Introductory Essay

On the Origin of Species Assemblages in Bornean Microsnail Communities

-

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ABSTRACT

Community assembly rules are dictated by the interactions between evolutionary history and community structure. It is believed that the core set of assembly rules should be general and overall applicable, yet we only just begin to get an understanding of them. Previous research in this field has focused on single or phylogenetically unrelated taxa, using mainly community data of a qualitative nature. In this PhD research, I focus on the relation between community diversity and diversification, using multiple, phylogenetically related, but "parallel" communities. I will test the hypotheses that more diverse communities show higher rates of species diversification, and that niche differentiation by food choice and microhabitat drive diversification further. The numerous microsnail communities of Bornean limestone outcrops offer a rich and relatively easy-to-mine set of phylogenetically related communities for which quantitative data can be gathered. Working together with fellow researchers that work with similar systems in the lab and in theoretical applications, I aim for my research to aid in the development of several important, general community assembly rules, valuable for both ecologists and conservationists.

1. INTRODUCTION

The formation of ecological communities in nature, or community assembly, is an intensely debated topic. Many natural communities are being threatened, virtually always by anthropogenic changes. It is often believed that many community assembly rules should be generic and widely applicable (e.g. Hubbell, 2001). Understanding how communities are formed is fundamental to making sound assessments in conservation biology and policy making. When correct predictions can be made, damage to communities we value most can be averted, or at least mitigated.

My PhD research focuses on microsnail communities in Sabah, Malaysian Borneo. These microsnail communities offer an exceptional study system with regard to community assembly. The communities are rich in trophically similar species, gathering quantitative community data is relatively straightforward, and many “parallel” communities exist on limestone “islands” in close proximity to each other, offering a natural laboratory with replication in temporal and spatial dimensions.

This research is embedded in the larger scientific work of prof. dr. Rampal Etienne of the University of Groningen, the Netherlands. The overarching subject of Etienne’s research is “The Origin of Biodiversity”. The value of his research has been well appreciated, as reflected by a Vici grant awarded to Etienne by the Netherlands Organisation for Scientific Research (NWO) in 2014 (NWO, 2013). Within the theoretical framework set out by Etienne (Figure 1), community assembly is studied from the perspectives of both evolutionary and ecological processes (Etienne, 2013). The community is studied at multiple related levels. At the higher level is the regional species pool, which is made up of several local species pools. Biotic and abiotic assembly processes result in an actual species community being distilled from such a local species pool. Currently, the main focus of Etienne’s work is on the influence of community diversity and community structure on diversification (the green arrow in Figure 1) (Etienne, 2013).

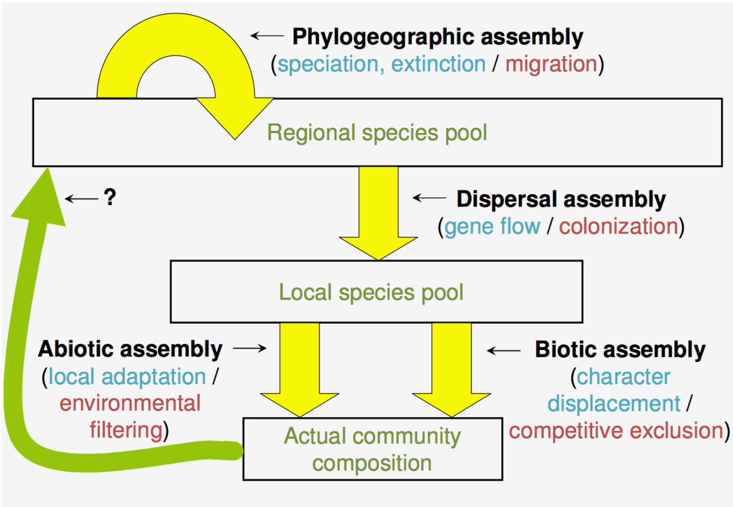

Figure 1 - Theoretical framework set out by Rampal Etienne to perform an integral study on biodiversity origins, focussing on both evolutionary (in blue) and ecological (in red) processes (from Etienne, 2013).

This Introductory Essay addresses the fundamental questions, objectives and ideas of my PhD research project, to be conducted between September 2014 and September 2019. The rest of Chapter 1 addresses fundamental considerations on the subject of the study of community assembly in Bornean microsnails. Chapter 2 introduces the main questions I try to answer. With each question, the rationale, background information, current knowledge, hypotheses and novelty of the study are presented. Chapter 3 describes the system I focus on, microsnail communities on limestone outcrops in Sabah, Malaysian Borneo. Unique characteristics of this system are emphasized. The species that form the communities, as well as the environment that hosts the communities, are delineated. Chapter 4 covers all the important practical issues to de dealt with, including fieldwork and materials and methods. To be able to work with consistent definitions throughout the project, I have provided a Glossary.

(1) WHY STUDY COMMUNITIES IN RELATION TO PHYLOGENETICS?

The temporal and spatial scales at with communities and phylogenetics are studied usually differ. The community is studied at a local scale, sometimes regional. Time involved is that of several to tens of generations. The unit of interest, the community itself, is formed by a group of populations of different species, closely connected by their habitat and feeding needs (consider the average food web). Phylogenetics, the history of species formations, is very often studied at a spatial scale of (part of) a continent or an oceanic basin. Even in tiny organisms such as microsnails, the spatial scale of study would likely be the Sunda Region or Southeast Asia as a whole. Temporal scale would be that of thousands to hundreds of thousands of generations, eras in which new species can form.

Consider the "community" of ships sailing the Mediterranean Sea on a single day (Figure 2). There is a clear pattern to be spotted. Luxurious yachts (in purple) sail along the shores or are at anchor, showing off to the people along the seashores. Passenger ferries (in green) sail either along the shores or up and down between the region's numerous islands. Local freighters (in blue) sail between local ports, and the large cargo vessels (in yellow and orange) sail between the region's main ports, Atlantic ports and beyond. Within this community, spatial structure is determined foremost by function. Niche occupation has arisen due to local circumstances, while historical influence is negligible.

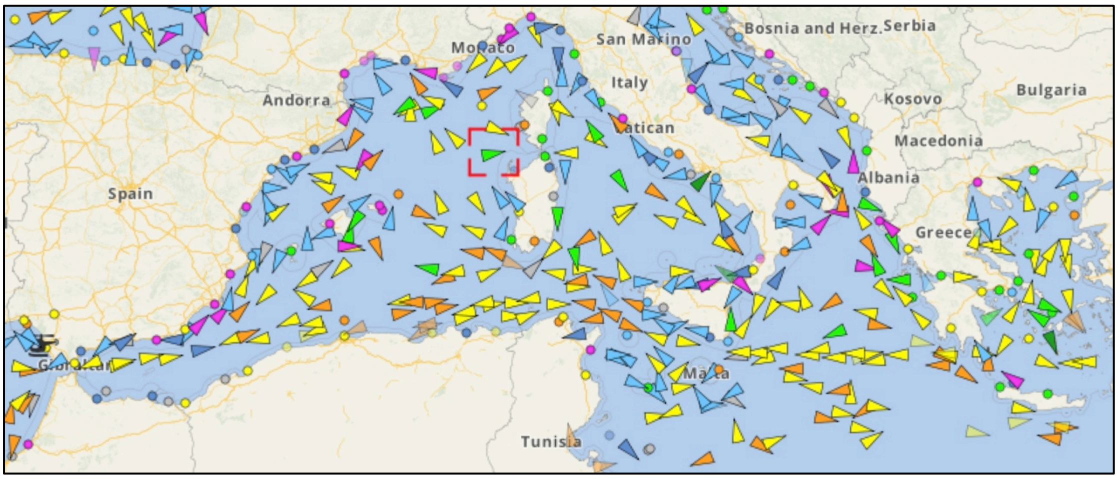

Figure 2 – A "community" of ships sailing the Mediterranean Sea. Spatial community structure is determined foremost by function (niche). Yachts in purple, passenger ferries in green, local freighters in blue and different cargo vessels in yellow and orange. Source: Vesselfinder.com, 23 April 2015.

Now, consider the "community" of inhabitants in the United States of America (Figure 3), ordered by descent. It is immediately clear that the people living in the north and centre of the country are mostly descendants from German immigrants. In the southwest, most people have descended from Mexican ancestors. Other clear ancestral pockets in the country are African Americans (southeast), English (Midwest), Irish, Finnish, Dutch, and so forth. We could try to explain this community structure from a functional (niche) perspective alone, but would end up finding that all people on average have the same capabilities to fulfil jobs around the country and so on. Contrary to the example of the "community" of vessels sailing the Mediterranean, we here see a clear case of how spatial community structure is determined foremost by history (phylogeny).

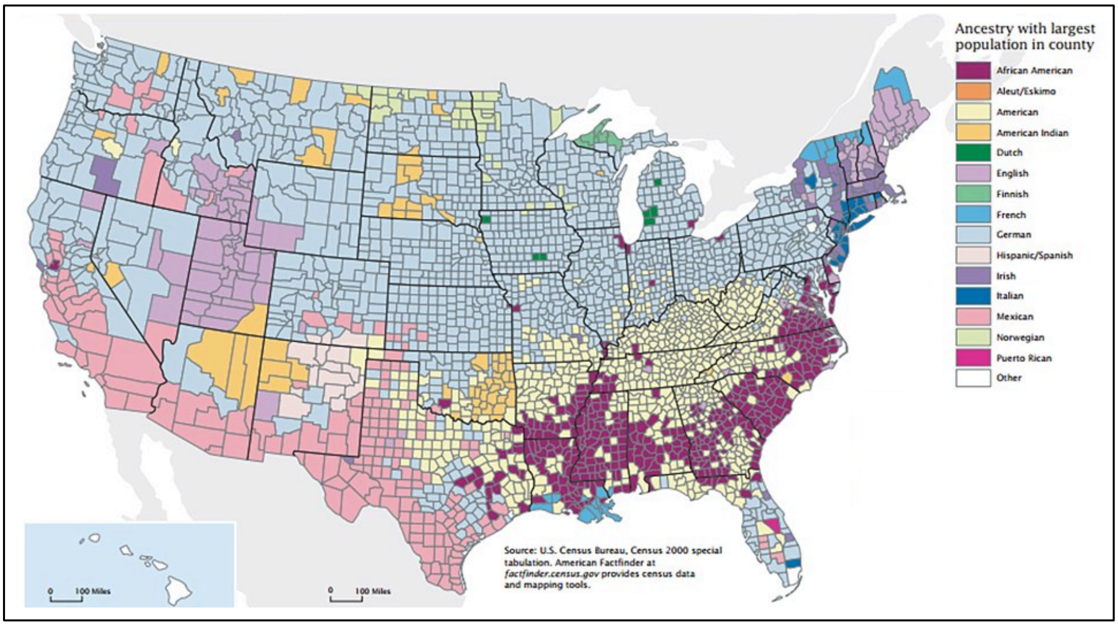

Figure 3 - A "community" of human inhabitants of the United States of America. Spatial community structure is determined by history (phylogeny) first. Source: http://www.dailymail.co.uk/news/article-2408591/American-ethnicity-map-shows-melting-pot-ethnicities-make-USA-today.html.

Back to the ecological community, the assemblage of organisms. When considering the Bornean microsnails living on limestone outcrops, it might be found that any community composition and structure within the community is determined by niche occupation, or maybe to a large extent by neutral processes. At the same time, as in the case of the "community" of inhabitants of the USA, historical processes may be at work, but less obvious. Of course, the game of community formation, or the assembling of species, can only be played by species having reached the (local) community. As such, history, and phylogeny as a result, are closely related to the formation of the species assemblages. Studying the two simultaneously thus makes proper sense.

Webb et al. (2002) (Figure 4) give an overview of how different approaches to the study of the community, phylogenetics and traits can be related. Highlighted in red are the approaches I undertake during my PhD research.

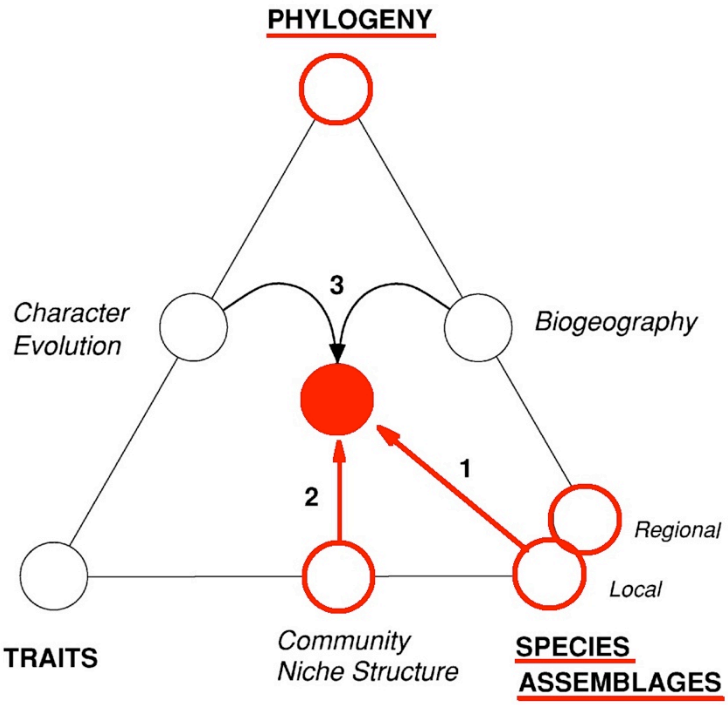

Figure 4 – How different approaches to the integration of phylogeny, traits and communities are related (from Webb et al., 2002). Highlighted in red are the approaches I take during my PhD research on community assembly of Bornean microsnails.

(2) Why study Microsnails?

Birds and mammals, as well as many other vertebrate taxa, have been well studied during the past 200 years. The body of scientific literature covering the ecology and evolution of birds, for example, is enormous. Data on distribution have been gathered over the last decades by thousands of enthusiastic volunteers. Proper, reliable phylogenies based on genetics are now available for most bird families. So, why focus on a group of little studied, tropical gastropods instead?

1. Since Darwin's visits to the Galapagos Islands, it has been realized that islands are "nature's test tubes" of species formation: "Islands are small, have distinct boundaries and simplified biotas" (Losos and Ricklefs, 2009). Many species of microsnail are obligate calcicoles, strictly present on open limestone outcrops. Such outcrops are present in an island-like pattern across the Southeast Asian tropics. Even complete archipelagos of limestone islands can be identified in Sabah, Malaysian Borneo, such as the ones described in Chapter 4, Paragraph 1.
2. The general naturalist, with no special eye for tiny gastropods, could go through a tropical forest without ever observing a single microsnail individual. Once aiming for these microsnails, and focussing on specific substrates, one can easily find thousands them. Bornean microsnails abound in numbers. And collecting is just as easy, when focusing on shells from the soil (Schilthuizen & Vermeulen 2014, pers. comm.; personal experience, exploratory fieldwork March 2015).

3. Regional microsnail communities come at a small spatial scale (Schilthuizen et al., 2002). My first studies will focus on heterogeneity within each limestone outcrop. My initial impression, based on a small inventory study of the limestone outcrop of Gua Laing, Crocker Range, Sabah, Malaysian Borneo (Chapter 4, Paragraph 1), is that the diversity and geographic structure in microsnails on a single limestone outcrop might be comparable to those found in a bird community at the scale of a mountain range such as the Andes! This makes sense, because microsnails are small and their dispersal capacity is very low indeed. If this turns out to be a general pattern, this means that the study of just a few (say, five) limestone outcrops for microsnail communities on Sabah could yield data comparable to the study of all the birds living in all mountainous areas in the Americas. Or, a few years of work by a single student of microsnails may yield data similar to that collected by hundreds of volunteers and several scientists studying birds across a continent over decades.
4. The current study will give special attention to the influence of community diversity on diversification. With microsnail communities holding between 20 and 60 species each, they are likely to offer the variation in species composition and diversity needed for a comparative analysis between communities.

(3) Is it valid to study Microsnail Communities in Isolation?

The current study is titled "On the origin of Species Assemblages in Bornean Microsnail Communities." But how should this community be defined? And is it valid to study such a community in isolation?

In every study of any natural system, choices have to be made on how to delineate the system of interest and the study of it. These choices are often implicit and can feel so natural they are no longer the issue of debate. In the study of animal behaviour, ethology, the unit of interest is the individual (or sometimes a pair or family). But, each individual (or pair, or family) is embedded in a population, with each population having a different influence on the individual. In return, each population is embedded in the meta-population, the genetics of each population being influenced by neighbouring populations. The community is the common unit of interest in community ecology and conservation biology. The community, as in the examples above, cannot be considered fully in isolation, but is embedded in a meta-community, "a set of interacting communities which are linked by the dispersal of multiple, potentially interacting species."

In my research, when I study the "limestone microsnail community", I focus on the community of microsnails that is part of a bigger limestone community, as depicted in Figure 5 and enclosed by a red dashed line. In fact, it would be more appropriate to talk of the "microsnail guild", where the guild is defined as "an association of functionally equivalent species in a community." For the time being, with no further knowledge at hand, I consider the limestone-inhabiting microsnails to form a single separate guild. Over time, after further collections and field studies, it may well turn out that, in fact, more than one guild can be distinguished, based for example on the occupation of clearly different microhabitats.

Is it valid to study a single guild in isolation? This is a very difficult question to answer beforehand. The overall best community ecology study ever would determine every relation between every species for every community. This is not possible. To work on manageable species relations, scientists have often focussed on single, species-to-species relations of predation, commensalism and mutualism. For my PhD study, I choose to focus on the guild as a unit, with the argument that the guild is composed of a limited number of sympatric species, closely connected by (almost) functional identity.

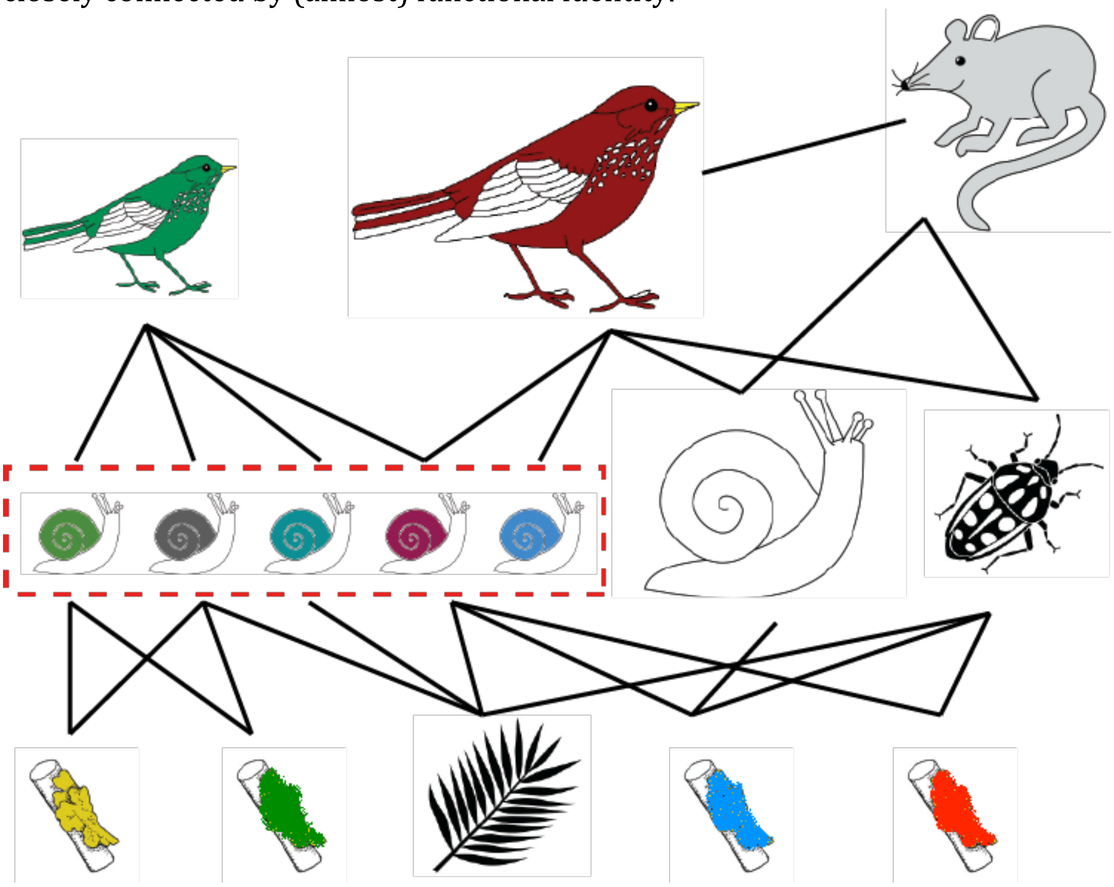

Figure 5 – A simplified and schematic representation of a community of limestone inhabiting organisms in three trophic levels and their relations. Enclosed by a red dashed line is a hypothetical guild of microsnails.

(4) Do Microsnail Shells in the Soil offer a True Representation of the Limestone Community?

Due to their tiny size and preference for shadowy and dark habitat, microsnails on limestone are notoriously hard to find alive (personal experience, exploratory fieldwork March 2015). Several species, such as those belonging to the genus *Plectostoma*, live on the bare rock and can be found alive in good numbers within several hours of searching time. Others, such as many members of the genus *Opisthostoma*, have almost never been found alive, and some species are known only from their shells (Schilthuizen & Vermeulen 2014, pers. comm.). Most taxa can be found alive, but search time would be many hours to days for several individuals (Liew 2015, pers. comm.). Census data on actual numbers present at a site is difficult to obtain in such a way of sampling.

It has been found decades ago that hundreds to thousands of shells of deceased microsnails end up in the soil below limestone outcrops. After dying, they are flushed out of the crevices, pits and holes of the rocks, from the patches of moss and from the leaves of nearby plants. With heavy tropical rains, this is not difficult to imagine. In the soil, these shells remain intact for several years (Schilthuizen 2011). The shell numbers found from the soil easily outnumber the animals found alive by hundreds to thousands of times.

Can we be certain that the representation of the animals found from the soil is representative for the local species community? One way to find out would be a complete overhaul of a plot. From a one by one meter plot, all soil would have to be collected and all shells sought out. Also, the complete vegetation would have to be cut down and taken to the lab. All the rock would have to be cut away up to a depth at with microsnails can live in the numerous limestone crevices and brought along. Moreover, a reconstruction of the community composition from the soil would include individuals living several meters higher up, having been flushed down by the rains, complicating matters further. The total exercise would make for an immense project.

It needs to be stressed that the representation of the community from soil data is very likely to be much closer to reality than the representation of the community that can ever be found from live specimens (Schilthuizen 2015, pers. comm.). Schilthuizen (2011) compares the efficiency of sampling the soil with that of fogging in canopy insects, which is an accepted method for sampling forest canopy insect communities. However, for the sake of scientific integrity, it would be demanded to set up several tests, preferably based on data to be collected to answer the main research questions (Chapter 2 and Chapter 4, Paragraph 2). Here I present several initial thoughts.

1. Do we find species alive which are absent from the soil data? If yes, the soil data should be distrusted. If no, the soil data can still be trusted as a source of information.
2. In species found alive in high numbers, consider their relative species abundance. Is the relative species abundance of the subset from the soil data (containing only the same species) equal? If yes, the soil data can still be trusted as a source of information. If no, than the soil data must be distrusted.

More ideas are to be formed and tested in the near future.

(5) The Geology of Borneo and its influence on Microsnails

The geology of the habitat of interest for the current study, limestone outcrops in Sabah, Malaysian Bornean, is complex. It has not yet been fully reconstructed by geologists. The following list covers the phenomena with the most influence on limestone outcrop geology, as based on personal comments by Willem Renema (2015) and the literature (Vermeulen and Whitten, 1999).

1. Formation: limestone originates from millions of years old corals, shells, algae and other marine organisms. However, some are much younger, raised coral reefs that have not undergone much post-depositional deformation.
2. Tectonics: vertical earth movements lifted up the deposited limestone, often above sea level.
3. Mechanical erosion: sediments from the top of the limestone were removed and the limestone rock was sculpted by mechanical wear in the process.
4. Dissolving action from water: limestone dissolves in water.
5. Chemical erosion: mainly humic acid from leaf litter, bacterial processes, etc.
6. Sea water level: throughout time, limestone outcrops may have seen periods of submergence by rising sea water levels.

Although the age of the limestone outcrop determines the maximum age of the community living on that outcrop, unfortunately, it is the complex interplay of all issues mentioned above that determines the actual age of each community at every limestone outcrop. In an archipelago of limestone outcrops close to each other, such as along Sungai Kinabatangan, it can be expected that outcrops of the same size and height have seen a similar geological history. However, if the origins of nearby outcrops turn out to differ, this is likely to have left a strong influence on community age and origins, too. This has to be kept in mind throughout the current PhD project, and more information on the geology of Bornean limestone outcrops should be collected.

2. RESEARCH QUESTIONS, OBJECTIVES, AND RESEARCH PLANS

In this chapter, I present my four main research questions, A to D. I give a summary of background information, link the subject to my study system, pose hypotheses and explain the novelty of my research. Related materials and methods needed for the resulting research, as well as first ideas on analytical tools and data analysis, are described in Chapter 4, Paragraph 2.

(A) Can Neutral Processes explain Microsnail Community Composition?

An ecological community is defined by the collection of species that live in the same location and engage in certain ecological relations with one another. Moreover, within many communities, phylogenetically closely related species co-occur. This has been shown to be the case in, for example, New World warblers (Parulidae; Collins et al., 1982, Parnell, 1969), Lake Malawi cichlid fish (Genner et al., 1999) and Bornean microsnails (Gastropoda; Schilthuizen et al., 2003a). Classical niche theory describes niche space as being multi-dimensional, and states that different species cannot co-occur when they inhabit exactly the same niche space (Gause, 1934, Hutchinson, 1953, Whittaker, 1972). In other words, similar species that co-occur must differ in the position on at least one dimension of niche space, even though this may not be readily apparent to the observer. Many studies have focussed on the identification of differences in niche occupation between highly similar species (e.g. Genner et al., 1999, Mumladze, 2014).

Neutral Theory also aims to explain the diversity of species in ecological communities. The theory assumes any niche differences between members of an ecological community as “neutral”, or irrelevant to their respective success (Hubbell, 2001). Hubbell writes: “I use neutral to describe the assumption of per capita ecological equivalence of all individuals of all species in a trophically defined community.” Neutral Theory has made many “rich predictions” in community ecology (Rosindell et al., 2011), such as those related to relative species abundances, species-area relationships and β-diversities.

Microsnails on Borneo offer relatively easy access to data on species diversity, relative species abundance, the meta-community and, above all, a high (> 10) number of “parallel” microsnail communities. On the other hand, data on species traits and functions of importance in community assembly is not easily obtained. To understand community structure in Bornean microsnails, I suggest starting with a study in which neutral predictions on the relative abundances and species diversities are tested on field data. Deviations between predicted community characteristics (such as community diversity) using Neutral Theory and field data is likely to offer valuable insights into community structure and community formation processes.

H_0: Neutral Theory alone can explain Bornean microsnail community patterns.
H_1: Neutral Theory alone cannot explain Bornean microsnail community patterns.

Novelty of the study

The scale at which microsnail community data can be made available (high number of species, high number of individuals, many communities) offers a chance to perform the same analyses multiple times and compare results. This study can offer many new insights for further research in both my own PhD and that of fellow PhD-students working on the subject of Neutral Theory.

(B) Does an Increase in Diversity increase Diversification?

Microsnails are a polyphyletic group, characterised by the homoplastic character state of a very small size, i.e. about five millimetres or less in shell diameter in adult stage, and sympatric occurrence. At different locations, microsnail community composition differs to a large extent. Also, diversity of the community is one of the varying characteristics between the various communities (Liew et al., 2008).

Systematics of Bornean microsnails has so far been based mainly on morphology, but molecular techniques to reconstruct evolutionary history have been applied over the past decade on a small number of genera. Studies on the genus *Plectostoma* (Liew et al., 2014b) and *Everettia* (Liew et al., 2009b) in Malaysia have shown the usefulness of applying molecular techniques as a basis for phylogenetic and ancestral state reconstructions.

I propose to reconstruct the phylogenetic histories of several further Bornean microsnail clades. Community history can be analysed based on the combination of phylogenetic and community data (Pigot and Etienne, 2015) (Figure 6). Tracing community diversity and diversification rates through evolutionary time for several different microsnail communities will offer valuable insight into one of Etienne's main questions: what is the influence of species diversity on diversification (Figure 1).

H_0: More diverse communities have not shown higher rates of diversification.
H_1: More diverse communities have shown higher rates of diversification.

Novelty of the study

The number of taxa for which both community and phylogenetic data are available, is still limited. The current study will be the first to gather datasets on a high number (> 10) of related, "parallel" communities. With the availability of fossil data for several taxa, calibration of phylogenies will be possible, too. As a result, the age and time of assembly of the multiple communities can be estimated. For the first time in microsnails, combining community with phylogenetic data, diversity numbers and diversification rates can be reconstructed for multiple similar communities, and related to each other.

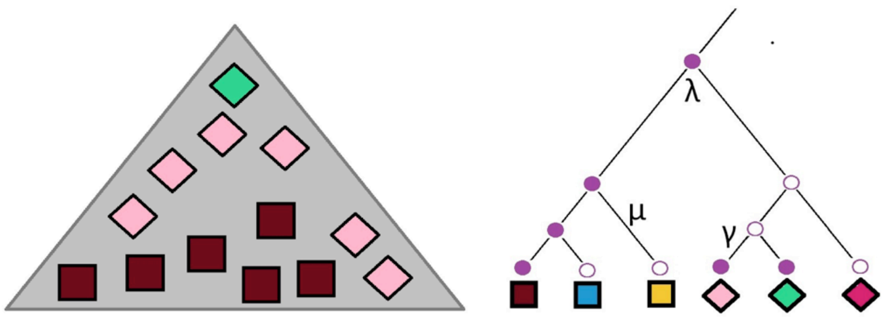

Figure 6 - Graphic of a hypothetical microsnail community (left) and phylogeny (right), showing presence/absence (circles filled/unfilled) of species and the evolution thereof. For the first time, the same analysis can be repeated for all of c. 10 parallel communities.

(C) Do Niches structure Community Composition?

Of the many dimensions that can make up niche space, microhabitat and food preferences may be some of the most important (Whittaker, 1972). Where we might see a single type of habitat upon first (human) sight, a multitude of microhabitats might be available for smaller organisms. However, even within a microhabitat, multiple food types can be available. By this, even different sympatric species can manage to live next to each other within the same microhabitat.

Most terrestrial gastropods are microphagous, feeding on live and decaying vegetation (Barker, 2001). The exact Bornean microsnails' food preferences are unknown, but for the rock dwelling species, the diet is assumed to consist mainly of lichen (algae; Cyanobacteria; Fungi; Figure 7) and mosses (Bryophyta) (Schilthuizen & Vermeulen 2014, pers. comm.). It seems not unlikely that species inhabiting plants around limestone outcrops take plant (Plantae) materials, too.

I propose to study microhabitat preferences in Bornean microsnail species. This could give a first insight into community structure of limestone inhabiting snail species. Additionally, I propose to sequence stomach contents of Bornean microsnails to qualify any food preferences. This could be done using a single or two generic, easy-to-amplify genetic markers for likely food to be taken by the microsnails.

H_0: The different species in a community of microsnails do not differ in the occupation of niche space as defined by microhabitat and food preference.
H_1: The different species in a community of microsnails differ in the occupation of niche space as defined by microhabitat and food preference.

Novelty of the study

Using molecular techniques to fully identify food taken is an upcoming technique with much potential and is likely to yield new and unexpected insights. I shall combine microhabitat and food niche data with both community and evolutionary data, by which it will be possible to reconstruct evolutionary shifts in niche space. A comparable study was done with spiny leg spiders (Tetragnatha; Gillespie, 2004) and included food preference data next to phylogenetic data. I now have the possibility to also qualify food taken and include quantitative community data in the analysis, and make use of several replicates of "parallel" communities.

Figure 7 - Lichen diversity on limestone rocks. Source: https://natureinfocus.wordpress.com/tag/lichen-patterns/.

(D) What is the Influence of the Community on Population Structure?

A number of different units of evolution and possible resultant speciation have been advocated. Next to popular views of the gene and the species, is the view of the population as a unit of evolution (Mayr, 1970). However, at what scale should we study a microsnail population to be able to detect any possible speciation events? On a continent, a mountain range, a single hill or even a single patch of rock? And how about the community? That consists of multiple species, and thus of multiple populations of these species. Surely, the community will also have an influence on how the population can evolve.

The genus *Plectostoma* is arguably the best-studied Southeast Asian microsnail genus. The occurrences of the different species are quite well known. Liew et al. (2014b) write: "[...] all species and populations are restricted to isolated limestone outcrops. This island-like (allopatric) distribution pattern suggests very limited gene flow between populations." If gene flow is indeed limited, the influence of the community each population is part of on the microsnail population is likely to be strong. Compare, for example, a migrating bird and its breeding community. Any community influence experienced by the bird population on the breeding grounds can easily be nullified by exchange of individuals from year to year. Not so in these microsnails.

After discussion with Alex Pigot (March 2015), I suggest studying population level data on a single species of *Plectostoma* in conjunction with community data on all microsnails for the same locations. By performing the same study for multiple locations on a number of limestone outcrops, the influence of the community on the population can be reconstructed. For example, a certain local population can turn out to be a sink of individuals (i.e. locally more deaths than offspring surviving up to adulthood for the species under study; immigration from nearby populations is important) due to the presence of one or more competitive species in the community. Contrary, a local population can be a source if the species is dominant in the local community (i.e. a local surplus of offspring surviving up to adulthood). Using techniques from population genetics, it will also be possible to infer migration rates between local populations. These can be compared to values estimated using different techniques, such as described by Pigot and Etienne (2015) (see research question B).

H_0: Community structure has no influence on population structure.
H_1: Community structure has an influence on population structure.

Novelty of the study

The influence of the community on the population seems straightforward, but studies that integrate these themes are rare or even unique. Still, a fundamental understanding of the general rules of how communities influence the population can be of great importance in conservation and conservation planning. Here, we have a chance to perform such a study with relative ease, including a number of repetitions for multiple limestone outcrops.

3. THE SYSTEM

This chapter introduces the system of interest during my PhD work; communities of subsets of the more than 200 species of microsnails inhabiting limestone outcrops in the tropics of Borneo. Explained are the species, the communities they make up, and the environment in which they live.

(1) The Species

Southeast Asia is inhabited by a large number of snail species. For the island of Bali it was shown that the snail species with an adult size in the range of 2 to 5 mm show the highest level of species diversity (Vermeulen and Whitten, 1998) (Figure 8), and it seems highly likely that this distribution in shell size would be similar in nearby Southeast Asian regions such as Borneo. From here onwards, I will refer to all the snail species with adult shell sizes < 5 mm as "microsnails".

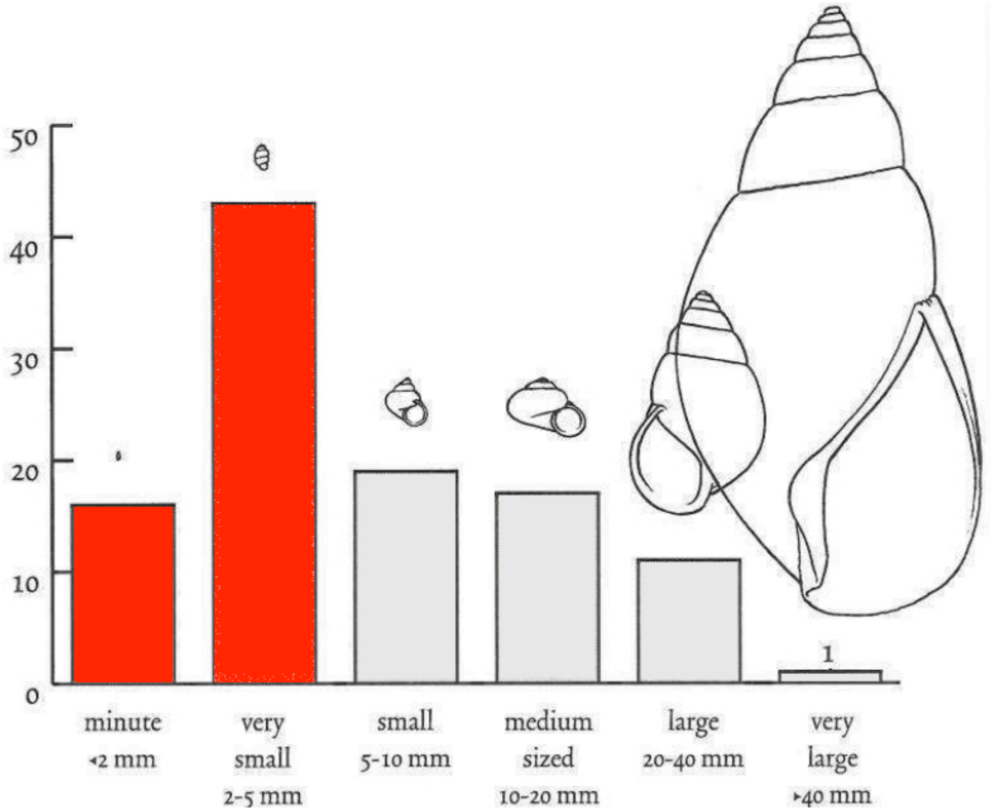

Figure 8 - Size distribution of land snails on Bali (from Vermeulen and Whitten, 1998); "microsnails" in red.

In a previous study, 27 limestone outcrops in East Malaysia (i.e. Sabah and Sarawak) were sampled, with 173 microsnail species from 64 genera registered (Clements et al., 2008). Table 1 presents an overview of all land snail families with representatives in Sabah, Malaysian Borneo, following Vermeulen et al. (2005). Clearly, some families are represented by a high number of taxa, such as the Diplommatinidae (c. 205 taxa), Cyclophoridae (47) and Euconulidae (23). Maybe not coincidentally, these larger families hold a relatively high number of microsnails.

Amongst the microsnails found on Borneo, a relatively large proportion is considered endemic. Endemism can be defined in a number of spatial and temporal dimensions. The definitions I will adhere to in current study are given in Table 2 and follow Vermeulen and Whitten (1999).

Based on molecular data, a high number of morphologically cryptic species were found for the genus *Everettia* in mountainous parts of Sabah (Liew et al., 2009b), showing that 'hidden biodiversity' in Sabah may still be considerable.

Due to their tiny size and their choice of habitat, live specimens of many microsnail species are hard to find in the field. Others can be collected alive, sometimes in good quantities, such as representatives of the prosobranch genera *Arinia*, *Diplommatina*, *Georissa*, and *Plectostoma* (Schilthuizen & Vermeulen 2014, pers. comm.; personal experience, exploratory fieldwork March 2015). Shells of dead specimens are found in high numbers in the soil below limestone outcrops (Schilthuizen, 2011). Due to weathering of the microsnails' shells in the soil, the shells found can be considered to be of recent age, having lived not more than 5 to 10 years ago (Schilthuizen, 2011; unpublised results from radiocarbon dating).

Table 1 - Land snail families recorded from Sabah, Malaysian Borneo, following Vermeulen et al. (2005). The number of taxa is, in general, equal to the number of species, though in several families taxonomy appears to be under continuous revision, with a pending demarcation of some species as a result.

Family	Genera	Taxa
Achatinellidae	2	3
Achatinidae	1	1
Ariophantidae	11	29
Assimineidae	4	14
Bradybaenidae	3	4
Camaenidae	7	15
Carychiidae	2	2
Charopidae	4	10
Clausiliidae	1	1
Cyclophoridae	9	47
Diplommatinidae	4	205
Endodontidae	1	7
Euconulidae	3	23
Ferussaciidae	1	1
Helicarionidae	1	2
Helicinidae	3	5
Helicodiscidae	1	1
Hydrocenidae	1	17
Punctidae	1	4
Pupinidae	1	1
Rhytididae	1	2
Streptaxidae	2	4
Subulinidae	5	6
Trochomorphidae	3	17
Truncatellidae	1	1
Vertiginidae	6	14
Zonitidae	1	1
TOTALS for Sabah	80	437

Table 2 - Definitions of endemism in spatial and temporal dimensions as used in the current study, following Vermeulen and Whitten (1999).

Term	Definition
Spatial dimensions	
Site endemic	Species can have a range of up to about 100 square kilometres, but sometimes has a range down to much less than 1 square kilometre; typically, the range covers a single limestone hill, or a group of karst hills on the same body of limestone bedrock.
Local endemic	Species has a range of about 100 to 10,000 square kilometres; the range typically covers two or more geologically separated bodies of limestone bedrock, which are often embedded in larger topographical units, such as a mountain chain.
Regional endemic	Species has a range covering 10,000 to 1 million square kilometres (for example, the island of Borneo).
Widespread species	Has a range larger than 1 million square kilometres.
Temporal dimension	
Ancient endemic	Species is a last surviving or relict species of often large groups in the distant geological past; often, no evolutionary relatives are extant, and the species is the sole living representative of a high-ranking taxonomic group.
Recent endemic	Species belongs to a cluster of closely related species that have evolved from a single ancestral species in the recent geological history.

(2) The Community

Darwin used the term "community" over 30 times in the 6th edition of On The Origin. For example:

> In social animals [natural selection] will adapt the structure of each individual for the benefit of the whole community; if the community profits by the selected change (Darwin, 1872).

Clearly, the definition of the community was something totally different for Darwin than our current understanding of the term in ecology. He seems to be dealing with the unit of the population instead. Hubbell defined an ecological community as:

> A group of trophically similar, sympatric species that actually or potentially compete in a local area for the same or similar resources (Hubbell, 2001).

Hubbell's definition adds the valuable notion of trophic similarity and this is the definition I will adhere to during my research on communities.

The community is one of the main subjects of research in the school of ecology, and one that has seen an exponential increase in interest over the past decades (Figure 9). At the rank above the community, we can define the meta-community: a set of interacting communities, linked by the dispersal of multiple, potentially interacting species. In Bornean microsnails, the meta-community is thought to consist of all communities found on a number of limestone outcrops in a limited region (e.g. all limestone outcrops along Sungai Kinabatangan). At the rank below the community, we can define the guild: an association of functionally equivalent species in a community. In Bornean microsnails, a guild could be formed by a limited number of species living on the same limestone rock and apparently sharing the same resources.

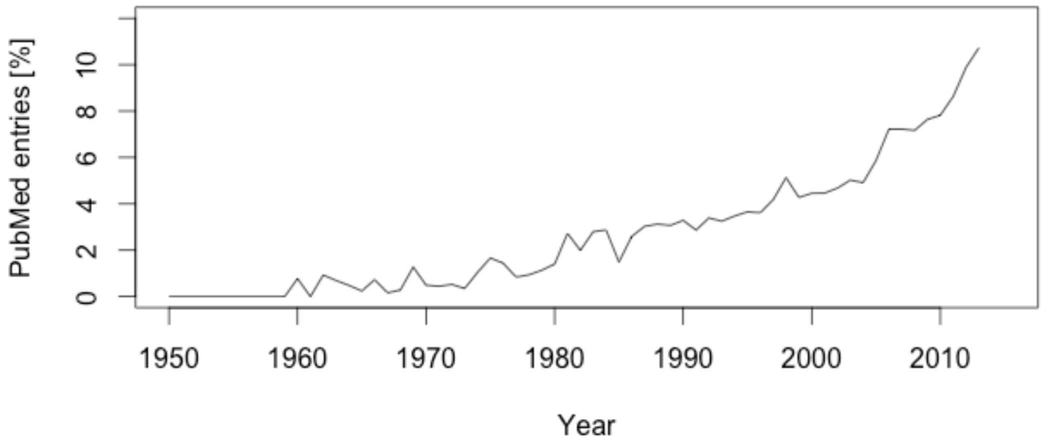

Figure 9 – Number of PubMed entries per year for the words "community ecology" (divided by the number of entries for the word "ecology") from 1950 up to the present, indicating an exponential increase in interest in community ecology research.

Depending on the research question at hand, studies should first focus on the community, the meta-community or the guild. For the moment, when discussing the more general "microsnail community", I mean the assemblage of trophically similar microsnail species that co-occur in a single location, on a single limestone outcrop.

Bornean microsnail communities form an ideal system for the study of community ecology. The following list of characters describing the microsnail communities explains.

1. Each limestone outcrop on Borneo holds a community of microsnails. Mostly, these communities are characterized by high species diversity. For example, Clements et al. (2008) recount snail sampling results from Sabah and finds that between 25 and 40 species were found on most outcrops in Sabah (Figure 10).

2. Many taxa are restricted to the limestone outcrops and show a distributional pattern related to a so-called island system (see Chapter 3, Paragraph 3).

3. The proportion of range-restricted species (endemics) is high and comes at a very small scale (Schilthuizen et al., 2003b). Figure 10 shows the high number of endemics found on limestone outcrops in Sabah. For 22 out of the 30 outcrops, over half of the species encountered shows a level of endemism as defined previously (Table 2). Using data from Clements et al. (2008), a rough, conservative, calculation of the endemic richness of land snails in Sabah (following the method proposed by Kier and Barthlott, 2001) results in a value of around 10. This shows the endemic richness of Sabah land snails is higher than

that found for mammals (1.83), birds (4.11), and reptiles (4.40) on oceanic islands (Kier et al., 2009).
4. Densities of microsnail shells are very high in the soil below the limestone outcrops, as is known from previous work (Dance, 1970, Schilthuizen, 2011). As a result, collecting in the field can be done very efficiently (see Chapter 4, Paragraph 1). It is assumed that the species distribution of shells in the soil is a good proxy for the species distribution of the snails present alive (but see Chapter 1, Paragraph 4).

Ecological studies on tropical microsnails have, up to this day, focused mainly on the comparison of abundances and diversities between localities (e.g. Liew et al., 2009a, Schilthuizen et al., 2002, Schilthuizen et al., 2003a, Schilthuizen et al., 2013). In his summarizing paper, Schilthuizen (2011) highlights the limited knowledge of “the niches and trophic levels of tropical forest snails”. Furthermore, past research has focused on restricted ecological interactions, such as carnivory (e.g. Liew and Schilthuizen, 2014). These studies, however, do not focus on the complete suite of interactions within microsnail guilds, the interactions that together have been part of structuring processes that formed the communities that we find today.

Surely, microsnails alone do not form a complete, single community. Instead, communities in which the microsnails are prevalent contain more taxonomic groups. These may include other herbivorous animals in the same trophic level as the microsnails, such as land snails larger than microsnails and species of beetle (Coleoptera). Further consideration on this topic can be found in Chapter 1, Paragraph 4.

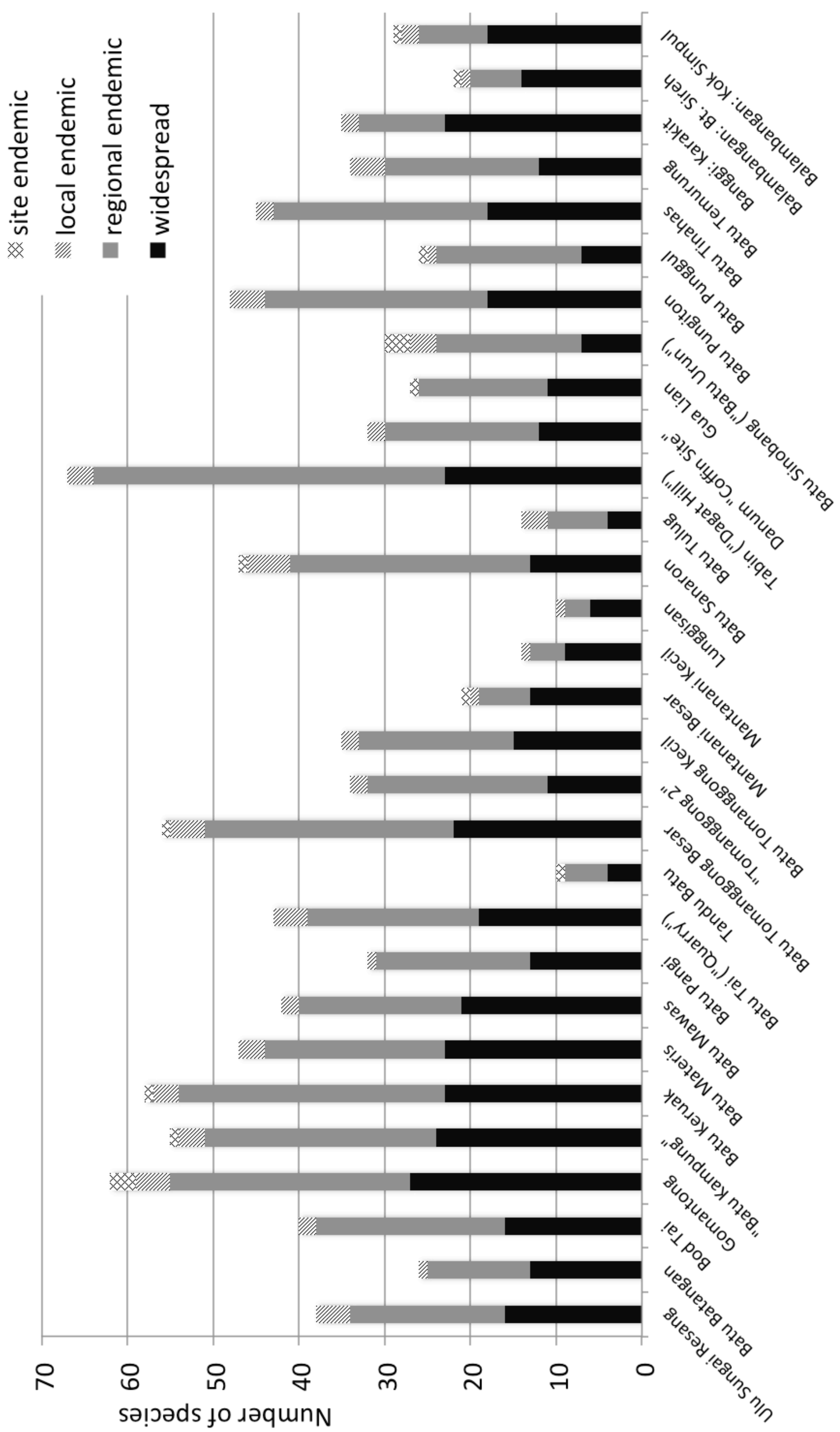

Figure 10 – Land snail species numbers in Sabah, Malaysian Borneo, showing the number of endemic (site, local or regional) and widespread species per limestone outcrop. Data from Clements et al. (2008).

(3) The Environment

Large communities of microsnails on Borneo can be found on limestone outcrops (Figure 11). The calcareous limestone substrate offers plenty of the calcium carbonate necessary for the snails to build their shells.

Figure 11 - Typical limestone outcrop along Sungai Kinabatangan, Borneo (photo by Kasper Hendriks, March 2015).

Hotspots of limestone outcrops of various dimensions in Sabah are found, amongst others, along Sungai Kinabatangan and in the Tabin Wildlife Reserve, Malaysian Borneo. Distances of a few hundred meters to a few kilometres separate the outcrops. The calcareous rocks themselves are rather barren, covered only in mosses, algae and smaller plants. In contrast, lowland rainforests, rivers and their tributaries, and vast oil palm plantations cover the landscape surrounding these outcrops. From a microsnail's perspective, often being an obligate calcicole, the result is an island-like landscape. "Islands" of limestone are discrete habitable instances within a "sea" of impenetrable forest (Clements et al., 2008) (Figure 12). It is exactly this island-like landscape (c.f. MacArthur and Wilson, 1967) that puts the areas rich in limestone outcrops up front as an ideal fieldwork location for my research on community ecology and evolution.

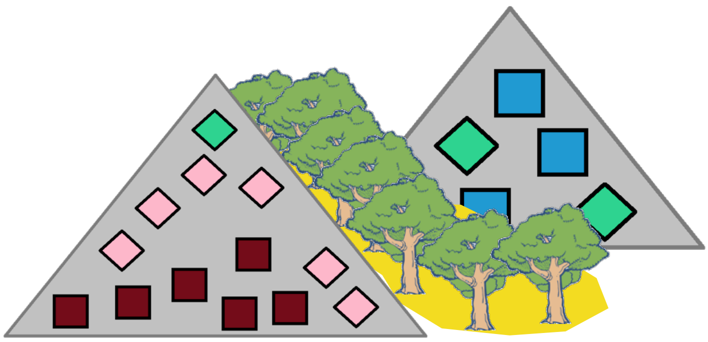

Figure 12 – Microsnail individuals (coloured squares) on limestone "islands" in a "sea" of forest.

4. Practical Considerations

This chapter deals with all practicalities that follow from the research proposed in Chapter 2. Where possible, issues are discussed in a general way, such as on travelling; when the various research questions stated in Chapter 2 need separate consideration, practicalities are tabulated to give a clear overview.

(1) Fieldwork

Most of the data required for current research will be collected from the field. Regions of interest should have multiple limestone outcrops, forming an island-like pattern within tropical rainforest or oil palm plantations. Preferably, multiple outcrops can be visited within several weeks of fieldwork. Each outcrop should hold a community of microsnails, for which it has to be possible to collect a fair number within a reasonable amount of time.

Geographical Details

In Southeast Asia, the geography of Sabah, Malaysian Borneo, is characterized by a relatively high number of limestone outcrops (Figure 13). Several publications have listed these outcrops, usually with the intention to catalyse conservation practices (Kiew and Lim, 1996, Lim and Kiew, 1997).

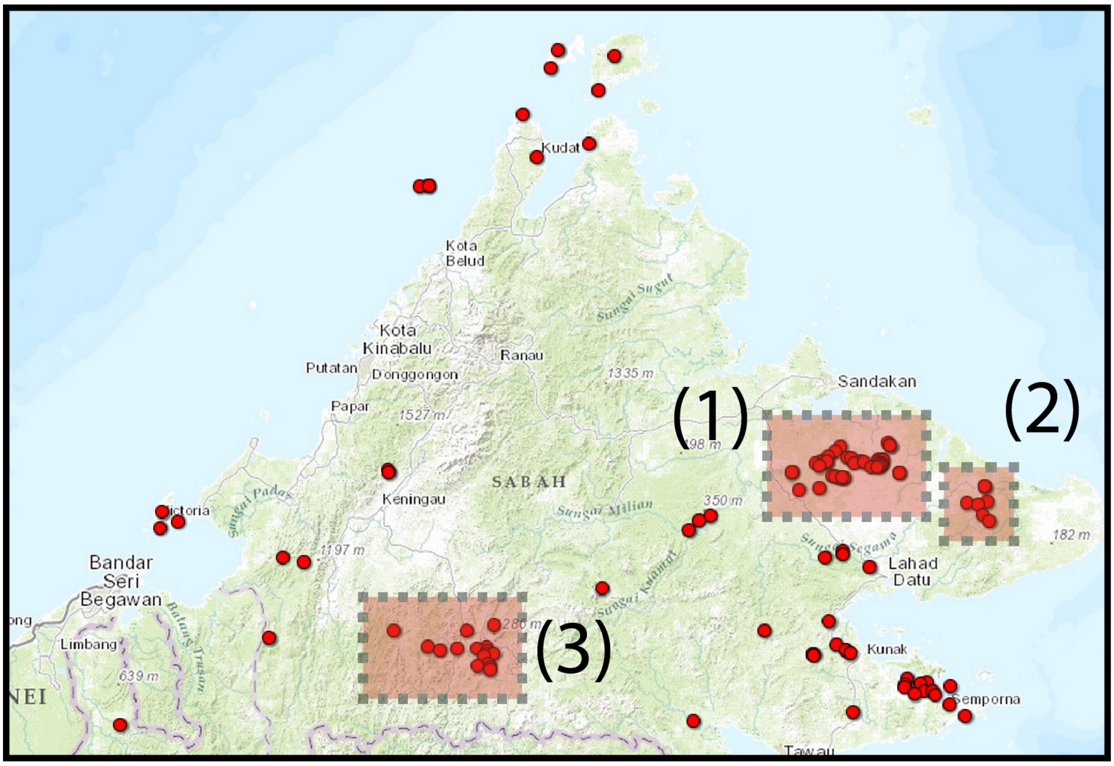

Figure 13 - Overview of known limestone outcrops (red dots) on Sabah, Malaysian Borneo. Highlighted are three inland regions showing a high density of limestone outcrops: (1) Sungai Kinabatangan region, (2) Tabin Wildlife Reserve and (3) Sapulot region. Map generated with the use of GPSVizualiser.com, based on an ArcGIS map.

The Sungai Kinabatangan region makes for a good first study location (numbered (1) in Figure 13), mainly because of the high number of limestone outcrops present (well over 30 known so far). But, also because the region has been the focus of several studies on microsnail evolution and ecology in the recent past (e.g. Schilthuizen et al., 2003b, Schilthuizen et al., 2006, Vermeulen, 1991). As a result, the outcrops and their access are relatively well known. Figure 14 shows the distribution of the known limestone outcrops along the river and their position relative to the small village of Sukau, which is the single road-based gateway to the region. New limestone outcrops are still being found in this region, though, as was shown during my exploratory field study in March 2015. It is advantageous that the species occurring in this region are quite well known, aiding in the identification of the species to be studied in current research. Also, it is well known that community composition differs from location to location (Clements et al., 2008), which is a prerequisite for current research, too.

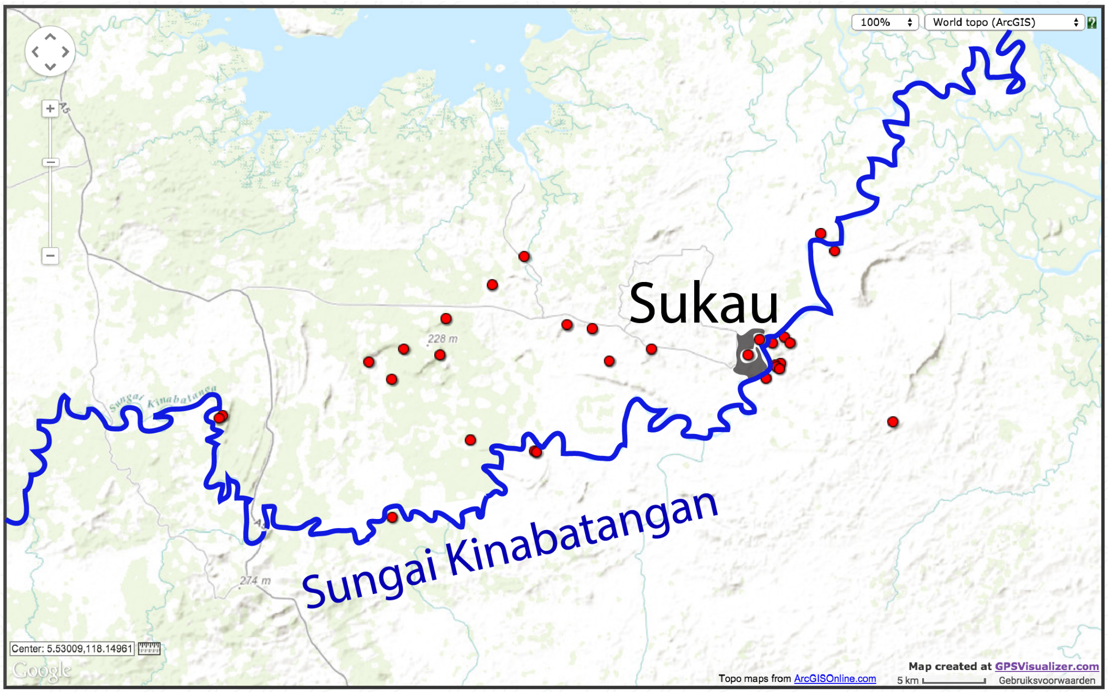

Figure 14 – Overview of the known limestone outcrops (red dots) of the Sungai Kinabatangan region. Map generated with the use of GPSVizualiser.com.

A second study region is Tabin Wildlife Reserve on the Dent Peninsula, in the far northeast of Sabah (numbered (2) in Figure 13). For this region, I am aware of about six limestone outcrops, most of them along Sungai Segama and its tributaries. Tabin has been studied for microsnails before (Schilthuizen et al., 2003a, Schilthuizen and Vermeulen, 2003, Clements et al., 2008), but has, so far, seen less scientific microsnail attention than Sungai Kinabatangan. Still, the Tabin region could make for an interesting extension to any community ecology study undertaken in the Sungai Kinabatangan region. Complicating factor with regard to fieldwork in Tabin is the recent security issue reported by the Dutch government (Rijksoverheid, 2015).

A third region in Sabah in which many microsnails have been collected in the past, is the area around the village of Sapulot (numbered (3) in Figure 13). For this region, no less than 12 limestone outcrops have been listed and many more are expected to be found. These outcrops are not situated within a protected area, but in commercial forest reserves, and their state should be checked and considered before any further research activities can be undertaken. The sheer number of outcrops and knowledge on their microsnail species (see species descriptions in Vermeulen et al., 2005) suggest the Sapulot region may make for a great additional research location to the Sungai Kinabatangan region.

Transport and Facilities

Transportation to and from Sabah is relatively easy. Flights from Singapore and Kuala Lumpur, Malaysia, connect to flights from Amsterdam. Arrival on Sabah is in the capital of Kota Kinabalu. Kota Kinabalu offers the possibility to stock up on field materials and food supply. Menno Schilthuizen, second supervisor to current PhD research, owns a comfortable house on the town border. Before and after fieldwork, use can be made of this house, as well as the small on-site laboratory. Menno also owns a 4WD car on Sabah. This car is available for research for which Menno shall charge usage on a per kilometre basis.

During meetings with Liew Thor-Seng and Bakhtiar Yahya of Universiti Malaysia Sabah (UMS), Malaysia, in March 2015, possibilities of using UMS facilities in the Sungai Kinabatangan region were discussed. UMS owns a small and simple house and laboratory at Sukau, including a boat to sail along the river Kinabatangan. After cooperation with students and researchers of UMS is set up, making use of these facilities should be possible.

During exploratory fieldwork in March 2015, use was made of the Danau Girang Field Centre of Cardiff University and Sabah Wildlife Department. The field centre offers access to several limestone outcrops further upriver from Sukau and has a laboratory available to sort samples.

Collecting Data

Here I discuss the general ways to collect community ecology and evolutionary data from the field, which are twofold.

The first method focuses on gathering community ecology data alone, and does so by collecting so-called “soil samples” from the floor down a limestone outcrop holding any microsnail community (cf. Liew et al., 2008, but see below for details). From experience it is known that deceased microsnails, having lived on the limestone before, end up in the soil below the outcrop, often in considerable numbers (Schilthuizen, 2011). The snail community in the soil is taken to be a good proxy for the snail community alive in the same plot.

The second method focuses on gathering community, microhabitat and genetic data. It involves the laborious search for live microsnail specimens on and around the limestone outcrop. This way of working is very intensive, especially given the local climate. It yields field data necessary for understanding microhabitat use and gathering genetic data for various applications.

Exploratory Fieldwork March 2015

After commencing my PhD, September 2014, the idea was put forward to visit Borneo within the first year. This has been accomplished, with an exploratory visit to both Crocker Range and Sungai Kinabatangan in March 2015. Our international fieldwork research team consisted of Menno Schilthuizen (Naturalis Biodiversity Center, the Netherlands), Iva Njunjić (University of Novi Sad, Serbia), Alex Pigot (University of Groningen, the Netherlands) and myself. Several targets were put on paper before taking off: getting to grips with the limestone habitat and data collecting; gathering initial samples, useful for at least the first year of research afterwards; strengthening bonds with allied scientists from Universiti Malaysia Sabah (UMS) and to discuss possible options for joint research. Looking back on a successful fieldwork period of several weeks, I conclude to have succeeded in all of the above.

A one-day visit was undertaken to a difficult-to-find cave of a limestone outcrop in the northern part of the Crocker Range, called Gua Laing, near the hamlet of Bingkor (Figure 15). After several hours of effort, the cave was located and eight sample plots were set up (see Paragraph 2 of this chapter for Materials & Methods). At each plot, soil data was collected and brought back to the Netherlands for further studies.

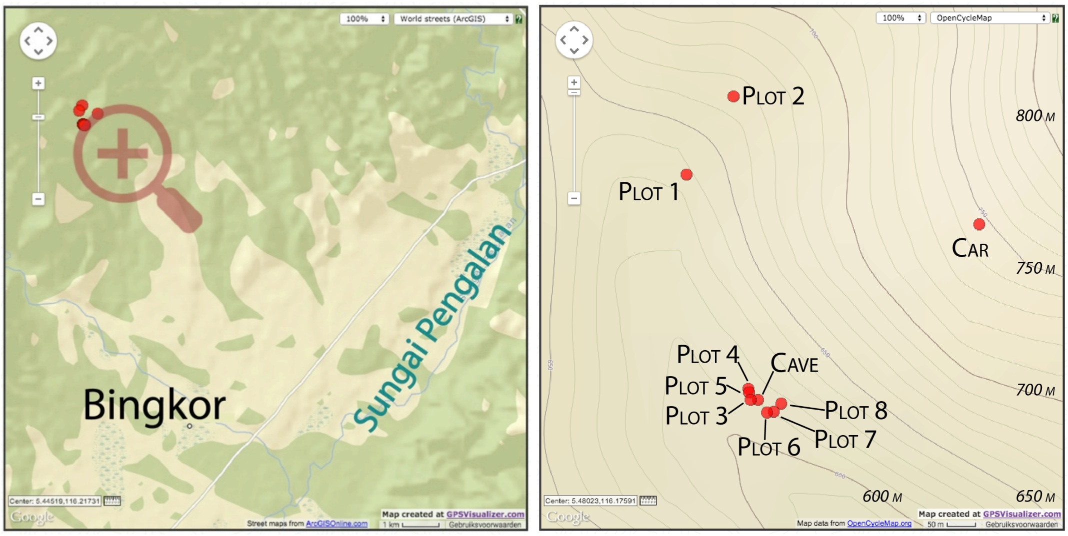

Figure 15 - Gua Laing limestone outcrop in the Crocker Range, Sabah, Malaysian Borneo, sampled March 2015. Overview of the area around the village of Bingkor (left) and details of the eight sampling plots, including reference to the location of the parked car and the nearby cave. Maps generated with the use of GPSVizualiser.com.

Following our visit to the Crocker Range, we moved to the Sungai Kinabatangan region. We stayed for a week at the Danau Girang Field Centre, owned and managed by Cardiff University (United Kingdom) and Sabah Wildlife Department. The field centre is located about two and a half hours travelling upriver from the village of Sukau. Hence, the strategy was to visit some limestone outcrops along the river that may be too far to visit from Sukau during a future fieldwork trip. Figure 16 shows a map of the area along the river and the four limestone outcrops sampled during the week. At each location, eight plots were set out and sampled for both soil data and live specimens. The locations

Batangan and Mawas were chosen to perform additional microhabitat studies. Six types of microhabitat were defined (bare rock; moss-covered rock; bottom of fresh leaves; top of fresh leaves; bark; dead leaves) and exhaustively searched for live specimens (2 times 30 minutes per plot by two different researchers; all microsnails collected on alcohol for further studies). All data (soil samples and live specimens collected on alcohol) from the Kinabatangan region were brought back to the Netherlands and are currently being studied.

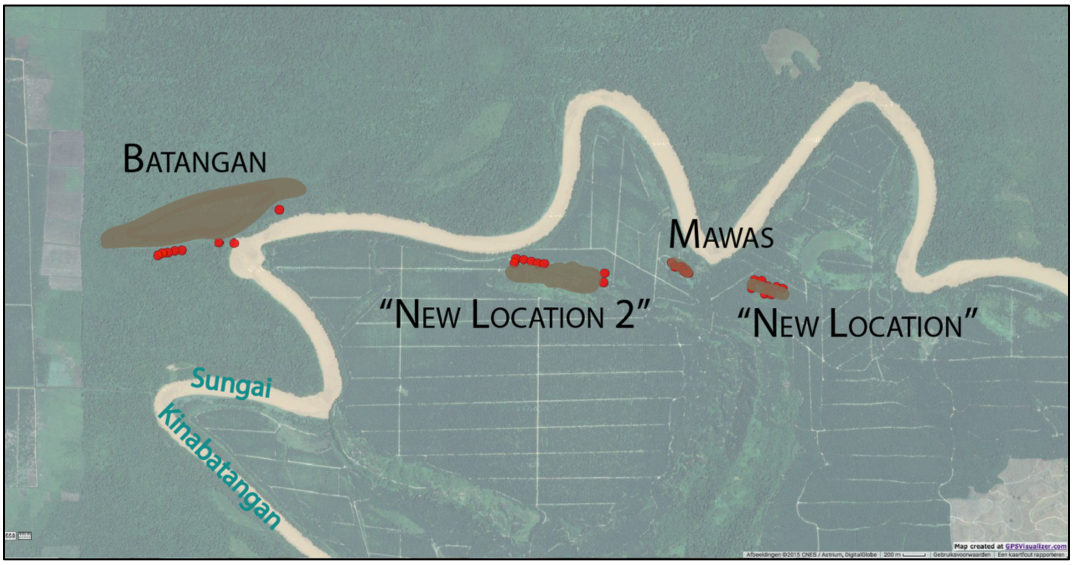

Figure 16 – Sungai Kinabatangan limestone outcrops, Sabah, Malaysian Borneo, sampled March 2015. For each location (i.e. "Batangan", "Mawas", "New Location 1" and "New Location 2") seven or eight plots were sampled. Map generated with the use of GPSVisualizer.com and satellite images by Google.

(2) Materials & Methods

To answer my research questions I aim to collect both soil samples (empty shells) and live samples from several limestone-inhabiting microsnail guilds. Limestone outcrops will be chosen based on previous studies and accessibility of the site, which is known to be variable (personal observations, March 2015). The Sungai Kinabatangan region will be studied first (target of at least 10 sample outcrops), while the Tabin Wildlife Reserve and Sapulot region will be studied if time and financial resources allow.

Community data on microsnails living on and around limestone outcrops in the tropics has often been collected from soil samples. Microsnails living on and around the limestone are washed down by the almost daily, heavy tropical rains and end up on the forest floor. The soil below a limestone slope usually contains tens to hundreds deceased microsnails per litre of soil (e.g. Clements et al., 2008). It is widely assumed that the community of deceased individuals is a good proxy for the community present alive. But, with live specimens notoriously hard to find, this is an assumption that might need further consideration (see Chapter 1, Paragraph 4).

Next to the community data, genetic data is needed to work out the answers to the research questions. First of all, genetic data is needed for each species, preferably per outcrop. Second of all, genetic data of stomach contents is needed to identify any possible niche partitioning on food resources, which is hard to impossible to derive from live observations. Thirdly, genetic data is needed on a population level to reconstruct any population genetics phenomena that take place within the microsnail species under study.

COLLECTING DATA

At each limestone outcrop, eight sampling plots are defined at the foot of the limestone outcrop's slope (Figure 17). Plot length (L_{plot}) and plot width (W_{plot}) are both 1 m. If local circumstances (limestone rocks overgrown or covered by mud, dense thickets, high water levels, etc.) impede reaching the foot of the slope, plots can be shifted up the slope, but not more than a few metres. In that case, all eight plots are to be located at a the same elevation above sea level. Five "neighbouring" plots are located on one side of the outcrop at a standardized distance of 50 metres from each other ($\Delta plot_{neighb.} = 50$ m; Figure 18). For smaller outcrops, the outcrop-to-outcrop distance can be reduced, but shall never be smaller than 10 times the plot length ($\Delta plot_{neighb.} \geq 10\ L_{plot}$). Several other plots ("outliers") are defined around the outcrop, at a distance of at least five times the distance between the neighbouring plots ($\Delta plot_{outlier} \geq 5\ \Delta plot_{neighb.}$). The outlier plots serve to identify and quantify any heterogeneity in the data at spatial scales larger than covered by the neighbouring plots alone.

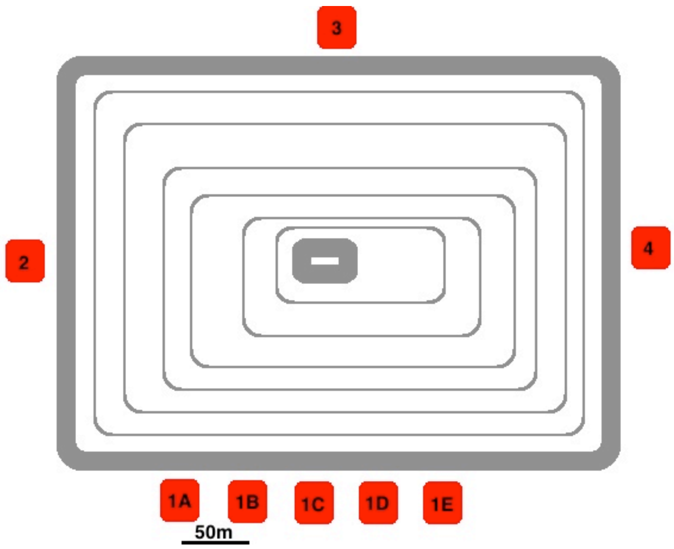

Figure 17 - Schematic top view of a limestone outcrop (grey) and sampling plot distribution (red).

For the collection of community data, soil samples are taken at each plot. The surface should be rich enough in soil (i.e. the loose top layer should hold at least the amount of soil defined here), or otherwise the plot is moved a few metres along the slope. For each plot, accurate GPS coordinates are taken. From the plot, five litres of soil are collected in a plastic bucket. To enrich the sampled soil, a first round of sieving (screen openings of 10 mm) is performed in the field. The debris left in the sieve is discarded, while the rest is brought to the laboratory for community composition analysis.

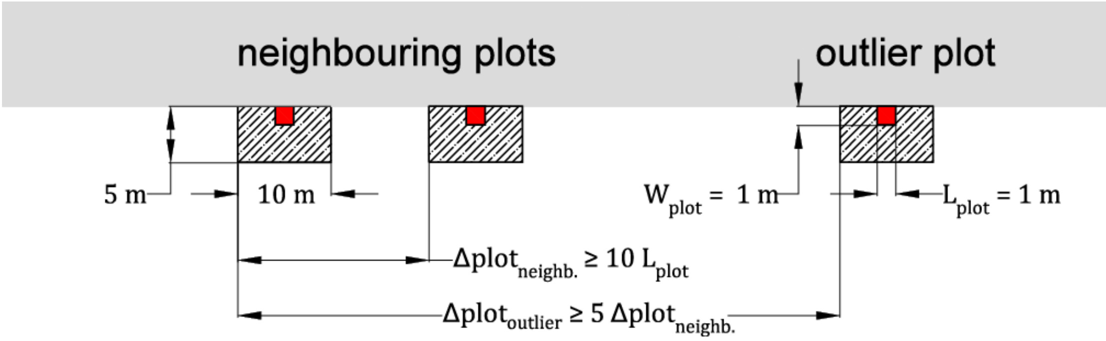

Figure 18 - Schematic top view of a limestone slope (grey) and sampling plot distribution. Sample plots are shown in red. Sample plot areas for collecting live specimens are shown as hatches around the sample plot.

Live specimens will be collected from the same sample plots, using inspection by eye, with the aid of a headlight torch (Figure 19). In order to obtain genetic data on as many species as possible, the area for searching live specimens is extended around the sample plot. The sizes of this extended plot are a length of 10 m and a width of 5 m (Figure 18). Other locations rich in live specimens will be used to sample species not found alive from designated sample plots, if necessary. Additionally, genetic data from previous studies will be incorporated where possible (as discussed with Liew Thor-Seng and Menno Schilthuizen, March 2015, UMS, Malaysia). For stomach content and population genetic studies, live specimens from only the sample plots will be taken, allowing for direct comparison studies with local community data. All live specimens will be collected in 98% alcohol and labelled per plot.

Table 3 summarizes sampling strategies, including target numbers to collect, according to the research questions stated in Chapter 2.

Figure 19 - Alex Pigot collecting live microsnail specimens from a limestone outcrop along Sungai Kinabatangan, Borneo, during exploratory fieldwork (photo by Kasper Hendriks, March 2015).

Table 3 - Sampling strategies per research question; research questions as stated in Chapter 2.

Research question	Sample strategy per location	Numbers collected
A (page 18)	Five litres of soil sample containing microsnail shells taken from each plot.	10 to 1 000 empty shells per plot
B (page 19)	Live specimens collected on alcohol from each plot and, if needed, in between (plus community data from A).	1 to 5 live specimens per species per plot
C (page 20)	Live specimens collected on alcohol from a selected guild, collected from each plot, but never in between (plus community data from A).	20 live specimens per species per plot
D (page 21)	Live specimens collected on alcohol, from one or two selected, common species from each plot, but never in between (plus community data from A).	20 live specimens from one or two species per plot

TRANSPORT OF SAMPLES

Soil materials can be transported from the field to the laboratory in strong plastic bags, available at many local shops in Sabah. Live specimens will be collected on 98% alcohol and transported to the laboratory in small vials. Vials will be labelled per plot and saved in plastic bags per limestone outcrop and labelled as such.

LABORATORY WORK

The empty microsnail shells are filled with air and thus have a density lower than water. The next step in soil enrichment involves the so-called method of "flotation" (Schilthuizen et al., 2002). The soil brought from the field is put to float on the surface of a bucket of water. All debris with a density higher than water sinks to the bottom, after which all debris left afloat (including the empty snail shells) can be scooped from the surface and put to dry. After thorough drying, a nested column of sieves with wire mesh is used to sort debris by size range. The resultant debris is scanned using a dissection microscope and all snails taken out by hand (using soft tweezers) and collected. The snails are identified and counted, with results registered in a database.

Genetic work will be performed at Naturalis Biodiversity Center (Naturalis). After identification, all soft tissue will be used for DNA extraction, due to the tiny size and the amount of tissue needed for DNA amplification to yield sufficient DNA for sequencing. All shells will be saved as voucher material. After extraction and amplification of DNA, sequencing will be performed at MacroGen or BaseClear, both located near Naturalis, Leiden. To answer the different research questions, different genetic markers are needed. Markers need to be informative on a level relevant to answer each research question. This will be at a (sub)species level when the aim is to reconstruct a species phylogeny. It will be at an individual level when the aim is to reconstruct population genetic patterns. And, it will be at a species/genus level when the aim is to reconstruct snail diet from stomach contents, with genetic markers based on plant and algae tissue. Ideally, use will be made of the next generation sequencing platform Ion Torrent, available at Naturalis, which becomes economically attractive when multiple markers (say, 50 per individual) are to be sequenced (e.g. Wielstra et al., 2014).

For the sequencing of mainstay markers, a more economic option may be to use traditional Sanger sequencing instead. For each subproject, a cost analysis will be made beforehand. The type of genetic data already available from fellow researchers will also be taken into consideration, as well as the necessity or preference for the use of multiple markers to reconstruct a phylogeny.

Fossil material available to the author through fellow researchers offers a chance to have the microsnail phylogenies dated. The laboratory work that follows includes describing morphological and ontological characteristics of both fossil and contemporary material, using the microCT scanner at Naturalis (cf. Liew et al., 2014a).

Table 4 summarizes laboratory work according to the research questions stated in Chapter 2. Associated student projects (described in Chapter 4, Paragraph 6) that could benefit my laboratory work, are listed in the last column.

Table 4 - Laboratory work per research question; research questions as stated in Chapter 2.

Research question	Laboratory work per research question	Associated student project (see Paragraph 6)
A (page 18)	Soil enrichment by flotation*; identification and counting of samples*.	*multiple BSc projects on community composition
B (page 19)	Identification of live specimens collected on alcohol; DNA extraction, amplification and sequencing for selected samples; reconstruction of phylogenetic histories; identification and dating of fossil material from Borneo**; calibration of phylogenies**.	**MSc project The age of the Diplommatinids
C (page 20)	Identification of live specimens collected on alcohol; selection of suitable genetic markers to identify stomach contents; DNA extraction, amplification and sequencing of stomach contents; reconstruction of stomach contents based on genetic data.	
D (page 21)	Identification of live specimens collected on alcohol for one or two common species; selection of suitable genetic markers to analyse evolutionary patterns at a population level***; reconstruction of temporal and spatial patterns at a population level***.	***MSc project Populations at Microscales

Sample Deposition

Scientific integrity requires all samples to be deposited in a known and accessible collection, i.e. to be stored and labelled in such a way as to be easily retrievable by future scientists. Soil samples and snail shells retrieved from the soil, after analysis, will be stored at Naturalis, Leiden, until my PhD studies have finished. Afterwards, samples will be deposited in the BORNEENSIS Reference Collection Centre of UMS, Malaysia, and duplicates in the molluscan collection of Naturalis, Leiden, the Netherlands.

DATA ANALYSIS

The first aim of the soil sample microsnail data is the reconstruction of community composition for a guild of limestone microsnails. This analysis will be repeated for a number of parallel communities, each living in a separate location, i.e. on a separate limestone outcrop.

Table 5 highlights the data analysis that follows, per research question as stated in Chapter 2. Descriptions given are still rough; not all details and possibilities of the analyses to be performed in the next four years are known at the moment of writing, so mainly first thoughts are given.

Table 5 – Data analysis (highlights) per research question; research questions as stated in Chapter 2.

Research question	Data analysis (highlights)
A (page 18)	Community reconstruction; data analysis at the level of species abundances; description of community structure, with quantification of e.g. species turnover and/or variation between plots, locations (and regions) (Whittaker, 1972, Anderson et al., 2011). Fitting data to Neutral Model theoretical predictions (Hubbell, 2001).
B (page 19)	Community data analysis at the level of species presence/absence, with additional phylogenetic data from live samples (Pigot and Etienne, 2015); tests for relations between community diversity and diversification rates; estimating rates of local extinction and speciation, and migration rates; analytical tools developed in association with colleagues.
C (page 20)	Community data analysis as for (A), with additional data on microhabitat preferences and genetic data on stomach contents from live specimens; analysis of correlation between choice of microhabitat, food choice and the local community composition.
D (page 21)	Community data analysis as for (A), with additional population genetic data; analysis of correlation between the community structure and the population genetics of one or two target species.

(3) RESEARCH PERMITS

At the time of writing, I am rounding up my SaBC Application for Access, the permit needed to collect data from natural resources in Sabah, Malaysia. Additionally, I will request an Export Permit, needed to bring samples to the Netherlands to study, and back to Sabah later to be stored in the BORNEENSIS collection of UMS. For both permits, I need local counterparts to work with. During my exploratory fieldwork in March 2015, I have visited several senior researchers at UMS. They have offered me help in acquiring permits mentioned.

(4) Collaborations within the Institute

Within University of Groningen I intend to work together closely with fellow PhD students and postdocs of Rampal Etienne. His students and postdoctoral researchers work on related questions and similar community systems. Some of them perform theoretical exercises on community composition, as well as phylogenetic reconstruction techniques, which offers great opportunities for bringing together our knowledge. Some fellow students may be excited to join for fieldwork on Borneo, which would benefit both them and me.

To answer Research Question D on the relation between communities and population structure, I would be very happy to cooperate with fellow researchers at The University of Groningen with a background in population genetics. For this, I aim to team up with prof. dr. Per Palsbøll or one of his students.

(5) Collaborations outside the Institute

First and foremost is the collaboration with my second supervisor, prof. dr. Menno Schilthuizen, a specialist in Bornean microsnail evolution and ecology at Naturalis.

Dr. Willem Renema of Naturalis is a palaeontologist and has ample knowledge on Bornean geology. Furthermore, he runs the microCT scanner at Naturalis, a sophisticated tool I intend to use for an MSc project (Chapter 4, Paragraph 6).

Dr. Jaap Vermeulen is a Southeast Asian orchid specialist and geologist from Leiden, the Netherlands. Furthermore, he is an authority on the microsnails of Borneo, Bali and other regions in SE Asia. He has been co-author of many scientific articles on Bornean microsnails, including detailed descriptions of most of the land snail families.

Dr. Liew Thor-Seng of UMS at Kota Kinabalu, Sabah, Malaysia, is a former PhD student of Menno Schilthuizen at Naturalis. He has performed extensive fieldwork in Sabah, studying evolution, ecology and biogeography of Bornean microsnails. He has been involved in the descriptions of many snail species new to science. Collaborations with Liew will include the exchange of genetic data to reconstruct microsnail phylogenies, and the intended supervision of some of his students during BSc and MSc courses.

(6) Student Projects

Community Composition of Bornean Microsnails

BSc Community Ecology Research Course, University of Groningen, 2015 to 2018; BSc internship at University of Groningen or Naturalis Biodiversity Center, when extended with regard to the BSc course mentioned above, 2015 to 2018.

Students will determine from Bornean microsnail community data (obtained from the numerous soil samples; Figure 20) important characteristics of the community. They will calculate community diversity measures cf. Whittaker (1972), such as α, β, and γ diversities, and compare results with previous studies on tropical faunal communities. Also, they'll identify and analyse spatial scales relevant for the measurement of differences between communities in species diversity. Finally, they shall hypothesize on the logic of correspondences and differences between plots and locations, and any possible differences between microsnail and other faunal communities known from literature. A discussion on what environmental factors could be useful to explore further is expected and could benefit my future fieldwork (Anderson et al., 2011).

Figure 20 - A microsnail community from a limestone outcrop along Sungai Kinabatangan, Sabah, Malaysian Borneo, collected by Menno Schilthuizen in 2003 (photo by Kasper Hendriks, January 2015).

The Age of the Diplommatinids

MSc internship at University of Groningen/Naturalis Biodiversity Center, 2016.

Microsnails have inhabited limestone outcrops in Borneo for quite some time. But exactly how old are the species? We will try to answer this and other questions using fossil (Figure 21) and recent material from the Bornean microsnail genus *Arinia*. The student will describe in full detail the shells of all *Arinia* species available, based on morphology and ontogeny. For this, use can be made of traditional light microscopy and a state-of-the-art microCT 3D scanner at Naturalis Biodiversity Center, Leiden, the Netherlands. The description of the shells will be based on techniques developed by Liew et al. (2014a). Results will be used to calibrate the phylogeny of the Diplommatinid family, which will greatly benefit my phylogenetic work on microsnails.

For the student, there is the option of a fieldwork trip to Borneo (first half year 2016, or a year later if that suits better), in which further focus on the collection of *Arinia* species is possible.

Figure 21 - A 5 to 8 MY old fossil representative of the Bornean microsnail genus *Arinia*, collected by Jon Todd and colleagues in 2011 (photo by Kasper Hendriks, 2014).

Populations at Microscales

MSc internship at University of Groningen, 2016.

Bornean microsnails live on discrete limestone outcrops in the tropical rainforest. The microsnails form unique communities that differ from outcrop to outcrop. In this study, we will focus on one or two common microsnail species from the genera *Plectostoma* and *Georissa*. Liew et al. (2014b) wrote: "This island-like (allopatric) distribution pattern [in the microsnail genus *Plectostoma*] suggests very limited gene flow between populations." Hence, these microsnail communities would form a suitable case to study population genetic patterns on microsnails, while at the same time incorporating the influence of the local community on the population.

Bornean microsnail population structure can help us understand how populations formed on microscales. What are gene-flow rates? What is the genetic diversity within the population? What is the age of a population? Is the gene pool around a single limestone outcrop homogeneous? We will design genetic markers suitable to obtain high resolution genomic data (cf. Davey and Blaxter, 2010) on the genus *Plectostoma* (Figure 22).

Furthermore, community data is available for each sampled location. The datasets on the population and the surrounding community will be integrated. Can we infer historical relations between community composition and population development? What has been the influence of the community on the population structure? Can we identify population sources and sinks around a limestone outcrop? Is community diversity of influence on population diversity and diversification?

There is the option of a fieldwork trip to Borneo (first half year 2016, or a year later if that suits better), in which the student will further focus on the collection of "popgen" data for relevant species.

Figure 22 - Population of a species of microsnail from the genus *Plectostoma* on a limestone outcrop along Sungai Kinabatangan, Sabah, Malaysian Borneo (photo by Kasper Hendriks, March 2015).

(7) Outreach

Where possible, I shall present results in peer-reviewed journals and at symposia and congresses. Popularisation of science, through e.g. interviews with papers or magazines, should be a possibility.

(8) Significance to Sabah

The limestone karsts of Sabah have previously been called "Imperilled Arks of Biodiversity" (Clements et al., 2006), offering a temporary refuge for species when environments are degraded but the rough limestone hills spared. They show high levels of species diversity (in some phyla, karst areas can harbour up to 20% of a region's biodiversity) most likely due to the high number of ecological niches offered by the rugged terrain of both the surface and subsurface of the karst hills (Clements et al., 2006). This holds even more so for snails, who are in need of calcium carbonate to build their shells. In the microsnail genus *Plectostoma*, all species show obligate calcicoly, meaning that they cannot survive without the limestone karsts at all. The future of the limestone karst's biodiversity is thus directly dependent on the limestone karst's future. Many threats to the karst landscape exist, though, mostly due to anthropogenic impacts such as mining, tourism and logging (Vermeulen and Whitten, 1999).

With the current research I aim to further delineate the importance of Sabah's limestone karsts to their unique and rare inhabitants, aiding in their preservation. By collaborations with UMS researchers and students, I can aid in knowledge transfer and raising awareness in both Malaysia and the Netherlands.

(9) Time Schedule

Table 6 below shows the anticipated time schedule for my PhD research project. Since, by contract, I work at 80% capacity, total calendar time amounts to five years, starting September 2014 and finishing end of August 2019. During this time, next to my contract at University of Groningen, I will be employed as "research associate" at the department of Terrestrial Zoology of Naturalis, Leiden, the Netherlands. At Naturalis I will work on sample material and perform genetic studies, among others.

Table 6 - Time schedule for five year PhD research

2014	Sep	Start PhD	2017	Jan	symposia.
	Oct	Study of literature,		Feb	Fieldwork Borneo: locations
	Nov	initial samples; setting up		Mar	to be determined.
	Dec	fieldwork; networking;		Apr	Community data analysis;
2015	Jan	symposia.		May	DNA sequencing; data
	Feb			Jun	analysis.
	Mar	Fieldwork Borneo:		Jul	
	Apr	Kinabatangan.		Aug	
	May	Introductory Essay.		Sep	
	Jun	Community data analysis;		Oct	
	Jul	DNA sequencing.		Nov	Finish second paper on
	Aug			Dec	Research Question B.
	Sep		2018	Jan	DNA sequencing; data
	Oct			Feb	analysis; scientific outreach.
	Nov			Mar	
	Dec	Setting up fieldwork;		Apr	
2016	Jan	symposia.		May	
	Feb	Fieldwork Borneo: Tabin,		Jun	Finish third paper on
	Mar	Kinabatangan.		Jul	Research Question C.
	Apr	Community data analysis;		Aug	Data analysis; scientific
	May	DNA sequencing, data		Sep	outreach.
	Jun	analysis.		Oct	
	Jul			Nov	
	Aug			Dec	
	Sep		2019	Jan	
	Oct	Finish first paper on		Feb	Finish fourth paper on
	Nov	Research Question A.		Mar	Research Question D.
	Dec	Setting up fieldwork;		Apr	Write thesis.
				May	
				Jun	
				Jul	
				Aug	

GLOSSARY

Items marked * are (partly) based on lemmas from http://en.wikipedia.org/.

ALLOPATRY	in reference to two or more related species which have separate geographic distributions (Rivas, 1964)
ALLOTOPIC	in reference to two or more related species which do not occupy the same macrohabitat; the species are presumably not in close proximity, cannot interbreed, and do not occur together in the same locality, although they may have the same coarse-grained geographic distribution (sympatric) (Rivas, 1964)
ANCIENT ENDEMIC	a species that is a last surviving or relict species of often large groups in the distant geological past (Vermeulen and Whitten, 1999)
CLADE	a group of organisms consisting of an ancestor and all its descendants*
COMMUNITY	a group of trophically similar, sympatric species that actually or potentially compete in a local area for the same or similar resources (Hubbell, 2001)
COMPETITIVE EXCLUSION	the observation that two species will not persist as direct competitors in a stable community
CONVERGENT EVOLUTION	the independent evolution of similar features in species of different lineages*
DEME	a local, panmictic population (Mayr, 1970)
DIPLOMMATINID	member of the Diplommatinidae, a family of microscopic land snails, often characterized by beautiful ornamentation
GASTROPOD	member of the molluscan class Gastropoda, known for locomotion on flat crawling sole
GUILD	an association of functionally equivalent species in a community
HOMOPLASY	cladistics term for convergent or parallel evolution: the independent evolution of similar features in species of different lineages*
LOCAL ENDEMIC	species that has a range of about 100 to 10,000 square kilometres (Vermeulen and Whitten, 1999)
MACROHABITAT	the habitat at a larger scale, such as that of as island or a natural park
METACOMMUNITY	a set of interacting communities which are linked by the dispersal of multiple, potentially interacting species*
MICROHABITAT	the habitat at a smaller scale, such as that within a limestone outcrop, or even within a plot of ten by ten metres
MICROSNAIL	a representative of the gastropods with a maximum adult shell size of five millimetres
MOLLUSC	member of the large invertebrate phylum called Mollusca; includes important classes, such as Bivalvia and Gastropoda

MONOPHYLETIC	in reference to a group of organisms which form a clade, i.e. an ancestor with all its descendants
NEUTRAL THEORY	a hypothesis that aims to explain the diversity and relative abundance of species in ecological communities; the theory assumes any (ecological or morphological) differences between members of an ecological community as "neutral", or irrelevant to their success*
NICHE	the place or function of an organism within its ecosystem
OPISTHOBRANCH	a polyphyletic group of gastropods in which the gills are situated behind the heart; includes the Pulmonata*
POPULATION	a summation of all the organisms of the same group or species, which live in a particular geographical area, and have the capability of interbreeding*
PROSOBRANCH	a polyphyletic group of gastropods in which gills, mantle cavity and anus are situated in front of the heart (as opposed to the Opisthobranch)*
POLYPHENISM	the occurrence in a population of several phenotypes, the differences between which are not the result of genetic differences (Mayr, 1970)
POLYPHYLETIC	in reference to a group of organisms taxa characterized by one or more homoplasies*; not monophyletic
PULMONATE	member of the informal group of Pulmonata, part of the Gastropoda, characterized by the ability to breathe air using a pallial lung instead of a gill*
RECENT ENDEMIC	a species that belongs to a cluster of closely related species that have evolved from a single parental species in the recent geological history (Vermeulen and Whitten, 1999)
REGIONAL ENDEMIC	a species with a range covering 10,000 to 1 million square kilometres (for example, the island of Borneo) (Vermeulen and Whitten, 1999)
SITE ENDEMIC	a species with a range of up to about 100 square kilometres, but sometimes a range down to much less than 1 square kilometre (Vermeulen and Whitten, 1999)
SYMPATRY	in reference to two or more related species having the same or overlapping geographic distributions, regardless of whether or not they occupy the same macrohabitat, or occur in the same locality (Rivas, 1964)
SYNTOPY	in reference to two or more related species which occupy the same macrohabitat; the species occur in the same locality, are observably in close proximity and would interbreed if they were capable (Rivas, 1964)
TAXON	a group of one or more populations of an organism seen to form a unit*, a clade

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