient (Page 2016, Meier et al. 2025).

In the current study, we focus on the diversity of Ceraphronoidea (Hymenoptera), a vastly understudied superfamily of parasitoid wasps that serves as a case study for a dark taxon in the putatively well-studied State of Baden-Württemberg in south-western Germany. Globally, Ceraphronoidea are widespread and amongst the most commonly collected microhymenoptera (Masner 1993, Schmitt 2004, Haas-Renninger et al. 2024). With about 740 described species, the superfamily is moderately species-rich, though recent estimates suggest that the true species richness considerably exceeds this number (Salden and Peters 2023). As parasitoids and hyperparasitoids, ceraphronoids exhibit a broad host spectrum spanning nine insect orders (Moser et al. 2024). However, for over 80% of species, host information remains unknown, reflecting significant gaps in our understanding of their biology (Moser et al. 2024).

Most ceraphronoid wasps are minute with a body length ranging from 0.5 to 4 millimetres and their taxonomic study is further complicated by morphological challenges: their external morphology is relatively monotonous and lacks distinctive characters (Mikó et al. 2013, Moser et al. 2023). Additionally, the few potentially informative morphological traits available, such as body size and surface sculpture, are often affected by allometry (Mikó et al. 2013). Over the past decades, the dissection and examination of male genitalia have been considered the only reliable method for species identification and diagnosis (Mikó et al. 2013, Ulmer et al. 2018, Salden and Peters 2023). However, this approach is inherently limited by its applicability only to males, making females largely undiagnosable using morphological characters. Genetic barcoding represents a promising solution to overcome these limitations, yet an effective protocol for Ceraphronoidea has only recently been developed, with the introduction of customised primers specific to this taxon (Vasilița et al. 2022). Furthermore, the superfamily lacks specialists with in-depth knowledge of their taxonomy and biology. Notably, almost half of all species descriptions have been authored by only two taxonomists, Jean-Jacques Kieffer (1857–1925) and Paul Dessart (1931–2001) (Johnson and Musetti 2004). This shortage of expertise has led to a lack of well-curated and accessible identification resources (Masner 1993, Moser and Krogmann In press). Altogether, these challenges have fostered considerable taxonomic confusion and underscore why Ceraphronoidea

exemplifies the key characteristics of a dark taxon, with the majority of species undescribed and fundamental aspects of their taxonomy, ecology and systematics poorly understood (Hartop et al. 2022, Moser et al. 2024).

Currently, the known species richness of Ceraphronoidea in Germany comprises 36 species: 12 species of Ceraphronidae and 24 species of Megaspilidae (Dessart 2001, Ulmer et al. 2018, Moser et al. 2023). To approximate a more realistic representation of the total species number, we present a comprehensive dataset of 2,136 barcode sequences of the superfamily Ceraphronoidea from Baden-Württemberg. Our primary objectives are: (1) to estimate species richness using molecular operational taxonomic units (mOTUs) as a proxy; (2) to add new records to the German ceraphronoid fauna; (3) to calculate ecological key parameters that help illuminate the biodiversity of the superfamily Ceraphronoidea and (4) to make the COI-barcodes publicly available as a basis for further research in the taxonomy, ecology, evolutionary biology and conservation of this particularly dark taxon. By making these data available and analysing key taxonomic and ecological parameters, we aim to promote the consideration of parasitoid wasps in broader applications, such as biodiversity monitoring, phylogenetics and biological control, aligning with global initiatives to document and conserve the Planet's biodiversity.

Material and methods

Species sampling, barcoding and imaging

We generated barcode sequences of the mitochondrial cytochrome c oxidase subunit I (COI) for a total of 2,136 specimens through the megabarcoding approach (Wang et al. 2018). The specimens were collected across 18 localities in the State of Baden-Württemberg, encompassing the majority of its natural regions and ranging in elevation from 181 to 514 m a.s.l. (Fig. 1). The samples were collected between 2013 and 2022 and stored in 99.6% ethanol at the State Museum of Natural History Stuttgart (SMNS), Germany. Sampling was conducted with Malaise traps between the months of March and November and the samples were collected in 2-week intervals.

COI-barcoding was performed through HotSHOT DNA extractions as detailed in Vasiliţa et al. (In press) or by non-destructive extraction using the Qiagen DNeasy Blood and Tissue Kit, following the manufacturer's protocol with minor changes as in Cruaud et al. (2019). Amplicons were then sequenced either by conventional Sanger sequencing or express barcoding and megabarcoding via MinION sequencing following the protocol of Vasiliţa et al. (2024) with a customised forward primer specific to Ceraphronoidea (Vasiliţa et al. 2022). Basecalling and demultiplexing followed the workflow provided by Srivathsan et al. (2021). Raw reads obtained by Sanger sequencing were assembled, trimmed and proofread with Geneious Prime. Sequences were aligned in Mega12 (Kumar et al. 2024) using the ClustalW method. The same software was used to calculate mean and pairwise genetic distances. DNA barcodes and associated metadata have been released to BOLD (DOI: dx.doi.org/10.5883/DS-CERBW). Identifications of the

species recorded for the first time in Germany presented herein are based on results of the identification engine of BOLD V4 (https://v4.boldsystems.org/index.php/IDS_OpenIdEngine, last accessed: 02-03-2025) through comparison with published species-level barcode records and morphological identification.

Specimens were imaged using an MZ 16 APO Leica R microscope with an attached DXM 1200 Leica R camera and subsequent stacking of images in Helicon Focus (version 7.6.1; Helicon Soft Ltd, Kharkov, Ukraine).
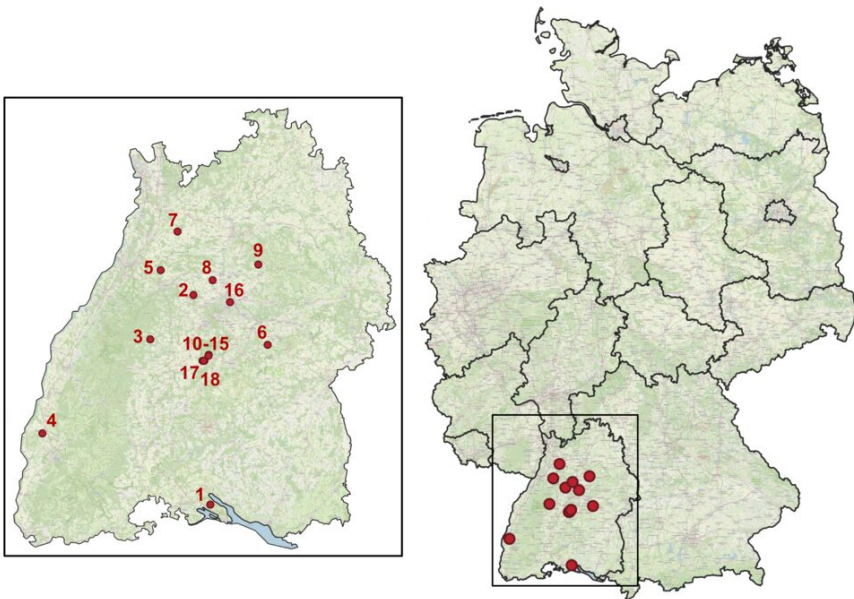

Figure 1. doi

Map of Germany displaying the geographic distribution of sampling sites across the country. The highlighted region indicates the State of Baden-Württemberg, with markers indicating precise locations of sampling sites.

Species delimitation

Species delimitation was performed using three complementary algorithms to estimate the species diversity of Ceraphronoidea in Baden-Württemberg, based on mOTUs of the 2,136 COI-barcode sequences: first, ASAP (Assemble Species by Automatic Partitioning), which builds species partitions from single locus alignments using pairwise genetic distances (Puillandre et al. 2020). For further analyses, we selected the partitioning with the lowest ASAP score. This score incorporates both the probability of the partitioning and the width of the barcode gap, with lower scores indicating a better fit to the data. Second, ABGD (Automatic Barcode Gap Discovery), which builds species partitions from pairwise genetic distances by detecting the barcode gap (Puillandre et al. 2011). Both were run through the Spart-Explorer platform (Miralles et al. 2021), selecting the Jukes-

Cantor (JC69) substitution model. Third, we used objective clustering in SpeciesIdentifier (Meier et al. 2006), using a 2% and 3% threshold, which corresponds to widely used thresholds for delineating mOTUs in DNA barcoding studies (e.g. Ratnasingham and Hebert (2007), Ratnasingham and Hebert (2013), Chimeno et al. (2023)), particularly in Braconidae (Hymenoptera) (Smith et al. 2012, Fernández-Flores et al. 2013, Fagan-Jeffries et al. 2018). Therefore, reporting the results at both the 2% and 3% thresholds allows for direct comparison with previous studies. For further analyses, we selected the less conservative 3% threshold, which aligns with the higher end of this standard range.

Diversity index calculation

Statistical analysis was performed using R (version 4.2.1) in RStudio (version 2023.06.1) (R Core Team 2023). The following packages were used: vegan (Oksanen et al. 2001), dplyr (Wickham et al. 2023), ggplot2 (Wickham 2009), reshape2 (Wickham 2007), iNEXT (Hsieh et al. 2022), SpadeR (Chao et al. 2016), SPECIES (Wang 2024), hilldiv (Alberdi and Gilbert 2019) and BiodiversityR (Kindt 2025).

Species richness and abundance indices were calculated, based on the mOTUs described above. Sampling coverage was assessed by extrapolating the number of undetected species through non-parametric richness indices, namely the Chao1 estimator (Chao 1984), which takes into consideration all abundances down to singletons, and ACE (Abundance-based coverage; Chao and Lee (1992)), which divides the dataset into abundant taxonomic units (i.e. those which occur more than 10 times) and rare taxonomic units. Species accumulation curves were calculated for the dataset, assigning consecutive location IDs to each geographic location (Fig. 1), thus clustering multiple collection events from the same location. Diversity profiles were analysed using Hill numbers, i.e. species richness ($q = 0$), Shannon diversity ($q = 1$) and Simpson diversity ($q = 2$) (Hill 1973, Chao et al. 2014), ensuring a comprehensive assessment of biodiversity while addressing potential biases due to incomplete sampling.

To assess patterns in species composition across sampled locations, we performed non-metric multidimensional scaling (NMDS, Suppl. material 2) using the Bray-Curtis dissimilarity index (Ricotta and Podani 2017) to quantify compositional differences amongst sites. NMDS ordination was calculated using the *vegan* package in R (Oksanen et al. 2001) with k = 2, restricting ordination to two dimensions and a maximum of 1000 iterations to ensure convergence on a stable calculation (Suppl. material 2). Stress values were examined to ensure the goodness of fit.

Results

The cluster analysis using ASAP returned a total of 193 subsets (ASAP score: 6.0) with a p-value of 9.999900e-06 and a threshold distance of 0.000018. The next two most likely ASAP partitionings return 193 and 185 subsets, both with an ASAP score of 14.5 (Table 1). The ten most likely partitions range from 185 to 214 clusters (average: 198.4). The three most probable ABGD partitioning all returned 211 initial partitions with prior maximum

divergences of intraspecific diversity of p = 0.059948, p = 0.035938 and p = 0.021544. In contrast, SpeciesIdentifier returned 291 clusters at a threshold of 2.0% and 259 clusters at 3.0% (Table 1).

Table 1.

Summary of the number of clusters identified by different approaches and respective parameters or indicators used for species delimitation. The methods compared are ASAP (Assemble Species by Automatic Partitioning, Puillandre et al. (2020)) with resulting ASAP scores, ABGD (Automatic Barcode Gap Discovery, Puillandre et al. (2011)) with respective barcode gaps and SpeciesIdentifier (Meier et al. 2006) with a 2% and 3% a priori threshold.

Species delimitation method	Number of clusters	Indices / Parameters
ASAP	193	asap score: 6.0
ASAP	193	asap score: 14.5
ASAP	185	asap score: 14.5
ABGD	211	barcode gap distance: 0.059948
ABGD	211	barcode gap distance: 0.035938
ABGD	211	barcode gap distance: 0.021544
SpeciesIdentifier	291	threshold: 2.0%
SpeciesIdentifier	259	threshold: 3.0%

Diversity indices

Ecological analyses were conducted, based on the 193 ASAP, 211 ABGD and 259 SpeciesIdentifier clusters resulting from COI-barcodes of 2,136 individuals, which were treated as mOTUs (Fig. 2). The mean estimated sample coverage was 0.968 (ASAP: 0.976; ABGD: 0.972; SpeciesIdentifier: 0.956), indicating that the dataset captures the majority of species present in the Ceraphronoidea community of Baden-Württemberg. Species richness estimators suggested a higher true diversity, with Chao1 estimating a mean of 292.7 species (ASAP: 241.1; ABGD: 267.1; SpeciesIdentifier: 369.8) and ACE yielding a slightly lower extrapolation of a mean 284.9 species (ASAP: 234.9; ABGD: 259.9; SpeciesIdentifier: 360.0).

Taking into account both species richness and evenness, mean Shannon entropy estimates are 4.257 (Maximum Likelihood estimation, MLE) or 4.349 (Chao & Shen), corresponding to an effective number of species (i.e. Shannon diversity) between 71.475 (MLE) and 78.540 (Chao & Shen). Simpson's diversity index results further supported high species evenness, with the inverse Simpson index estimated at 30.772 (minimum variance unbiased estimator, MVUE). The decrease in mean Hill numbers with increasing order, from 292.7 at q = 0 to 30.8 at q = 2, highlights the contribution of relatively few dominant species to overall community structure. These results provide a robust assessment of species diversity, accounting for both richness and evenness in the sampled population. Exact diversity indices are reported in Suppl. material 3.
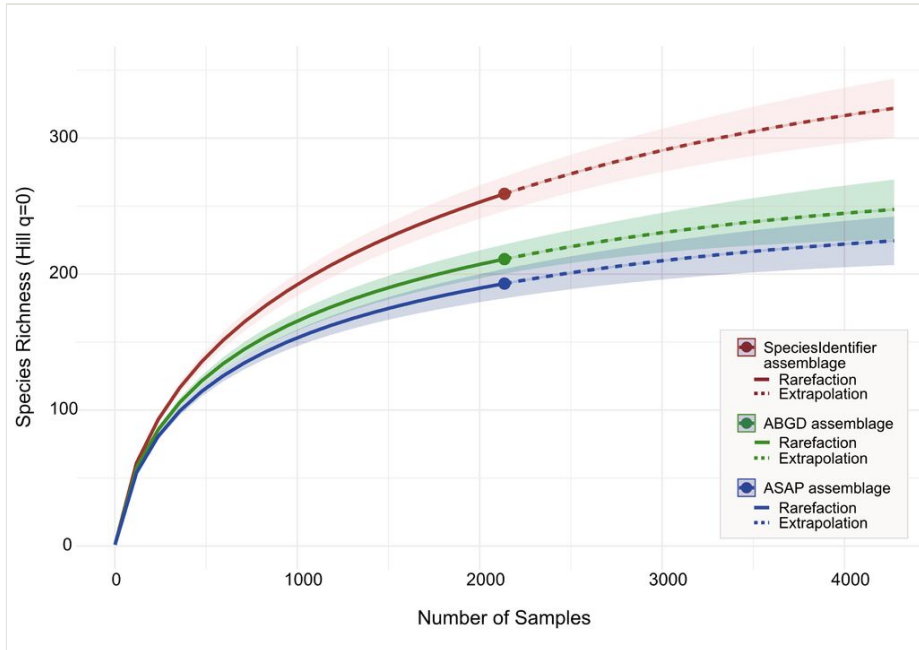
Figure 2. [doi]

Rarefaction and extrapolation curve showing species richness as a function of sampling effort. The x-axis represents the number of samples, while the y-axis indicates the estimated species richness, based on clustering with SpeciesIdentifier (red), ABGD (green) and ASAP (blue). The solid portion of the curve represents observed richness (rarefaction), while the dashed portion represents projected richness (extrapolation).

Taxonomic findings

The following species was identified morphologically and affirmed by comparison with identified sequences on BOLD and is a new record for the German fauna.

Creator spissicornis (Hellén, 1966)

Creator spissicornis was originally described as *Lygocerus spissicornis* from Jomala and Nystad (Finland) by Hellén (1966). Since its original description, the species has undergone taxonomic re-assessment, resulting in a transfer to *Dendrocerus* (Dessart 1972) and later to its own monotypic genus, *Creator* (Alekseev 1980). The reclassification is based mainly on the shape of the syntergum, which is only slightly narrowed anteriorly, forming a broad collar and which is covered in strigose sculpture anteriorly (Alekseev 1980). Further notable characters are the coarse, mostly rugose sculpture as well as the relatively large body size (Alekseev 1980, Fergusson 1980).

Following its Finnish records, *C. spissicornis* has since been reported from additional European countries, including England, Sweden (Fergusson 1980), Belgium, France

(Dessart 1972), Ukraine (Alekseev and Radchenko 2001), the Netherlands (observation by van Loon, M. (2023): https://waarneming.nl/observation/287666956/, accessed on 04-05-2025) and Norway (GBIF Secretariat 2023). The hosts of *C. spissicornis* are cyclorrhaphous Diptera (Moser et al. 2024).

The following three specimens were identified as *C. spissicornis*:

- Continent: Europe; country: Germany; countryCode: DE; stateProvince: Baden-Württemberg; locality: Lkr. Esslingen, Bissingen a. d. Teck, Eichhalde, Obstwiese; verbatimElevation: 513 m; decimalLatitude: 48.5070; decimalLongitude: 9.4926; samplingProtocol: Malaise trap; eventDate: 21/06/2014–10/08/2014; sex: female; lifeStage: adult; preparations: 99.6% ethanol; recordedBy: Rager, L.; identifiedBy: M. Moser; dateIdentified: 2025; accessionNumber: SMNS_Hym_Meg_000366.

- Continent: Europe; country: Germany; countryCode: DE; stateProvince: Baden-Württemberg; locality: Lkr. Karlsruhe, Östringen, NSG 2.217 Apfelberg; verbatimElevation: 181 m; decimalLatitude: 49.1675; decimalLongitude: 8.7903; samplingProtocol: Malaise trap; eventDate: 18/06/2019–02/07/2019; sex: female; lifeStage: adult; preparations: 99.6% ethanol; recordedBy: LUBW Insektenmonitoring; identifiedBy: M. Moser; dateIdentified: 2025; accessionNumber: SMNS_Hym_Meg_000565.

- Continent: Europe; country: Germany; countryCode: DE; stateProvince: Baden-Württemberg; locality: Lkr. Karlsruhe, Östringen, NSG 2.217 Apfelberg; verbatimElevation: 181 m; decimalLatitude: 49.1675; decimalLongitude: 8.7903; samplingProtocol: Malaise trap; eventDate: 30/07/2019–13/08/2019; sex: female; lifeStage: adult; preparations: 99.6% ethanol; recordedBy: LUBW Insektenmonitoring; identifiedBy: M. Moser; dateIdentified: 2025; accessionNumber: SMNS_Hym_Meg_000531.

The similarity between the COI-sequences in our dataset and the closest reference sequences on BOLD (BIN ID: "BOLD:ADT2227", sequence IDs: NOMEG358-21.COI-5P; NOMEG176-21.COI-5P) is 100% for both SMNS_Hym_Meg_000366 and SMNS_Hym_Meg_000531 (Fig. 3) and slightly lower at 99.83% for SMNS_Hym_Meg_000565. The overall mean distance between our COI-sequences and the BOLD sequences is 0.000653 with a maximum pairwise distance of 0.00167 between NOMEG176-21.COI-5P and SMNS_Hym_Meg_000565.

Discussion

The parasitoid wasp superfamily Ceraphronoidea is a prime example of a dark taxon that has not received much scientific attention in the past. As a result, most species cannot be identified through genetic barcoding due to the missing connection between barcodes and formal scientific names in sequence reference databases, such as GenBank or BOLD (Page 2016). In Ceraphronoidea, which comprises about 740 described species worldwide, only 53 species (7.2%) are currently associated with barcodes deposited in

BOLD (Megaspilidae: 47 species; Ceraphronidae: 6 species; https://v4.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=125, accessed: 02-03-2025). The lack of reliably identified reference sequences often stems from a lack of taxonomic capacity, along with a general inaccessibility of older taxonomic literature (Page 2016), which is not retrievable online or not available in machine-readable format.

Figure 3. doi

Female *Creator spissicornis* (Hellén, 1966); SMNS_Hym_Meg_000531. **a** lateral habitus, scale bar: 500 µm. **b** Dorsal habitus, scale bar: 500 µm. **c** Antennae and head in frontal view, scale bar: 200 µm.

Like in many other dark taxa, species richness is much higher in Ceraphronoidea than the current number of described species would suggest. The results of the current study attest to at least 193 species of Ceraphronoidea in the State of Baden-Württemberg alone. This number is in stark contrast to the 36 species that are listed either in the most recent checklist of German Ceraphronoidea (Dessart 2001) or were recently described from Germany (Ulmer et al. 2018, Moser et al. 2023). This discrepancy could, in part, be attributed to the death of Paul Dessart, the only active Ceraphronoidea specialist worldwide at that time, in March 2001 (Pauly 2001). His death preceded the printing of the current checklist of German Hymenoptera in August 2001 (Dathe et al. 2001), for which he compiled the chapter on Ceraphronoidea (Dessart 2001).

For the State of Baden-Württemberg, our extrapolated rarefaction curves plateau at about 224 species (ASAP), 247 species (ABGD) and 322 species (SpeciesIdentifier) at twice

the collection effort, i.e. 4,272 specimens (Fig. 2). According to our analysis, additional sampling would result in only little additional species detection beyond this point. These data, as well as the mean estimated sample coverage of 0.968, suggest that our sampling effort was sufficient to capture most of the community diversity. This is supported by the Chao1 estimator, which predicts a mean total of 292.7 species (ASAP: 241.1; ABGD: 267.1; SpeciesIdentifier: 369.8) species of Ceraphronoidea in Baden-Württemberg. However, it is important to acknowledge the limitations inherent to species richness analyses based on a single collection method. While Malaise traps are highly effective at sampling a broad spectrum of flying insect diversity and are well suited for investigating many parasitoid microhymenoptera (Darling and Packer 1988, Haas-Renninger et al. 2024), taxa that live mainly in leaf litter or canopy habitats could remain undetected. In particular, within the family Megaspilidae, some species of *Conostigmus* Dahlbom, 1858 and *Dendrocerus* Ratzeburg, 1852, as well as the females of *Lagynodes* Förster, 1841, are wingless and, therefore, unlikely to be captured effectively by Malaise traps. This suggests that further sampling with complementary methods such as pan traps, leaf litter extraction or targeted sweep netting would be required to capture the full species richness of Megaspilidae.

Notably, our mean Chao1 estimate of 292.7 species already exceeds the 259 described species of Ceraphronoidea known to occur in the whole Palaearctic (Johnson and Musetti 2004). However, this number is obviously an under-representation of the true species diversity of the Palaearctic: estimates for Europe have assumed about 400 species, cautioning that, for Ceraphronoidea, estimates are precarious at best due to the poor state of taxonomic knowledge (Ulrich 1999). As a model region diverse in habitats, geology and land-use types, Baden-Württemberg is estimated to be home to 75% of the Hymenoptera species found in Germany (Dathe et al. 2001, Dathe and Blank 2004). Based on the Chao1 estimations presented in the current study, which project that the number of Ceraphronoidea species in Baden-Württemerg lies somewhere between a minimum of 241.1 (following ASAP clustering) and a maximum of 369.8 (following SpeciesIdentifier clustering) species, we would expect a total of 321.5–493.1 species of Ceraphronoidea in Germany, provided that the overall ratio of all Hymenoptera (Dathe et al. 2001, Dathe and Blank 2004) is extrapolatable to Ceraphronoidea. Using the total number of 154,000 described species of Hymenoptera (Aguiar et al. 2013, Huber 2017) as a worldwide estimate and Germany being home to 9,625 (i.e. 6.25%) (Dathe and Blank 2004) of these species, the total number of Ceraphronoidea in the world could consequently be extrapolated to 5,143 – 7,889 species. This estimate seems conservative, especially compared to a recent estimate of 12,000 – 21,000 species, based on Afrotropical species (Salden and Peters 2023).

This discrepancy could, however, be informative in validating the hypothesis of Mikó et al. (2016), who raise the question of whether Ceraphronoidea show a reverse latitudinal diversity gradient in species richness or whether this phenomenon is merely the result of sampling bias. The comparison of 221 recorded and 292.7 expected species in the State of Baden-Württemberg (47° – 49° latitude) versus 88 recorded and 122 expected species in three regions of equatorial Africa (-0.3° – 3° latitude) (Salden and Peters 2023) gives

evidence for a reverse latitudinal diversity gradient. This would align with recent findings of an "anomalous" latitudinal gradient in Ichneumonoidea, which peaks at 50° latitude (Castellanos-Labarcena et al. 2024). However, to draw any definitive conclusions on global distribution patterns of Ceraphronoidea, more comprehensive sampling, especially across latitudes and with standardised methods, is required.

The key findings of the current study are the unrecognised species richness of Ceraphronoidea in Baden-Württemberg and the addition of a new species record to the German checklist. Furthermore, the analysis of the current dataset provides crucial insights into the ecology of Ceraphronoidea: species distribution throughout the community is moderately even, leading to a mean Shannon diversity (i.e. effective number of species) of 77.2 species across the three clustering methods and the community is dominated by 30.8 species as denoted by the inverse Simpson's index (Suppl. materials 1, 3). These indicators of community structure emphasise the need to move beyond purely taxonomic approaches and integrate macroecological methodologies to uncover hidden patterns of biodiversity and distribution.

Despite their taxonomic and ecological significance, these findings most likely do not fully represent the whole diversity of Ceraphronoidea in Baden-Württemberg. Similar sampling across more habitat types and with complementary sampling methods could help address potential sampling biases, as well as put the ecological indicators presented herein into a larger context. This larger dataset would not only refine current diversity estimates, but also allow for the calculation of robust β-diversity metrics to assess differences in species richness and composition across sites. Further, continuous or periodic sampling would provide a more complete picture of community diversity over time and allow for analyses of how changes in land use and environmental factors influence biodiversity.

Taxonomically, an interesting next step would be the identification of as many of the mOTUs as possible. On the one hand, this approach could reveal a wealth of previously undescribed species, which require formal naming and description in order to be made available for databasing, further taxonomic and ecological research, as well as conservation strategies. On the other hand, this work would enable the assignment of existing species names to mOTUs, allowing for the linkage of ecological and taxonomic knowledge acquired through previous research. In combination with the results of the current study, this next step would improve our understanding of the distribution and abundance of ceraphronoid wasps throughout Germany, which is the basis for a comprehensive assessment of the extent to which they are threatened by the same drivers that cause overall insect declines (Shaw and Hochberg 2001, Haas-Renninger et al. 2024).

Conclusions

Altogether, our case study demonstrates that even allegedly well-studied regions in Central Europe still require basic taxonomic research. Only a fraction of the mOTU

clusters in the dataset were identifiable to species level through COI barcoding, which illustrates that future taxonomic work is urgently required.

The broader significance of this study lies in its implications for biodiversity conservation. Traditionally, conservation efforts have focused mainly on well-studied taxa with sufficient data available to enable assessment under the IUCN Red List Criteria. Within this framework, lesser-known dark taxa, including many groups of parasitoid wasps and nematoceran Diptera, are frequently neglected due to their taxonomic disarray and the consequent lack of robust data. Yet, parasitoid wasps, such as Ceraphronoidea, play a major role in ecosystems, as indicated by the combination of high species diversity and moderately even community structure found in the current study. Given their high trophic level in food webs as highly specialised (hyper-)parasitoids, future biodiversity management and conservation frameworks should integrate parasitoids like Ceraphronoidea, moving beyond the obsolete dogma of waiting for complete taxonomic resolution before implementing conservation measures.

The discovery of unrecognised species richness amongst ceraphronoid wasps in our case study, conducted in a region previously believed to be well-documented, underscores the necessity for more comprehensive conservation strategies. Moving forward, integrating taxonomic and ecological analyses will provide valuable insights into patterns of biodiversity and distribution, bridging the knowledge gaps that have historically excluded these taxa from targeted conservation measures. Since filling these knowledge gaps requires substantial time, resources and expertise, it would be pragmatic for conservation strategies to prioritise overall habitat and ecosystem preservation. Focusing on maintaining habitat quality would benefit and protect the whole spectrum of biodiversity, covering both known and yet-to-be-discovered elements of biodiversity, ultimately fostering a comprehensive approach to maintaining ecosystem resilience.

Acknowledgements

Funding program

Conflicts of interest

The authors have declared that no competing interests exist.

References

- Aguiar AP, Deans A, Engel M, Forshage M, Huber J, Jennings J, Johnson N, Lelej AS, Longino J, Lohrmann V, Mikó I, Ohl M, Rasmussen C, Taeger A, Yu DSK (2013) Order Hymenoptera. In: Zhang, Z-Q (Ed.) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness (Addenda 2013). Zootaxa 3703 (1): 51-62. https://doi.org/10.11646/zootaxa.3703.1.12
- Alberdi A, Gilbert MTP (2019) hilldiv: an R package for the integral analysis of diversity based on Hill numbers. bioRxiv1-12. https://doi.org/10.1101/545665
- Alekseev VN (1980) Systematic position of *Dendrocerus spissicornis* Hellen, 1966 and notes on the systematics of the genus *Dendrocerus* Ratzeburg (Hymenoptera, Ceraphronoidea, Megaspilidae). Entomologicheskoye Obozreniye 59: 385-390. [In Russian].
- Alekseev VN, Radchenko TD (2001) Ceraphronoid wasps (Hymenoptera, Ceraphronoidea) of the fauna of the Ukraine. Communication 1. Vestnik Zoologii 35 (3): 3-16. URL: https://www.v-zool.kiev.ua/pdfs/2001/3/01.pdf
- Blaimer B, Santos B, Cruaud A, Gates M, Kula R, Mikó I, Rasplus J, Smith D, Talamas E, Brady S, Buffington M (2023) Key innovations and the diversification of Hymenoptera. Nature Communications 14 (1): 1-18. https://doi.org/10.1038/s41467-023-36868-4
- Castellanos-Labarcena J, Steinke D, Adamowicz S (2024) Anomalous latitudinal gradients in parasitoid wasp diversity—Hotspots in regions with larger temperature range. Journal of Animal Ecology 94 (3): 410-422. https://doi.org/10.1111/1365-2656.14196
- Ceballos G, Ehrlich P, Barnosky A, García A, Pringle R, Palmer T (2015) Accelerated modern human–induced species losses: Entering the sixth mass extinction. Science Advances 1 (5): 1-5. https://doi.org/10.1126/sciadv.1400253
- Chao A (1984) Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics 11 (4): 265-270. URL: https://www.jstor.org/stable/4615964
- Chao A, Lee S (1992) Estimating the number of classes via sample coverage. Journal of the American Statistical Association 87 (417): 210-217. https://doi.org/10.1080/01621459.1992.10475194
- Chao A, Gotelli N, Hsieh TC, Sander E, Ma KH, Colwell R, Ellison A (2014) Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecological Monographs 84 (1): 45-67. https://doi.org/10.1890/13-0133.1
- Chao A, Ma K, Hsieh T, Chiu C (2016) SpadeR: Species-richness prediction and diversity estimation with R. 0.1.1. Release date: 2016-9-06. URL: https://CRAN.R-project.org/package=SpadeR

- Chesters D, Wang Y, Yu F, Bai M, Zhang T, Hu H, Zhu C, Li C, Zhang Y (2012) The integrative taxonomic approach reveals host specific species in an encyrtid parasitoid species complex. PLOS One 7 (5): 1-11. https://doi.org/10.1371/journal.pone.0037655
- Chimeno C, Schmidt S, Cancian de Araujo B, Perez K, von Rintelen T, Schmidt O, Hamid H, Pramesa Narakusumo R, Balke M (2023) Abundant, diverse, unknown: Extreme species richness and turnover despite drastic undersampling in two closely placed tropical Malaise traps. PLOS One 18 (8): 1-18. https://doi.org/10.1371/journal.pone.0290173
- Cowie R, Bouchet P, Fontaine B (2022) The sixth mass extinction: fact, fiction or speculation? Biological Reviews 97 (2): 640-663. https://doi.org/10.1111/brv.12816
- Cruaud A, Nidelet S, Arnal P, Weber A, Fusu L, Gumovsky A, Huber J, Polaszek A, Rasplus J (2019) Optimized DNA extraction and library preparation for minute arthropods: Application to target enrichment in chalcid wasps used for biocontrol. Molecular Ecology Resources 19 (3): 702-710. https://doi.org/10.1111/1755-0998.13006
- Darling DC, Packer L (1988) Effectiveness of Malaise traps in collecting Hymenoptera: the influence of trap design, mesh size, and location. The Canadian Entomologist 120 (8-9): 787-796. https://doi.org/10.4039/Ent120787-8
- Dathe H, Blank S (2004) Nachträge zum Verzeichnis der Hautflügler Deutschlands, Entomofauna Germanica Band 4 (Hymenoptera). Entomologische Nachrichten und Berichte 48: 179-183. URL: ISSN 0232-5535
- Dathe HH, Taeger A, Blank SM (Eds) (2001) Verzeichnis der Hautflügler Deutschlands (Entomofauna Germanica 4). [Catalogue of Hymenoptera of Germany (Entomofauna Germanica 4)]. 4. Entomologische Nachrichten und Berichte, Dresden, 178 pp. [ISBN 0232-5535]
- Derocles SP, Plantegenest M, Rasplus J, Marie A, Evans D, Lunt D, Le Ralec A (2015) Are generalist Aphidiinae (Hym. Braconidae) mostly cryptic species complexes? Systematic Entomology 41 (2): 379-391. https://doi.org/10.1111/syen.12160
- Dessart P (1972) Revision des espèces européennes du genre *Dendrocerus* Ratzeburg, 1852 (Hymenoptera Ceraphronoidea). Memoires de la Société Royale Belge d'Entomologie 32: 1-310.
- Dessart P (2001) Ceraphronoidea. In: Dathe H, Taeger A, Blank S (Eds) Verzeichnis der Hautflügler Deutschlands (Entomofauna Germanica 4). 7. Entomologische Nachrichten und Berichte, Dresden, 178 pp.
- Fagan-Jeffries E, Cooper SB, Bertozzi T, Bradford T, Austin A (2018) DNA barcoding of microgastrine parasitoid wasps (Hymenoptera: Braconidae) using high-throughput methods more than doubles the number of species known for Australia. Molecular Ecology Resources 18 (5): 1132-1143. https://doi.org/10.1111/1755-0998.12904
- Fergusson NDM (1980) A revision of the British species of *Dendrocerus* Ratzeburg (Hymenoptera: Ceraphronoidea) with a review of their biology as aphid hyperparasites. Bulletin of the British Museum (Natural History) Entomology 41: 255-314. https://doi.org/10.5962/bhl.part.28549
- Fernández-Flores S, Fernández-Triana JL, Martínez JJ, Zaldívar-Riverón A (2013) DNA barcoding species inventory of Microgastrinae wasps (Hymenoptera, Braconidae) from a Mexican tropical dry forest. Molecular Ecology Resources 13 (6): 1146-1150. https://doi.org/10.1111/1755-0998.12102
- GBIF Secretariat (2023) *Creator spissicornis* (Hellen, 1966) in: GBIF Backbone Taxonomy. Checklist dataset. Accessed via GBIF.org on 2025-05-04.

- Gebiola M, Monti M, Johnson R, Woolley J, Hunter M, Giorgini M, Pedata P (2016) A revision of the *Encarsia pergandiella* species complex (Hymenoptera: Aphelinidae) shows cryptic diversity in parasitoids of whitefly pests. Systematic Entomology 42 (1): 31-59. https://doi.org/10.1111/syen.12187
- Godfray H (1994) Parasitoids. Behavioral and evolutionary ecology. Princeton University Press, New Jersey, 488 pp. [ISBN 978-0-691-00047-3] https://doi.org/10.1515/9780691207025
- Gokhman VE (2018) Dimensions and borderlines of parasitoid Hymenoptera species: A paradigm shift? Biology Bulletin Reviews 8 (3): 227-233. https://doi.org/10.1134/s2079086418030052
- Haas-Renninger M, Bigalk S, Frenzel T, Gamba R, Görn S, Haas M, Haselböck A, Hörren T, Sorg M, Wendt I, Jansta P, Zimmermann O, Steidle JM, Krogmann L (2024) Phenology of microhymenoptera and their potential threat by insect decline. Journal of Hymenoptera Research 97: 699-720. https://doi.org/10.3897/jhr.97.128234
- Habel JC, Samways M, Schmitt T (2019) Mitigating the precipitous decline of terrestrial European insects: Requirements for a new strategy. Biodiversity and Conservation 28 (6): 1343-1360. https://doi.org/10.1007/s10531-019-01741-8
- Hallmann C, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, Stenmans W, Müller A, Sumser H, Hörren T, Goulson D, de Kroon H (2017) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLOS One 12 (10). https://doi.org/10.1371/journal.pone.0185809
- Hartop E, Srivathsan A, Ronquist F, Meier R (2022) Towards large-scale integrative taxonomy (LIT): Resolving the data conundrum for dark taxa. Systematic Biology 71 (6): 1404-1422. https://doi.org/10.1093/sysbio/syac033
- Hebert PN, Cywinska A, Ball S, deWaard J (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences 270 (1512): 313-321. https://doi.org/10.1098/rspb.2002.2218
- Hellén W (1966) Die Ceraphroniden Finnlands (Hymenoptera Proctotrupoidea). Fauna Fennica 20: 1-45.
- Heraty J (2017) Parasitoid biodiversity and insect pest management. In: Foottit R, Adler P (Eds) Insect biodiversity: Science and society. 2, 1. Wiley-Blackwell, Hoboken, New Jersey, 603-625 pp. [ISBN 9781118945537]. https://doi.org/10.1002/9781118945568.ch19
- Hill MO (1973) Diversity and evenness: A unifying notation and its consequences. Ecology 54 (2): 427-432. https://doi.org/10.2307/1934352
- Hsieh TC, Ma KH, Chao A (2022) iNEXT: Interpolation and extrapolation for species diversity. 3.0.1. Release date: 2024-3-24. URL: 10.32614/CRAN.package.iNEXT
- Huber J (2017) Biodiversity of Hymenoptera. In: Foottit R, Adler P (Eds) Insect Biodiversity: Science and Society. 2, 1. Wiley-Blackwell, Hoboken, New Jersey, 419-461 pp. [ISBN 9781118945537]. https://doi.org/10.1002/9781118945568.ch12
- Johnson NF, Musetti L (2004) Catalog of the systematic literature of the superfamily Ceraphronoidea (Hymenoptera). Contributions of the American Entomological Institute 33: 1-149.
- Kim KC, Byrne L (2006) Biodiversity loss and the taxonomic bottleneck: emerging biodiversity science. Ecological Research 21 (6): 794-810. https://doi.org/10.1007/s11284-006-0035-7
- Kindt R (2025) BiodiversityR: Package for community ecology and suitability analysis. 2.17-2. Release date: 2025-4-17. URL: 10.32614/CRAN.package.BiodiversityR

- Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S, Tamura K (2024) MEGA12: Molecular evolutionary genetic analysis version 12 for adaptive and green computing. Molecular Biology and Evolution 41 (12): 1-9. https://doi.org/10.1093/molbev/msae263
- Masner L (1993) Superfamily Ceraphronoidea. In: Goulet H, Huber J (Eds) Hymenoptera of the World. Agriculture Canada Publication, Ottawa, 668 pp. [ISBN 0-660-14933-8].
- Meier R, Shiyang K, Vaidya G, Ng PL (2006) DNA barcoding and taxonomy in Diptera: A tale of high intraspecific variability and low identification success. Systematic Biology 55 (5): 715-728. https://doi.org/10.1080/10635150600969864
- Meier R, Lawniczak MN, Srivathsan A (2025) Illuminating entomological dark matter with DNA barcodes in an era of insect decline, deep learning, and genomics. Annual Review of Entomology 70 (1): 185-204. https://doi.org/10.1146/annurev-ento-040124-014001
- Mikó I, Masner L, Johannes E, Yoder M, Deans A (2013) Male terminalia of Ceraphronoidea: morphological diversity in an otherwise monotonous taxon. Insect Systematics & Evolution 44: 261-347. https://doi.org/10.1163/1876312x-04402002
- Mikó I, Trietsch C, Sandall E, Yoder MJ, Hines H, Deans AR (2016) Malagasy *Conostigmus* (Hymenoptera: Ceraphronoidea) and the secret of scutes. PeerJ 4 (e2682): 1-87. https://doi.org/10.7717/peerj.2682
- Miralles A, Ducasse J, Brouillet S, Flouri T, Fujisawa T, Kapli P, Knowles LL, Kumari S, Stamatakis A, Sukumaran J, Lutteropp S, Vences M, Puillandre N (2021) SPART: A versatile and standardized data exchange format for species partition information. Molecular Ecology Resources 22 (1): 430-438. https://doi.org/10.1111/1755-0998.13470
- Moser M, Ulmer J, Van de Kamp T, Vasilița C, Renninger M, Mikó I, Krogmann L (2023) Surprising morphological diversity in ceraphronid wasps revealed by a distinctive new species of *Aphanogmus* (Hymenoptera: Ceraphronoidea). European Journal of Taxonomy 864: 146-166. https://doi.org/10.5852/ejt.2023.864.2095
- Moser M, Salden T, Mikó I, Krogmann L (2024) Synthesis of the host associations of Ceraphronoidea (Hymenoptera): a key to illuminating a dark taxon. Insect Systematics and Diversity 8 (6): 1-12. https://doi.org/10.1093/isd/ixae039
- Moser M, Krogmann L (In press) Illustrated identification key to Palaearctic genera of Ceraphronoidea (Hymenoptera: Apocrita). Integrative Systematics.
- Oksanen J, Simpson G, Blanchet FG, Kindt R, Legendre P, Minchin P, O'Hara RB, Solymos P, Stevens MHH, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista HBA, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak CF, Weedon J, Borman T (2001) vegan: Community ecology package. CRAN: Contributed Packages https://doi.org/10.32614/cran.package.vegan
- Page RM (2016) DNA barcoding and taxonomy: dark taxa and dark texts. Philosophical Transactions of the Royal Society B: Biological Sciences 371 (1702): 1-7. https://doi.org/10.1098/rstb.2015.0334
- Pauly A (2001) In Memory Paul Dessart (9th June 1931 - 26th March 2001). Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Entomologie 137: 186-192.
- Peters R, Krogmann L, Mayer C, Donath A, Gunkel S, Meusemann K, Kozlov A, Podsiadlowski L, Petersen M, Lanfear R, Diez P, Heraty J, Kjer K, Klopfstein S, Meier R, Polidori C, Schmitt T, Liu S, Zhou X, Wappler T, Rust J, Misof B, Niehuis O (2017) Evolutionary history of the Hymenoptera. Current Biology 27 (7): 1013-1018. https://doi.org/10.1016/j.cub.2017.01.027

- Pimm S, Raven P, Peterson A, Şekercioğlu Ç, Ehrlich P (2006) Human impacts on the rates of recent, present, and future bird extinctions. Proceedings of the National Academy of Sciences 103 (29): 10941-10946. https://doi.org/10.1073/pnas.0604181103
- Puillandre N, Lambert A, Brouillet S, Achaz G (2011) ABGD, automatic barcode gap discovery for primary species delimitation. Molecular Ecology 21 (8): 1864-1877. https://doi.org/10.1111/j.1365-294x.2011.05239.x
- Puillandre N, Brouillet S, Achaz G (2020) ASAP: assemble species by automatic partitioning. Molecular Ecology Resources 21 (2): 609-620. https://doi.org/10.1111/1755-0998.13281
- Quicke DJ (2012) We know too little about parasitoid wasp distributions to draw any conclusions about latitudinal trends in species richness, body size and biology. PLOS One 7 (2): 1-9. https://doi.org/10.1371/journal.pone.0032101
- Ratnasingham S, Hebert PN (2007) BOLD: The barcode of life data system (http://www.barcodinglife.org). Molecular Ecology Notes 7 (3): 355-364. https://doi.org/10.1111/j.1471-8286.2007.01678.x
- Ratnasingham S, Hebert PN (2013) A DNA-based registry for all animal species: The barcode index number (BIN) system. PLOS One 8 (7): 1-16. https://doi.org/10.1371/journal.pone.0066213
- R Core Team (2023) R: A language and environment for statistical computing. 4.2.1. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org
- Ricotta C, Podani J (2017) On some properties of the Bray-Curtis dissimilarity and their ecological meaning. Ecological Complexity 31: 201-205. https://doi.org/10.1016/j.ecocom.2017.07.003
- Salden T, Peters R (2023) Afrotropical Ceraphronoidea (Insecta: Hymenoptera) put back on the map with the description of 88 new species. European Journal of Taxonomy 884 (1): 1-386. https://doi.org/10.5852/ejt.2023.884.2181
- Sánchez-Bayo F, Wyckhuys KG (2019) Worldwide decline of the entomofauna: A review of its drivers. Biological Conservation 232: 8-27. https://doi.org/10.1016/j.biocon.2019.01.020
- Schmitt G (2004) Die Parasitoidengemeinschaft (Hymenoptera) in einer Agrarlandschaft: Effekte von Nutzungstypen und Bewirtschaftungsmaßnahmen auf strukturelle Parameter. [The parasitoid community (Hymenoptera) in an agricultural landscape: Effects of land use types and management measures on structural parameters]. Technische Universität Dresden, Dresden, 249 pp.
- Shaw M, Hochberg M (2001) The neglect of parasitic Hymenoptera in insect conservation strategies: The British fauna as a prime example. Journal of Insect Conservation 5 (4): 253-263. https://doi.org/10.1023/a:1013393229923
- Shaw M (2006) Habitat considerations for parasitic wasps (Hymenoptera). Journal of Insect Conservation 10 (2): 117-127. https://doi.org/10.1007/s10841-006-6288-1
- Shaw R, Farquharson K, Bruford M, Coates D, Elliott C, Mergeay J, Ottewell K, Segelbacher G, Hoban S, Hvilsom C, Pérez-Espona S, Ruņģis D, Aravanopoulos F, Bertola L, Cotrim H, Cox K, Cubric-Curik V, Ekblom R, Godoy J, Konopiński M, Laikre L, Russo I, Veličković N, Vergeer P, Vilà C, Brajkovic V, Field D, Goodall-Copestake W, Hailer F, Hopley T, Zachos F, Alves P, Biedrzycka A, Binks R, Buiteveld J, Buzan E, Byrne M, Huntley B, Iacolina L, Keehnen NP, Klinga P, Kopatz A, Kurland S, Leonard J, Manfrin C, Marchesini A, Millar M, Orozco-terWengel P, Ottenburghs J, Posledovich D, Spencer P, Tourvas N, Unuk Nahberger T, van Hooft P, Verbylaite R, Vernesi C, Grueber

C (2025) Global meta-analysis shows action is needed to halt genetic diversity loss. Nature 638 (8051): 704-710. https://doi.org/10.1038/s41586-024-08458-x

- Smith AM, Fernández-Triana JL, Eveleigh E, Gómez J, Guclu C, Hallwachs W, Hebert PDN, Hrcek J, Huber JT, Janzen D, Mason PG, Miller S, Quicke DLJ, Rodriguez JJ, Rougerie R, Shaw MR, Várkonyi G, Ward DF, Whitfield JB, Zaldívar-Riverón A (2012) DNA barcoding and the taxonomy of Microgastrinae wasps (Hymenoptera, Braconidae): impacts after 8 years and nearly 20 000 sequences. Molecular Ecology Resources 13 (2): 168-176. https://doi.org/10.1111/1755-0998.12038
- Smith MA, Rodriguez J, Whitfield J, Deans A, Janzen D, Hallwachs W, Hebert PN (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. Proceedings of the National Academy of Sciences 105 (34): 12359-12364. https://doi.org/10.1073/pnas.0805319105
- Srivathsan A, Lee L, Katoh K, Hartop E, Kutty SN, Wong J, Yeo D, Meier R (2021) ONTbarcoder and MinION barcodes aid biodiversity discovery and identification by everyone, for everyone. BMC Biology 19 (1): 1-21. https://doi.org/10.1186/s12915-021-01141-x
- Ulmer J, Miko I, Deans A (2018) *Ceraphron krogmanni* (Hymenoptera: Ceraphronidae), a new species from Lower Saxony with unusual male genitalia. Biodiversity Data Journal 6 (e24173): 1-15. https://doi.org/10.3897/bdj.6.e24173
- Ulrich W (1999) The number of species of Hymenoptera in Europe and assessment of the total number of Hymenoptera in the world. Polish Journal of Entomology 68: 151-164.
- Vasiliţa C, Moser M, Krogmann L (2022) Mission possible: an optimised protocol for the unbarcodable Ceraphronoidea (Hymenoptera). Biodiversity Data Journal 10 (e84860): 1-8. https://doi.org/10.3897/bdj.10.e84860
- Vasiliţa C, Feng V, Hansen AK, Hartop E, Srivathsan A, Struijk R, Meier R (2024) Express barcoding with NextGenPCR and MinION for species-level sorting of ecological samples. Molecular Ecology Resources 24 (3): 1-7. https://doi.org/10.1111/1755-0998.13922
- Vasiliţa C, Bremer J, Popovici O, Krogmann L (In press) From shadows to clarity: Resolving the taxonomy of *Gryon* (Hymenoptera: Scelionidae) by integrating modern and historic data. Proceedings of the Entomological Society of Washington.
- Wang J (2024) SPECIES: Statistical package for species richness estimation. 1.2.0. Release date: 2024-9-23. URL: 10.32614/CRAN.package.SPECIES
- Wang W, Srivathsan A, Foo M, Yamane S, Meier R (2018) Sorting specimen-rich invertebrate samples with cost-effective NGS barcodes: Validating a reverse workflow for specimen processing. Molecular Ecology Resources 18 (3): 490-501. https://doi.org/10.1111/1755-0998.12751
- Whitfield JB (1998) Phylogeny and evolution of host-parasitoid interactions in Hymenoptera. Annual Review of Entomology 43 (1): 129-151. https://doi.org/10.1146/annurev.ento.43.1.129
- Wickham H (2007) Reshaping data with the reshape package. Journal of Statistical Software 21 (12): 1-20. https://doi.org/10.18637/jss.v021.i12
- Wickham H (2009) ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York. [ISBN 978-3-319-24277-4] https://doi.org/10.1007/978-0-387-98141-3
- Wickham H, François R, Henry L, Müller K, Vaughan D, Posit Software, PBC (2023) dplyr: A grammar of data manipulation. 1.1.4. Release date: 2023-11-17. URL: 10.32614/CRAN.package.dplyr

Supplementary materials

Suppl. material 1: Supplementary Figure 1 `doi`

Authors: Moser, M; Vasilița, C; Haas-Renninger, M; Pirvu, E; Haas, M; Krogmann, L
Data type: Image
Brief description: Biodiversity indices of the Ceraphronoidea community of Baden-Württemberg using ASAP clustering (a,b), ABGD clustering (c,d) and SpeciesIdentifier clustering (e,f). Species accumulation boxplots (a,c,e) plot the number of sampling sites (x-axis) against the cumulative number of species observed (y-axis). Boxplots represent variability (95%-confidence intervals) in species richness at each sampling effort level. Diversity profiles (b,d,f) are based on Hill numbers for different orders of diversity: q = 0 (i.e. species richness), 1 (Shannon diversity), 2 (Simpson diversity) (Chao et al. 2014), illustrating how diversity estimates change with varying levels of sensitivity to species abundances.
Download file (795.24 kb)

Suppl. material 2: Supplementary Figure 2 `doi`

Authors: Moser, M; Vasilița, C; Haas-Renninger, M; Pirvu, E; Haas, M; Krogmann, L
Data type: Image
Brief description: Non-metric multidimensional scaling (NMDS) ordination of species composition across sampled sites, based on Bray-Curtis dissimilarity of ASAP clustering. Each point represents a sampling site and the distance between points reflects differences in community composition. The ordination was performed with a maximum of 1000 iterations in two dimensions (k = 2), achieving a final stress value of 0.088. Points are colour-coded by habitat type to illustrate differences in species composition across habitats.
Download file (369.36 kb)

Suppl. material 3: Supplementary Table 1 `doi`

Authors: Moser, M; Vasilița, C; Haas-Renninger, M; Pirvu, E; Haas, M; Krogmann, L
Data type: Table
Brief description: Comparative analysis of diversity indices for varying q values using Chao & Jost (2015) and empirical Maximum Likelihood estimators, along with 95% confidence intervals.
Download file (136.74 kb)