Unprecedented ichneumonid parasitoid wasp diversity in tropical forests

Anu Veijalainen1,2,*, Niklas Wahlberg1, Gavin R. Broad3, Terry L. Erwin4, John T. Longino5 and Ilari E. Sääksjärvi1

1Department of Biology, University of Turku, 20014 Turku, Finland
2Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA
3Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK
4Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013-7012, USA
5Department of Biology, University of Utah, Salt Lake City, UT 84112, USA

The megadiverse parasitoid wasp family Ichneumonidae (Hymenoptera) is classically considered an exception to the extensively studied latitudinal diversity gradient: the majority of ichneumonid species are described from temperate regions. The gradient has been hypothesized to be dependent on the biology of the wasps, but recently questions of sampling and description biases have been raised. Here, we show with primary data that the species richness of Ichneumonidae is markedly underestimated in tropical areas and that latitudinal diversity patterns in the family remain uncharacterized. We discovered a startling 177 likely undescribed orthocentrine species with relatively low sampling effort in the forests of Central America and Amazonian Ecuador, over three times the previously known orthocentrine diversity in the world’s tropics. Species accumulation curves reveal that we are just beginning to unveil the true extent of tropical orthocentrine diversity. We also found evidence for cryptic species; our DNA analysis revealed additional species not easily distinguishable using morphological characteristics. The difficulty in establishing species richness patterns of Ichneumonidae probably follows from the relative lack of taxonomic expertise and the low density of ichneumonid species throughout the landscape.

Keywords: cryptic species; DNA barcoding; latitudinal diversity gradient; Neotropics; Orthocentrinae

1. INTRODUCTION

Explaining large-scale latitudinal gradients of species richness contributes to a better understanding of ecological and evolutionary factors that foster global biodiversity patterns. For well over a century, ecologists have recognized that species richness increases with decreasing latitude in most taxa [1,2]; however, the mechanisms underlying this pattern are still contentious [3–6]. One notable exception to the common trend has been the parasitoid wasp family Ichneumonidae (Hymenoptera)—possibly the world’s largest animal family—which has repeatedly been shown to be more species-rich in northern temperate latitudes than in tropical areas [7–11]. The vast tropical species richness of another major parasitoid taxon, the Chalcidoidea, is well established [12], which has meant that the supposedly lower tropical species richness of Ichneumonidae is all the more anomalous and must be intrinsically associated with the ichneumonid way of life [13].

That Ichneumonidae are supposed to be more species-poor in lower latitudes has been ascribed to limitations emerging from host predation, location and utilization [14]. This is thought to be the case especially for ichneumonid koinobiont groups (i.e. parasitoids that arrest their hosts’ development at oviposition; [15]). Nevertheless, the view that this anomalous latitudinal species richness gradient applies to large components of the Ichneumonidae is persistent [17]. However, recent evidence from a database and catalogue of Ichneumonoidae [18] and Western Hemisphere subfamily abundance studies suggest that the assumption of low tropical ichneumonid diversity may follow from sampling and description biases, and particularly that the small-bodied koinobiont species might be overlooked or undersampled in the tropics [11,14,19,20].

Sufficient primary species-level data on tropical small-bodied koinobiont ichneumonids have thus far been lacking. Because parasitoids play key roles in multitrophic interactions, underestimated tropical ichneumonid diversity would not only bring into question any conclusions drawn about the family’s latitudinal trends but also the level of impact that ichneumonid species have on tropical ecosystem dynamics. Given that (i) most ichneumonids remain undescribed, (ii) this is especially true of tropical faunas, and (iii) there is limited revisionary taxonomic work ongoing, we will need to employ methods beyond mining catalogues and taxonomic monographs if we are to gain a better understanding of the size and composition of tropical ichneumonid faunas.

Here, we hypothesized that additional sampling in Neotropical forests could reveal reservoirs of unknown ichneumonid species diversity. We examined whether the small-bodied koinobiont subfamily Orthocentrinae—cosmopolitan and with all reliable host records from larval...
fungus gnats (Diptera: Sciaroidea)—is more species-rich in the tropics than indicated by the current totals of described species (48 tropical species of a total of 463 described species) [18,21]. We further employed integrative morphological and molecular species identification methods to search for complexes of cryptic species. Molecular identification methods may greatly advance species delimitation in parasitoids and other organisms and reveal complexes of morphologically similar species (bearing in mind the limitations of the procedures; [19,22,23]).

2. MATERIAL AND METHODS

We collected orthocentrines with relatively low sampling effort from the Amazonian lowland rain forest canopy in Ecuador (using canopy fogging methods; [24]) and from various altitudes and vegetation types in Central America (using Malaise traps; electronic supplementary material, table S1). We sorted the specimens into morphologically similar groups representing the minimum number of morphospecies, and independently studied the differences in the mitochondrial gene cytochrome oxidase c subunit I of selected individuals to estimate the cryptic species diversity in the sample.

(a) Field sampling

The Central American specimens were collected by the LLAMA project (J. T. Longino) with ground-level Malaise traps at 25 study sites ranging from 30 to 2625 m.a.s.l. in Guatemala, Honduras and Nicaragua in May–July 2009, 2010 and 2011, respectively. Traps were generally located on forest edges or in small clearings. Sampling took place during the transition from dry season to wet season. Total sample size was 313 Malaise trap days, which collected a total of 471 orthocentrine individuals (see the electronic supplementary material, table S1).

The Western Amazonian canopy fogging samples were collected in eastern Ecuadorian non-inundated lowland rain forest sites near Yasuni National Park at Onkone Gare (0°39'25.685" S, 76°27'10.813" W) and Tiputini Biological Station (0°37'55.397" S, 76°08'39.204" W), Orellana Province, adjacent to the Yasuni National Park, 21 km from each other and approximately 220 m.a.s.l. Both sites were 1 ha in size: a 100 × 1000 m area divided into 10 transects of 10 × 100 m where 10 randomly positioned collecting stations (sheets each 9 m²) were placed on each sampling occasion in February, June–July and September–October of 1994–1996, 1998–1999 and 2005 [20,24]. The vegetation at the sites is old and diverse, primary lowland terra firme rain forest, and the climate is somewhat aseasonal (least rain falling in February and most in the June–July field season). Orthocentrines were found in 599 of the 1079 canopy fogging samples available for this study [20]. Of a total of 2731 individuals, 1078 were assigned to morphospecies (see below).

(b) DNA barcoding and mini-barcoding

A total of 226 randomly chosen specimens were studied genetically. We used the DNA barcoding method for the Honduran (ca 600 bp; DNA barcoding) and the Guatemalan and Ecuadorian (ca 300 bp; DNA mini-barcoding) specimens to find differences in the mitochondrial gene cytochrome oxidase c subunit I (COI) sequences. First, we removed three legs of one side from each specimen and extracted their DNA using the DNeasy Blood & Tissue Kit (QIAGEN). We followed the standard bench protocol for animal tissue in DNeasy Blood & Tissue Handbook 07/2006 with additional incubation at 70°C for 10 min after adding the Buffer AL and vortexing. Next, we amplified the fragments of the 5’ end of the COI by PCR (20 µl volume; 1 µl of DNA extract and 19 µl of master mix: 12.5 µl dH2O, 2.0 µl 10 × PCR Gold Buffer, 2.0 µl MgCl2 solution, 2 × 1.0 µl primer, 0.4 µl dNTP, 0.1 µl Ampli Taq Gold). We used the primers LCO (5’GGTTCAAAATCATATAGATTTGG 3’) and HCO (5’TAAACTTCTACGGGATCACCCTATAATCA 3’) [25] in DNA barcoding and K698 (5’TACATTTCGCGCCCAAACCCACG 3’) and K699 (5’WGCGGTGTAAC TGTTCATCC 3’) [26] in DNA mini-barcoding. The PCRs were run for 40 cycles with an annealing temperature of 50°C. Macrogen (South Korea) cleaned and sequenced the successful PCRs before we edited and aligned the sequences. Finally, we constructed the neighbour-joining tree, based on the Maximum Composite Likelihood using MEGA v. 4 [27]. The sequences are found under accession numbers JX657848–JX658074 in GenBank.

(c) Morphological identification

We identified 1549 specimens to genera according to Broad [21] and Townes [28] after first excluding 464 Ecuadorian specimens that were in a condition that prevented their confident identification, and then excluding 1189 non-barcoded Ecuadorian Orthocentrus genus group males. Matching the sexes of an ichneumonid species may be challenging; thus we excluded the non-barcoded Ecuadorian Orthocentrus genus group males to minimize the chance of incorrectly classifying the two sexes of one species as two distinct ones. We compared the morphospecies to type material or photos of type material of the described Neotropical species (Chilocerus carinatus Townes, Megastylus panamensis Wahl, Orthocentrus insularis Ashmead, Stenomacrus variabilis (Ashmead)) and Nearctic species found in the southern US (Stenomacrus californicus (Ashmead), Stenomacrus hastatus Davis, Stenomacrus pinnatus (Davis), Stenomacrus umbiola (Ashmead), Plectiscus sulfus (Davis), Orthocentrus decoratus Townes, Orthocentrus tetrazonatus (Ashmead)), and ran the Helictes genus group specimens through the key in [29], concluding that all the morphospecies are likely undescribed.

3. RESULTS

We examined 1549 individuals (1078 from Ecuador and 471 from Central America) that included a minimum of 177 morphospecies (figure 1 and table 1), which is over three times the number of currently described tropical orthocentrine species. We applied non-parametric methods (ACE, Chao1 and Jack2) for species richness estimation [30]. The morphospecies numbers were shown to be underestimates of the true species richness of both areas (figure 2 and table 1; calculated by using ESTATES v. 8.2.0). All morphospecies appear to represent undescribed species. All genera except two (Apodima, Catatenus) that have been reported from the Neotropics were represented in the samples (figure 1). The numbers of morphospecies of the orthocentrine genera Chilocerus, Gnathochorisis, Orthocentrus and Megastylus were close to or higher than their global described species numbers (figure 1). The voucher specimens are currently on loan to the Zoological Museum, University of Turku (ZMUT) and later to be deposited in the collaborative institutions of the LLAMA project and National Museum of Natural History, Smithsonian Institution.
DNA sequence data were obtained for 226 individuals of 57 morphospecies. For 43 morphospecies (124 individuals, of which 28 were singletons), genetic clusters corresponded to morphospecies. For 14 morphospecies (102 individuals), there were deep genetic divergences, with individuals of single morphospecies clustering in two or three groups. The 14 morphospecies contained 31 potential cryptic species not easily distinguishable using morphological features (see the electronic supplementary material, figure S1).

4. DISCUSSION

The high species richness that we discovered demonstrates that tropical ichneumonids have been vastly underestimated when considering latitudinal diversity gradients. When the minimum number of Neotropical orthocentrine species is considered to be 177 rather than the currently described 14, and is compared with the 151 known species for all of the Nearctic ([18]; Nearctic fauna revised in large part by Dasch [29]), the need to examine the true ichneumonid species richness at different latitudes becomes apparent. Additional research is almost certain to substantially increase the species number for at least three reasons: (i) our sampling covered only a tiny fraction of the spatial and temporal tropical habitat complexity, (ii) the integrated identification results pointed to the presence of species complexes that are difficult to separate using morphological characters (e.g. *Chilocyrtus* sp. 12 and *Orthocentrus* sp. 10), and (iii) the species number estimations indicated clearly higher-than-observed total local diversity in both study areas. The Central American data presumably consist of species specialized to a variety of environmental conditions, as the specimens were collected from 25 ecologically and altitudinally distinct study sites across Guatemala, Honduras and Nicaragua. The specimens from Ecuador came from two sites at the same altitude and only 21 km apart, yet showing high beta-diversity ([24]; e.g. the two transects have 75% different tree species: [31]).

Most described Neotropical orthocentrines are from Central America and the Caribbean ([18, 32]; here we reveal that the subfamily is species-rich also in Western Amazonia. The 95 Ecuadorian morphospecies were collected from only two hectares of rain forest canopy ([20, 24]), which prompts the question of how many more unknown ichneumonid species does the entire Amazonia harbour? We also expect Orthocentrinae to be diverse in the tropics of other continents; for example, no species have been described from southeast Asia ([18] although Orthocentrinae is one of the most abundant ichneumonid subfamilies in that region’s rain forests [33]).

Reliable host records for Orthocentrinae are all from Sciaroidea (Diptera), the larvae of which are fungivores or root eaters (or sometimes predatory, e.g. Keroplatidae) and rarely reared in the tropics to look for parasitoids. This is thus a very large sector of parasitoid diversity that has been mostly overlooked in the Neotropics (and other tropical regions). There is also the intriguing problem that species richness of some orthocentrine genera has potentially been massively underestimated in the north temperate and subarctic zones too ([34], further obscuring any latitudinal gradients in species-richness. Given that the Orthocentrinae comprises a great many species in many biomes, and that a large proportion of the genera are essentially cosmopolitan in distribution, the subfamily would repay large-scale quantitative sampling efforts at different latitudes.

Table 1. Morphospecies numbers per study area showing numbers of morphospecies, including females or distinguished based only on males (see §2). The estimated numbers of species (ACE, Chao1 and Jack2) were calculated based on the number of species in ‘All material’ (i.e. Ecuador: 95, Central America: 88).

<table>
<thead>
<tr>
<th>morphospecies</th>
<th>only females or both sexes</th>
<th>only males</th>
<th>all material</th>
<th>ACE</th>
<th>Chao1</th>
<th>Jack2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecuador</td>
<td>73</td>
<td>16</td>
<td>89</td>
<td>118</td>
<td>111</td>
<td>134</td>
</tr>
<tr>
<td>Central America</td>
<td>68</td>
<td>14</td>
<td>82</td>
<td>139</td>
<td>134</td>
<td>157</td>
</tr>
<tr>
<td>common to both sites</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>147</td>
<td>30</td>
<td>177</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Figure 1. Number of morphospecies in the total dataset (grey bars), and described orthocentrine species in the Neotropics (black bars) and the world (white bars) (after [18, 21]).](http://rsb.resm.lsu.edu/)
This study made use of ‘residual’ material from biodiversity inventories directed at other taxa. It impressively demonstrates how such residual material can be used to facilitate the discovery of tropical arthropod diversity. Our Ecuadorean Amazonian morphospecies alone would account for about 78 per cent of the total number of new aquatic and terrestrial plant and vertebrate species described on average per year from the entire Amazon biome (1220 species in 1999–2009; [35]). It is no surprise that laboriously identifiable species from remote environments with unknown socio-economic potential have not yet caught much scientific attention, yet their systematic underestimation biases our understanding of multi-trophic tropical interactions and determination of large-scale biodiversity patterns. Increasing anthropogenic pressure on natural tropical environments should encourage us to promptly address the true extent of tropical ichneumonid diversity [36].

We conclude with primary data that tropical sampling and description has indeed been inadequate to draw any conclusions about the species richness patterns in Ichneumonidae. We revealed very high undescribed diversity in Orthocentrinae, a group of koinobiont ichneumonid parasitoid wasps, in tropical America, where the group had previously been rarely reported. The results are significant because Ichneumonidae is a very large family and a classic example of a group with an atypical latitudinal diversity gradient. This study demonstrates that our understanding of the latitudinal diversity gradient of Ichneumonidae is incomplete, and additional sampling will likely require us to redefine the current understanding of large-scale diversity patterns in the family.

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