Hymenoptera functional groups’ shifts in disturbance gradients at Andean forests in Southern Ecuador

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Abstract
Ecosystems under ecological restoration should be monitored in order to investigate if the ecosystem is being functionally recovered, especially in highly vulnerable biodiversity hotspots like Andean forests. Here we sampled Hymenoptera families in four Andean forest reserves above 1800 masl from Southern Ecuador, in three conservation levels in each forest: low (degraded), medium (10–15 years of recovery) and high (well-conserved forest). All Hymenoptera families were classified into four functional groups: predators, herbivores, pollinators and parasitoids. A total of 32 hymenopteran families were collected, with parasitoids clearly dominating in the samples. Family assemblages were not statistically different, neither in abundance nor family richness. Assemblages were more similar between them in the high and medium areas than in low conservation areas, where assemblages were very variable and showed a higher functional diversity in two of the reserves. The low presence of pollinators may be due to the high humidity during the sampling and the sampling method. Although some results are promising for the restoring trajectory, especially for parasitoids, we should keep in mind that this is at family level, so it would be interesting to know if these patterns persist at lower taxonomic levels.

Keywords
Herbivores, Hymenoptera families, Parasitoids, pollinators, predators
**Introduction**

Ecosystems provide a wide range of services that are at risk because of biodiversity loss derived from demographic growth and land use changes (De Groot et al. 2012). Ecosystems do have an ability to recover previous biodiversity up to a certain point, beyond which they need some assistance. The process of “assisting the recovery of an ecosystem that has been degraded, damaged or destroyed” is called ecological restoration (SER 2004). However, since ecosystems may progress in different ways after restoration efforts, depending on many different environmental variables (Bullock et al. 2011), monitoring is essential to know how ecosystem attributes are recovering.

Andean forests are considered as a highly vulnerable biodiversity hotspot (Myers et al. 2000; Mathez-Stiefel et al. 2017; Murcia et al. 2017), with most of its plant species already experiencing shifts in their distribution because of global warming (Fadrique et al. 2018). Although there are some initiatives for restoring Andean forests, they are not being monitored comprehensively (Mazón et al. 2019), with plant structure and diversity being evaluated in most cases (Yepes et al. 2010; Szejner et al. 2011; Camelo et al. 2017). Therefore, little is known about whether many other ecosystem services are being recovered in these forests.

Hymenoptera is one of the most important and diverse insect orders, including representative groups of two of the most essential ecosystem services: pollination (i.e., bees) and natural pest control (i.e., parasitoid wasps). Both pollinators and parasitoids (whose hosts are mostly herbivorous insects), because of their close relationship with plants, have been used as indicators for ecosystem disturbances (Vos et al. 2001; Requier 2019) and their recovery (Maeto et al. 2008; Ferronato et al. 2018). Pollinators have received much attention in recent years because of their worrying decreasing populations and their high vulnerability to climate change (Potts et al. 2010; Powney et al. 2019). However, other hymenopterans like parasitoid wasps and ants are also sensitive to ecosystem degradation and fragmentation (de Sassi and Tylianakis 2012). Since hymenopteran families may have different responses to habitat degradation (Banks et al. 2013), they may also have different responses when being monitored in ecological restoration projects, as well as the roles they play in the ecosystem. For instance, bee and parasitoid diversity has been shown to be higher in restored areas than in disturbed ones (Barbieri Junior and Penteado-Dias 2012; Marrec et al. 2018) but the species interaction and functions appear to take a longer time to recover (Albrecht et al. 2007). Therefore, species diversity per se may not be the best predictor of ecosystem services, but, rather, diversity at the functional level (Griffin et al. 2013). In consequence, measuring functional diversity may predict ecosystem functioning better than the traditional species diversity measures (Loreau et al. 2001).

In this study we aim to 1) identify the Hymenoptera families present in different disturbance levels in Andean forests, and 2) evaluate how functional groups within Hymenoptera are shifting as disturbance increases.
Methods

Study area

The research was done in four protected areas of Andean forest located in Loja and Zamora Chinchipe provinces, in the buffer area of Podocarpus National Park, at southern Ecuador (Fig. 1), with altitudes ranging 1840–2630 masl (Table 1). Andean forests are ecosystems located between 1200 and 3600 masl at Los Andes mountains (Quintero et al. 2017), characterized by their elevated humidity and a high diversity of ferns, lichens, mosses, palms and orchids (Bravo 2014; Kattan 2017).

Sampling and identification

In each reserve, we identified three areas of different conservation status with the help of their personal staff: a well-conserved area, an area under about 10–15 years of recovery, either naturally or assisted, and a degraded area (Table 1). In each area, we installed a white Townes style Malaise trap (Townes 1972) for six weeks, from December 2015 to January 2016 in most reserves, except for Tapichalaca, which was sampled from March to April 2018; in all cases, sampling was carried out during the rainy season. We filled pots with ethanol 70% and replaced them every two weeks, i.e., having three samples per area.

We sorted and identified all hymenopteran specimens to family level, and then we classified them into four major functional groups: predators, herbivores, pollinators and parasitoids, following Fernández and Sharkey (2006).

Data analyses

We compared the family assemblages in the three conservation levels by a non-metric multidimensional scaling (NMDS) and a PERMANOVA with 9999 permutations, using Jaccard index for similarity, which considers presence/absence of families, regardless of the relative abundances of each one. We did the same analyses to check for differences in the hymenopteran families assemblages related to the reserves.

Regarding the functional groups, we calculated both richness (i.e., number of families) and abundance (i.e., number of individuals) for every functional group, and we compared them across the conservation levels by means of a KRUSKAL-WALLIS test and a post-hoc DUNN test.

Additionally, we evaluated functional diversity with the Shannon index, considering the abundance of individuals belonging to every functional group. Since some samples were damaged and lost, we considered the mean abundance values for the two or three samples in every sampling site. Then, we compared Shannon indices in two ways: by the KRUSKAL-WALLIS with the four reserves as replicates, and in the four reserves treated as independent samples, compared by a randomization test with 1000 random partitions (Solow 1993).
The NMDS, PERMANOVA, KRUSKAL-WALLIS and post-hoc tests were run with software Past version 3.0 (Hammer et al. 2001). Shannon diversity index and randomization tests were performed with software Species Diversity and Richness 3.02 (Pisces Conservation, Ltd., Pennington, Lymington, UK).

Results

We collected a total of 32 hymenopteran families, mostly belonging to the parasitoid functional group, with family Ichneumonidae clearly dominating the sampling (1930 individuals). We only collected seven specimens belonging to three families from the pollinator functional group (Table 2).

Families assemblages were not statistically different, neither regarding the conservation level ($F = 1.354, p = 0.156$) nor the reserve ($F = 1.382, p = 0.118$). In the NMDS, although the samples were distributed from high to low conservation level (Fig. 2A), those from the low level were the most variable, especially the sample from Madrigal reserve, which was more different than the others in terms of hymenopteran family composition (Fig. 2B). In both cases, axis 1 explained more of the data (50.14% and 49.87%, respectively) than axis 2.
### Table 1. Location of trapping sites in the three conservation levels of the four Andean forest reserves.

| Reserve | Conservation level | Coordinates X | Coordinates Y | Altitud (masl) |
|---------|--------------------|---------------|---------------|---------------|
| ECSF    | high              | -79.077       | -3.973        | ca 1870       |
|         | medium             | -79.078       | -3.975        | ca 1860       |
|         | low                | -79.079       | -3.972        | ca 1840       |
| Arcoiris| high              | -79.095       | -3.988        | ca 2160       |
|         | medium             | -79.093       | -3.988        | ca 2160       |
|         | low                | -79.093       | -3.989        | ca 2160       |
| Madrigal| high              | -79.168       | -4.051        | ca 2520       |
|         | medium             | -79.175       | -4.045        | ca 2350       |
|         | low                | -79.176       | -4.047        | ca 2400       |
| Tapichalaca| high          | -79.126       | -4.489        | ca 2570       |
|         | medium             | -79.130       | -4.493        | ca 2520       |
|         | low                | -79.126       | -4.490        | ca 2620       |

### Table 2. Abundances and functional groups of every collected hymenopteran family in the three conservation levels (high, medium and low) from the four Andean forest reserves in southern Ecuador. PRED = predators, HERB = herbivores, PAR = parasitoids, POL = pollinators.

| Family            | Functional groups | ECSF high | medium | low | Arcoiris high | medium | low | Madrigal high | medium | low | Tapichalaca high | medium | low | Total |
|-------------------|-------------------|-----------|--------|-----|---------------|--------|-----|---------------|--------|-----|------------------|--------|-----|-------|
| Pompilidae        | PRED              | 0         | 0      | 0   | 0             | 1      | 0   | 0             | 0      | 0   | 0                | 1      | 0   | 1     |
| Sphecidae         | PRED              | 0         | 0      | 0   | 0             | 0      | 0   | 0             | 0      | 0   | 0                | 0      | 0   | 0     |
| Vespidae          | PRED              | 1         | 0      | 0   | 1             | 4      | 1   | 1             | 0      | 0   | 2                | 5      | 27  |
| Formicidae        | PRED              | 19        | 4      | 2   | 2             | 1      | 3   | 1             | 0      | 0   | 2                | 3      | 65  |
| Pergidae          | HERB              | 0         | 0      | 0   | 0             | 0      | 4   | 0             | 0      | 0   | 1                | 1      | 0   | 6     |
| Tenthredinidae    | HERB              | 0         | 0      | 2   | 0             | 0      | 3   | 0             | 0      | 0   | 1                | 2      | 4   | 12    |
| Xiphydriidae      | HERB              | 0         | 0      | 2   | 1             | 0      | 0   | 0             | 0      | 0   | 2                | 0      | 0   | 5     |
| Bethylidae        | PAR                | 10        | 0      | 1   | 0             | 1      | 1   | 1             | 0      | 0   | 1                | 6      | 21  |
| Braconidae        | PAR                | 167       | 9      | 12  | 60            | 15     | 6   | 134           | 25     | 2   | 38               | 31     | 76  | 577   |
| Diapriidae        | PAR                | 48        | 1      | 1   | 6             | 1      | 14  | 3             | 0      | 6   | 17               | 36     | 134 |
| Dryinidae         | PAR                | 0         | 1      | 1   | 0             | 0      | 1   | 0             | 1      | 0   | 2                | 10     | 4   | 19    |
| Embolomoridae     | PAR                | 2         | 0      | 0   | 0             | 0      | 0   | 0             | 0      | 0   | 0                | 0      | 0   | 2     |
| Eucharitidae      | PAR                | 0         | 0      | 0   | 1             | 0      | 0   | 0             | 0      | 0   | 0                | 0      | 0   | 1     |
| Euolpididae       | PAR                | 5         | 0      | 0   | 0             | 0      | 0   | 0             | 0      | 0   | 0                | 1      | 2   | 11    |
| Euplomidae        | PAR                | 0         | 1      | 1   | 0             | 0      | 0   | 0             | 0      | 0   | 0                | 1      | 0   | 4     |
| Eurytomidae       | PAR                | 3         | 0      | 0   | 0             | 0      | 0   | 0             | 0      | 0   | 0                | 0      | 0   | 3     |
| Evaniidae         | PAR                | 25        | 2      | 0   | 14            | 4      | 2   | 11            | 3      | 0   | 1                | 14     | 9   | 92    |
| Figitidae         | PAR                | 1         | 0      | 2   | 0             | 0      | 3   | 0             | 0      | 4   | 2                | 5      | 5   | 17    |
| Ichneumonidae     | PAR                | 300       | 33     | 6   | 188           | 72     | 23  | 248           | 101    | 6   | 195              | 233    | 401 | 1930  |
| Lioperidae        | PAR                | 0         | 0      | 0   | 0             | 0      | 0   | 0             | 0      | 0   | 0                | 2      | 0   | 2     |
| Muntingidae       | PAR                | 5         | 0      | 0   | 0             | 0      | 0   | 0             | 0      | 0   | 0                | 0      | 0   | 5     |
| Mymaridae         | PAR                | 0         | 0      | 0   | 0             | 0      | 0   | 0             | 0      | 0   | 0                | 0      | 0   | 1     |
| Orussidae         | PAR                | 0         | 0      | 0   | 0             | 1      | 0   | 0             | 1      | 0   | 2                | 1      | 0   | 5     |
| Perilampidae      | PAR                | 3         | 0      | 0   | 0             | 0      | 0   | 1             | 0      | 0   | 0                | 0      | 0   | 4     |
| Platygastridae    | PAR                | 13        | 2      | 2   | 5             | 0      | 3   | 0             | 0      | 5   | 6                | 8      | 44  |
| Proctotrupidae    | PAR                | 2         | 0      | 0   | 0             | 3      | 0   | 0             | 0      | 2   | 1                | 2      | 10  |
| Pteromalidae      | PAR                | 6         | 0      | 0   | 2             | 0      | 1   | 0             | 0      | 0   | 0                | 6      | 0   | 16    |
| Sapygidae         | PAR                | 1         | 0      | 0   | 1             | 4      | 1   | 0             | 1      | 0   | 0                | 1      | 0   | 9     |
| Tiphidae          | PAR                | 5         | 0      | 0   | 0             | 0      | 0   | 0             | 3      | 0   | 0                | 12     | 3   | 23    |
| Agonidae          | POL                | 0         | 0      | 0   | 1             | 0      | 0   | 0             | 0      | 0   | 0                | 0      | 0   | 1     |
| Apidae            | POL                | 0         | 1      | 1   | 0             | 0      | 1   | 0             | 0      | 0   | 0                | 0      | 0   | 4     |
| Halictidae        | POL                | 0         | 0      | 0   | 0             | 0      | 0   | 0             | 0      | 0   | 0                | 2      | 0   | 3     |
When comparing mean abundance and total richness of every functional group with the conservation levels no significant differences were found either (Table 3).

Regarding functional diversity, no significant results were obtained in the KRUSKAL-WALLIS test ($H = 0.3462$, $p = 0.841$). However, when treating every reserve independently, permutational tests gave very different results in the four reserves (Fig. 3). Surprisingly, functional diversity was significantly higher in the more perturbed areas in ECSF and Arcoiris. However, diversity in the areas under restoration (i.e., medium conservation level) was more similar to those in the conserved areas than in the highly perturbed ones.
In the present research we found 32 hymenopteran families, which represents 36.31% of Neotropical families (Fernández and Sharkey 2006). Ichneumonidae and Bracconidae were the most abundant, which are usually amongst the main hymenopteran families collected by Malaise trap in many ecosystems, as in preserved sandbanks (Oliveira et al. 2009), open pastures (Castiglioni et al. 2017) or cacao plantations (Mazón 2016). The high abundance of these two families is also reflected in the dominance registered for parasitoid functional groups. Actually, parasitoids are the most abundant and diverse hymenopteran group (Fernández and Sharkey 2006) and are able to adapt

**Discussion**

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However, the low occurrence of hymenopteran pollinators was unexpected. Bees are abundant in Andes even above 2500 masl (Gonzalez and Engel 2004), and at least 51 bee species have been recorded from Southern Ecuador (Rasmussen 2004). Since one of the main factors limiting bee nesting is high humidity (Michener 2007; Reyes-Novelo et al. 2009), the season when sampling was done may have influenced these results. Furthermore, Malaise traps may not be the most appropriate sampling method for bees. Although showed as highly effective for parasitoids (Mazón and Bordera 2008) and aculeates in general (Volpato et al. 2020), with some reported bias either towards males (Aguiar and Santos 2010) or females (Mazón et al. 2020), scent-baited traps or entomological nets seem to work better for bees (Santos Júnior et al. 2014; Ferronato et al. 2018; Alvarenga et al. 2020). Other sampling methods should be used to complement Malaise traps, especially when time for field work is brief (McGravy et al. 2016; Saunders and Ward 2018). Sampling should also include different weather seasons in order to have a better representation of all Hymenoptera families and to clarify if bees have reduced populations in these forests.

No differences among conservation levels were found. Restoration may favour the presence of wild bees (Araújo et al. 2018; Taki et al. 2018; Alvarenga et al. 2020) and parasitoids (Marrec et al. 2018), but some hymenopteran assemblages from restored areas may be functionally similar to those from forests (Montoya-Pfeiffer et al. 2020), and their diversity has been found to be lower in forests compared to more open areas (Pardo and Gonzalez 2007). Therefore, it seems that, even when the areas labeled as low conservation presented a high degree of perturbation, being embedded into a preserved area matrix helped to increase diversity in these areas, since the hymenopteran diversity is positively affected by the proximity to forest (Banks et al. 2013). Furthermore, not all types of forest restoration will equally favour Hymenopterans, with ecological restoration, as applied in all the studied reserves, being more effective than monoculture tree plantations (de Araújo et al. 2019). This indicates that ecological restoration in these areas has a positive effect on hymenopteran diversity, and the more disturbed areas within the reserve matrix do not significantly impact these assemblages, at least at family level.

Another aspect that may have masked the effect of conservation level on Hymenoptera richness and abundance are the altitudinal differences amongst some of the reserves. Parasitoids (van Noort 2004; Veijalainen et al. 2014; Hall et al. 2015), wasps and bees (Perillo et al. 2017; Widhiono et al. 2017) and ants (Guerrero and Sarmiento 2010; Burwell and Nakamura 2011) have been found to be sensitive to altitude in tropical and subtropical ecosystems. However, to see more clearly how Hymenoptera are responding to both altitude and conservation level it would be necessary to use lower taxa. Higher taxa have been used as surrogates for species to assess biodiversity in highly species-rich ecosystems or when sampling time is limited. For this purpose, genus (Derraik et al. 2010; Vieira et al. 2012) or subfamily level (Mazón 2016) have proved to be useful surrogates in Hymenoptera. Although family level may not be such
a good surrogate for species diversity than other lower taxonomic levels (Balmford et al. 1996), it can provide important ecological information, especially when grouped into functional groups (Bragança et al. 1998).

When looking at assemblage composition, samples were grouped according to conservation level rather than to reserves, although samples were very separated one from another, showing that assemblages, even in the same conservation level, were highly variable. The type of disturbance may be influencing these results, since it will affect the way the ecosystem responds to it (Jones and Schmitz 2009; Pandit et al. 2018), and therefore insect communities will also change accordingly. However, that variable was not considered in this study, so it is difficult to draw those conclusions. On the other hand, most of the assemblages from conserved areas were very similar amongst themselves except for the Tapichalaca reserve, that was clearly separate from the others, indicating the Andes may be acting as an ecological barrier for these insects, even at a local scale.

Regarding overall functional diversity, it was significantly higher in the low conservation areas of two reserves. Although it can not be seen as a consistent pattern, open areas may serve as corridors for insects (Mazón and Bordera 2014; Gutiérrez-Chacón et al. 2020) connecting well-conserved forests, and therefore these more disturbed areas would have more diverse assemblages. Also, by being embedded in a conserved matrix, these areas may be acting as ecotones, which may harbor a higher diversity of Hymenoptera than the forests, as seen for ants (Coelho and Ribeiro 2006), bees (Polatto and Alves 2020; Theodorou et al. 2020) and parasitoids (Rossetti et al. 2013). However, the occurrence of insects in these ecotones would be limited by some traits as the presence of trees (especially for nesting bees and wasps, Polatto and Alves 2020) or the dispersal ability of each species (Hamm and Drossel 2017), therefore functional diversity based on the genus or species level, with more detailed functional groups, would provide a better information.

Ecological restoration is an effective way to recover the structure and function of ecosystems, but comprehensive monitoring should be carried out in order to investigate if its functions are becoming similar to those from a healthy well conserved reference ecosystem. Here we show promising results for parasitoid wasps, but not so for bees. It would be interesting to see what happens when identification goes to genus or species level, and whether trends observed for families are persisting for these lower taxonomic levels.

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