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**SPECIES RICHNESS  
OF NEOTROPICAL PARASITOID WASPS  
(HYMENOPTERA: ICHNEUMONIDAE)  
REVISITED**

by

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## LIST OF ORIGINAL PAPERS

This thesis is based on the following publications and manuscript which are referred to in the text by their Roman numerals:

- I** Veijalainen A, Sääksjärvi IE, Erwin TL, Gómez IC & Longino JT (2012, published online) Subfamily composition of Ichneumonidae (Hymenoptera) from western Amazonia: insights into diversity of tropical parasitoid wasps. *Insect Conservation and Diversity*. doi: 10.1111/j.1752-4598.2012.00185.x
- II** Veijalainen A, Sääksjärvi IE, Broad GR, Bordera S, Jussila R & Longino JT. – Productive short-term tropical inventory clarifies altitudinal trends of species richness in ichneumonid parasitoid wasps (Hymenoptera). Manuscript.
- III** Veijalainen A, Wahlberg N, Broad GR, Erwin TL, Longino JT & Sääksjärvi IE (2012) Unprecedented ichneumonid parasitoid wasp diversity in tropical forests. *Proceedings of the Royal Society B* 279: 4694-4698.
- IV** Veijalainen A, Broad GR, Wahlberg N, Sääksjärvi IE & Longino JT (2011) DNA barcoding and morphology reveal two common species in one: *Pimpla molesta* **stat. rev.** separated from *P. croceipes* (Hymenoptera, Ichneumonidae). *ZooKeys*, 124, 59–70.

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	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
Original idea	AV, IES	AV, IES	AV	AV, IES
Field work	IES, TLE, ICG	JTL	TLE, JTL	JTL
Preparation of material	AV, IES, ICG	AV	AV	AV
Identification of specimens	AV, IES, ICG	IES, GB, SB, AV, RJ	AV, GB	AV, GB
DNA analyses	-	-	AV, NW	AV, NW
Other analyses	AV	AV	IES	-
Writing of the manuscript	AV	AV	AV, NW, GB	AV, GB
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# 1. INTRODUCTION

It has taken over three billion years for life on Earth to evolve to such high complexity that we see today as biodiversity. At the same time, modern human behaviour is reducing biodiversity at an alarming pace, and the world's biota is facing its sixth mass extinction (Barnosky et al. 2011). Encouragingly, humans attempt to conserve biodiversity for its intrinsic value and for the ecosystem services biodiversity provides for our survival and well-being (Cardinale et al. 2012). For nature conservation to be successful, it must be based on extensive and multidisciplinary scientific knowledge. In ecology, it is particularly the numerous small organisms of hyperdiverse groups found in remote locations that preclude us from forming a more complete understanding of the natural world. This dissertation is a tribute to the tropical “little things that run the world”, which so often go overlooked by the human eye.

## 1.1 Obscurity of species diversity and distribution

Some major shortfalls hampering our understanding of biodiversity carry the names of famous past naturalists (see Cardoso et al. 2011). First, the term Linnean shortfall refers to how few of the co-inhabitants of our planet have been formally described and given a scientific identity. The Wallacean shortfall, on the other hand, describes the incomplete understanding of species' geographic distribution ranges as extensive areas of the globe remain unexplored. The Prestonian shortfall highlights the dynamic nature of biological systems; the commonness and rarity of species and their changes in space and time are largely unknown. Lastly, the Hutchinsonian shortfall refers to the inadequate understanding of the multiple ways of life on this planet and of how resources limit the distribution and abundance of species. These aspects influence how we understand the complexity of life on this planet.

The seemingly simple question “how many species are there on Earth?” is in fact a particularly tricky one to answer. One significant theoretical problem is to define what a species is. Despite being a fundamental unit in all biological studies, there is no one all-encompassing theoretical definition for species (Wheeler & Meier 2000). A more practical problem is that simply counting the species on Earth is not possible because a majority of the world's species have not yet been discovered and formally described – nor is it clear how many species are currently described (Erwin 1982, Chapman 2009, Mora et al. 2011). After nearly 300 years of taxonomic work and about 1.7 million currently described species (see IUCN 2009), at least 10,000 (May 2000), if not closer to 20,000 new species are still being described annually. At the same time, numerous described species are being synonymised every year (e.g., Jussila et al. 2010). Estimates of the total global number of species vary hugely between 3–100 million (May 2010), 8.7 million total species being one of the latest estimates (Mora et al. 2011). A majority of the unknown species are invertebrates, especially insects and their relatives (Chapman

2009). The question then becomes: “how to accurately *estimate* the total number of species on Earth?”

Some significant variables in estimating species numbers are species’ level of specialisation and turnover of species composition in space and time (Erwin 1982, Novotny et al. 2007, Stork 2007, Dyer et al. 2007, Hamilton et al. 2010). Again, scientific knowledge to reliably indicate the correct values is seriously wanting. Many species records are from few point observations, impeding the determination of species’ habitat requirements and extent of occurrence (Nelson et al. 1990, Schulman et al. 2007, Puhakka et al. 2011). While new species are still continually being described from relatively well-studied regions such as Europe (Fontaine et al. 2012), it is particularly deep-sea, soil, and tropical rain forest ecosystems (especially the forest canopies) that are massively undersampled environments, entitling them to be referred to as the “last biotic frontiers” (Erwin 1983, André et al. 1994, Lamshead & Boucher 2003). Paradoxically, species richness of most terrestrial groups of organisms is highest (Gaston 2000), and the rate of habitat conversion is alarming (Laurance & Peres 2006), in the inadequately studied tropical rain forests.

## **1.2 Large-scale patterns of parasitoid species richness**

Insecta is one of the most species rich groups of organisms, and those with a parasitoid lifestyle have become exceptionally successful (Gauld et al. 2002, see Hamilton et al. 2010). Parasitoids are insects whose larvae develop by feeding in or on other arthropods (usually other insects), which results in the death of the parasitoid’s host (Godfray 1994). Parasitoids are species rich in the orders Hymenoptera (bees and wasps) and Diptera (flies), and a few are encountered in, e.g., Coleoptera (beetles), Neuroptera (net-winged insects) and Trichoptera (caddisflies). Following from their biology, parasitoids play important roles in trophic interactions and food web dynamics as they influence or regulate the population densities of their hosts. Their close and typically specialised association with their hosts make parasitoid populations dependant on the abundance and distribution of suitable hosts, which also makes parasitoid species particularly vulnerable to different levels of extinctions if host availability drops significantly (Shaw & Hochberg 2001, Hance et al. 2007). From the human perspective, the biological control that parasitoids provide is an important ecosystem service, and accordingly, parasitoids have become widely applied in pest control in agriculture and forestry (Heraty 2009). However, the potential benefit of biological control can be only partially exploited in human endeavours since most parasitoid groups are taxonomically and ecologically poorly known – especially in the tropics.

The species rich parasitoid wasp taxa Chalcidoidea and Braconidae are known to be well represented in tropical regions (Noyes 1989, Wharton et al. 1997, Horstmann et al. 1999), but intriguingly, most species of the large family Ichneumonidae have been sampled and described from temperate rather than tropical regions (Owen & Owen

1974, Janzen 1981, Skillen et al. 2000, Jones et al. 2012; but see Gauld 1987, Sääksjärvi et al. 2004a). In particular, the ichneumonid fauna of tropical rain forest canopies have been claimed to be scarce (Noyes 1989, but see Horstmann et al. 1999, Querino et al. 2011). The latitudinal diversity distribution of Ichneumonidae is puzzling because many arthropod groups that ichneumonids utilize as hosts show their highest species richness in the tropics (Robbins & Opler 1997, Höfer & Brescovit 2001, Erwin et al. 2005, but see Kouki et al. 1994). Despite the family's proclaimed poor representation in the tropics, Ichneumonidae may still be the largest of all animal families with over 100,000 estimated species worldwide (Gauld et al. 2002; Yu et al. 2005: >23,000 described species). However, recent studies highlight that sampling and description biases have likely led to underestimating the true tropical ichneumonid diversity (Sääksjärvi 2003, Santos & Quicke 2011, Quicke 2012). There is thus great need to address the species richness of tropical Ichneumonidae more accurately.

Even though exact estimation and mapping of biodiversity is not feasible in the near future, it has long been clear that species richness is unevenly distributed among taxa and over geographical areas at local to global scale. The latitudinal species richness gradient is the longest recognized large-scale ecological pattern (Wallace 1878, Hawkins 2001), followed by extensive investigations on other species richness gradients, e.g. altitudinal and benthic. Generally, species richness decreases with increasing latitude, altitude, and depth. However, still after centuries of active research on latitudinal differences in species richness, there is no consensus on the factors creating and maintaining higher tropical versus temperate diversity in most groups of organisms. Hypotheses proposed to explain the pattern can be classified under climatic, ecological, evolutionary, historical, and spatial themes (Erwin 1985, Hillebrand 2004, Willig 2004, Wiens & Graham 2005, Mittelbach et al. 2007, Condamine et al. 2012). Understanding why some groups of organisms are more species rich than others, and why some groups do not follow the typical latitudinal species richness gradient, can ultimately help us define evolutionary processes that create and maintain biodiversity.

The parasitoid wasp family Ichneumonidae is a suitable study group because it is extremely species rich, yet a classic example of a group with an anomalous latitudinal species richness gradient. The family's origin dates back to the Early Cretaceous (Grimaldi & Engel 2005), and the groups within Ichneumonidae have different evolutionary histories (see e.g., Townes 1963). For example, Labeninae has a Gondwanan origin (Gauld & Wahl 2000), Pimpliformes has likely arisen in the northern temperate regions (Wahl & Gauld 1998), and the pantropical pimpline *Camptotypus* genus group is an example of a group that originated and radiated in South America, from where different lineages have dispersed to the Old World and Australia (Sääksjärvi et al. 2004b). Porter (1978, 1980) recognised three major evolutionary centres for Neotropical ichneumonids: the southeast Brazilian subtropical and tropical wet forests, Andean subtropical and tropical cloud forests, and the Mesoamerican tropical, subtropical and warm-temperate wet forests, while claiming that the Amazon Basin and the West Indian faunas are comparatively depauperate. Quicke et al. (2009) recently constructed a large phylogeny

of the Ichneumonidae based on morphological and molecular data for about 1,000 exemplar taxa.

Over time, different ichneumonid lineages have evolved to exploit various host groups, especially Lepidoptera (butterflies and moths), Coleoptera, Diptera, other Hymenoptera, and Araneae (spiders). Koinobiont ichneumonid species generally allow the host to continue its development after the wasp's oviposition and they have become specialised to tolerate or overcome (e.g., via polydnviruses) the host's immunological and chemical defences, whereas the more generalist idiobionts paralyze the host permanently in order to circumvent its immune system (Askew & Shaw 1986, see Santos & Quicke 2011). Tropical species are generally considered to be more specialised than their temperate counterparts, but it is not completely understood whether this also holds for Ichneumonidae (Gauld et al. 1992). That particularly koinobiont ichneumonids are supposed to be more species poor at lower latitudes has been ascribed to limitations emerging from host predation, location and utilization. The hypotheses proposed to explain the anomalous gradient were recently reviewed by Santos and Quicke (2011), and they are summarized in Table 1.

Later, high tropical species richness has been discovered in certain ichneumonid groups, which has prompted speculation over the true nature of the family's latitudinal gradient. Particularly some idiobiont and nocturnal koinobiont taxa have been found to be at their most species rich in tropical areas (Gauld 1987, Sääksjärvi et al. 2004a), but the assumption of especially low koinobiont ichneumonid species richness is still persistent. However, relatively little sampling and description effort has been addressed on tropical versus temperate ichneumonid species (Horstmann et al. 1999, Sääksjärvi et al. 2004a, van Noort 2004). Nearly 30 years since the first notions of the anomalous gradient were reported (Owen & Owen 1974), it was proposed that large scale patterns of parasitoid insects may be artefacts due to biases in species sampling and description, and that we know too little about the distributions of ichneumonid species to draw any conclusions about their latitudinal trends in species richness (Santos & Quicke 2011, Quicke 2012). However, these recent studies were based on the world Ichneumonoidea database records (Yu et al. 2005) and did not include any primary data on tropical ichneumonids. Similarly, Townes and Townes (1966) had already identified the high diversity and the lack of knowledge of Neotropical ichneumonids by stating that the species known so far comprised about 10% of the total Neotropical ichneumonid species richness. However, it took almost 40 years before the first tropical South American study locality was included in the latitudinal diversity gradient studies of the family (Sääksjärvi et al. 2004a). In summary, tropical ichneumonids are poorly known, and multiple latitudinal species richness trends may exist within Ichneumonidae depending on the taxon's biology and evolutionary history.

**Table 1.** Hypotheses proposed to explain low tropical ichneumonid (especially koinobiont) diversity (modified from Santos & Quicke 2011).

Hypothesis	By	Presumption	Prediction
<b>Resource fragmentation</b>	Janzen & Pond 1975, Janzen 1981	Host population densities are too low in the diverse tropics to support specialist species (koinobionts).	Tropical parasitoids are more generalist (idiobionts) or better at finding suitable hosts.
<b>Predation on hosts</b>	Rathcke & Price 1976	Juvenile mortality of groups parasitizing larval herbivores (many koinobionts) is higher in the tropics as predation on hosts is higher in the tropics.	Parasitoids on other host groups (e.g., pupal herbivores, concealed hosts) are more successful in the tropics (many idiobionts).
<b>Predation on parasitoids</b>	Gauld 1987	Due to low host population densities, koinobionts spend more time than idiobionts searching for scarce suitable hosts in the tropics.	Koinobionts will be more exposed to predation than idiobionts in the tropics.
<b>Interphyletic competition</b>	Eggleton & Gaston 1990	Diversity of other parasitic organisms is higher in the tropics than temperate areas.	Competition for hosts is higher in the tropics resulting in reduced parasitoid diversity.
<b>Nasty host</b>	Gauld et al. 1992, Gauld & Gaston 1994	Tropical plants are more toxic than temperate plants. Tropical herbivores obtain secondary chemicals from plants. Thus tropical parasitoids on herbivores must be specialised to tolerate host chemical defences.	Parasitoids attacking exposed hosts or chemically well-protected life-stages (many koinobionts) are more affected by the chemicals than those attacking concealed hosts or less-protected life-stages (many idiobionts).

### 1.3 Identification of tropical parasitoid wasp diversity

Correct species identification underpins good ecological studies. Ichneumonidae is generally regarded as a taxonomically challenging group. One factor significantly limiting tropical ichneumonid studies is the lack of user-friendly and geographically extensive identification keys. The Costa Rican Ichneumonidae is a good starting-point for morphological identification of Neotropical ichneumonids (however, the revisions do not cover all subfamilies and many South American species are not found in Costa Rica; Gauld 1988, 1991, 1997, 2000, Gauld et al. 1998, 2002) and among other works on Neotropical ichneumonids (e.g., Dasch 1974, Porter 1970, 1978, 1980, 1998). To overcome the limitations of species identification and to gather data for ecological studies, one can identify specimens first to genera, and then to “morphospecies”, i.e., one may create hypothetical groups of species based on their external characteristics

without formally assigning the specimens to species. However, the description of new Neotropical ichneumonid genera is frequent (Sääksjärvi et al. 2003, Palacio et al. 2007, Broad et al. 2011, Khalaim et al. 2012), demonstrating that the work on revealing the region's true ichneumonid diversity is in progress.

Integrating morphological species recognition with molecular methods can accelerate biodiversity inventories and lead to species identifications. The most widely used molecular recognition method in animals is DNA barcoding of the c. 600 bp mtDNA sequence of *cytochrome oxidase subunit I* (COI). If the DNA has become very fragmented because, for example, the specimen is old or the sample has been preserved in weak alcohol, it is possible to use shorter, e.g., 300 bp sequences (DNA mini-barcoding). Right after the launch of the method about ten years ago (Hebert et al. 2003), DNA barcoding was welcomed with excitement by the scientific community. DNA barcoding is a global enterprise with a desire to tackle the taxonomic impediment and ultimately the biodiversity crisis, however, the critics suspect the mission may fall short of its ambitious goals if it fails to follow the quickly developing fields of molecular taxonomy and systematics (Taylor & Harris 2012). DNA barcoding has been discovered to assist species separation successfully in many, but not all (Elias et al. 2007, Whitworth 2007, Dasmahapatra 2010), animal taxa. In tropical parasitoid studies, integrating molecular and morphological (and sometimes ecological) species delineation has facilitated revealing complexes of cryptic species (two or more distinct species classified as one due to morphological similarity; e.g., Smith et al. 2008). The method can assign ichneumonids to subfamilies fairly reliably (Quicke et al. 2012), but it is still little studied whether DNA barcoding can significantly promote separating and quantifying the frequency of cryptic tropical ichneumonid species.

#### **1.4 Aims of this study**

My all-embracing goal was to study whether additional research can reveal reservoirs of unknown ichneumonid diversity in Neotropical forests. I wanted to address which ichneumonid groups might be abundant but greatly underestimated in the Neotropical region, particularly in western Amazonia and Central America, and to study how the subfamily and species compositions varied over geographic areas. I was also interested in investigating how large an ichneumonid community could be found in the diverse western Amazonian lowland rain forest canopies – which have never been studied extensively for ichneumonids – and what new taxonomic and ecological insights short-term sampling in the Central American mountains might provide. Last, my intention was to investigate whether molecular species delineation methods (DNA barcoding of COI) could assist in the separation of large ichneumonid samples to species. I approached these topics in four case studies:

- I** I compare ichneumonid subfamily compositions in western Amazonia, Central America, and southern United States to demonstrate which subfamilies may be species rich but understudied in the Neotropics. Surprisingly, some subfamilies

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consisting of mainly small-bodied species or koinobiont parasitoids of Lepidoptera were relatively equally abundant in tropical and temperate areas. These subfamilies would be interesting for future latitudinal ichneumonid species richness gradient studies in the Western Hemisphere because their Neotropical species richness may be greatly understudied.

- II** I study altitudinal trends of species richness and interesting taxonomic findings in a geographically extensive but temporally small dataset from Central America. I conclude that such sampling may boost the description of new species remarkably, but addressing large-scale ichneumonid diversity patterns should make use of representative samples of the actual local ichneumonid fauna obtained through extensive spatial and temporal sampling.
- III** I study whether high abundance of individuals corresponds to a high number of species in orthocentrine samples from Amazonian Ecuador and Central America. I further investigate whether molecular and morphological species separation methods group the specimens similarly. The results show that the species richness of some ichneumonid groups is radically underestimated in the Neotropics.
- IV** I demonstrate how subtle morphological differences some ichneumonid species have, and how molecular species separation methods can aid in finding these differences. I conclude that integrating molecular and morphological methods is recommended when studying the true species richness of tropical ichneumonids.

## 2. MATERIAL AND METHODS

I made use of material from previous biodiversity inventories targeting either ichneumonids (I. E. Sääksjärvi and I. C. Gómez) or general biodiversity (T. L. Erwin) in western Amazonia, or which had been collected as a part of a leaf-litter arthropod sampling programme in Central America (J. T. Longino/Project LLAMA; Table 2). The Central American study area is located in one of Porter's (1980) evolutionary centres, while the western Amazonian study areas lie somewhat in the ecotone of the supposedly depauperate (for ichneumonids) Amazon Basin and the Andean evolutionary centre. For additional data, I referred to previously published records of Ichneumonidae subfamily abundances in the southern United States and Costa Rica (Gaasch et al. 1998, Shapiro & Pickering 2000; case study I), and used natural history museum collections (BMNH: Natural History Museum, London, NMNH: Smithsonian Institution, National Museum of Natural History, Washington, DC, and ANSP: Academy of Natural Sciences, Philadelphia, PA) to confirm species identifications and to understand the range of intraspecific morphological variation in different species (III, IV). (See Fig. 1.)

**Table 2.** The primary data sets: collectors, study areas and specific locations, original focus groups, collecting methods, sample sizes (MTM: Malaise trap months), and case studies where the data sets were investigated.

	<b>LLAMA</b>	<b>Erwin</b>	<b>Sääksjärvi</b>	<b>Gómez</b>
<b>Area</b>	Guatemala, Honduras, Nicaragua	Orellana (Ecuador)	Loreto (NE Peru)	Madre de Dios (SE Peru)
<b>Place</b>	25 sites	Onkone Gare, Tiputini	National Reserve of Allpahuayo Mishana	Los Amigos Conservation Concession
<b>Habitat</b>	Sites at 20–2625 m	Lowland rain forest	Lowland rain forest	Lowland rain forest
<b>Focus</b>	Leaf-litter arthropods	General biodiversity	Ichneumonidae: Pimplinae, Rhyssinae	Ichneumonidae: Pimplinae
<b>Method</b>	Malaise trapping	Canopy fogging	Malaise trapping	Malaise trapping
<b>Size</b>	15 MTM	1079 foggings	185 MTM	27 MTM
<b>Study</b>	II, III, IV	I, III	I	I





**Figure 1.** Areas studied for ichneumonid data. Primary data were sampled at areas 2 and 4–6, and additional published records (Gaasch et al. 1998, Shapiro & Pickering 2000) were reported from 1 and 3.

## 2.1 Study region

The Neotropical ecozone is a biogeographic region typically classified to encompass all of South and Central America, the Caribbean, and parts of southern Mexico, and in some categorizations, also the southern parts of Florida (USA). The region includes a great variety of environments from high mountain ranges and vast areas of lowland rain forests to remote oceanic islands. Some areas of the Neotropics are exceptional in terms of very high species richness (e.g., western Amazonia; Gentry 1988, Bass et al. 2010) and high levels of endemism (e.g., the Andes and the Cordilleras; Myers et al. 2000, Laurance et al. 2011).

The distinct geological histories of Central and South America explain some of the faunal and floral differences in their biota. The Amazonian landscapes and ecosystems have been significantly influenced by the Andean uplift. The subduction of the oceanic Nazca tectonic plate under the South American plate has resulted in the rise of the Andes; erosion and river dynamics have deposited Andean mineral material across the Amazon Basin supporting the formation of very species rich communities especially in western Amazonia (Hoorn et al. 2010). On the other hand, what probably was first a range of oceanic volcanic islands gradually became the Isthmus of Panama: as the Pacific plate slid under the Caribbean plate, water-borne sediment started accumulating between the

islands, and the isthmus closed about three million years ago facilitating the interchange of northern and southern taxa (Cody et al. 2010).

## **2.2 Study areas and field sampling**

Specimens were collected by Malaise traps and insecticidal canopy fogging. Most of the original studies were designed to meet the needs of ecological research and used standardized sampling: Project LLAMA focused on investigating, e.g., altitudinal differences in species composition, Erwin's research aimed at understanding the impacts of road-building and consequent changes in the rain forest canopy fauna, and Sääksjärvi examined the impacts of habitat heterogeneity on parasitoid community structure. Making use of material collected by standardized sampling enables a wider range of research topics to be studied than museum specimens which are usually point observations lacking detailed documentation of, e.g., the habitat and climate.

The most commonly used method to sample ichneumonids and other parasitoid Hymenoptera is Malaise trapping (Fig. 2), while other methods are also applied especially to catch certain targeted groups (e.g., canopy fogging, pan traps, light traps). Malaise traps are typically set up at ground-level on supposed insect flight routes. They are tent-like flight-intercept traps; the flying individual faces a vertical wall typically made of thin black mesh textile, moves up towards light, is funnelled by a diagonal roof-like construction usually made of white mesh textile, and eventually falls into a collecting chamber placed in the uppermost corner of the trap. Malaise traps are usually emptied every week or fortnight.

The canopy fogging method is based on fogging rain forest vegetation with pyrethroid insecticide; the pyrethroid enters arthropod tissues causing the individuals to die and fall down onto sheets spread near the ground (Figs. 3, 4; Lucky et al. 2002, Erwin et al. 2005). There have been contradictory results published concerning the efficacy of canopy fogging for collecting tropical ichneumonids. Noyes (1989) found canopy fogging to be an excellent means of sampling most Hymenoptera – except Ichneumonidae and Aculeata – present in the canopy of a tropical forest in Indonesia. Perhaps those two groups were poorly represented in the canopy, or, the method failed to collect them (possibly because these species are typically strong fliers). However, Horstmann and others (1999, 2005) collected ichneumonids successfully in the lowland rain forests of Malaysia, suggesting that ichneumonids do occur in tropical rain forest canopies, and that they can be sampled successfully by the canopy fogging method.



**Figure 2.** Malaise trap. Photo: I. C. Gómez.



**Figure 3.** Rain forest canopy near Tiputini Biological Station, Ecuador.



**Figure 4.** T. L. Erwin and M. C. Pimienta collecting canopy arthropods with insecticidal canopy fogging. Photo: C. J. Geraci.

### 2.2.1 Guatemala, Honduras, and Nicaragua: Project LLAMA sites

Samples were collected as part of the Leaf Litter Arthropods of Mesoamerica (LLAMA) project in Guatemala, Honduras and Nicaragua, during the transition from dry to wet season in May–June of 2009, 2010 and 2011, respectively. The project had also collected samples in Mexico in 2008, but those samples were excluded from my studies. The project placed emphasis on sampling in evergreen wet forest habitats across multiple altitudes, and the 26 sites consisted of multiple vegetation types, e.g., primary and secondary lowland rain forests, cloud forest, and old plantations. The main goal of the project was to collect leaf litter arthropods by Winkler samples along two transects at each site, but additional sampling methods, e.g., Malaise traps and beating of vegetation, were also applied. The project visited each site for 3–7 days, and typically set up four ground-level Malaise traps in the vicinity of the transects for this period, usually at forest edges or in small clearings. I studied ichneumonids from 25 sites (no Malaise traps were set up in Pico Bonito, Honduras) at 20 to 2625 m.a.s.l. collected by Malaise traps. The total sample size was 436 Malaise trap days (MTD), or approximately 15 Malaise trap months (MTM). I sorted the specimens to subfamilies and of the Orthocentrinae (*Orthocentrus* genus group; Figs. 5, 6) and some Pimplinae (e.g., *Pimpla*) to morphospecies, while a number of ichneumonid specialists, S. Bordera (Cryptinae), G. R. Broad (Campopleginae and Orthocentrinae: *Helictes* genus group), R. Jussila (Mesochorinae), and I. E. Sääksjärvi (remaining groups excluding some Ichneumoninae, Tersilochinae and Tryphoninae: *Netelia*), contributed to the morphospecies identification of the rest of the material.

### 2.2.2 Ecuador: Onkone Gare and Tiputini Biological Station

The two Ecuadorean study sites, Onkone Gare (OG) and Tiputini Biological Station (TBS), are located near Yasuni National Park, Orellana Province (formerly part of Napo Province), in the Amazonian lowland rain forests of eastern Ecuador. The Yasuni region is acknowledged to be one of the most biodiverse areas in the entire world, however, the active petroleum industry in the area is considered a threat to the area's biodiversity (Bass et al. 2010). Each of the sites was 1 ha in size and they are located 21 km apart from each other. The vegetation in and between the sites consists of primary lowland rain forest (Fig. 3). The climate is somewhat aseasonal with some changes in the amount of precipitation. The samples were collected in three times of the year (wettest: January–February, driest: July, and transition: September–October) across five field work years during 1994–96 (OG), 1998–99 (TBS), and 2005–06 (OG).

T. L. Erwin and his team have collected altogether 2100 canopy fogging samples from the two sites in general exploration of biodiversity (Lucky et al. 2002, Erwin et al. 2005), and 1079 of them were available for my studies (Fig. 7). The study areas in both OG and TBS were 100 m × 1000 m in size and included ten 10 m × 100 m transects. Within each of these transects, ten collecting sheets each 9 m<sup>2</sup> in size were placed at the same spot on every sampling event. Each canopy fogging sample was obtained with a single fogging from a column of vegetation on the collecting surface. In the middle of each collecting sheet, a jar filled with 80% ethanol was placed in the middle and underneath it as large



funnel. A two-hour drop time was allowed before sample harvesting. The sampling was carried out in the early morning hours (4–5 a.m.) when air currents are nil or relatively low in tropical rain forests and most insects least active.



**Figure 5.** A specimen of *Orthocentrus* sp. 57 (see III).



**Figure 6.** *Orthocentrus* sp. 57 face (III).



**Figure 7.** Sample of canopy Hymenoptera.

### 2.2.3 Peru: National Reserve of Allpahuayo Mishana and Los Amigos Conservation Concession

I studied samples from two Peruvian sites: the National Reserve of Allpahuayo Mishana (NRAM) in the north-east of the country, and Los Amigos Conservation Concession (ACC) in south-eastern Peru. NRAM (ca. 57 000 ha) is located about 25 km southwest of the large city of Iquitos, department of Loreto. The inventories were conducted by I. E. Sääksjärvi to study the ichneumonid subfamilies Pimplinae and Rhyssinae for his

dissertation project (Sääksjärvi 2003, Sääksjärvi et al. 2004, 2006). The area is lowland tropical rain forest terrain where seasonality is low and the forests are extremely heterogeneous in structure and species composition (Whitney & Alvarez 1998). The NRAM area is composed of various rain forest types growing on clayish, white sand or loamy soils that differ from each other in physiognomy and floristic composition (Kalliola & Flores Paitán 1998). There were four different sampling areas, each containing several rain forest types. In total, five Malaise traps were placed in each study area: two in forests on clayish to loamy soils, and three in forests on nutrient poor white sand soil with differing humidity and forest structure. The NRAM sampling was conducted in August 1998–January 1999, and in January 2000–January 2001. The total sample size was 185 MTM: 44 in 1998 and 141 in 2000.

The other Peruvian site, ACC (ca. 146 000 ha), is located in the department of Madre de Dios; it is also a lowland rain forest area where the soil types show a mosaic of clayey, sandy, silty and gravel materials. The climate is humid and seasonal with a pronounced 3–4-month dry season in June–September and wet season in October–May. I.C. Gómez carried out the sampling as a part of his M.Sc. thesis project. Nine Malaise traps were set up mainly in primary forests, some in secondary forests, and the distance between the two most distant traps was approximately five kilometres (I. C. Gómez, pers. comm.). The traps were run in the dry season of May–July, 2008, making the total sample size 27 MTM.

### **2.3 Species delimitation, identification, and species richness estimation**

Morphological identification was used to sort the specimens to subfamilies (case study **I**), morphospecies (**II**, **III**), or described species (**IV**). In papers **III** and **IV**, specimens were compared to type material or to photographs of type material of described species to confirm their identity, and in paper **II**, morphospecies were compared to described species from Costa Rica. I used molecular species recognition methods in case studies **III** and **IV**. In the former, I tried to form an understanding of how similar were the results of morphological and molecular species recognition, and in the latter, I studied how subtle morphological differences may be in genetically clearly distinct species. I applied DNA barcoding to search for differences in the mtDNA COI sequences. In the laboratory, I first removed three legs of one side from each specimen and extracted their DNA. Next, I amplified the COI gene by PCR with the primers LCO and HCO (DNA barcoding; ca. 600 bp), or K698 and K699 (DNA mini-barcoding; ca. 300 bp). The cleaning and sequencing of the successful PCR products was conducted by Macrogen (South Korea). I edited and aligned the sequences in BioEdit, and lastly, constructed the neighbour-joining trees in MEGA 5.05 to illustrate the genetic distances between different haplotypes (Saitou & Nei 1987).

When evaluating the sufficiency of sampling effort, I calculated a species accumulation curve by studying the number of observed species as a function of sampling effort using EstimateS 8.2.0. A curve reaching an asymptote would indicate that additional sampling

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would not add many new species to the species list, i.e., the sampling effort had been adequate to collect most of the species of the area (Gotelli & Colwell 2011). When studying tropical biodiversity inventory data sets, sampling is virtually never complete because tropical communities are so diverse and many species are rare. In these cases, when the species accumulation curve does not reach an asymptote, i.e., the total number of species is not known because not all the species were sampled, species richness can still be estimated using, e.g. non-parametric estimators (bearing in mind that the estimation becomes more inaccurate as, for example, study area and complexity increase; Gotelli & Colwell 2011). In EstimateS, appropriate estimators for abundance data (number of individuals observed for a species in a sample) are ACE, Chao1 and jackknife 1 and 2 (Jack1 and Jack2), while estimators requiring replicated incidence data (a species is detected or undetected in a sample) are Chao2 and ICE (Gotelli & Colwell 2011).

### **3. RESULTS AND DISCUSSION**

This dissertation demonstrates that an abundant and largely undescribed ichneumonid fauna resides in the forests of Central America and western Amazonia. I discovered the Orthocentrinae is generally the most abundant neotropical koinobiont ichneumonid subfamily and a massively underestimated group in terms of tropical species richness. A number of other subfamilies are also very abundant and presumably species rich in the Neotropical region, including many koinobiont groups parasitizing exophytic herbivores which have traditionally been thought to be poorly represented in the tropics. Many taxonomically and biogeographically interesting findings can be made for Neotropical ichneumonids with relatively small sampling-effort, but extensive and long-term sampling is recommended for studies attempting to elucidate large-scale ichneumonid species richness patterns. On a number of occasions, I encountered groups of individuals where morphological differences were too subtle to readily separate individuals to species, but which molecular data recognized as distinct genetic clusters. These findings revise the directions for future tropical ichneumonid studies.

#### **3.1 Neotropical ichneumonid subfamilies**

My studies made use of a large dataset: I included over 30,000 ichneumonid individuals from western Amazonia, and over 3,700 from Central America. The individuals belonged to 23 subfamilies – 20 subfamilies in both data sets – and 17 subfamilies were collected in both western Amazonia and Central America. In case studies **I** and **II**, I studied ichneumonid subfamily compositions across different latitudes and altitudes to demonstrate how their diversity is distributed over temperate and tropical areas. In summary, the groups that are abundant in lowland tropical areas may significantly influence the understanding of large-scale diversity patterns in Ichneumonidae and the importance ichneumonid species have in tropical food-web dynamics.

Paper **I** clarifies which ichneumonid subfamilies are abundant and expectedly species rich in the lowland Neotropics and particularly in western Amazonia. The large sample size gives reason to expect that the data represent relative western Amazonian ichneumonid subfamily abundances well (however, some groups may be under represented due to the collecting methods used, or the patchy distribution of specific micro-habitats, e.g., dead wood-material; cf. Ophioninae, Labeninae). Contrary to the common assumption, a number of ichneumonid subfamilies are very abundant in the western Amazon Basin. Most subfamilies accounted for similar proportional abundances in the three study areas (OG and TBS, NRAM, ACC), suggesting the subfamily composition is fairly alike in different parts of the region. Despite similar relative subfamily compositions, the species compositions are likely to be different between the areas because western Amazonia supports a mosaic of multiple rain forest types and a variety of different habitats. Sampling efficiency and the distribution of the subfamilies probably explain why few



subfamilies were not found in all three western Amazonian study areas, or why some were slightly under or over-represented in one area compared to the other two. When expanding the subfamily composition comparison to lowland forests of Costa Rica and the south-eastern USA, I detected more variation in the subfamilies present, yet many subfamilies still showed rather similar proportional abundances across the areas.

In paper **I**, the most notable result was the high Amazonian abundance of many koinobiont subfamilies, especially of those parasitising Lepidoptera (Anomaloninae, Banchinae, Campopleginae, Cremastinae, Ichneumoninae, Metopiinae), which have generally been considered relatively poorly represented in tropical lowland rain forests. Also, the subfamilies dominated by small-bodied species (Mesochorinae, Orthocentrinae, Tersilochinae) are abundant, likely species rich, and unquestionably understudied in the Neotropics (see also **III**). The study gave further support to earlier speculation; some idiobiont ectoparasitoid groups are diverse in the tropics (e.g., Cryptinae, Pimplinae), and some subfamilies that are relatively common in temperate areas (e.g., Ctenopelmatinae, Tryphoninae, Diplazontinae, Xoridae) are rare in the lowland Neotropics probably because their hosts are centred in temperate environments.

In paper **II**, I expanded the study area from lowland forests to an altitudinally wide study area, and from equatorial latitudes to Central America. I found that the abundance of Central American ichneumonids seems to be generally highest at middle and high elevations, and together with the well-studied Costa Rican ichneumonid fauna (Gauld 1988, 1991, 1997, 2000, Gauld et al. 1998, 2002), these studies suggest that elevational trends vary at the subfamily level. Further, many of the subfamilies that were found to be more abundant in temperate latitudes in paper **I** seem to be centred in highlands rather than lowlands in Central America. Particularly for Ctenopelmatinae and Diplazontinae (and the majority of Tryphoninae), additional research in the tropical Western Hemisphere is unlikely to discover such high, currently unknown species richness that it would challenge the impression of an anomalous latitudinal diversity gradient in these subfamilies.

Concluding from the subfamilies' different altitudinal distributions in Central America, some subfamilies that are common in the South American highlands are not necessarily encountered in large numbers in lowland Amazonia, and vice versa. This is supported by material collected from other western Amazonian localities. While Neotropical diplazontine diversity is centred in the highlands (Gauld 1997), I discovered one diplazontine individual (*Woldstedtius* sp.n.) when going through T. L. Erwin's lowland rain forest canopy samples from north-eastern Peru (Loreto: Sucusari). Also, C. Castillo's field work in the Peruvian Andes has sampled single individuals of Poemeniinae and Acaenitinae usually encountered in the temperate areas in the Western Hemisphere, but apparently never before in western Amazonia (Castillo et al. 2012). These findings suggest that although the subfamilies are generally restricted to temperate environments, some lineages have diverged and species have adapted to areas and living conditions atypical of the subfamily. Which ichneumonid groups have temperate origins, which of their lineages (e.g., genera) have successfully exploited new niches in tropical

areas, and which attributes have enabled these niche shifts, are fundamental questions for future tropical ichneumonid studies.

### 3.2 Neotropical ichneumonid species richness and distribution

In papers **II** and **III**, I studied ichneumonids identified to the species level. The former study covered nearly all sampled ichneumonid taxa, i.e., 2,843 Central American individuals identified to 750 morphospecies in 19 subfamilies, and the latter included 1,549 Central American and Ecuadorean orthocentrine individuals identified to 177 morphospecies. In paper **II**, I examined whether short-term sampling collects more species in highland than lowland areas and whether the species show a general tendency to be restricted to certain altitudes. In paper **III**, I familiarized myself with the unknown species as I addressed the numbers of undescribed and cryptic orthocentrine species in the samples. I further investigated differences in species compositions between study areas (**III**) to form a preliminary idea of species' habitat preferences and the extent of their distribution ranges. Answering these questions about ichneumonid species numbers and distribution ranges may advance our understanding of the family's diversity in different Neotropical environments and in the region as a whole.

Even a rapid inventory like that carried out in Central America can produce taxonomically very informative Neotropical ichneumonid material (**II**). Discoveries of just a few Neotropical specimens may require re-definitions of the morphological characteristics of ichneumonid genera (Palacio et al. 2010), and even subfamilies (Broad et al. 2011). However, addressing large-scale ichneumonid diversity patterns is a complex task that should make use of representative samples of the actual local ichneumonid fauna obtained through spatially and temporally extensive sampling. These observations are certainly not new for tropical arthropod studies in general (see Coddington et al. 2009), or ichneumonid studies in tropical and temperate regions specifically (Sääksjärvi et al. 2004, Fraser et al. 2008). However, the importance of extensive sampling is worth highlighting here because the classic impression of low Neotropical ichneumonid diversity relies largely on incomplete point-observation records rather than on large-scale sampling programmes (Porter 1980, Gupta 1983, Yu et al. 2005; excluding Gauld and others' work in Costa Rica, Sääksjärvi et al. 2004 in north-eastern Peru). Large-scale species richness patterns remain obscure especially for those groups observed to be abundant in middle and low elevation tropical forests (**I**, **II**), which should encourage future research to emphasise the lowland Neotropical ichneumonid fauna.

When focusing only on Orthocentrinae and expanding the species composition comparison over a wider geographical region from Central America to Amazonian Ecuador, I discovered that the study areas had few species in common, both faunas were species rich, and they were comprised exclusively of undescribed species (**III**). The 177 morphospecies are over 12 times the the 14 currently described Neotropical, over three times the 48 tropical, and even more than the total 151 currently described Nearctic orthocentrine species (Yu et al. 2005). Some morphospecies also contained potential

cryptic species not easily distinguishable using morphological features (see below 3.3). Neotropical orthocentrine diversity will not total 177 species; the species number estimators indicated higher total local diversity for both study areas, and the sampling covered only a small fraction of the spatial and temporal habitat complexity. There is also reason to expect Orthocentrinae to be diverse and understudied in the tropics of other continents. These findings give no evidence of an anomalous trend of decreased orthocentrine diversity in the tropics. On the contrary, they indicate that orthocentrine species richness may in fact be higher in tropical than north temperate areas of the Western Hemisphere. However, there is also the possibility that species richness of some orthocentrine genera has been underestimated in the temperate and sub-arctic regions too, further obscuring any latitudinal gradients in species-richness.

Such drastic underestimation of tropical Orthocentrinae diversity may be largely explained by the species' inconspicuous habitus and a lack of taxonomic expertise in the group. Parasitoids of Diptera are generally a very large sector of parasitoid diversity which has been overlooked (Jussila et al. 2010), including in the Neotropics; for example, Arias-Penna et al. (2012) describe 24 new Neotropical species of *Trichacis* Foerster (Hymenoptera: Platygasteridae) from only 145 individuals. Like Orthocentrinae, the species of *Trichacis* are presumed to be koinobiont endoparasitoids of Diptera, but attacking gall midge larvae (Diptera: Cecidomyiidae) rather than Sciaroidea (the presumed hosts of most orthocentrines). Costa Rican Orthocentrinae have not been revised but such work might give a boost to incorporating Orthocentrinae into ecological studies of the region.

### 3.3 Integrating species identification methods

The results of papers **III** and **IV** confirm that integrating multiple identification methods can greatly aid the discovery of morphologically very similar ichneumonid species. In paper **III**, I obtained COI sequence data for 226 individuals of 57 morphospecies to estimate cryptic species diversity in the sample. For 14 morphospecies there were deep genetic divergences, with individuals of single morphospecies clustering in two or three groups, potentially containing a total of 31 species not easily distinguishable using morphological features. The results of paper **III** demonstrate that morphologically similar but molecularly distinct species are not uncommon in Neotropical ichneumonid data. In paper **IV**, I took one step further and used COI data to help separate one species into two; the pimpline species *Pimpla croceipes* Cresson (Ichneumonidae: Pimplinae) was discovered to be *P. croceipes* and *P. molesta* **stat. nov.** First, COI sequence data helped divide the 77 sequenced Guatemalan and Honduran Project LLAMA specimens into two clusters that were genetically deeply divergent. When expanding the study to cover altogether 361 specimens (Project LLAMA and BMNH collections), some subtle morphological characters separating the two species could be confirmed. The study demonstrated that even relatively large, common, and widespread co-occurring ichneumonid species may have been defined incorrectly if the identification was based

solely on morphological identification methods. These observations encourage the application of molecular species identification methods along with morphology in future ichneumonid studies.

Paper **IV** was based only on the Honduran and Guatemalan LLAMA project samples. I received samples from Nicaragua after completing the study and found that they included 45 individuals of *P. croceipes* but not a single individual of *P. molesta*. The morphological characters listed in paper **IV** apply well in distinguishing the species when considering also the Nicaraguan material. This observation, together with the fact that *P. molesta* was shown to occur on average at somewhat different altitudes than *P. molesta*, suggest that the species have somewhat dissimilar geographic distribution ranges (however, the sampling periods were too short to detect all the species of the areas, see **II**). The species were also collected in exactly the same samples. This sympatric occurrence confirms that the species' morphological and molecular differences are not just intraspecific variation between distinct populations of one species, but that they are actually two distinct entities.

Concluding whether the observed differences represent intra- and interspecific variation may be difficult if the specimens are not sampled from the same localities. For example, slight differences in the molecular or morphological structures may represent variation between geographically distant populations and not between two species. In situations like these, the identity of the species might be confirmed with more extensive data (e.g., multiple genes and/or specimens from intermediate localities). Another problem may arise if morphologically similar individuals show relatively high intraspecific variation in the COI sequence. For example, in paper **III**, I identified *Chilocyrtus* sp. 15, *Gnathochoris* sp. 3, *Orthocentrus* sp. 44 as distinct morphospecies supported by both morphological and molecular data, but alternatively, the individuals might represent complexes of morphologically similar yet molecularly different cryptic species. These uncertainties in determining correct species boundaries address the practical difficulty of deciding when the "barcoding gap" (a threshold to define distinct species in the neighbour-joining tree; Wiemers & Fiedler 2007) is significant with the COI data. Thus, increasing sample size and sampling intensity, and including additional information on, for example, sampling localities and hosts, may give new informative insights into determining correct species boundaries.

Any improved species identification methods are good news for accelerating biodiversity research and for understanding hyperdiverse, taxonomically challenging and poorly known groups such as Ichneumonidae. DNA barcoding is currently the most widely applied molecular method in species identification. However, for informative results, COI sequence data has to be compared with morphological data, which again requires taxonomic expertise. Genetic research methods are advancing rapidly, and soon new molecular species identification methods may prove more efficient than DNA barcoding (Shendure & Ji 2008). Adopting the most effective research methods would certainly be an asset when trying to understand the diversity of ichneumonids, and in a broader context, when battling biodiversity loss and taxonomic shortfalls.

### 3.4 Some challenges for uncovering the true extent of tropical ichneumonid diversity

The results presented here indicate which ichneumonid subfamilies would be interesting for future tropical studies and highlight the need for integrating different species identification methods in obtaining correct data. While these advances are important, there is still plenty of work to do before ichneumonid diversity can be understood well at different spatial and temporal scales. The difficulty in establishing species richness patterns of Ichneumonidae and understanding species' roles in ecological interactions probably has a lot to do with the relative lack of taxonomic expertise and scarcity of research projects given the family's large size. The taxonomic impediment – generally referring to the lack of trained personnel and funding to discover and comprehend the unknown biodiversity – is widely acknowledged as hampering biodiversity conservation and management. Next, I will briefly discuss how the Linnean, Wallacean, Prestonian and Hutchinsonian shortfalls mentioned in 1.1. are linked with the results presented here and with ichneumonid research in a wider context.

#### 3.4.1 *The unknown nature of species, their distribution ranges, and level of specialisation*

Most species are undescribed (the Linnean shortfall), and a demonstrative example of that is the startling number of undescribed orthocentrines discovered in paper **III**. If detecting morphologically distinct species was not demanding enough, addressing which species are actually cryptic complexes complicates the matter even further (**III**, **IV**). Studies integrating morphology, DNA barcoding, and ecological data, have revealed morphologically very similar parasitoid species complexes from both tropical and temperate regions (Smith et al. 2008, Fernandez-Triana et al. 2011). Whether cryptic parasitoid species are prevalent enough to significantly raise the already high estimates of the total number of ichneumonid species remains to be seen.

Because species distributions are mostly unknown (the Wallacean shortfall), basic ecological data are limited for ichneumonids when focusing only on described species. Further, previously gathered ecological information may be difficult to trace back to correct species when species are shown to be complexes of several species (**IV**), or to have been misidentified (often the case in poorly characterized faunas). Tropical (koinobiont) parasitoid species are generally regarded as highly specialised in exploiting certain resources (cf. the nasty host hypothesis; Gauld et al. 1992, Dyer et al. 2007), which would indicate that their potential to occupy wide geographic areas is limited. Papers **II** and **III** give some insights into the distribution of tropical ichneumonids. Paper **II** supports previous observations for Costa Rica (see above) that relatively few ichneumonid species may occur across a wide altitudinal gradient, while paper **III** indicates that relatively few orthocentrine species may be shared over large Neotropical areas, namely between Central America and western Amazonia. Information on species distributions is necessary for understanding their habitat preferences and estimating total species richness.

Level of specialisation is one of the key variables when estimating the number and distribution of ichneumonid and other parasitoid species in temperate and tropical regions. As noted above, tropical parasitoids are generally regarded as highly specialised to exploit certain resources. However, host records are available for very few tropical ichneumonid species, and thus the level of specialisation in tropical ichneumonids is largely unknown (cf. the Hutchinsonian shortfall). Parasitoid host data are often gathered as a part of Lepidoptera rearing projects (e.g., Costa Rica: D. H. Janzen and L. A. Dyer; Ecuador: L. A. Dyer; South East Asia: V. Novotny; Uganda: H. Roininen; and a newly started project focusing on Ichneumonidae in north-eastern Peru: I. E. Sääksjärvi). Because rearing is laborious and produces information mainly about the groups parasitizing larval Lepidoptera, molecular methods such as DNA barcoding of the parasitoid gut contents have been employed to gather parasitoid host data to increase the accuracy of food web studies (Rougerie et al. 2011).

Some tropical ichneumonid studies have investigated the level of specialisation of species to certain habitats (cf. the Hutchinsonian and Prestonian shortfalls). Sääksjärvi et al. (2006) did not find the mainly idiobiont Pimplinae and Rhyssinae ichneumonids to be clearly specialised to particular forest types in north-eastern Peru. At OG and TBS – which represented high beta diversity in tree species composition – the observed orthocentrine fauna shared many species (39 out of 95 morphospecies were collected at both sites; **III**). These findings encourage the tentative proposal that spatial turnover of ichneumonid subfamilies may not be as high as that of certain plant groups in western Amazonia, however, other biologically different ichneumonid groups must naturally be studied before drawing general conclusions about the spatial distribution of ichneumonids in the tropics. Aridity, or scarcity of humid and shady forested habitats, has been proposed several times to affect ichneumonid diversity at different spatial scales, as discussed in paper **I**. Also, as many ichneumonid species are strong fliers, they may move some distances between different forest types but prefer only some for reproduction. In summary, future ichneumonid research should embrace multiple tropical rain forest types and vegetation strata to reveal how ichneumonids are distributed spatially.

In Paper **I**, I showed that koinobiont ichneumonids parasitising Lepidoptera are relatively abundant in western Amazonia. Because high abundance is often an indication of a high number of species in tropical ichneumonid studies (Coddington et al. 2009), the koinobiont ichneumonid parasitoids of Lepidoptera can thus be assumed also to be species rich in the samples. This assumption is supported by the results of paper **III** where the 1,078 Ecuadorean orthocentrines (koinobiont endoparasitoids of Diptera) included a minimum of 95 morphospecies, 27 of them being singletons (28%) and 18 doubletons (19%). Koinobiont endoparasitoids of Lepidoptera are a classic example of ichneumonids thought to have an anomalous latitudinal diversity gradient (Sime & Brower 1998). If their tropical diversity were proven to be remarkably higher than assumed, it would require a re-evaluation of the role of specialisation in the evolution of high species diversity and distribution patterns of Ichneumonidae, and ecological ichneumonid studies in general.

### 3.4.2 Turnover of species composition in space and time

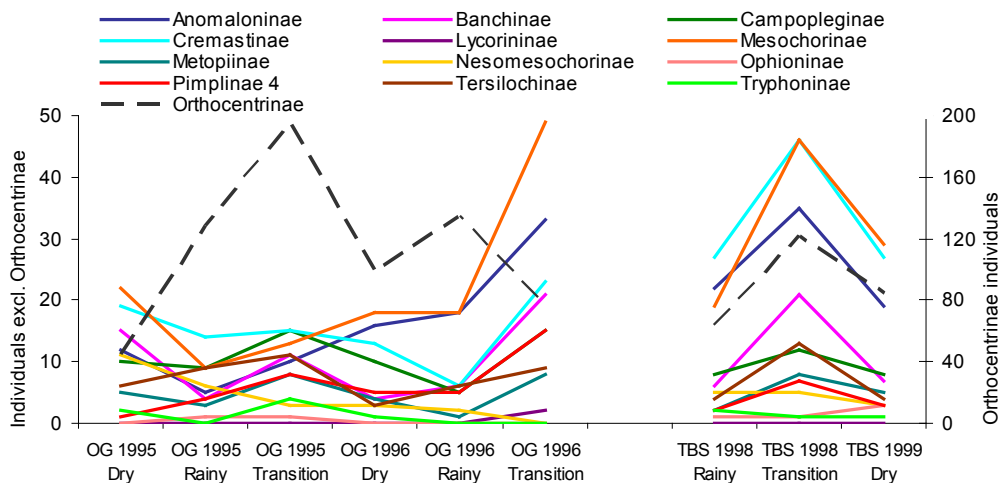
Yet another aspect complicating ecological research in general is the dynamic nature of biological systems; the abundance of ichneumonid species and their spatial and temporal turnover is largely unknown (cf. the Prestonian shortfall). Most neotropical ichneumonids are either rare or rarely collected, as even extensive sampling produces only a few specimens of most species (Sääksjärvi et al. 2004). The sampling of the Ecuadorean canopy fauna included multiple replicates, and still the number of singleton and doubleton orthocentrine specimens remained quite high (see above). However, as in any fauna, there are still some commonly encountered species, such as the characteristic pimplines of paper **IV**. In the Ecuadorean Orthocentrinae data there were also some relatively very abundant morphospecies, like *Orthocentrus* sp. 57 (Figs. 5, 6) which comprised 11% of the total 491 *Orthocentrus* genus group female specimens. Since the goal in paper **III** was to identify the *minimum* number of morphospecies, further research (e.g., sequencing additional genes) might separate some of these abundant morphospecies into multiple species.

The Prestonian shortfall takes into account the temporal aspect of species turnover, which is little studied for tropical ichneumonids. The few available studies on tropical ichneumonid seasonal flight activity suggest that seasonal rainfall may affect the numbers of observed ichneumonids, their community structure, and trophic interactions, possibly through resource availability (Owen & Chanter 1970, Gauld 1991, Shapiro & Pickering 2000). Interestingly, months of peak abundance differ somewhat for koinobionts and idiobionts (Gauld 1991), and ecologically different subfamilies (Shapiro & Pickering 2000), perhaps because the necessary host stages for koinobionts are more temporally limited than for idiobionts (idiobionts typically attack late instar larvae or pupae, which may stay in that stage until the conditions become more favourable). In Costa Rica, the majority of koinobionts are active at the beginning of the rainy season, while most idiobionts appear slightly later in the season and remain active into the dry season (Gauld 1991). What the situation is for ichneumonids in the less seasonal equatorial Amazonia has not previously been demonstrated.

To study whether similar temporal patterns as in Costa Rica pertain also for the less seasonal western Amazonia, for koinobionts and idiobionts, I observed seasonal differences of abundance for 20 subfamilies (Pimplinae was further divided into four biologically distinct groups, see Sääksjärvi et al. 2004; thus 23 parasitoid groups) in about one half of the Ecuadorean OG and TBS canopy fogging material (2,882 individuals). Most parasitoid groups peaked in abundance in the transition season (transition: 16, driest: 5, wettest: 1, wettest and transition equally abundant: 1). Koinobiont individuals were also most abundant in transition season in each study year (transition 862, wettest 554, driest 516 individuals), and less clearly so for idiobionts (transition 346, driest 320, wettest 284). Koinobiont abundance was most often highest in the transition season especially in the OG 1996 and TBS 1998–99 study years (Fig. 8), whereas that of idiobionts did not usually peak in any season, or only for the larger parasitoid groups which were mainly endoparasitoids of concealed Lepidoptera

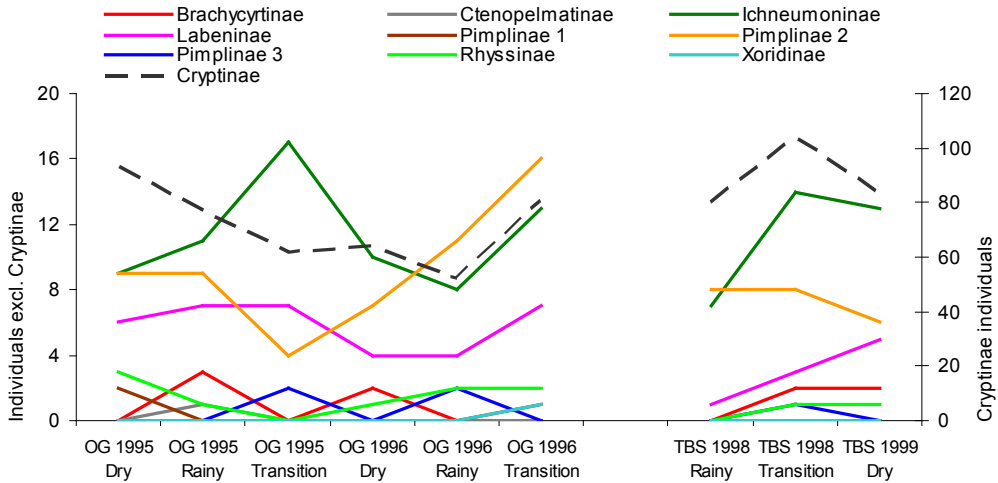
(Ichneumoninae, Pimplinae 2: represented in the data by *Neotheronia*, *Xanthopimpla*, and non-endoparasitoid *Camptotypus* genus group; Fig. 9). Interestingly, contrary to most koinobiont groups, the abundance of the Orthocentrinae drops in the OG 1996 transition season (Fig. 8). Orthocentrines parasitise fungivorous Diptera that feed on organic material in moist habitats, and their rareness in the OG 1996 samples may be due to the El Niño Southern Oscillation (ENSO) causing drought in that particular period (see Lucky et al. 2002, Horstmann et al. 2005, Shapiro & Pickering 2000). Overall, the results suggest that Ichneumonidae diversity exhibits seasonal patterns also in the equatorial rain forests where seasonality is low, and parasitoid abundances may be influenced by host availability.

Gauld (1991) suggests that koinobionts synchronise their active periods with their mainly herbivorous larval hosts. The more generalist idiobionts, many of which have wider host range and are pupal parasitoids, would remain active for a longer period of time. In Owen & Chanter's (1970) study, the highest numbers of ichneumonids coincided with highest amount of average rainfall and highest numbers of Lepidoptera, one of the most common host groups of Ichneumonidae. Both of the studies propose that adult ichneumonids are observed in highest numbers when their food availability is increased. When hosts are not available, ichneumonids would pass the unfavourable period in dormancy or as juvenile stages. In general, recognising how insect abundances change temporally is important for arriving at correct conclusions about species diversity and ecology. Studying the western Amazonian canopy ichneumonid subfamily composition suggests that tropical ichneumonid diversity is best understood through inventories that extend over multiple years and seasons.



**Figure 8.** For several koinobiont parasitoid groups, the observed abundances increase in the transition seasons. Orthocentrinae abundance is illustrated on the secondary y-axis.



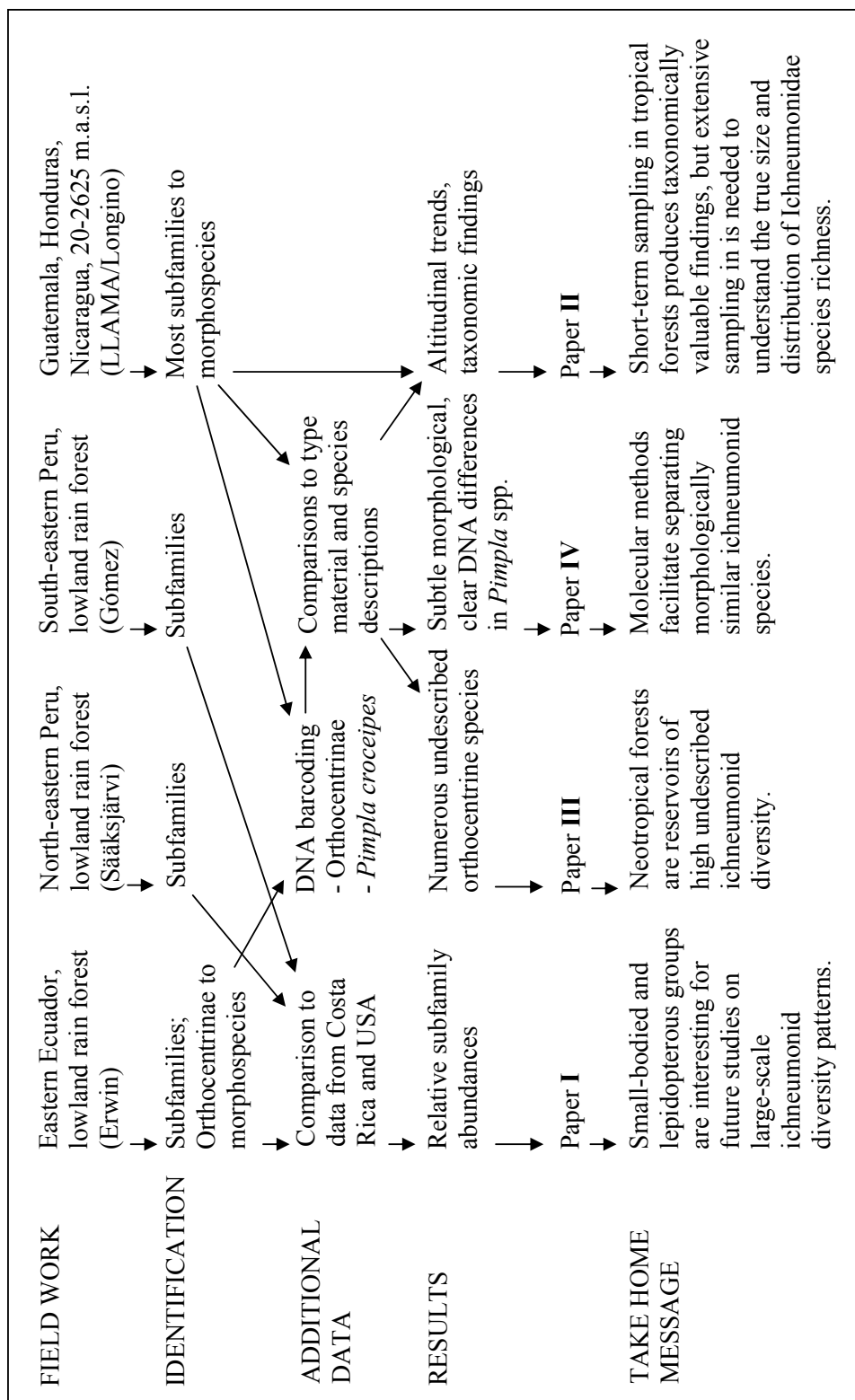


**Figure 9.** Observed idiobiont abundances are not significantly higher in the transition seasons. Cryptinae abundance is illustrated on the secondary y-axis.

### 3.5 Conclusions and future directions

In this dissertation, I have shown that additional research in Neotropical rain forest environments can increase the number of known tropical ichneumonid species remarkably (Fig. 10). Some of the most important findings were that: 1) Neotropical ichneumonid species richness is probably most radically overlooked for certain small-bodied and koinobiont groups that parasitise Lepidoptera; 2) western Amazonian lowland rain forest canopies support a species rich ichneumonid fauna; and 3) integrating morphological and molecular species identification methods is advisable for attaining correct estimates of tropical ichneumonid diversity. The results also support some wider conclusions and guidelines for future research discussed below.

The tropical Ichneumonidae fauna is likely to be much more species rich than is currently reported, and samples from the species rich primary tropical rain forests ought to be incorporated into future temperate-tropical comparisons. As the family is of enormous size, encompasses a great variety of parasitoid life history strategies, and all lineages have distinct evolutionary histories, it seems best to investigate the latitudinal species richness gradient of Ichneumonidae with smaller clades or functional groups within the family. Once large-scale diversity patterns become better known at the subfamily or biological group level, it will be easier to distinguish factors creating and maintaining them. I suggest that the subfamilies potentially exhibiting a regular latitudinal diversity gradient in the western Hemisphere are the small-bodied Mesochorinae, Orthocentrinae and Tersilochinae, and the koinobiont lepidopteran parasitoids of the Anomaloninae, Banchinae, Campopleginae, Cremastinae, Ichneumoninae and Metopiinae, but only future research can clarify this supposition. Just collecting primary data on these groups in the tropics is not sufficient, but the results should be compared with reliable and representative data from temperate regions and with biologically distinct ichneumonid groups.



**Figure 10.** An illustration of the PhD research project.

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Overall, the results presented here call for a rigorous evaluation of the true diversity, distribution, and ecological importance of Ichneumonidae in the tropics and worldwide, ideally using spatially, temporally and methodologically extensive standardised sampling and latest research methods. Studies should embrace, for example, multiple biogeographical regions, tropical rain forest types, vegetation strata, years, seasons, collecting methods, and identification techniques. Host records are vital for exposing the level of specialisation and food web dynamics. Morphologically very similar species complexes may bias ichneumonid study results to some degree if cryptic diversity is not addressed by, particularly, molecular species delimitation methods. The widely applied DNA barcoding can give a general overview of ichneumonid diversity in a sample but due to the limitations of the procedure, more advanced genetic methods may prove more useful in the future. Finally, using “residual” material from biodiversity inventories directed at other taxa can facilitate the discovery of tropical arthropod diversity. Increasing anthropogenic pressure on natural tropical environments encourages the adoption of new, productive research methods and the prompt addressing of the true extent of tropical ichneumonid diversity at different scales.

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