**Title**: Anomalous latitudinal gradients in parasitoid wasp diversity - hotspots in regions with larger temperature range.

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**Data Availability:** The data and code that support the findings of this study are openly available on GitHub at <https://github.com/Labarcena-Jessica/Assignment-Reproducibility>.

**Conflict of Interest statement**

No conflicts of interest

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# Abstract

Parasitoid wasps are among the shortlist of taxa showing an anomalous latitudinal diversity gradient. Using the largest georeferenced molecular dataset, we used a macrogenetics approach to explore the spatial relationship between intra- and interspecific levels of diversity and potential environmental variables influencing the anomalous diversity pattern. Nucleotide diversity values were consistently higher at temperate latitudes, peaking at 50 degrees. We found a positive but weak relationship between intraspecific diversity and the latitude, between intra- and interspecific diversity, and a significant positive effect of the temperature range. Examining the spatial relationship between these levels of biodiversity and its drivers is particularly relevant considering climate change and its impact on species distribution. Yet, in insects, it has been challenging to integrate ecological, evolutionary, and geographical components when analyzing the processes leading to species richness gradients. Our study demonstrates how macrogenetics analyses of large-scale patterns of diversity provide insights into potential causes.

# Introduction

The emergence of comprehensive global datasets of the spatial distribution of species, as well as molecular and fossil record information, has facilitated global analyses of diversity gradients. However, most studies testing the latitudinal diversity gradient (LDG) have focused on vertebrates and woody plants, as those are the taxa with the most data available. A lack of data for most invertebrate groups has limited the number of analyses, although several pioneering studies have examined the LDG in insects [Condamine et al. (2012)](Owen and Owen, 1974).

The most notable pattern observed is the one of species richness increasing from the poles to the equator (Saupe et al., 2019). However, some groups, e.g., ichneumonid wasps, show an anomalous latitudinal diversity gradient, which is characterized by higher diversity in some temperate regions compared to tropical biomes (Owen and Owen, 1974). These parasitoid wasps (Ichneumonoidea: Ichneumonidae, Braconidae and the recently described Trachypetidae family (Quicke et al., 2020) constitute a large and ecologically important group within the Hymenoptera, with approximately 41,000 described species, likely representing just a fraction of its true diversity. Ichneumonids play a key role in regulating populations of many groups of insects, including pests that affect agriculture, horticulture, and forestry. However, in particular, the tropical diversity of ichneumonoids is still poorly understood, as most sampling and species-level taxonomic descriptions have been focusing on temperate regions. Several studies highlight that the understanding of large-scale diversity patterns in these families is hindered by inadequate sampling of tropical species (Quicke, 2012).

Yet, this scenario is changing as large-scale studies are being conducted in Southeast Asia , South America, Uganda, and South Africa; revealing and characterizing the vast diversity of southern parasitoid wasps. Additionally, a new research program in Costa Rica aims to conduct a comprehensive, nationwide Malaise trapping program to barcode the nation’s multicellular biodiversity. Nevertheless, based on current knowledge, these families show strong latitudinal patterns of species richness peaking at temperate latitudes and declining near the equator (Quicke, 2014).

This study used a macrogenetics approach to explore large-scale patterns and predictors of intraspecific genetic diversity in parasitoid wasps. To account for the taxonomic debt at lower latitudes, (Freeman and Pennell, 2021) we used molecular clusters as a proxy for species, as in this highly diverse and less-studied group, a high number of cryptic species can be expected (Smith et al., 2008). With very limited taxonomic expertise available worldwide, the use of DNA-based clustering of specimens into species has shown to be a low-cost and low-risk methodology in most taxa, especially in insects (Cariou et al., 2020). This approach constitutes an area of active debate, so we implemented several quality control steps to account for taxonomic misclassification, contaminations, and sequencing errors within each molecular cluster. We used a publicly available genetic dataset for the DNA barcoding region cytochrome C oxidase subunit I (COI). Mitochondrial loci have long been used for exploring correlations between species and genetic diversity in global-scale macrogenetic studies, and despite the limitations associated with using a single gene, this is likely the best way to conduct large-scale biodiversity studies of largely unexplored taxonomic groups.

We also explored the effect of sampling coverage to determine the minimum number of sequences needed to calculate accurate intraspecific diversity estimates. The data were grouped at three different geographic scales to account for the fact that the geographic distance between pairs of conspecific sequences influences the expected genetic diversity in a set of DNA sequences. Therefore, we used grid cells, latitudinal bands, and climatic zones to explore global patterns of intraspecific genetic diversity. We examined the spatial relationship between intra- and interspecific levels of diversity and explored environmental predictor variables influencing the anomalous diversity pattern reported for this group.

# Methods

**Data Acquisition, filtering, and quality control**.

We downloaded the data directly into R (R Core Team, 2023) using the Barcode Of Life Data System (BOLD) (Ratnasingham and Hebert, 2013) API (Application-Platform Interface) on October 19, 2021. The combined data set for Ichneumonidae and Braconidae consisted of 243,870 sequences of the 5’ region of the cytochrome c oxidase subunit I gene (*COI*-5P), the barcode gene for animals.

**Intraspecific diversity estimation**

We calculated the nucleotide diversity per molecular cluster after grouping the data into three different spatial resolution levels: hexagonal grid cells, latitudinal bands, and climatic zones.  We used the R package dggridR (version 3.00) (Barnes and Sahr, 2017) to build a global grid of hexagonal cells of 209,904 km2, all of which with identical sizes. The data were grouped by cells using the geographic coordinates associated with each record. We aligned the sequences of each molecular cluster in each cell using the R package muscle. The nucleotide diversity (Π) was calculated as the average number of variable sites in each pairwise sequence comparison using the R package PopGenome (verison 2.7.5) (Pfeifer et al., 2014). Average pairwise nucleotide differences (using Nei’s calculation of pi were divided by the number of base pairs to obtain diversity per site values. The function used, diversity.Stats, incorporates the calculated number of comparisons per site, which results in a Π value weighted by the number of comparisons. The average genetic diversity per cell was calculated as the mean of all nucleotide diversity values per molecular cluster in each cell. Considering that the intensity of sampling, both in terms of the number of molecular clusters per cell and the number of sequences in each molecular cluster, varied substantially among cells, we also evaluated the impact of this variation. Molecular clusters with 10 or more sequences were used for sensitivity tests following (Miraldo Andreia et al., 2016). These clusters and only cells with three or more molecular clusters were retained for further analysis. We mapped the nucleotide diversity values per cell using the R package tmap (version 3.3-3).

# Results

**Global pattern of intra- and interspecific diversity in parasitoid wasps**. The nucleotide diversity values for parasitoid wasps were found to be higher at temperate latitudes based on data analyzed at three geographical scales. We analyzed 85 cells and found that those with higher nucleotide diversity values were located between 40- and 60 degrees latitude (Figure 1).  We found a weak yet significant positive correlation between the absolute value of latitude and nucleotide diversity per cell (R2 = 0.094, p = 0.004).

## Reading layer `sf\_grid\_mpre10' from data source   
## `/Users/jessica/Documents/FALL 2023/CIEE courses/Reproducibility/Assignment\_Reproducibility/04\_manuscript/sf\_grid\_mpre10.shp'   
## using driver `ESRI Shapefile'  
## Simple feature collection with 595 features and 10 fields  
## Geometry type: POLYGON  
## Dimension: XY  
## Bounding box: xmin: -153.9464 ymin: -48.25852 xmax: 179.5306 ymax: 81.58269  
## Geodetic CRS: WGS 84

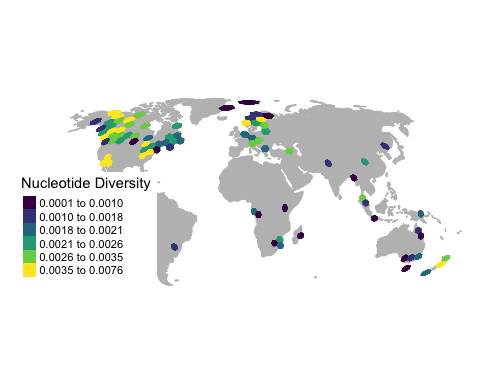


Figure 1: Global patterns of nucleotide diversity for parasitoid wasps. (a) Hexagonal grid cells of approximately 209,904 km2 show the mean nucleotide diversity values in a colour gradient where yellow represents higher values. Using a dataset of 209,382 COI records grouped in 15,584 molecular clusters, nucleotide diversity (Π) per molecular cluster was calculated as the average number of variable sites in each pairwise sequence comparison. After filtering, 85 cells were retained for a minimum of three molecular clusters per cell and 10 COI sequences per molecular cluster.

# Discussion

Our results show that intra and interspecific diversity of parasitoid wasps covary and follow an atypical latitudinal diversity pattern with a peak at temperate latitudes. The weak but significant correlation between these two dimensions of biodiversity indicates common underlying evolutionary and ecological processes acting along a latitudinal gradient. While it has been previously noted that the latitudinal pattern of species richness for parasitoid wasps differs from the ones seen in other well-studied groups [Condamine et al. (2012)](Lawrence et al., n.d.), this study is the first to test the relationship between genetic diversity and a proxy for species richness. Our findings support the notion that the unexpected decline in diversity towards the tropics, which was previously identified through morphological data, is also evident in molecular data. The congruence between spatial variation of genetic diversity and species richness has been described for other groups of animals and was explained by well-supported hypotheses such as the evolutionary speed theory, the time and area, and the Red Queen hypothesis (Theodoridis et al., 2020). Nevertheless, none of these can explain the anomalous pattern found for parasitoid wasps.

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