UNIVERSITY OF TURKU

ON THE PARASITOID WASPS OF THE AFRICAN TROPICAL FOREST

and how their species richness compares to Amazonia

Tapani Hopkins

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. AII OSA – TOM. 378 | BIOLOGICA – GEOGRAPHICA – GEOLOGICA | TURKU 2021

UNIVERSITY OF TURKU

ON THE PARASITOID WASPS OF THE AFRICAN TROPICAL FOREST

and how their species richness compares to Amazonia

Tapani Hopkins

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. AII OSA – TOM. 378 | BIOLOGICA – GEOGRAPHICA – GEOLOGICA | TURKU 2021

University of Turku

Faculty of Science
Department of Biology & Biodiversity Unit
Doctoral Programme in Biology, Geography and Geology (BGG)

Supervised by

Ilari E. Sääksjärvi
Biodiversity Unit
University of Turku
Finland

Heikki Roininen
Department of Environmental and
Biological Sciences
University of Eastern Finland
Finland

Gavin R. Broad
Department of Life Sciences
The Natural History Museum
London
United Kingdom

Reviewed by

Seraina Klopfstein
Natural History Museum Basel
Switzerland

Marko Mutanen
Ecology and Genetics Research Unit
University of Oulu
Finland

Opponent

Atte Komonen
Department of Biological and
Environmental Science
University of Jyväskylä
Finland

The originality of this publication has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

Cover Image: Trap CCT1 in Uganda. Own image.

ISBN 978-951-29-8361-2 (PRINT)
ISBN 978-951-29-8362-9 (PDF)
ISSN 0082-6979 (Painettu/Print)
ISSN 2343-3183 (Sähköinen/Online)
Painosalama Oy, Turku, Finland 2021

To all God's creatures on Earth and beyond;
past, present or future;
and especially to those who would learn to know them.

TURUN YLIOPISTO
Matemaattis-luonnontieteellinen tiedekunta
Biologian laitos & Biodiversiteettiyksikkö
TAPANI HOPKINS: On the parasitoid wasps of the African tropical forest – and how their species richness compares to Amazonia
Väitöskirja, 137 s.
Biologian, maantieteen ja geologian tohtoriohjelma (BGG)
Helmikuu 2021

Tiivistelmä

Planeettaamme asuttavat miljoonat eliölajit, joista useimpia ei vielä ole löydetty. Tämä ällistyttävä lajirunsaus on korkeimmillaan tropiikissa, ja trooppiset hyönteiset muodostavat siitä suuren osan. Maailman kenties lajirikkain eläinheimo, ahmaspistiäiset (Hymenoptera: Ichneumonidae), tunnetaan erityisen huonosti tropiikissa. On jopa epäilty sen olevan poikkeuksellisen vähälajinen tropiikissa.

Väitöskirjaani varten keräsin ahmaspistiäisiä vuoden ajan lukuisilla Malaisepyydyksillä Ugandan trooppisessa metsässä. Tarkastelin erään alaheimon (porapistiäiset, Rhyssinae) lajirunsautta, ja selvitin miten lajit jakautuvat eri metsätyyppeihin sekä milloin ne lentävät. Lisäksi vertasin lajirunsautta Amazonin sademetsään, jossa oli kerätty ahmaspistiäisiä samalla menetelmällä.

Pyydykset saivat vuoden aikana saaliiksi kuusi porapistiäislajia. Näistä kaksi oli tieteelle uusia. Nämä tulokset (ja erään aiemman keräyksen tulokset) osoittavat että Afrikassa elää runsas ja suurelta osin tuntematon loispistiäislajisto.

Porapistiäisiä saatiin Ugandassa eniten sateettomalla säällä, lahopuun lähellä koskemattomassa metsässä. Tämä viittaa siihen, että ne loisivat lahopuussa eläviä toukkia. Saimme myös viitteitä siitä, kuinka pitkään ne elävät aikuisena. Tropiikista saadaan harvoin tällaista tietoa, ja suosittelenkin menetelmieni käyttämistä myös muiden trooppisten pyydystysten yhteydessä.

Verratessani kahta trooppista aluetta jotka sijaitsevat eri mantereilla sain viitteitä siitä, että vertailukohtana toimiva Amazonin sademetsän alue olisi afrikkalaista tutkimusaluettanikin lajirunsaampi. Kummatkin alueet vaikuttivat kuitenkin kuuluvan samaan lajirunsauden jatkumoon. Suosittelen vastaavaa pyydystystä useammalla tutkimusalueella. Näin selviäisi, ovatko ahmaspistiäiset lajirikkaita koko tropiikissa, ja onko Amazonin sademetsä lajirikkaammasta päästä.

Keruumenetelmämme, jossa lukuisia Malaisepyydyksiä käytetään vuoden ajan, antoi runsaan aineiston. Se tuotti tietoa erään trooppisen metsän lajistosta, lajien elintavoista ja siitä miten ne vertautuvat toisen mantereen vastaaviin lajeihin. Olemme vasta pääsemässä alkuun tropiikin ällistyttävän monimuotoisten ahmaspistiäisten kartoittamisessa.

Avainsanat: Lajirikkaus, lajirunsauden maantieteellinen jakautuminen, Ichneumonidae, Malaisepyydys

UNIVERSITY OF TURKU
Faculty of Science
Department of Biology & Biodiversity Unit
Tapani Hopkins: On the parasitoid wasps of the African tropical forest – and how their species richness compares to Amazonia
Doctoral Dissertation, 137 pp.
Doctoral Programme in Biology, Geography and Geology (BGG)
February 2021

Abstract

Our planet is inhabited by an astounding diversity of species, most of which have not yet been discovered. This species richness is at its highest in the tropics and particularly in tropical insects. The tropical Darwin wasps (Hymenoptera: Ichneumonidae), which represent one of the largest animal families on Earth, are especially poorly known. So much so, that doubts persist as to their being species rich in the tropics.

In this PhD, I collected Darwin wasps in tropical forest in Uganda, using large numbers of Malaise traps for a year. I then examined the species richness of one of the subfamilies (Rhyssinae), and also investigated the phenology and habitat use of the species. I compared the species richness of my Ugandan site with that of Amazonia, which had been sampled using the same methods.

A year of Malaise trapping discovered six rhyssine species in Uganda. Two of these species were new to science. These results, together with the results of previous sampling at the site, clearly demonstrated that there is a diverse Darwin wasp fauna still awaiting discovery in Africa.

Ugandan rhyssines were caught in largest numbers in dry weather, near decaying wood in primary forest. The results suggested they prey on wood-boring larvae, and also gave an indication of their adult lifespan. This kind of data is rarely obtained in the tropics, and I suggest applying the same methods to the results of other sampling programs.

Comparing the rhyssines of two tropical forest sites on different continents suggested that the Amazonian site I compared to may have even more species than my African site. However, Amazonia did not stand out as anomalously diverse. I suggest collecting Darwin wasps from further sites using the same methods. This would answer the question of whether or not Darwin wasps are species rich throughout the tropics, with Amazonia being among the more diverse areas.

Using large numbers of Malaise traps for a year gave a wealth of information on what species were present at a tropical site, how they lead their lives, and how they compare to their counterparts on another continent. Current work has only scratched the surface of the astounding diversity of tropical Darwin wasps.

Keywords: Species richness, diversity gradient, Ichneumonidae, Malaise trapping

Table of Contents

List of original publications

This dissertation is based on the following original publications. They are referred to in the text by their Roman numerals.

I Hopkins T, Roininen H & Sääksjärvi IE 2018. Assessing the species richness of Afrotropical ichneumonid wasps with randomly placed traps provides ecologically informative data.
African Entomology 26: 350–359. https://doi.org/10.4001/003.026.0350

II Hopkins T, Roininen H & Sääksjärvi IE 2019. Extensive sampling reveals the phenology and habitat use of Afrotropical parasitoid wasps (Hymenoptera: Ichneumonidae: Rhyssinae).
Royal Society Open Science 6: 190913. https://doi.org/10.1098/rsos.190913

III Hopkins T, Roininen H, van Noort S, Broad GR, Kaunisto K & Sääksjärvi IE 2019. Extensive sampling and thorough taxonomic assessment of Afrotropical Rhyssinae (Hymenoptera, Ichneumonidae) reveals two new species and demonstrates the limitations of previous sampling efforts.
ZooKeys 878: 33–71. https://doi.org/10.3897/zookeys.878.37845

IV Hopkins T, Tuomisto H, Gómez I & Sääksjärvi IE. A comparison of the parasitoid wasp species richness of Amazonian and African sites – subfamily Rhyssinae (Hymenoptera: Ichneumonidae).
Manuscript

1 Background and aims of the study

In 1746, Linné's pupil Christoffer Tärnström set sail in a Swedish East India Company ship towards China. It was the continuation of numerous short expeditions carried out by Linné and his pupils in and near Sweden. During the next decades, dozens of Linné's pupils were to explore the world and bring back huge collections of the Earth's plants and animals. Some, such as Turku student Pehr Kalm (Finland being a part of Sweden at the time), made their careers on their expeditions. Others, such as Christoffer Tärnström, never returned: he caught a fever when his ship was becalmed for half a year off the coast of what is now Vietnam. (Lehikoinen et al. 2009 pp. 376–387, Broberg 2019 pp. 303–311)

The aim of these expeditions has not changed during the past 274 years. It is to find out who we share our planet with. Linné and his pupils were fascinated by the world that the age of exploration had revealed, especially its plants and animals. Their work was continued by people like the Sahlbergs (expedition to Alaska and Siberia 1839-1843, then during the next hundred years Brazil, Siberia, the Middle East, North Africa, Caucasia, etc.: *Nordisk familjebok. 24. Ryssläder - Sekretär* 1916 pp. 345–347, Saalas 1952), Carl Gustaf Mannerheim (20 000 beetle species from all over the world, mainly through exchanges in the early 19th century: Muona 2004), and many others with links to Turku. The shelves at the Turku Natural History Museum are overflowing with specimens like these, a rich document of the plants and animals who have lived on our planet during the past centuries. Each and every one collected and painstakingly prepared by someone motivated by a childlike curiosity about the world and its inhabitants.

Almost 300 years after Christoffer Tärnström set sail for China, the methods have become easier but the goal is the same. We still do not know who we share our planet with. We still have a fascinating, unknown planet to explore.

1.1 How many species are there?

There are about 8.7 million eukaryote species on Earth (Mora et al. 2011). Or perhaps only 5 million, or even over 100 million (May 1988, May 2010). Estimates vary, but all agree on a simple but astounding fact: the vast majority of our planet's species are still undiscovered. Only about 14% of eukaryote species are believed to have been described (Mora et al. 2011). Nor are all these missing species obscure, tiny creatures in far-off places. Sweden, the country of Linné, whose nature is among the best documented in the world, still gives hundreds or even thousands of new species (Johansson & Cederberg 2019, Karlsson et al. 2020). Our museum in Turku has a monitor lizard one metre long, the holotype of a new species collected in the far off days of eight years ago (Weijola et al. 2016).

Millions of these species, perhaps even most of them, are insects (May 1986, Hamilton et al. 2010). There may be about a million described insect species (Chapman 2009); "may be", because there is currently no global centralised database that contains all described species (although the COL+ project is getting close: *The Catalogue of Life plus project* 2017/2020). There are millions more undescribed species, with tropical insects especially poorly known (Erwin 1982, May 1986, Hamilton et al. 2010). For most of the world's insects, we do not even know their species *exists*, let alone how they live their life or interact with other species.

This lack of knowledge of the species we share a planet with is not only, as Robert May put it, "embarrassing" (May 2010). It is also a grave problem, because species are one of the fundamental units of biology. When we predict how a forest will recover from logging, we predict what happens to its *species* (e.g. Daïnou et al. 2011). When we observe a male chimpanzee kill an infant (Arcadi & Wrangham 1999), we assume that we have observed more than the behaviour of just one individual; that the behaviour is partly generalisable to the *species* as a whole. The less we know about species, the less we are able to do in almost any field of biology.

1.2 More species in the tropics

Species are not evenly distributed on Earth. Instead, the Earth's species richness is distributed unevenly in different latitudes and habitats. Factors such as latitude and elevation, precipitation and temperature, and surface area all affect how many species an area has (Gaston 2000).

One of the earliest observed patterns was for species richness to increase towards the equator. Early explorers were astounded by the vast diversity of plants and animals they saw in the tropics (e.g. Vespucci 1503, although it is unclear if this was written by Vespucci himself), and by the late 18th century it seems to have been taken for granted that the tropics have the most species (Hawkins 2001). The debate on *why* there are more species the closer you get to the equator has continued ever since. Possible explanations can be roughly divided (Mittelbach et al. 2007) into *ecological* hypotheses, where the higher temperature, rain and/or productivity of the tropics give more niches for species (e.g. Humboldt et al. 1850 p. 217, numerous hypotheses summarised by Willig et al. 2003), *evolutionary* hypotheses, where species evolve faster or are less likely to go extinct in the tropics (e.g. Condamine et al. 2012), and *historical* hypotheses, where tropical species have had more time to develop or have not been wiped out by ice ages (Mittelbach et al. 2007). Many proposed explanations overlap these divisions (e.g. Wallace 1878 p. 66), and it is likely that more than one factor contributes to the pattern (Gaston 2000, Willig et al. 2003, Condamine et al. 2012).

The trend for more species at low latitudes is surprisingly general and seems to apply to most taxa or species groups. Trees (Novotny 2006), molluscs, snakes, dragonflies and mammals (Stevens 1989), ants, fishes and birds (Gaston 1996) and many other taxa seem all to be at their most species rich in the tropics. There are very few exceptions apart from the obvious ones, i.e. very small or specialised taxa ('It is probably not very profitable to dwell long on why there are not lots of kinds of penguins in the tropics', Gaston 1996). The few notable exceptions include aquatic plants (Hillebrand 2004), sawflies (Kouki et al. 1994) and Darwin wasps or ichneumonids (Owen & Owen 1974), and even some of them are doubtful (Quicke 2012).

1.3 Darwin wasps (or ichneumonids)

The Darwin wasps (family Ichneumonidae, also known as ichneumonids) are an extremely diverse group of parasitoid wasps found throughout the planet, on all continents except Antarctica. They are parasitoids who lay their eggs on or inside other arthropods; the egg later hatches and consumes the host alive. This makes them ecologically important, since they regulate the populations of other insects and spiders (Gauld & Bolton 1988, and e.g. Várkonyi et al. 2002, Morris et al. 2004, Roslin et al. 2013). They are also potentially useful indicators of what species are present in an area, since their numbers and diversity likely reflect that of their hosts and indirectly, the plants that many of the hosts feed on (May 1988, Sääksjärvi et al. 2006).

If insects are both extremely species rich and poorly known, Darwin wasps are even more so. They may number over 100 000 species, which would make Ichneumonidae the most species rich family on Earth (Gauld et al. 2002). About 24 000 – 25 000 species have been described (Bennett et al. 2019, Roskov et al. 2019). They are grouped into up to 42 subfamilies, but the exact limits of these subfamilies and how they are related is still unclear (Quicke et al. 2009, Bennett et al. 2019). Some of the subfamilies, such as Ctenopelmatinae and Tryphoninae, are commonly held not to be monophyletic and will likely some day be merged with other subfamilies (Bennett et al. 2019). A broad classification into three informal groups is often also used: Ichneumoniformes (including e.g. the subfamilies Ichneumoninae and Cryptinae), Ophioniformes (including e.g. the subfamily Ophioninae) and Pimpliformes (including e.g. the subfamilies Pimplinae and Rhyssinae)(Bennett et al. 2019, Klopfstein, Santos, et al. 2019), although not all species are included in these groups (Klopfstein, Langille, et al. 2019). The Darwin wasp fossil record stretches back about 150 million years, to the early Cretaceous or late Jurassic (Klopfstein, Santos, et al. 2019). Very little is known of the family's evolutionary history, but there are signs that it originated during the Early Jurassic, and that the pimpliforms originated during the Middle Jurassic and radiated during the early Cretaceous (Spasojevic et al. 2020).

Darwin wasps were long known as one of the most important exceptions to the latitudinal diversity gradient: it was proposed that they were at their most species rich in mid latitudes instead of the tropics (Owen & Owen 1974, Janzen & Pond 1975, Janzen 1981; but see Morrison et al. 1979 for an early critique). Over the next decades, a large body of literature either tried to explain this fascinating anomaly (Janzen & Pond 1975, Rathcke & Price 1976, Janzen 1981, Gauld 1987, Gauld et al. 1992, Sime & Brower 1998), or cited it as an interesting exception when trying to explain the general trend for highest species richness in the tropics (Gaston 1996, Gaston 2000, Willig et al. 2003, Hillebrand 2004, Mittelbach et al. 2007).

The later discovery of extremely species rich Darwin wasp faunas in Costa Rica (Gauld 1991, Gaston & Gauld 1993) and Amazonia (Sääksjärvi et al. 2004, Veijalainen et al. 2012) cast doubt on this anomaly. These discoveries were the result of extensive, long-term Malaise trapping, unlike the earlier results which were typically based on very small-scale sampling or patchy catalog data. The "anomalous latitudinal diversity gradient" of Darwin wasps may thus have been an artefact of insufficient sampling, and we currently do not have enough data to say how Darwin wasp species richness is distributed on Earth (Quicke 2012).

1.4 Malaise trapping

Malaise traps are tent-like traps that collect flying insects. They were invented by Swedish entomologist René Malaise (Malaise 1937, Vårdal & Taeger 2011), and have become one of the standard methods for collecting large numbers of insects with relative ease. They can be left in place for days or even weeks, and collect insects into a bottle typically filled with a killing agent and preservative such as ethanol.

The most common use for Malaise traps is in taxonomy. Typically, a small number of traps are used for some weeks or months (or at most a year), with the aim of sampling the species present in an area. The advantage of Malaise traps for this purpose is that they can collect a large sample size with relatively little effort. Darwin wasps have been sampled in this way throughout the world (e.g. Owen & Chanter 1970, Owen & Owen 1974, van Noort et al. 2000, Bartlett 2000, van Noort 2004, Barahoei et al. 2015, several sites listed in Timms et al. 2016, several sites listed in Gómez et al. 2017, Pham et al. 2018).

The problem with using only a small number of traps, and/or sampling for only a short time, is that the results often cannot be used to investigate the ecology of the species. Getting a representative sample of tropical Darwin wasps, for example, seems to require extensive long-term sampling (Sääksjärvi et al. 2004, Gómez et al. 2017). This is presumably due to many tropical Darwin wasps being seasonal (Gauld & Mitchell 1978, Shapiro & Pickering 2000) and their population sizes being small (or at least, they are rarely caught, Morrison et al. 1979, Sääksjärvi et al. 2004, Gómez et al. 2017). Also, a tropical inventory that only uses a few traps faces the challenge that tropical forests are a rich mosaic of different habitat types (Tuomisto et al. 1995, Whitney & Alonso 1998). Even outside the tropics, there is a need to use a large number of traps if wanting to e.g. compare different habitat types, since Malaise traps typically have very variable Darwin wasp catches even when in the same habitat (Fraser et al. 2008, Saunders & Ward 2018). Long-term Malaise sampling of Darwin wasps with large numbers of traps has only rarely been conducted in the tropics (Costa Rica, over 1200 trap months of which 576 were in the most sampled site: Gauld 1991, Costa Rica and Panama, about 190 trap months: Shapiro & Pickering 2000, Amazonia, 185 trap months: Sääksjärvi et al. 2004, Amazonia, at least 72 additional trap months: Gómez et al. 2017). The results have been used to investigate Darwin wasp phenology (Gauld 1991 p. 31, Shapiro & Pickering 2000) and the association between Darwin wasps and vegetation (Sääksjärvi et al. 2006). Outside of the tropics, extensive sampling has been conducted in e.g. Sweden (1785 trap months, albeit scattered throughout Sweden: Karlsson et al. 2020).

Malaise trapping can also be used to compare the species richness of different sites. This was done by the study that first suggested that Darwin wasps are relatively scarce in the tropics: Owen and Owen (1974) placed a trap in each of Uganda, Sierra Leone, England and Sweden, then compared the number of species (and diversity as measured by an index) caught in the four traps. Two other studies have attempted to compare the Darwin wasp species richness of different sites, although both got limited results. Timms et al. (2016) compared 38 datasets which had been collected by Malaise traps between latitudes 82°N and 25°S. On average, each dataset had been collected with four traps for a total of 22 trap months (Timms et al. 2015). Gómez et al. (2017) compared the data of 97 sites on three different continents. On average, each site had been sampled for a total of 34 trap months. Both of these studies found some interesting latitudinal patterns, but were unable to reach firm conclusions due to too low sample sizes. The latter study also suffered from variability in how the Malaise trapping had been conducted (e.g. the Ugandan site was sampled with unusually small traps). The general picture emerging from these studies is that most datasets collected by Malaise trapping cannot be used for comparing sites, due to too small sample sizes and a lack of standardisation. Although Malaise trapping has great potential for global comparisons of Darwin wasp species richness, it has not yet realised this potential.

The best solution to the lack of standardisation in Malaise trapping is to collect new datasets in a standard fashion. There are at least two ongoing projects doing just that. The Global Malaise Trap Program has sampled 158 sites in 33 countries with one Malaise trap at each site ('Global Malaise Trap Program' n.d.). The samples are sorted into orders then DNA barcoded. Project LIFEPLAN aims to sample at least 100 global sites, and 125 sites each in Sweden and Madagascar, with several methods including Malaise trapping ('Lifeplan' 2019). As in the Global Malaise Trap Program, there will be one trap at each global site and sample processing will be speeded up with DNA methods. The sampling period will be six years. Both of these projects are likely to get good, comparable data, which should allow them to e.g. make rough comparisons of the species richness of different latitudes. However, the use of only one trap at each site will prevent them from being able to compare the Darwin wasps of these sites at any finer scale – although the LIFEPLAN sampling of Sweden and Madagascar may give interesting results.

1.5 Aims of this study

In this study, I collected Darwin wasps with a large number of Malaise traps for a year in tropical Africa, using the same methods as earlier used in Amazonia (Sääksjärvi et al. 2004). There were three main aims of the study:

1. To find out what species are present at the African site.
2. To find out how the species live, i.e. what time of the year they are active, and what habitat they are associated with.
3. To compare the species richness of the two different tropical forest sites, which are located near the same latitude but on different continents.

By extending the earlier Amazonian inventories to tropical Africa, I hoped to get more data on how Darwin wasp species richness is distributed on Earth. I especially wanted to find out if the unexpectedly rich Amazonian Darwin wasp faunas are typical of the tropics.

Due to the large number of Darwin wasps I collected, I was forced to focus on one subfamily, the Rhyssinae. The results are thus not yet generalisable to the family as a whole. My additional aim in this PhD was therefore to develop methods for analysing Rhyssinae which can later be easily applied to the other subfamilies.

2 Methods

There were three main phases of this study. Before the fieldwork, I studied the Darwin wasps collected by an earlier inventory at the African study site. During the fieldwork, in 2014–2015, I collected Darwin wasps in Kibale National Park, Uganda. After returning from the fieldwork I started processing the Ugandan samples (this is still ongoing), compiled the data of earlier Amazonian inventories, and analysed the results.

2.1 Previous Ugandan data (2014)

Before I started my PhD, the Zoological Museum of the University of Turku already contained a small sample of 1212 Darwin wasps from Kibale National Park. These had been collected by Malaise trapping in 2011. The Malaise trapping had been extensive, with thirty traps regularly moved between randomly selected locations for a whole year, but had given unexpectedly few Darwin wasps.

To get a first indication of the Darwin wasp diversity of the site, I labelled these wasps and sorted them into subfamilies. I also sorted two of the subfamilies (Cryptinae and Ophioninae) into preliminary morphospecies. The results allowed me to assess the suitability of Afrotropical Malaise trap data for studying Darwin wasp diversity and ecology (paper I).

As well as giving data on the site's diversity, the results also indicated whether or not it was worth revisiting the site. Because the 2011 Malaise trapping had caught so few Darwin wasps, we suspected that the study site might be very species poor, and were seriously considering other African sites instead. The preliminary results convinced us that it was worth trying a second year of Malaise trapping at Kibale National Park.

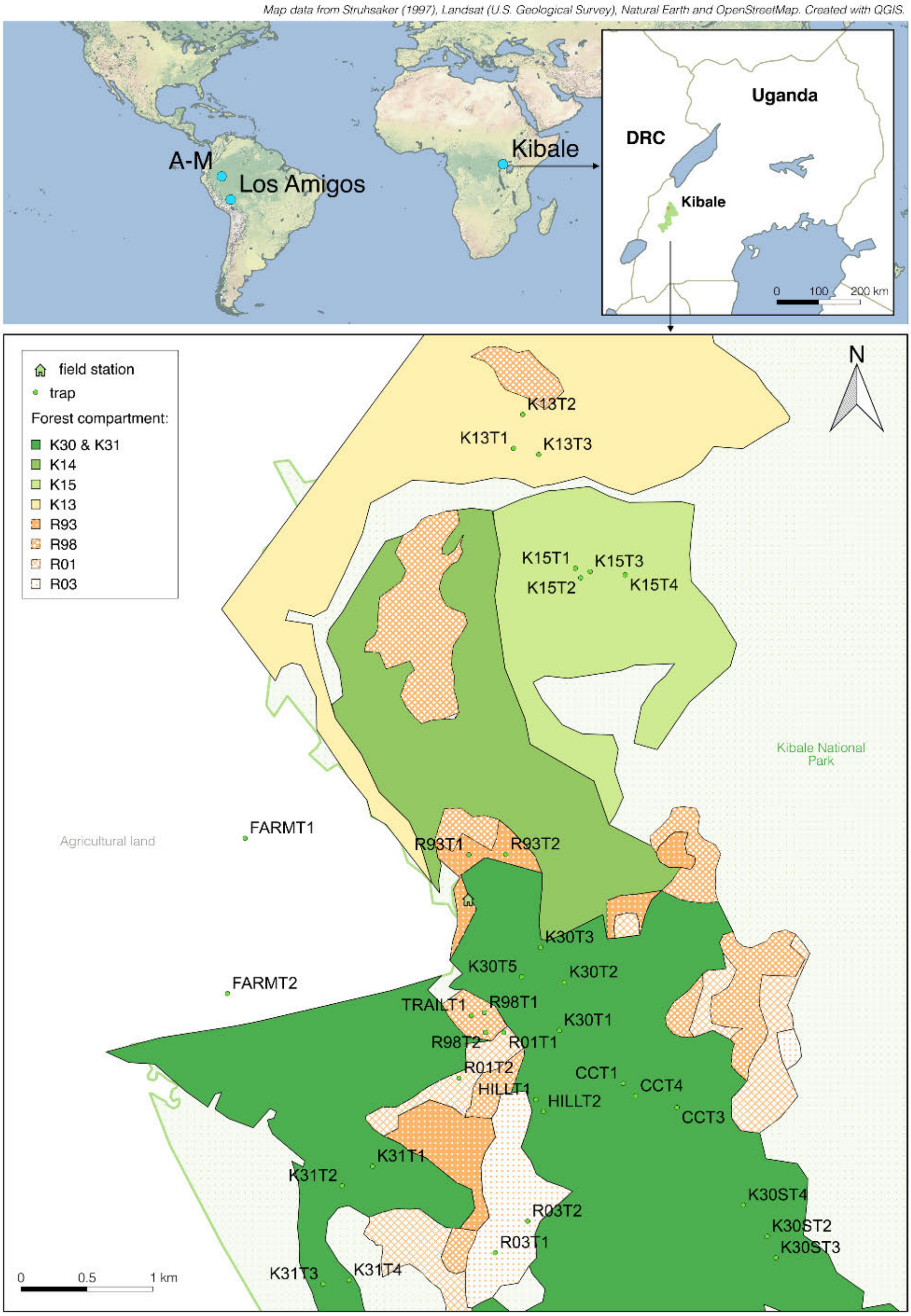

Figure 1. Map of the main study site, Kibale National Park in western Uganda. I placed traps in primary forest (K30&31), disturbed forest where about half the trees were logged 1968–1969 (K14, K15, K13), clearcut plantation, i.e. former exotic plantation logged 1993–2003 (R93–R03) and farmland outside the national park. Also shown are the two Amazonian sites, Allpahuayo-Mishana and Los Amigos. Modified from paper II.

2.2 Uganda Malaise trapping 2014-2015

I collected Darwin wasps in Kibale National Park (western Uganda, figure 1) using the same methodology as in Amazonia: large numbers of Malaise traps, a whole year of trapping (September 2014 – September 2015), and traps placed in a variety of habitats on the likely flight paths of insects. The trap locations were not randomly selected, partly to make the results more comparable with Amazonia, but mainly because the earlier 2011 inventory caught very few Darwin wasps.

Kibale National Park is an approximately 795 km^2 large protected area in western Uganda, near the border of the Democratic Republic of the Congo (figure 1), which contains a mix of medium altitude moist evergreen forest, swamps, grasslands, woodland thickets and colonizing shrubs (Struhsaker 1997, Chapman & Lambert 2000). It has earlier been connected to the forest of the Congo Basin, but the connection has been cut by human activity and the park is nowadays surrounded by agricultural land (Naughton-Treves 1998). Mean annual rainfall is approximately 1700 mm, maximum daily temperature 24°C and minimum daily temperature 16°C (Chapman et al. 1999). I placed my Malaise traps near the Makerere University Biological Field Station (0.5625° N, 30.3561° E; approx. 1500 m.a.s.l.).

I placed 34 Malaise traps in five different habitat types organised by successional status: primary forest, swampy primary forest, disturbed forest, clearcut plantation and farmland (figure 1). The traps were the same size and of the same design as in Amazonia: black with a white roof (figure 2), approximately 170 cm long with two 1.6 m^2 openings, supplied by B&S Entomological Services. They collected flying insects into about 80% ethanol, and I and a field assistant (Isaiah Mwesige) emptied them at approximately two week intervals. The variation in emptying times was largely due to our having to avoid elephants. After emptying traps, we transferred the samples to cloth pouches and placed them in a bucket filled with 80% ethanol (figure 2). This allowed us to easily change the ethanol, which was often quite diluted after soaking water from the insects. It also made it easy to transfer the samples to Finland: we simply lifted the pouches out of the ethanol and most of the ethanol drained away like tea out of teabags.

As well as collecting insects with Malaise traps, I also carried a hand net with me and collected any Darwin wasps or braconids in sight. This gave about 3400 wasps, most of which are large-sized Darwin wasps (e.g. in subfamilies Pimplinae, Cryptinae, Ichneumoninae, Ophioninae). I placed the hand-netted wasps in 96% undenatured ethanol, in individual tubes. The wasps are now stored in the Zoological Museum freezer at -18°C, mostly waiting for someone to have the time to study them. Their data includes GPS coordinates and the GPS tracks followed when collecting them.

Figure 2. Workflow of the Ugandan Malaise trapping. In Uganda, I collected insects with 34 Malaise traps for a year. Samples were stored in cloth pouches. In Finland, we separated the parasitoid wasps (families Ichneumonidae and Braconidae) from the other insects, then separated and pinned the subfamily Rhyssinae, and sorted the rhyssines into species. Other data, such as hand-netted wasps, weather data and vegetation data, were also collected.

I also obtained background data such as weather and the vegetation surrounding the traps. The weather data were kindly provided by the Makerere

University Biological Field Station: they consisted of daily rainfall, maximum temperature and minimum temperature at the station, and had been gathered by Yosinta Tumusiime. The vegetation data were collected by me and two field assistants (Isaiah Mwesige and Richard Sabiiti) in two 50 x 2.5 metre transects centred on each trap. (As a sidenote for future inventories, I later came to the conclusion that a circle would have been better than transects) We documented all trees with a diameter of over five centimetres in the transects, including dead fallen logs. We also noted what plant species were present in a five metre square centred on the trap. The species were mostly identified by Richard Sabiiti. Many of the traps were near the trap sites of the 2011 trapping (see previous chapter), during which vegetation data were also gathered, but in the event we did not use these earlier vegetation data in this study. The background data are available online as a supplement of paper II (Hopkins et al. 2019).

Since Uganda (like most tropical countries) operates a research permit system, there were several permit applications and fees involved alongside the fieldwork. Permission to carry out research in the country was granted by the Uganda Wildlife Authority (UWA) and the Uganda National Council for Science and Technology (UNCST), and security clearance by the Office of the President. Permission to enter Kibale National Park, and to transfer samples from the country, were granted by UWA and UNCST, and Customs, Uganda Revenue Authority were informed of the transfer. All official fees (such as permit application fees, monthly stay in Kibale National Park etc) were paid. Unofficial fees (such as requests for bribes) were rare and were turned down.

2.3 Processing the Ugandan samples (2015-)

The Ugandan samples were transferred to Finland in two batches, one in March 2015 and the other in September 2015. After the fieldwork, I and many others processed the samples by separating the ichneumonoid wasps (Ichneumonidae and Braconidae) from the other insects, then separating the subfamily Rhyssinae, pinning and databasing it and sorting it into species (figure 2).

Samples were processed by a large number of people at the Zoological Museum of the University of Turku, including staff and students of the university and school pupils from the Turku region. We first transferred the Malaise samples to long-term storage in glass jars filled with approximately 80% ethanol. There were 876 samples with about 1½ decilitres of insects in each. We separated the ichneumonoid wasps from these samples by placing each sample in small portions in a dish then picking out ichneumonoids under a microscope. Students and school pupils who processed the samples were taught to recognise ichneumonoids and their first samples were double-checked. I estimate that at least 95% of the ichneumonoids have been separated from the samples (based on double-checking some samples and seeing how many wasps were missed the first time). At least 100 000 wasps were separated, most of which are Darwin wasps.

Once the ichneumonoids had been separated (in 2017), we separated the rhyssine wasps (subfamily Rhyssinae) from the other ichneumonoids. This was again done under the microscope, with students advised to look for the typical ridges on the mesopleuron. We then pinned the rhyssines and databased them in the Kotka Collection Management System, giving each wasp an online identifier (e.g. http://mus.utu.fi/ZMUT.4920). There were 456 rhyssines in total of which eight were caught by hand-netting instead of by Malaise trap. I estimate that almost all rhyssines were separated from the ichneumonoid samples, although I have found one more *Epirhyssa quagga* after publishing the species.

Once the rhyssines were pinned and databased, I sorted them into species. This is a much more subjective process than is often appreciated. The most common definition of what a species is, a population that breeds with itself but not with others (Mayr 1999 p. 120), is hard to use for dead insects. Instead, species tend to be separated by their outward appearance or genetic dissimilarity. I split the Ugandan rhyssines into species following roughly the same principle as used for the Amazonian rhyssines: each species should have a unique morphological character (or combination of characters), and preferably also a unique colour pattern. I did not use colour on its own since in our experience the colour of tropical wasps often varies within species. Having split the rhyssines into species, I compared them to existing species descriptions and to the type specimens of described species in other museums. This gave me four described species and two species new to science (and also led to synonymising one existing species). It is worth noting that the Turku style of delimiting species is fairly conservative and may lump some species together. *Epirhyssa uelensis* in particular could benefit from DNA analysis to find out if it is actually more than one species.

2.4 Compiling the Amazonian data (2019-2020)

To be able to compare the Ugandan and Amazonian rhyssine wasps, we needed data on what Malaise samples and what rhyssines were caught in Amazonia. Unfortunately, the main Amazonian collecting event was in 2000, before the era of omnipresent laptops and online databases. The data had got fragmented during the past two decades and had to be compiled from multiple sources.

To find out what *wasps* were collected in Amazonia, Ilari Sääksjärvi searched the zoological museum for Amazonian rhyssines (a process that sounds easier than it actually was). He found 150 rhyssines. I databased these rhyssines (e.g. http://mus.utu.fi/ZMUT.9422) and compared their labels to earlier publications and various old data files. This allowed me to add seven more rhyssines to the database; these had been mentioned in Gómez et al. (2015) but have not yet been found at the museum. The total was 157 Amazonian rhyssines, of which 94 have been collected by the four collecting events (in 1998, 2000, 2008, 2011) to which I compared the Ugandan data. I checked that this total of 94 wasps more or less matched earlier publications and data files (these gave a range of 93–96 wasps).

To find out what Malaise *samples* were collected in Amazonia, I gathered together data from a large variety of sources. Sources included old computer files kindly donated by former coauthors, the labels of some Malaise samples at the museum, the labels of several thousand pinned wasps which I examined in the museum's cellar, a multitude of files found on computers at the museum, and several publications. I combined all of these data, then corrected errors and inconsistencies. The result was a complete list of what Malaise traps were used in 1998, 2000, 2008 and 2011, what samples were collected and when they were collected. The combined sampling efforts (in trap months) were close to what had been published earlier.

As well as gathering together the existing data of the Amazonian Malaise trapping, I got some new weather data. These came from two Amazonian meteorological stations whose data is available online.

We published the entire compiled dataset online (all data except rhyssines: Hopkins et al. 2020, rhyssines in https://laji.fi).

2.5 Analyses

When analysing the results of biodiversity inventories, the KISS principle ("Keep it simple, stupid") is a good guideline. Biological systems are extremely complex with a lot of inherent noise, and no amount of mathematical trickery will change this. In this PhD, I tried a variety of different statistical methods, but the main results of the PhD can be deduced without them. All the analyses presented here are subsidiary to simply showing what wasps were caught, when and where (c.f. figure 2 of paper II).

When analysing the results of the previous Ugandan Malaise trapping (paper I), I used a mix of distance-based methods and modelling to find out how wasps were distributed in different habitats. Both of these were somewhat overkill, since I was simply trying to find out if the wasps in forest are different to those found in clearcut plantation, but I wanted to test the suitability of various commonly used methods for this kind of data. The distance-based methods boiled down to my calculating how dissimilar the wasp assemblages of different traps were, then seeing if traps in forest were more similar to each other than to traps in clearcut plantation. I used the Bray-Curtis index as a measure of dissimilarity; like most such indices, this is merely a simplified way of expressing the similarity of two faunas, with a number ranging from 0 (same wasps in both sites) to 1 (completely different wasps).

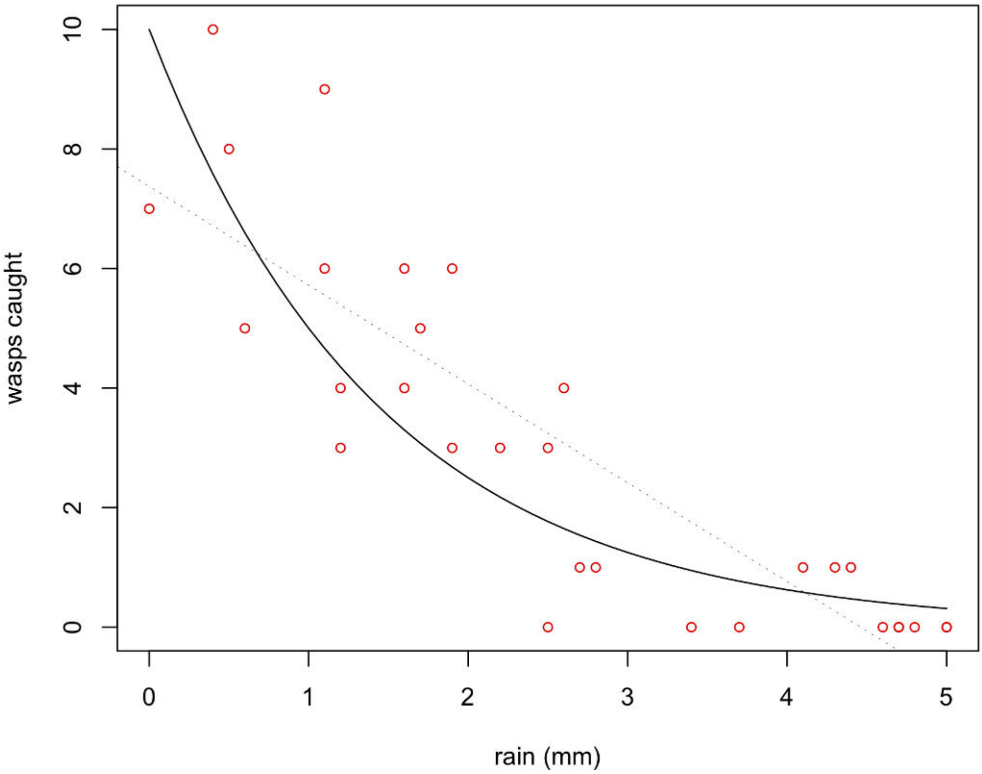

Figure 3. Simplified example of how I estimated how factors such as rainfall affect the number of wasps caught. My data consisted of samples which had caught a specific number of wasps during specific rainfall (red circles). I fitted an exponential curve to this data (black line): in this simplified case, ten wasps are caught on average when it does not rain, and each millimete of rain halves the catch. This is more realistic than fitting a straight line (faint dotted line), since that would eventually predict that less than zero wasps are caught (e.g. -0.9 wasps when it rains 5 millimetres).

I used generalised linear models to compare the Darwin wasps of forest and clearcut plantation (paper I), to find out how rain and habitat affect the number of rhyssines caught by traps (paper II) and to estimate how many Ugandan rhyssines would have been caught if it had rained as much in Uganda as in Amazonia (paper IV). These models boil down to fitting an exponential curve to the data (figure 3). Although the main point of modelling is to describe how catches vary with rainfall, habitat type and other such factors, it is also possible to test statistical significances by resampling. To test if e.g. rainfall genuinely affected Ugandan catches, I (or rather, my computer) resampled the residuals of the fitted exponential curves (Warton et al. 2017). One disadvantage of this kind of modelling is that the methods available are not always user-friendly: in papers I and II, for example, I almost certainly performed an unecessary calculation due to not fully understanding what the computer was doing. I tried to prevent resampling from mixing the samples of different traps, despite this apparently not being necessary in the statistical package I used (which performs residual resampling instead of case resampling, Warton et al. 2017).

When comparing the species richness of Amazonia and Uganda, I faced a fundamental challenge: we do not actually know how many species either of these sites has. Indeed, one of the results of my PhD is showing that not even a year of sampling with dozens of traps will get all the species present at a tropical site. Instead, I had to estimate the relative species richness of Amazonia and Uganda from species accumulation curves. For each trap, I displayed the rate at which the trap caught species (by drawing a curve with the number of individuals caught on the x axis and number of species on the y axis). The assumption is that the more species there are present in an area, the faster the rate at which species will be caught. This approach relies on a large number of assumptions, including that the relative abundances of the species follow a similar distribution at each site. For reasons summarised by Gotelli and Colwell (2011), I decided to place the number of individuals on the x axis, instead of the number of trap days. Although this helps correct for the tendency of Malaise traps (Saunders & Ward 2018) to catch very varied sample sizes, I am not entirely satisfied with it since it allows variation in the abundance of the species (even of a single species) to strongly affect the results. It is, however, the least biased method currently available.

There were several commonly used methods I did not use. These include ways of estimating the total number of species in an area (e.g. the Chao 1 estimator: Chao 1984), and measuring species diversity with a diversity index instead of measuring species richness. The reason for not using species richness estimators was simple: the sample sizes in this PhD are simply too small to give a reliable estimate. The only thing that the estimators were able to show was that there are lots of species still undiscovered in both Uganda and Amazonia – something that is completely obvious anyway. (In general, I have a sneaking suspicion that species richness estimators work best when sample sizes are so large that they are not actually needed, c.f. Gotelli & Colwell 2011) As for diversity indices, a lot has been written about their use and misuse (e.g. Hurlbert 1971, Peet 1974, Hamilton 2005, Tuomisto 2010). In this PhD, I calculated some diversity indices, but was unable to find any actual use for them. I was able to assign a number to Ugandan forest and another number to clearcut plantation, but these numbers lacked meaning. Whereas species richness is interesting in its own right, "diversity" as measured by indices is not, and I came to the conclusion that diversity indices were not relevant to my study.

3 Results

There were four main results of my PhD. Rather conveniently, these match the four papers that form this thesis. I found that even small sample sizes of Malaise-trapped Darwin wasps can give ecological information (I), and found out what rhyssine species inhabit my Ugandan site (III), how they live (II), and how they compare to their Amazonian counterparts (IV).

3.1 Even a very small sample size can reveal habitat use (I)

In many ways, the previous Malaise trapping in Uganda (in 2011) became a pilot study for my fieldwork. Despite using 30 Malaise traps, it caught only 1212 Darwin wasps during the whole year. Even this ridiculously small sample size was enough for detecting habitat use patterns: I found that forest had a different mix of Darwin wasp subfamilies to clearcut plantation. One subfamily in particular (Ichneumoninae) was much more common in forest than in clearcut plantation.

The results also demonstrated that there were (and still are) lots of undiscovered Darwin wasps in African forest. There were two subfamilies for which we only caught a single individual, and several new species had already been described from the material.

We never found out for certain why so few wasps were caught, but suspected that the sampling design played a part. The traps were unusually small, were moved once a week, and were placed in random locations: all of this made statistical sense, but seems to have led to a small sample size. In our main Malaise trapping (in 2014–2015) we thus decided to play safe and used larger Malaise traps which we placed more conventionally.

3.2 Rhyssines prefer dry weather and primary forest (II)

The 2014–2015 Malaise trapping caught over 400 rhyssine wasps (subfamily Rhyssinae), and so overwhelmingly many other Darwin wasps that they are still being processed. This sample size allowed us to get information on how the rhyssine species live, instead of merely documenting that the species exist.

We found that the greatest number of rhyssines are caught in dry weather, near decaying wood in primary forest. There was a gradient from primary forest (most rhyssines) to farmland (no rhyssines), with disturbed forest and clearcut plantation intermediate. Interestingly, the rhyssine abundance in the samples also increased with time. We fitted a model to the data which can predict how many rhyssines of each species to expect for a given rainfall, time of year, forest type and amount of decaying wood.

This information allowed us to make several deductions on the biology of the rhyssine species. Since the rhyssines were mainly to be found near decaying wood, we deduced that they probably prey on wood-boring larvae. They have always been assumed to do so, but there was no data supporting this for the tropics. We also found signs that the rhyssines preferentially emerge as adults twice a year, in the middle of the dry seasons, and that the adults only live for a few weeks. The reason for their being caught in smaller numbers when it rains is unclear, but it seems to be a mix of their not flying when it rains (i.e. they are not flying into the traps) and of their adult abundances being lower during the rainy seasons.

As well as giving fascinating information on how the rhyssine wasps of an African forest lead their lives, our results also highlighted how useful our sampling design can be. Large numbers of Malaise traps, operated for a whole year, gave a wealth of information not available when sampling on a smaller scale. Interestingly, there are some other tropical surveys (in Costa Rica and Amazonia) which have used the same or similar methods. Since they have reported very little ecological data, we suggested taking a fresh look at their data to see if the methods we used for Uganda could also be applied to them.

3.3 A third of Ugandan rhyssine species are new to science (III)

I sorted the Ugandan rhyssines into six species. Two of these were new to science, so we described and named them: *Epirhyssa johanna* Hopkins and *E. quagga* Hopkins et al. We also reviewed all the Afrotropical rhyssine species.

After our review, there are a total of 13 rhyssine species and 490 individual wasps known from the Afrotropics. Almost all the 490 collected individuals are from Uganda. Amazingly, only thirty African individuals were known before this study (we found four more old specimens in the London Natural History Museum). We also described the male of *Epirhyssa overlaeti* Seyrig, and synonymised *E. gavinbroadi* Rousse & van Noort with *E. uelensis* Benoit. The synonymisation was made possible by our large sample size: when compared to our 160 Ugandan *E. uelensis*, the sole female *E. gavinbroadi* turned out to fit inside the other species' intraspecific variation.

Amazingly, not even a year's worth of extensive Malaise trapping was enough to catch all the rhyssine species at the site. One of our species (*E. johanna*) consists of a sole female. We do not know what the male looks like, nor have the males of *E. tombeaodiba* ever been caught (six females from Uganda, no males). This clearly demonstrated that we have only scratched the surface of African rhyssine diversity. As a further (non-Ugandan) example, our knowledge of the rhyssines of Madagascar turned out to be based on just eight wasps, all belonging to the same species and collected in the 1930s.

3.4 Even more rhyssine species in Amazonia than Uganda (IV)

Comparing the results of our Amazonian and Ugandan sampling events for rhyssines revealed two major differences: more rhyssine species were caught in Amazonia, but far fewer individuals.

The difference in the number of individuals seemed to largely be due to different rainfall. An average Amazonian trap caught 0.4 rhyssines per month during the main sampling event, which is much less than the average for Ugandan traps (1.2 rhyssines per month). But it also rained more in Amazonia, and rain often decreases the catches of Malaise traps. When I used the model in paper III to estimate how many Ugandan rhyssines I would have caught if it had rained as much in Uganda as in Amazonia, the difference became much smaller: 0.4 *versus* 0.6 rhyssines per month.

Amazonian traps caught species at a faster rate than Ugandan traps, which suggests there are more species there. This was the case for all Amazonian and Ugandan forest types. However, the Amazonian sample sizes were low, which means that the difference between our second Amazonian site Los Amigos and Uganda is uncertain. The difference in the number of species could possibly be due to our Amazonian sites having a more varied habitat.

Since we only sampled one site in Africa and two in Amazonia, we weren't able to draw any conclusions on which continent has more species. However, the methods we used would answer this question if more sites were sampled with our style of Malaise trapping. For future Malaise trapping, we recommended getting a better coverage of different habitat types, since many of our Amazonian habitats had too low a sample size. Using models to standardise the data (which we did to account for differences in rainfall) also seemed promising, and we especially recommended it for other subfamilies which are more abundant than Rhyssinae.

4 Discussion

The results of this PhD give valuable information on the Darwin wasp species richness of Africa and how one of the subfamilies compares to similar sites in Amazonia. They also demonstrate the advantage of our sampling design for studying the ecology of Darwin wasp species, and making global comparisons of the species richness of different sites. The hypothesis that tropical Darwin wasps would be relatively species poor receives no support from this study.

4.1 Africa has lots of species, most still undiscovered

Collecting insects at a forest site in Uganda demonstrated that the whole of Africa is severely undersampled. With hindsight, this should not be surprising: my fieldwork represents the first time African Darwin wasps have been collected long-term with large numbers of traps. Or rather, the first time the collecting has been successful and results have been published: the Ugandan Malaise trapping in 2011 caught only 1212 individuals, and there is one other sampling program, in South Africa, whose results are still being processed (see paper II). Earlier Darwin wasp collecting in Africa has largely been short-term and has typically involved only hand nets or at most a few Malaise traps, or other traps such as yellow pans (e.g. Owen & Owen 1974, several collections mentioned by Gauld & Mitchell 1978 p. 6, van Noort et al. 2000, van Noort 2004). With my Ugandan primary forest traps typically catching two rhyssine wasps *a month*, and my other traps catching even less, it is scarce surprising that such small-scale sampling has failed to get a representative catch. One additional factor is that Darwin wasps have generally been collected during the wet seasons (on the unspoken assumption that they are most abundant then) which are the seasons when my traps caught the least. This is not to say that the wet season catches least everywhere in Africa: in dry areas, the wet season could well be the best time to catch many insect taxa, whereas for moist areas such as forest it could be the other way round (Janzen 1973).

Although in this PhD I have focused on one Darwin wasp subfamily (Rhyssinae), it is obvious that examining the other subfamilies in my material will reveal the same pattern of severely undersampled African diversity. What are we to make of the Ophioninae, for example, whose African classification is largely based on about 4000–5000 individuals (Gauld & Mitchell 1978 p. 6, Rousse & Noort 2014)? My Ugandan material seems to have almost as many individuals, and there is at least one probable new species. The subfamily Brachycyrtinae has never been described from mainland Africa; the only two African females I know of were caught in Madagascar in 1936 and 1937 (van Noort 2019). This in itself is a sign of undersampling, and resembles the situation for Rhyssinae before this PhD. Since I have seen quite a few brachycyrtines in my Ugandan material, I refuse to believe that the subfamily has been adequately sampled in Africa. Importantly, it is also a fact that there are still undiscovered species even at my study site. In paper III we found that two rhyssine species were caught in very small numbers (six *E. tombeaodiba*, one *E. johanna*), a sure sign that the sample size has been too low to catch all species. There were also several described species which I did not catch: I strongly suspect that at least some of the species from neighbouring DRC are present at my study site. These could include *Megarhyssa babaulti* Seyrig, whose sole representative was collected in 1932 just 340 km from my site. In paper IV, the species accumulation curves of my traps did not reach an asymptote. Quite the contrary, they were straight lines showing little sign of peaking, and not a single one of my traps caught all six rhyssine species. If the diversity of a single Ugandan site is so high that a whole year of operating 34 traps does not catch all the species, what must the situation be like for the rest of Africa!

Not only does undersampling result in our not knowing all the species that inhabit Africa, it also means that what little knowledge we have may be inaccurate. In paper III, we synonymised *Epirhyssa gavinbroadi*. This species had been described in 2014 on the basis of one single female from Cameroon (Rousse & van Noort 2014). At a time when our entire knowledge of African rhyssines was based on just 30 specimens, this low sample size was the norm: even the species that it most resembled, *E. uelensis*, consisted of just five females and a male. It was only when my Ugandan traps caught 160 *E. uelensis*, some of which resembled *E. gavinbroadi* at least as much as other members of their own species, that the status of *E. gavinbroadi* as a separate species became suspect. The ideal species delimitation based on museum specimens uses large sample sizes for each species (to find out how much intraspecific variation there is), something which is impossible if there are only 30 specimens, divided into 12 species, for an entire continent. For the African rhyssines, my gut feeling is that there are no more synonymisations needed (with a tiny question mark for *E. leroyi* Benoit), because the remaining species are clearly distinct and many like *E. shaka* Rousse & van Noort are thousands of kilometres from the nearest other known rhyssine. However, some species may need to be split once new material is collected elsewhere in Africa. I am particularly suspicious of the high variability of

E. uelensis: although there is currently no way to reliably split it, I suspect that DNA analysis of the Ugandan specimens might reveal more than one species (and perhaps even reinstate *E. gavinbroadi*).

4.2 Amazonia has even more species?

Amazonia seems to have even more Darwin wasp species than tropical Africa. That is what I would *like* to be able to state. But of course it is far too early to make such a bold statement, on the basis of sampling 4 x 4 km in South America and 4 x 7 km in Africa (paper IV), and not even catching all the species present. What I have shown, however, is that our style of extensive Malaise sampling can (and hopefully will) eventually answer this question. Once I get round to looking at the other subfamilies in our Ugandan and Amazonian material (some of which are much more abundant than Rhyssinae), and once a broad range of sites in both Amazonia and Africa are sampled in the same way, we will know which of the tropical continents has more species.

All the few signs we currently have point in the direction of Amazonia being the richer. Traps catch rhyssine species at a faster rate in Amazonia, western Amazonian habitat is likely to be more varied (which could partly explain *why* there are more species), and it is what we would half-expect based on other plant and animal taxa (e.g. Pearson 1977, Gentry 1982, Gentry 1988). However, it is worth remembering that only a few decades ago all the signs pointed towards Darwin wasps being relatively species poor in the tropics, a hypothesis which is starting to look ever more suspect (Quicke 2012). This is an inevitable problem in tropical entomology: sample sizes and geographic coverage are never large enough. I personally suspect that Darwin wasps are more species rich in Amazonia, based not only on my published results but what I have seen of the remaining unpublished material, and I would be very surprised if there were noticeably more species in Africa.

4.3 Extensive sampling tells us how species live

One of the most frustrating aspects of our current knowledge of the species we share a planet with is that, in practice, we know nothing about them. I have looked at species after species in museum collections, and most of the time they are just a (pseudo-Latin) name: we do not know how they live, what they eat, how long they live.. we only know that the species exists. This is particularly frustrating when contrasted to the fascinating details of the few species which we do know something about. We have wasps which make felt out of spider silk (Fritzén & Sääksjärvi 2016), wasps which may mimic ants as a form of psychological warfare against the spiders they prey on (Sääksjärvi et al. 2015), and wasps which lay their eggs inside the wasp larvae parasitising other insects (e.g. Ashfaq et al. 2005). How much are we missing by knowing virtually nothing about the small minority of species we have discovered?

My results show that extensive sampling with Malaise traps can be used to find out how Darwin wasps lead their lives (paper II). This is made possible by the sampling design, which gives large sample sizes, collected by traps that span both space (i.e. forest type) and time. We now know that African rhyssines avoid rainy weather, prefer primary forest, and can even guess at what they eat (larvae in decaying wood) and how long they live (some weeks as adults, likely six or twelve months as larvae). Not only is this information interesting, it can also be of practical use. The protected status of Kibale National Park, for example, appears to be justified: its rhyssine species prefer primary forest and would presumably go locally extinct without this protected status.

What is particularly interesting is that obtaining this wealth of information did not require very much extra work. I acquired very basic Ugandan weather data, inventoried the vegetation surrounding my traps, and this was enough to transform what would otherwise have been a purely taxonomic sampling program into an investigation of both what species are present and how they lead their lives. This immediately suggests doing the same elsewhere: not only in future studies, but also for previous work. There are several other sampling programs which have collected insects long-term with large numbers of Malaise traps (Costa Rica: Gauld 1991, Amazonia: Sääksjärvi et al. 2004, Sweden: Karlsson et al. 2020). Although some work has been done on the phenology or habitat use of the species (Shapiro & Pickering 2000, Sääksjärvi et al. 2006), the focus has largely been taxonomic. Very little work would be needed to add background data such as vegetation or weather to the results, potentially opening up a wealth of new information on the species. In this PhD, I have compiled such data for the Amazonian Malaise trapping (paper IV). Unfortunately, rhyssine sample sizes were too low to allow this data to be used to full effect, but other more abundant subfamilies may well give interesting results.

4.4 Extensive sampling allows global comparisons of species richness

My results strongly suggest that extensive, standardised sampling is needed to be able to draw any conclusions on how Darwin wasp species richness is distributed on Earth. Despite collecting for a total of 373 trap months in Uganda and 151 trap months in Amazonia (during the main trapping in 2000), our sample sizes were too low to allow a straightforward comparison of species counts. Instead, we had to estimate relative species richness by comparing rates of species accumulation (paper IV). This kind of comparison is impossible without standardised data and reasonable sample sizes. In paper II, I also found that tropical rhyssines (and presumably other Darwin wasps) are strongly seasonal, so sampling for less than a year is likely not to get a representative sample of a site's species. Given that even the Ugandan and Amazonian sample sizes were barely sufficient, it is scarce surprising that earlier attempts at global comparisons have achieved only limited success (average of four traps per site: Timms et al. 2016, average of 34 trap months per site, varied trap size and placement: Gómez et al. 2017).

There are many potential ways to extensively sample Darwin wasps, but the Turku style used in this study (with large numbers of Malaise traps used for at least a year) shows promise. It gets an adequate sample of the species without becoming as overwhelmingly laborious as sampling designs that use a mix of different collecting methods (e.g. Longino et al. 2002 for ants). There is one other style of collecting which is currently relevant in global comparisons of species richness: both the Global Malaise Trap Program and project LIFEPLAN aim to have just one trap at each site, but to cover a broad range of sites throughout the Earth (‘Lifeplan’ 2019, ‘Global Malaise Trap Program’ n.d.). They also aim to automatise sample processing by DNA-barcoding the samples. I see these two styles as complementing each other. GMTP/LIFEPLAN will get the broad scale (but low resolution) global patterns, and may for example be able to conclusively demonstrate whether or not Darwin wasp diversity peaks in the tropics. The more intensive Turku style Malaise trapping will get the detailed comparisons of different sites, giving high resolution at the expense of only being able to sample a few sites at a time. This may turn out to be essential for anything but very broad scale conclusions on species richness. It is even possible that the single traps per site of GMTP/LIFEPLAN will fail to find out where Darwin wasp diversity peaks (c.f. the Amazonian and Ugandan traps in paper IV, where the sample size of individual traps would not have been large enough for species accumulation curves to detect differences). In this case, the more laborious Turku style will have to be employed.

In any case, one good next step for the style of Malaise trapping used in this PhD would be to sample the Darwin wasps of Southeast Asia, preferably at several different sites, and to sample more sites in Africa and South America. This would allow comparisons of the tropical forest Darwin wasps of three continents, and give a good basis for expanding to higher latitudes. Adding DNA barcoding to the methods used to process samples would also be useful (or almost essential), since current methods are so slow that most Amazonian and Ugandan data is still unavailable.

4.5 Are Darwin wasps at their most diverse in the tropics?

The old idea that Darwin wasps would be relatively species poor in the tropics has in recent decades started to look ever more unlikely. This "anomalous latitudinal diversity gradient" was proposed in the 1970s (Owen & Owen 1974), raised Darwin wasps to the forefront of research on latitudinal patterns in diversity (with numerous attempts to explain the prominent exception, e.g. Rathcke & Price 1976, Gauld 1987, Sime & Brower 1998), and seems to have quietly faded in the 2000s after ever more tropical species were discovered (e.g. Sääksjärvi et al. 2004). Currently the consensus seems to be a somewhat silent "let's wait for more data" (Quicke 2012).

Although it is still too early to bury this idea, my results are one more tiny nail in its probable future coffin. This is mostly because of what the results say about the evidence used to first advance this idea (which I will get back to in later paragraphs), but also because of what species I found. I discovered a rich rhyssine fauna in tropical Uganda, with a third of the species new to science, and signs that there are still undiscovered species (of both rhyssines and other subfamilies) at the study site. To say nothing of what the results suggest for the rest of Africa, which compared to Uganda is almost entirely unsampled. This suggests that the unexpectedly rich Amazonian Darwin wasp faunas (e.g. Sääksjärvi et al. 2004, Veijalainen et al. 2012) are not a hyperdiverse exception, not to be found anywhere else and unrepresentative of the tropics as a whole. Instead, it is starting to look as if tropical forests in general have rich Darwin wasp faunas. Western Amazonia could be among the more diverse tropical areas (and paper IV suggests it could be for rhyssines), but there is no evidence for it being an anomaly.

If the idea of relatively low tropical diversity seems likely to be false, why did it arise in the first place? The answer, as has been pointed out before (Morrison et al. 1979, Quicke 2012), is insufficient data. Owen and Owen (1974), when they proposed the anomaly, were relying on the data of just four Malaise traps; one each in Uganda, Sierra Leone, England and Sweden. They noted that all the traps caught species at similar rates, and stated that there was "no evidence of the expected greater diversity in the two tropical localities". The problem is, with such sample sizes one would not expect there to be. By their very nature, species accumulation curves look the same until a substantial fraction of the area's species have been caught (figure 4). None of the four traps caught anywhere near all the species: about a third of the species were represented by just one individual. Other complicating factors, such as the traps being in gardens instead of natural habitat, are serious enough, but pale before the fundamental issue of too low a sample size.

The same problem of insufficient data plagued two other studies that supported the idea of relatively low tropical species richness. Janzen and Pond (1975) compared sweep samples collected in England and Michigan (5+1 samples), and

Costa Rica (six samples), and noted that the highest species count was in one of the English samples instead of in the tropics. However, their total catch, of *all* parasitoid wasps, was 4062 (668 for Costa Rican secondary habitat, table 4a–f in Janzen 1973, 3394 for England + Michigan, table 1 in 1975). I have over 100 000 of these wasps, and it is barely enough for comparisons (paper IV). I am tempted to state "case closed" and leave it at that, but could note that it is not surprising that some of Janzen and Pond's other taxa seemed to show a latitudinal gradient, since a smaller sample size may be enough for other, less diverse taxa. Janzen (1981) later examined the North American catalogue data of eight Darwin wasp subfamilies, and calculated how many of the species had been found in latitudinal bands stretching from 25°N to 72.5°N. He found a peak around 40°N, and concluded that this was additional support for Darwin wasps not peaking in species richness in the tropics. One problem with this conclusion is that catalogues share the same weakness as the small scale inventories which originally collected their data: sample sizes are locally small. It has been later found that Townes' catalogue which Janzen used was not as complete as he believed (Timms et al. 2016). Even without going into the questions of how representative a sample stretching north of 25°N is of the tropics, or how usable miscellaneous museum specimens, collected piecemeal over several centuries by varying methods, are for global comparisons, it is questionable if the sample sizes were sufficient for reliable conclusions.

My results do not give much additional information on the distribution of Darwin wasp diversity at a global scale. They do, however, strongly suggest that large scale, standardised sampling is needed to be able to draw any conclusions on how Darwin wasp species richness is distributed on Earth (see 4.4). This casts further doubt on the data that created the anomalous diversity gradient of Darwin wasps, to say the least. The sample sizes were far too small for this kind of global comparison (Owen & Owen 1974, Janzen & Pond 1975), or where the sample sizes were better the data had not been collected in a standardised fashion (Janzen 1981). Also, later comparisons which had sample sizes that the 1970s could only dream of have found no support for this anomaly (Timms et al. 2016, Gómez et al. 2017), while still having too little data. On the whole, there is no longer any reason to believe that Darwin wasps do not peak in species richness in the tropics, and many reasons to suspect they do.

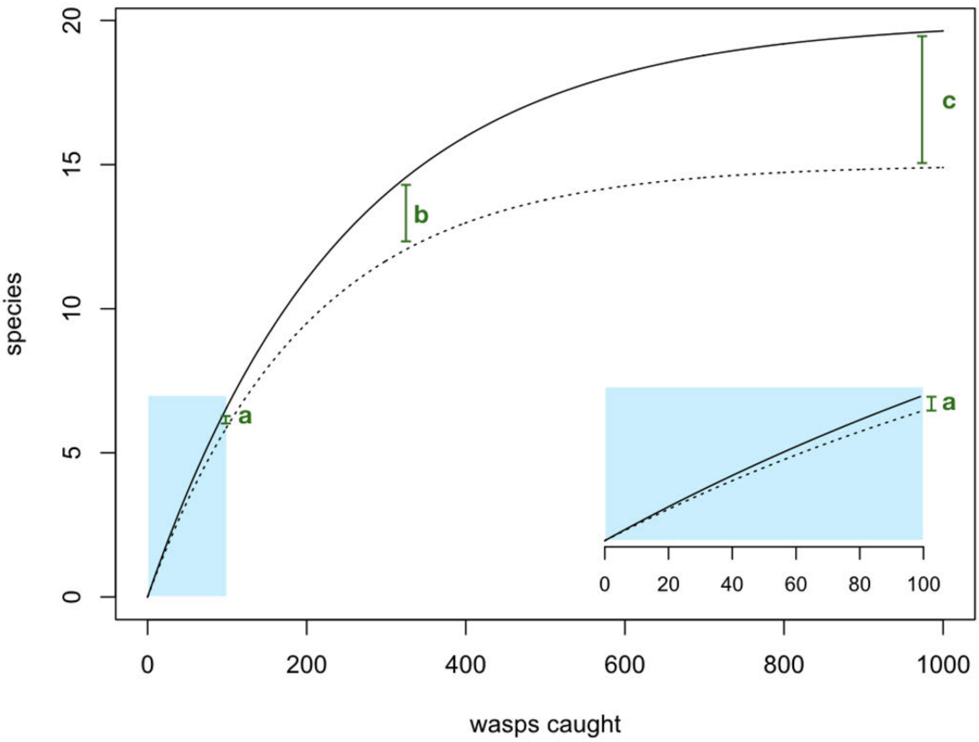

Figure 4. Species accumulation curves of two sites, illustrating how such curves can be used to compare the species richness of sites. In an ideal world, all species have been caught and comparing sites is a simple matter of comparing species counts (15 *versus* 20 species, **c**). This is wishful thinking for tropical Darwin wasps. On occasion, sample sizes are large enough to show a difference between rates of species accumulation (**b**). This PhD was lucky enough to get these kind of sample sizes. Usually, however, sample sizes are so small that curves are identical and it is impossible to tell which site has more species (**a**). This is the situation for Owen and Owen's paper (1974).

5 Conclusions

Despite centuries of biodiversity research, we have still only scratched the surface of Darwin wasp diversity. In this PhD, I found a species rich Darwin wasp community in the tropical forest of Uganda. The results demonstrate that there is an impressive diversity of rhyssines, and presumably also other Darwin wasps, still awaiting discovery throughout Africa. Although Amazonian diversity may be even higher, high Darwin wasp species richness could be the norm for the tropics, and the results cast further doubt on the evidence for the old hypothesis that these wasps would be exceptional in the global distribution of their species richness.

My results also demonstrate the advantages of the Turku sampling design. Collecting insects with a large number of Malaise traps for a whole year, and combining the results with ecological data, gave a wealth of information of a kind that is rarely obtained in the tropics. Such sampling revealed six rhyssine species in Uganda, gave information on their phenology and habitat use, and allowed their species richness to be compared to that of their Amazonian counterparts. It also gave an overwhelming sample size of other Darwin wasp subfamilies, whose results once processed will likely equal or surpass those of this PhD. Future sampling of this sort in Southeast Asia, possibly combined with the data of other global sampling programs, would give a good tropical baseline for comparisons with other latitudes.

The results of this PhD also highlight how poorly we know who we share our planet with. Almost 300 years after Linné sent his pupils out to explore the world, Turku students are still hauling in massive treasure troves of new species and information about them. We still have a fascinating, unknown planet to explore.

Acknowledgements

I'm going to forget someone, aren't I? It's inevitable. This project has directly involved countless people, and indirectly even more.

So let me start by acknowledging you, whoever you are, whom I inadvertently forgot to mention. Rest assured it wasn't deliberate, and that your contribution was every bit as important.

I must also acknowledge all the countless people who indirectly contributed to this work, often without even realising it. I didn't just walk on my own to Uganda, with traps, ideas and food appearing from nowhere. Any successes of this work are partly the successes of society as a whole; of its many teachers, shopkeepers, boda boda drivers, healthworkers and others.

On the topic of society as a whole, I must especially acknowledge the Finnish welfare state. Long may people be free to study or do what they want, not what they can afford or their social rank allows! It is no coincidence that the Nordic countries punch above their weight when it comes to science, music, and other excuses not to get a real job.

Some income is needed even when not doing productive work. I am very grateful to the Finnish Cultural Foundation, Oskar Öflunds Stiftelse sr, the Helsinki Entomology Society (Societas Entomologica Helsingforsiensis), Waldemar von Frenckells stiftelse and Kela (the Social Insurance Institution of Finland) for seeing to it I could concentrate on looking at small tropical wasps without worrying about where the next meal comes from. And I should not forget the university itself, whose funds covered any number of miscellaneous costs, such as posting insect traps to Uganda – I wonder what the Ugandan Customs made of that one.

Collaborative writing is not really my thing. Whenever anyone *dares* touch one comma of "my text", I feel deeply and personally offended for a few hours. Then I look at it again and realise it's actually a good idea. My co-authors Ilari, Heikki, Gavin, Simon, Kari, Hanna and Isrrael have all greatly improved this work by offending me in this way. So have Seraina and Marko in their pre-examination of the thesis, several reviewers and editors, and so I am sure will Atte when acting as opponent. I must also thank Bernardo Santos for taking the trouble to start pre-examining the thesis, before it became clear who the final examiners would be.

Hanging out in tropical forest for almost a year is much more fun if you're not the only human there. I am deeply indebted to everyone at MUBFS (the biological field station), staff, scientists and visitors alike. Isaiah, you know the Ugandan forest better than I ever will, and this work would never have been possible without you! Thank you Cloud, Ed, Maureen, Nigel, Margaret, Kris, Drew, Averee, Jess, Niwaeli, Yaelle and Cecily for candlelit evening meals and daily updates on what the chimps, baboons, wasps etc respective study creatures had been up to. I realise as I write this that someone's name is missing and I'm going to be ever so embarrassed when I find out whose.. Juce, thank you for getting me safely to Fort Portal for shopping and contact home most Saturdays (weather permitting). Nor would this list be complete without thanking those UWA rangers who got me, Niwaeli and Jess out of that tree which the elephants had chased us up.

Although almost never acknowledged, it is a fact that biological research does not involve just the human species. I had the pleasure, and the honour, of living for almost a year amongst an immensely diverse community of living creatures. Every meeting was a source of interest, be it with the turacos calling in the tree tops, the black and white colobus growling in the morning, bumping into the Kanyawara group of chimpanzees building hammocks, observing with fascination the braconids drilling down into a rotten log, seeing the Aframomums flower then tasting the delicious fruit, even those ants despite their getting under my shirt. Although I must confess to a slight irritation with the Tsetse fly habit of going for the soles of the feet, given half a chance.. I must especially acknowledge the over 100 000 wasps and millions of other invertebrates who lost their lives as a result of this work. Yes, I probably only shortened your lives by a few days, and made the end less painful than being torn to pieces by ants, but I do not like killing unless I have to. I mean to see to it that your involuntary sacrifice was not in vain.

Although no one who has heard my interminable monologues on "Uganda this" and "Uganda that" would believe it, I actually spent most of my PhD in Finland. Countless people at the university have been involved in this work, some of them directly, such as all the students who have sorted my insects, some simply by being there and nattering on with me in the coffee room or in seminars. Not to mention the many school pupils who have helped me sort the samples! The Amazon research team has been especially welcoming of this weird team member who's never been within a thousand miles of Amazonia. I must especially thank Hanna for directing me towards Ilari and the museum seven years ago, when I contacted her with the well thought out PhD plan of "I want an excuse to go to the tropics, and also to find out why there are so many species, any ideas?".

Our Biodiversity Unit has been (and hopefully will continue to be) that epitome of all that universities should stand for: a gathering point for people fascinatedly exploring the world we inhabit. There's always something going on, often very enthusiastically so, ranging from dead spiders and monitor lizards arriving by post, via the care and growing of tropical orchids, to trying to extract plant fossils from bags of soil. It's been a particular pleasure to be at the museum, that memory bank of centuries of enthusiastic exploration; how many people spend their days next to a stuffed crocodile? Or find that someone visited the exact same patch of Ugandan forest the previous century and left the insects they caught in the cellar?

Oh, and when I write "unit and "museum", I of course actually mean the people who form those entities, all those people with whom I've interacted the past years. I really wanted to mention you all by name, but there are so many people who have contributed so much to this work. I found myself muttering things like "Vexi, Anssi, Ari and Varpu have to be mentioned of course, but is Carlos more important than Inkeri, or do I have space for both?". The only solution is to apologise for the lack of names, you know who you are and how important you've been!

I have benefited from having a whole of three supervisors. This work would have been completely impossible without you, Heikki, especially your extensive Ugandan experience. It's amazing how much the statement "Heikki's student" helped make all the practicalities flow oh-so-much easier; I hope I have done my part in building trust (and a reputation for incorruptibility), and thus making life just that little bit easier for the next Finn in Uganda. Gavin, fate seems to have decreed we must never meet in person during my PhD :) Either I'm in Uganda, or you are off the Antarctic coast, or a pandemic closes all the borders... never mind, we managed to show off our taxonomic objectivity anyway, here's hoping you can have *E. gavinbroadi* back some day when we get round to DNA barcoding! Ilari, you have a knack for getting a passing-by student enthusiastic about ridiculously small wasps, then constantly encouraging that enthusiasm throughout the resulting years. You gave me a lot of independence – more than you intended I dare say – and I appreciate it. This whole thesis is a continuation of the work you started twenty years ago; now we need to brainwash even more victims into thinking it's fun to collect tropical insects for a year, so the work continues even further :)

Finally, and most importantly, there is my family. You are a large part of the reason why I got into this in the first place, and why I've been able to do this work. Veikko and Tauno, thanks for providing me a home base in Loimaa at the start of the PhD! Juhani, you most impolitely finished your PhD before me, but I forgive you. Are you sure you don't have space for some Ugandan plants before they swamp our house? My parents are very much to blame for all this unproductive biology and natural world interest (c.f. Hopkins J PhD thesis 2018). At least I didn't dump any snails in the cellar, but on the other hand you have had a nice active time seeing to it the grandchildren give Daddy some time to do a bit of writing. Nor can very many PhD students claim to have been helped by Mummy in emptying Ugandan forest traps! Eivor and Erik, you have been a great help to Daddy; I'm not

entirely certain what effect you've had on the speed of completion, but it's been much more fun. I'm also almost certain you never actually managed to eat any of the type specimens. Johanna, I now know the best place to meet one's future wife is in a museum cellar, surrounded by smelly ethanol-soaked insects you've just brought back from Africa. I am very fortunate in more ways than I can express, not least in having a wife who actually appreciates her name being attached to a murderous tropical creature that devours its victim alive :)

Kållby, 20.1.2021
Tapani Hopkins

References

Arcadi AC & Wrangham RW 1999. Infanticide in chimpanzees: Review of cases and a new within-group observation from the Kanyawara study group in Kibale National Park. *Primates* 40: 337–351. https://doi.org/10.1007/BF02557557

Ashfaq M, Erlandson M & Braun L 2005. Hyperparasitism by Mesochorus spp. (Hymenoptera: Ichneumonidae) in Peristenus sp. (Hymenoptera: Braconidae) and development of PCR primers for hyperparasitoid detection. *Biological Control* 32: 371–377. https://doi.org/10.1016/j.biocontrol.2004.11.007

Barahoei H, Nader E & Rakhshani E 2015. Cryptinae (Hymenoptera: Ichneumonidae) fauna of Isfahan Province of central Iran. *Turk J Zool* 6.

Bartlett RP 2000, August. *Efficiency of collection methods and flight activity of Ichneumonidae (Hymenoptera) in three sites in Guanacaste, Costa Rica* (Thesis). University of Georgia. Retrieved from http://athenaeum.libs.uga.edu/handle/10724/20088

Bennett AMR, Cardinal S, Gauld ID & Wahl DB 2019. Phylogeny of the subfamilies of Ichneumonidae (Hymenoptera). *Journal of Hymenoptera Research* 71: 1–156. https://doi.org/10.3897/jhr.71.32375

Broberg G 2019. *Mannen som ordnade naturen : En biografi över Carl von Linné* (1st ed.). Natur & Kultur.

Chao A 1984. Nonparametric Estimation of the Number of Classes in a Population. *Scandinavian Journal of Statistics* 11: 265–270.

Chapman AD 2009. *Numbers of Living Species in Australia and the World, 2nd edition* (Australian Biological Resources Study). Retrieved from https://www.environment.gov.au/system/files/pages/2ee3f4a1-f130-465b-9c7a-79373680a067/files/nlsaw-2nd-complete.pdf

Chapman CA & Lambert JE 2000. Habitat alteration and the conservation of African primates: A case study of Kibale National Park, Uganda. *American Journal of Primatology* 50: 169–185.

Chapman CA, Wrangham RW, Chapman LJ, Kennard DK & Zanne AE 1999. Fruit and flower phenology at two sites in Kibale National Park, Uganda. *Journal of Tropical Ecology* 15: 189–211. https://doi.org/10.1017/S0266467499000759

Condamine FL, Sperling FA, Wahlberg N, Rasplus J-Y & Kergoat GJ 2012. What causes latitudinal gradients in species diversity? Evolutionary processes and ecological constraints on swallowtail biodiversity. *Ecology Letters* 15: 267–277.

Daïnou K, Bauduin A, Bourland N, Gillet J-F, Fétéké F & Doucet J-L 2011. Soil seed bank characteristics in Cameroonian rainforests and implications for post-logging forest recovery. *Ecological Engineering* 37: 1499–1506. https://doi.org/10.1016/j.ecoleng.2011.05.004

Erwin TL 1982. Tropical forests: their richness in Coleoptera and other arthropod species. *Coleopterists Bulletin* 36: 74–75.

Fraser SE, Dytham C & Mayhew PJ 2008. The effectiveness and optimal use of Malaise traps for monitoring parasitoid wasps. *Insect Conservation and Diversity* 1: 22–31. https://doi.org/10.1111/j.1752-4598.2007.00003.x

Fritzén NR & Sääksjärvi IE 2016. Spider silk felting—functional morphology of the ovipositor tip of Clistopyga sp. (Ichneumonidae) reveals a novel use of the hymenopteran ovipositor. *Biology Letters* 12: 20160350. https://doi.org/10.1098/rsbl.2016.0350

Gaston KJ 1996. Biodiversity - latitudinal gradients. *Progress in Physical Geography: Earth and Environment* 20: 466–476. https://doi.org/10.1177/030913339602000406

Gaston KJ 2000. Global patterns in biodiversity. *Nature* 405: 220–227. https://doi.org/10.1038/35012228

Gaston KJ & Gauld ID 1993. How many species of pimplines (Hymenoptera: Ichneumonidae) are there in Costa Rica? *Journal of Tropical Ecology* 9: 491–499. https://doi.org/10.1017/S0266467400007550

Gauld I & Bolton B 1988. *The Hymenoptera*. London: Oxford University Press & British Museum (Natural History).

Gauld ID 1987. Some factors affecting the composition of tropical ichneumonid faunas. *Biological Journal of the Linnean Society* 30: 299–312.

Gauld ID 1991. The Ichneumonidae of Costa Rica, 1. *Memoirs of the American Entomological Institute* 47: 1–589.

Gauld ID, Gaston KJ & Janzen DH 1992. Plant allelochemicals, tritrophic interactions and the anomalous diversity of tropical parasitoids: the 'nasty' host hypothesis. *Oikos* 65: 353–357.

Gauld ID & Mitchell PA 1978. *The taxonomy, distribution, and host preferences of African parasitic wasps of the subfamily Ophioninae*. Farnham Royal, Slough, SL2 3BN, England: Commonwealth Agricultural Bureaux.

Gauld ID, Sithole R, Gomez JU & Godoy C 2002. The Ichneumonidae of Costa Rica, 4. *Memoirs of the American Entomological Institute* 66: 1–768.

Gentry AH 1982. Neotropical Floristic Diversity: Phytogeographical Connections Between Central and South America, Pleistocene Climatic Fluctuations, or an Accident of the Andean Orogeny? *Annals of the Missouri Botanical Garden* 69: 557. https://doi.org/10.2307/2399084

Gentry AH 1988. Tree species richness of upper Amazonian forests. *Proceedings of the National Academy of Sciences* 85: 156–159. https://doi.org/10.1073/pnas.85.1.156

Global Malaise Trap Program | Centre for Biodiversity Genomics (n.d.). Retrieved 15 February 2020, from https://biodiversitygenomics.net/projects/gmp/

Gómez IC, Sääksjärvi IE, Mayhew PJ, Pollet M, Rey del Castillo C, Nieves-Aldrey J-L, … Tuomisto H 2017. Variation in the species richness of parasitoid wasps (Ichneumonidae: Pimplinae and Rhyssinae) across sites on different continents. *Insect Conservation and Diversity*. https://doi.org/10.1111/icad.12281

Gómez IC, Sääksjärvi IE, Puhakka L, Castillo C & Bordera S 2015. The Peruvian Amazonian species of *Epirhyssa* Cresson (Hymenoptera: Ichneumonidae: Rhyssinae), with notes on tropical species richness. *Zootaxa* 3937: 311–336. https://doi.org/10.11646/zootaxa.3937.2.4

Gotelli NJ & Colwell RK 2011. Estimating species richness. *Biological Diversity: Frontiers in Measurement and Assessment* 39–54.

Hamilton AJ 2005. Species diversity or biodiversity? *Journal of Environmental Management* 75: 89–92. https://doi.org/10.1016/j.jenvman.2004.11.012

Hamilton AJ, Basset Y, Benke KK, Grimbacher PS, Miller SE, Novotný V, … Yen JDL 2010. Quantifying Uncertainty in Estimation of Tropical Arthropod Species Richness. *The American Naturalist* 176: 90–95. https://doi.org/10.1086/652998

Hawkins BA 2001. Ecology’s oldest pattern? *Trends in Ecology & Evolution* 16: 470.

Hillebrand H 2004. On the generality of the latitudinal diversity gradient. *The American Naturalist* 163: 192–211.

Hopkins T, Gómez I, Tuomisto H, Ruokolainen K, Cárdenas GG, Mesones I, … Sääksjärvi IE 2020. Amazon Malaise trapping data 1998 2000 2008 2011 [Dataset]. *Zenodo*. https://doi.org/10.5281/zenodo.4030168

Hopkins T, Roininen H & Sääksjärvi IE 2019. Uganda Malaise trapping 2014–2015 background data [Dataset]. *Zenodo*. https://doi.org/10.5281/zenodo.2225643

Humboldt A von, Bohn HG & Otté EC 1850. *Views of nature, or, Contemplations on the sublime phenomena of creation, with scientific illustrations* (3rd ed.). London : H.G. Bohn. Retrieved from https://www.biodiversitylibrary.org/item/57775

Hurlbert SH 1971. The Nonconcept of Species Diversity: A Critique and Alternative Parameters. *Ecology* 52: 577–586. https://doi.org/10.2307/1934145

Janzen DH 1973. Sweep Samples of Tropical Foliage Insects: Effects of Seasons, Vegetation Types, Elevation, Time of Day, and Insularity. *Ecology* 54: 687–708. https://doi.org/10.2307/1935359

Janzen DH 1981. The peak in North American ichneumonid species richness lies between 38 degrees and 42 degrees N. *Ecology* 532–537.

Janzen DH & Pond CM 1975. A comparison, by sweep sampling, of the arthropod fauna of secondary vegetation in Michigan, England and Costa Rica. *Transactions of the Royal Entomological Society of London* 127: 33–50. https://doi.org/10.1111/j.1365-2311.1975.tb00551.x

Johansson N & Cederberg B 2019. Review of the Swedish species of Ophion (Hymenoptera: Ichneumonidae: Ophioninae), with the description of 18 new species and an illustrated key to Swedish species. *European Journal of Taxonomy* 0. https://doi.org/10.5852/ejt.2019.550

Karlsson D, Hartop E, Forshage M, Jaschhof M & Ronquist F 2020. The Swedish Malaise Trap Project: A 15 Year Retrospective on a Countrywide Insect Inventory. *Biodiversity Data Journal* 8: e47255. https://doi.org/10.3897/BDJ.8.e47255

Klopfstein S, Langille B, Spasojevic T, Broad GR, Cooper SJB, Austin AD & Niehuis O 2019. Hybrid capture data unravel a rapid radiation of pimpliform parasitoid wasps (Hymenoptera: Ichneumonidae: Pimpliformes): Rapid radiation of pimpliform parasitoid wasps. *Systematic Entomology* 44: 361–383. https://doi.org/10.1111/syen.12333

Klopfstein S, Santos BF, Shaw MR, Alvarado M, Bennett AMR, Pos DD, … Broad GR 2019. Darwin wasps: a new name heralds renewed efforts to unravel the evolutionary history of Ichneumonidae. *Entomological Communications* 1: ec01006–ec01006.

Kouki J, Niemelä P & Viitasaari M 1994. Reversed latitudinal gradient in species richness of sawflies (Hymenoptera, Symphyta). *Annales Zoologici Fennici* 83–88.

Lehikoinen E, Lemmetyinen R, Vuorisalo T & Kivistö S 2009. *Suomen lintutieteen synty — Turun Akatemian aika*. Faros-kustannus Oy.

Lifeplan: A Planetary inventory of life | University of Helsinki 2019, November 8. Retrieved 15 February 2020, from https://www.helsinki.fi/en/projects/lifeplan

Longino JT, Coddington J & Colwell RK 2002. The ant fauna of a tropical rain forest: estimating species richness three different ways. *Ecology* 83: 689–702. https://doi.org/10.1890/0012-9658(2002)083[0689:TAFOAT]2.0.CO;2

Malaise R 1937. A new insect-trap. *Entomologisk Tidskrift* 58: 148–160.

May R 1988. How many species are there on earth? *Science* 241: 1441–1449.

May RM 1986. How many species are there? *Nature* 324: 514–515.

May RM 2010. Tropical Arthropod Species, More or Less? *Science* 329: 41–42. https://doi.org/10.1126/science.1191058

Mayr E 1999. *Systematics and the Origin of Species, from the Viewpoint of a Zoologist*. Harvard University Press.

Mittelbach GG, Schemske DW, Cornell HV, Allen AP, Brown JM, Bush MB, … Turelli M 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology Letters* 10: 315–331. https://doi.org/10.1111/j.1461-0248.2007.01020.x

Mora C, Tittensor DP, Adl S, Simpson AGB & Worm B 2011. How Many Species Are There on Earth and in the Ocean? *PLOS Biology* 9: e1001127. https://doi.org/10.1371/journal.pbio.1001127

Morris RJ, Lewis OT & Godfray HCJ 2004. Experimental evidence for apparent competition in a tropical forest food web. *Nature* 428: 310–313. https://doi.org/10.1038/nature02394

Morrison G, Auerbach M & McCoy ED 1979. Anomalous Diversity of Tropical Parasitoids: A General Phenomenon? *The American Naturalist* 114: 303–307. https://doi.org/10.1086/283477

Muona J 2004. Kreivi Carl Gustav Mannerheim, entomologi. *Luonnon Tutkija* 108: 100–106.

Naughton-Treves L 1998. Predicting patterns of crop damage by wildlife around Kibale National Park, Uganda. *Conservation Biology* 12: 156–168.

Nordisk familjebok. 24. Ryssläder - Sekretär 1916. (2nd ed.). Retrieved from http://runeberg.org/nfcd/0195.html

Novotny V 2006. Why Are There So Many Species of Herbivorous Insects in Tropical Rainforests? *Science* 313: 1115–1118. https://doi.org/10.1126/science.1129237

Owen DF & Chanter DO 1970. Species Diversity and Seasonal Abundance in Tropical Ichneumonidae. *Oikos* 21: 142–144. https://doi.org/10.2307/3543849

Owen DF & Owen J 1974. Species diversity in temperate and tropical Ichneumonidae. *Nature* 249: 583–584. https://doi.org/10.1038/249583a0

Pearson DL 1977. A Pantropical Comparison of Bird Community Structure on Six Lowland Forest Sites. *The Condor* 79: 232. https://doi.org/10.2307/1367167

Peet RK 1974. The measurement of species diversity. *Annual Review of Ecology and Systematics* 5: 285–307.

Pham NT, Broad GR, Zhu C-D & Achterberg CV 2018. A review of the genus Triancyra Baltazar (Ichneumonidae: Rhyssinae) from Vietnam, with descriptions of three new species. *Zootaxa* 4377: 565. https://doi.org/10.11646/zootaxa.4377.4.5

Quicke DLJ 2012. We know too little about parasitoid wasp distributions to draw any conclusions about latitudinal trends in species richness, body size and biology. *PLoS ONE* 7: e32101. https://doi.org/10.1371/journal.pone.0032101

Quicke DLJ, Laurenne NM, Fitton MG & Broad GR 2009. A thousand and one wasps: a 28S rDNA and morphological phylogeny of the Ichneumonidae (Insecta: Hymenoptera) with an investigation into alignment parameter space and elision. *Journal of Natural History* 43: 1305–1421. https://doi.org/10.1080/00222930902807783

Rathcke BJ & Price PW 1976. Anomalous Diversity of Tropical Ichneumonid Parasitoids: A Predation Hypothesis. *The American Naturalist* 110: 889–893.

Roskov Y, Ower G, Orrell T, Nicolson D, Bailly N, Kirk PM, … Penev L (Eds.) 2019. Species 2000 & ITIS Catalogue of Life, 2019 Annual Checklist. *Species 2000: Naturalis, Leiden, the Netherlands*. Retrieved from http://www.catalogueoflife.org/annual-checklist/2019

Roslin T, Wirta H, Hopkins T, Hardwick B & Várkonyi G 2013. Indirect interactions in the high arctic. *PLoS One* 8: e67367. https://doi.org/10.1371/journal.pone.0067367

Rousse P & Noort S van 2014. Afrotropical Ophioninae (Hymenoptera, Ichneumonidae): an update of Gauld and Mitchell's revision, including two new species and an interactive matrix identification key. *ZooKeys* 456: 59–73. https://doi.org/10.3897/zookeys.456.8140

Rousse P & van Noort S 2014. A review of the Afrotropical Rhyssinae (Hymenoptera: Ichneumonidae) with the descriptions of five new species. *European Journal of Taxonomy* 91: 1–42. https://doi.org/10.5852/ejt.2014.91

Sääksjärvi IE, Castillo C, Bordera S & Broad GR 2015. Clistopyga caramba sp. nov.(Hymenoptera: Ichneumonidae; Pimplinae), an astonishing example of mimicry in spider-attacking parasitoid wasps. *Zootaxa* 4013: 287–292. https://doi.org/10.11646/zootaxa.4013.2.9

Sääksjärvi IE, Haataja S, Neuvonen S, Gauld ID, Jussila R, Salo J & Burgos AM 2004. High local species richness of parasitic wasps (Hymenoptera: Ichneumonidae; Pimplinae and Rhyssinae) from the lowland rainforests of Peruvian Amazonia. *Ecological Entomology* 29: 735–743. https://doi.org/10.1111/j.0307-6946.2004.00656.x

Sääksjärvi IE, Ruokolainen K, Tuomisto H, Haataja S, Fine PVA, Cárdenas G, … Vargas V 2006. Comparing composition and diversity of parasitoid wasps and plants in an Amazonian rain-forest mosaic. *Journal of Tropical Ecology* 22: 167–176. https://doi.org/10.1017/S0266467405002993

Saalas U 1952. *Retkeilyjä kolmessa maanosassa*. Werner Söderström OY.

Saunders TE & Ward DF 2018. Variation in the diversity and richness of parasitoid wasps based on sampling effort. *PeerJ* 6: e4642. https://doi.org/10.7717/peerj.4642

Shapiro BA & Pickering J 2000. Rainfall and parasitic wasp (Hymenoptera: Ichneumonoidea) activity in successional forest stages at Barro Colorado Nature Monument, Panama, and La Selva Biological Station, Costa Rica. *Agricultural and Forest Entomology* 2: 39–47. https://doi.org/10.1046/j.1461-9563.2000.00048.x

Sime KR & Brower AVZ 1998. Explaining the latitudinal gradient anomaly in ichneumonid species richness: evidence from butterflies. *Journal of Animal Ecology* 67: 387–399. https://doi.org/10.1046/j.1365-2656.1998.00198.x

Spasojevic T, Broad GR, Sääksjärvi IE, Schwarz M, Ito M, Korenko S & Klopfstein S 2020. Mind the Outgroup and Bare Branches in Total-Evidence Dating: a Case Study of Pimpliform Darwin Wasps (Hymenoptera, Ichneumonidae). *Systematic Biology*. https://doi.org/10.1093/sysbio/syaa079

Stevens GC 1989. The Latitudinal Gradient in Geographical Range: How so Many Species Coexist in the Tropics. *The American Naturalist* 133: 240–256.

Struhsaker TT 1997. *Ecology of an African rain forest: logging in Kibale and the conflict between conservation and exploitation*. Gainesville, Florida, USA: University Press of Florida.

The Catalogue of Life plus project 2020. sp2000. Retrieved from https://github.com/Sp2000/colplus (Original work published 2017)

Timms L, Schwarzfeld M & Sääksjärvi I 2015. Ichneumonid subfamily abundances from 38 datasets collected between 25°S and 81°N [Dataset]. *Figshare*. https://doi.org/10.6084/m9.figshare.1501519.v1

Timms LL, Schwarzfeld M & Sääksjärvi IE 2016. Extending understanding of latitudinal patterns in parasitoid wasp diversity. *Insect Conservation and Diversity* 9: 74–86. https://doi.org/10.1111/icad.12144

Tuomisto H 2010. A consistent terminology for quantifying species diversity? Yes, it does exist. *Oecologia* 164: 853–860. https://doi.org/10.1007/s00442-010-1812-0

Tuomisto H, Ruokolainen K, Kalliola R, Linna A, Danjoy W & Rodriguez Z 1995. Dissecting Amazonian Biodiversity. *Science* 269: 63–66. https://doi.org/10.1126/science.269.5220.63

van Noort S 2004. Ichneumonid (Hymenoptera: Ichneumonoidea) diversity across an elevational gradient on Monts Doudou in Southwestern Gabon. *California Academy of Sciences Memoir* 28: 187–216.

van Noort S 2019. WaspWeb: Hymenoptera of the Afrotropical region. Retrieved 27 February 2020, from www.waspweb.org

van Noort S, Prinsloo GL & Compton SG 2000. Hymenoptera, excluding Apoidea (Apiformes) & Formicidae (Insecta). *Cimbebasia Memoir* 9: 289–364.

Vårdal H & Taeger A 2011. The life of René Malaise: from the wild east to a sunken island. *Zootaxa* 3127: 38–52.

Várkonyi G, Hanski I, Rost M & Itämies J 2002. Host-parasitoid dynamics in periodic boreal moths. *Oikos* 98: 421–430. https://doi.org/10.1034/j.1600-0706.2002.980306.x

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: Biological Sciences* 279: 4694–4698. https://doi.org/10.1098/rspb.2012.1664

Vespucci A 1503. Letter on his Third Voyage from Amerigo Vespucci to Lorenzo Pietro Francesco di Medici. *Project Gutenberg E#36924* 42–52.

Wallace AR 1878. *Tropical Nature: And Other Essays*. Macmillan and Company, London.

Warton DI, Thibaut L & Wang YA 2017. The PIT-trap—A "model-free" bootstrap procedure for inference about regression models with discrete, multivariate responses. *PLOS ONE* 12: e0181790. https://doi.org/10.1371/journal.pone.0181790

Weijola V, Donnellan S & Lindqvist C 2016. A new blue-tailed Monitor lizard (Reptilia, Squamata, Varanus) of the Varanus indicus group from Mussau Island, Papua New Guinea. *ZooKeys* 568: 129–154. https://doi.org/10.3897/zookeys.568.6872

Whitney BM & Alonso JA 1998. A new Herpsilochmus antwren (Aves: Thamnophilidae) from northern Amazonian Peru and adjacent Ecuador: The role of edaphic heterogeneity of terra firme forest. *The Auk* 559–576.

Willig MR, Kaufman DM & Stevens RD 2003. Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. *Annual Review of Ecology, Evolution, and Systematics* 34: 273–309. https://doi.org/10.1146/annurev.ecolsys.34.012103.144032

What species inhabit our planet? Where are they to be found? In this thesis, I look at the Darwin wasps (Hymenoptera: Ichneumonidae) of African tropical forest. I describe how many species of the subfamily Rhyssinae I found at a study site in Uganda, when and where in the forest they are active, and how their species richness compares to that of study sites in western Amazonia.

Suomenkieliset tiivistelmät ja kuvatekstit löytyvät osoitteesta:
Sammandrag och bildtexter på ***svenska*** finns här:
https://doi.org/10.5281/zenodo.3708018

UNIVERSITY OF TURKU

Painosalama Oy, Turku, Finland 2021

ISBN 978-951-29-8361-2 (PRINT)
ISBN 978-951-29-8362-9 (PDF)
ISSN 0082-6979 (Painettu/Print)
ISSN 2343-3183 (Sähköinen/Online)