The causes of insect endemicity with the example of Madagascar

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Abstract

Biodiversity is disappearing at an exceptional rate, engendering a considerable effort of the scientific community to conserve the rarest and most threatened species. Biodiversity Hotspots were designed for this purpose and Madagascar, showing the highest levels of endemism and imbalance across taxa, rapidly became their flagship. The processes that lead to such exceptional biodiversity patterns on the island remain poorly understood and the lack of efficacy of traditional taxonomy to catalogue tropical endemic biodiversity hinders the scientific advances in this domain. This study tackles insect endemism in Madagascar, aiming at identifying the evolutionary and ecological factors responsible for their present diversity patterns. Aquatic beetles, living in standing or running water, submit to differences in habitat stability, potentially affecting dispersal patterns and hence endemism patterns. We chose them as a target group for these reasons, and first achieved a DNA level inventory of the fauna. We identified 169 species, showing high congruence with the morphological taxonomy and corresponding to 74% of the total estimated species richness. The mitochondrial dataset revealed high spatial turnover at the species and haplotype levels. A phylogenetic tree from three gene markers was used for ancestral state character reconstruction and suggested the dependent evolution of endemism and habitat use. Secondly, our results highlight a combination of low dispersal activity and important climatic constraints for the lotic fauna, explaining the local scale endemism in this group, whereas the lentic species turnover correlated the least to both climate and geographic distance. Last, a distribution modelling approach revealed the uniqueness of Malagasy climate niches and the absence of conservation of environmental envelopes at higher phylogenetic levels. We conclude that endemism in Madagascar is best explained locally by the dependence to environmental variability of the lotic species, and that dispersal capacities are the primary hindrance to colonising external suitable habitats.

Declaration

I hereby declare that the work in this thesis is my own. Co-authors on manuscripts to be submitted are listed in the respective chapters. Any additional help provided has been specifically acknowledged.

Benjamin Isambert, November 2009:



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CHAPTER 1

Introduction

1.1 THE STUDY AREA

'May I announce to you that Madagascar is the naturalist's promise land? Nature seems to have retreated there into a private sanctuary, where she could work on different models from any she has used elsewhere. There you meet bizarre and marvellous forms at every step…'

Philibert Commerson, XVIII century (Teissier, 1859)

1.1.1 Madagascar: the number one biodiversity hotspot

Biodiversity on earth is presently disappearing at such a high rate that little is known about what is being lost (Pimm *et al.*, 1995). In that context of race against species extinction, and given restricted budgets, choices for conservation priorities must be made. Norman Myers came first with the concept of "biodiversity hotspots" in 1988, by identifying areas characterised by high levels of plant endemism and remarkable habitat loss (Myers, 1988). One year later, Conservation International adopted the concept of hotspots and started to reassess their quantitative boundaries (www.biodiversityhotspots.org/). Today, 34 regions of the world, including Madagascar and the surrounding Indian Ocean islands, are listed as biodiversity hotspots (Myers *et al.*, 2000), with a habitat cover representing 2.3% of the Earth's surface area (Mittermeier *et al.*, 2005). About 50% of the world's plant species and more than 40% of terrestrial vertebrates are endemic to these regions, but Madagascar's outstanding levels of endemism and imbalance across taxa are unmatched by any other hotspot: according to Conservation International, the island totals 14 000 endemic plant species (90% endemism), 144 endemic mammal species (92.9% endemism), 367 endemic reptile species (95.6% endemism) and 229 endemic

amphibian species (99.6% endemism). Though, endemism doesn't occur only at the species level but concerns also higher taxonomic levels: primates are a particularly remarkable group in this regard, with five families and 15 genera endemic, and plants show the striking number of eight endemic families, which is unequalled by any other country. Moreover, Madagascar represents less than 0.4% of the Earth's land surface area, and has consequently one of the highest ratios of endemic species richness over land area (Myers *et al.*, 2000). Regrettably, recent human activities have considerably endangered the island's natural habitats, particularly the lowland humid and subhumid forests, resulting in major soil erosion (Green and Sussman, 1990, Consiglio *et al.*, 2006) and alarming estimates of biodiversity loss (Allnutt *et al.*, 2008). Madagascar is considered today as the most important reservoir of rare and threatened diversity, and is placed at the top of the list of biodiversity hotspots (Ganzhorn *et al.*, 2001, Mittermeier *et al.*, 2005, Brooks *et al.*, 2006).

1.1.2 Paleogeography and endemism

The biological concept of island refers not only to a landmass surrounded by water, but more generally to a system in isolation (MacArthur and Wilson, 1967). The events responsible for physical isolation can differ, leading to the notion of Darwinian islands, which have always been isolated, versus fragment islands, which have a history of connections with other land bodies (Gillespie and Roderick, 2002). This distinction is crucial when considering island endemism since it relates to different speciation processes: in the early stages, Darwinian islands see their species number increasing through immigration mainly, but long term isolation will allow the formation of new species referred to as neo-endemics. The species pool found on fragment islands constitute a subset of the biota originally part of the mainland, but

can evolve or become extinct in response to the physical and environmental conditions encountered on the newly formed island. The resulting endemic species are defined as paleo-endemics (Cox and Moore, 2000).

Approximately 160 million years ago, Madagascar was located in the centre of Gondwana, a supercontinent comprising Africa, India, South America, Antarctica and Australia (Upchurch, 2008). The split of eastern and western Gondwana isolated Madagascar, India, Antarctica and Australia from Africa and South America. Eastern Gondwana drifted for several million years in the ocean until India together with Madagascar separated from it, forming the Indomadagascar subcontinent (± 135 million years ago) (Yoder and Nowak, 2006). The separation of Madagascar from India began only 35 to 55 million years later, depending on the estimations considered (Plummer, 1996, Torsvik *et al.*, 2000), resulting in at least 80 million years of complete isolation (de Wit, 2003). Today Madagascar is the fourth largest island in the world and lies about 400 km away from Africa, 4000 km from India, 5000 km from Antarctica and 6000 km from Australia (Yoder and Nowak, 2006). Its paleogeographic history of isolation and contiguity with Gondwanan subcontinents is considered to have played a major role in promoting the exceptional biodiversity richness and endemism of the island.

1.1.3 An exceptional macrohabitat diversity

It is well accepted now that the present fauna of Madagascar must include groups whose presence is best explained by ancient vicariant events, and other groups that originally reached the island via transoceanic dispersal (Torres *et al.*, 2001, de Wit, 2003, Zakharov *et al.*, 2004, Monaghan *et al.*, 2005b, Poux *et al.*, 2005). In both cases, organisms on the island face a unique set of physical and environmental

conditions. First, Madagascar provides a very large territory: it is 1600 km long, and extends 14º in latitude, southward of the 12ºS. At the widest, 600 km separate the east coast form the west coast, which confers the island a total area of approximately 730 000 km^2 (larger than France) (de Wit, 2003). Second, the topography of the island is strongly asymmetric: a linear range of mountains culminating at more than 2800 m stretches from north to south along the east coast, within 100 km from the ocean, and drops progressively across 400 km towards the west coast, following a weaker slope (Pearson and Raxworthy, 2009). Madagascar is also characterised by unique climatic features (Ganzhorn, 1995): most of its precipitations are brought through the east coast by the numerous Indian Ocean cyclones or trade winds, but remain blocked by the eastern escarpment (Pearson and Raxworthy, 2009). As a result, the island presents an exceptional climate spatial heterogeneity and unpredictability, creating a hypervariable environment for its inhabitants (Dewar and Richard, 2007). The combination of topography, geology and climatic variability engendered a complex network of ecotones and a high macrohabitat diversity (Goodman and Benstead, 2003), dividing the island in seven distinct ecoregions (as defined by the WWF: http://www.panda.org/about_our_earth/ecoregions/ecoregion_list/): lowland forests on the east side, subhumid forests and ericoid thickets in the central high plateaux, central south and the north, and finally spiny thickets, dry deciduous forests, mangroves and succulent woodlands in the southern and western part of the island. These conditions offer a great potential for ecological niche differentiation, and ecological speciation is now strongly considered to explain the exceptional biodiversity patterns of Madagascar (Dewar and Richard, 2007, Raxworthy *et al.*, 2007, Pearson and Raxworthy, 2009).

1.2 FROM DNA TAXONOMY TO DELIMITING SPECIES

1.2.1 The emergence of DNA taxonomy and DNA barcoding

Taxonomists have the difficult duty of classifying living things and describing species. Their discipline dates back to 250 years in its modern era, and remained based until recently on Linnaeus' binomial classification system (Linnaeus, 1758). At first, two major issues arose from this system: 1) traditional taxonomy operated on the basis of individuals' expertise, and the lack of communication between taxonomists led to multiple descriptions of the same entity and to the misclassification of species (Godfray, 2002), and 2) identifications based essentially on morphological characters rely excessively on the observer's skills and limit the boundaries of species to humanly visible features. Yet, the most critical problem emerged from the fact that traditional taxonomy offers limited resources and an inadequate number of specialists to tackle increasing estimates of unknown biodiversity (Hammond, 1992, Hawksworth and Kalin-Arroyo, 1995, Blaxter, 2003, Wilson, 2003). This context combined to the current rate of species extinction appealed to a faster and more factual system to classify organisms (Blaxter, 2004). DNA taxonomy has been proposed as a new discipline aimed at facing these challenges (Tautz *et al.*, 2002, 2003): it puts forward the association of molecular data (in the form of DNA sequences) to type specimens (and ideally their taxonomic status), used afterwards as a reference for comparisons with other individuals' DNA sequences so as to determine their own taxonomic status. Hebert and colleagues proposed the cytochrome c oxydase I mitochondrial gene (*cox1*) as a universal animal bioidentifier or "barcode" for species delimitation but also higher taxonomical discrimination (Hebert *et al.*, 2003, Hebert and Gregory, 2005). Mitochondrial DNA (mtDNA)

became a good candidate for two main reasons: first because it evolves faster than nuclear DNA, which offers visible divergence even for lineages with a recent reproductive isolation, and secondly because of its limited exposure to recombination (due to the way it is inherited) (Saccone *et al.*, 1999). The efficacy of *cox1* as a barcode for distinguishing species has been proved within highly diverse groups, like birds, fish (Hebert *et al.*, 2004, Ward *et al.*, 2005) and insects (Balke *et al.*, 2004, Monaghan *et al.*, 2005a, Caesar *et al.*, 2006) but also appeared very powerful for revealing cryptic species, when sexual dimorphism and different life stages complicate the task of morphological identification (Goetze, 2003, Smith *et al.*, 2006, Vahtera and Muona, 2006). DNA barcoding and DNA taxonomy brought new hopes within the scientific community to face the challenge of identifying and classifying the estimated 10 million species on Earth (Savolainen *et al.*, 2005).

1.2.2 Towards the delimitation of species

The role of DNA barcodes was initially restricted to the identification of species, but *cox1* and its broad phylogenetic signal started to be used in the context of systematics. It provided a new tool for understanding biodiversity richness and endemism patterns, and appeared particularly useful at investigating the evolution and phylogeographic history of species (Chinn and Gemmell, 2004, Ribera and Vogler, 2004, Smith *et al.*, 2005, Orsini *et al.*, 2007). Additionally, mtDNA began to serve also at exploring intraspecific genetic variation (Vieites *et al.*, 2006), and made the thought of using mitochondrial sequence divergence as foremost criterion for species discrimination come forth (Vogler and Monaghan, 2007). Thus, DNA taxonomy evolved from complementing the concept of traditional taxonomy towards a discipline more autonomous regarding the delimitation of species. In the last 10 years,

multiple species delimitation methods have been developed based on the combination of mtDNA and morphological data (Puorto *et al.*, 2001, Templeton, 2001, Wiens and Penkrot, 2002) and more recently based on sequence divergence only (Morando *et al.*, 2003, Pons *et al.*, 2006). These new approaches were accompanied by multiple variations of the species concept, creating a shakedown in the field of systematic biology (De Queiroz, 2007, Wiens, 2007), but exhibit a basic conceptual concordance.

The General Mixed Yule Coalescent model (GMYC) developed by Pons *et al.* (2006) combines models of stochastic lineage growth with coalescent theory in order to define the threshold between species level and population level branching events on a phylogenetic tree. One of the main advantages of this sequence-based species delimitation method is that it doesn't require prior taxonomic knowledge and it is independent of morphological character identifications. Consequently, it can be applied for accelerated large-scale species discovery, and it is particularly well adapted to very diverse and poorly known faunas (Pons *et al.*, 2006). In this regard, it has proved particularly efficient at cataloguing insects faunas in both tropical (Monaghan *et al.*, 2009) and temperate regions (Papadopoulou *et al.*, 2008) and revealed a high congruence with existing taxonomic status in many groups (Monaghan *et al.*, 2009). The GMYC approach offers an undeniable potential for fast biodiversity inventorying and combined to ecological concepts, can ameliorate the comprehension of the evolutionary processes leading to species diversification, which makes its use particularly relevant in the present conservation context.

1.3 A MODEL SYSTEM WITHIN THE MALAGASY INSECT FAUNA

1.3.1 Insect inventories in Madagascar

Despite the considerable scientific effort to understand Madagascar's exceptional biodiversity richness and endemism patterns, it appears that not all organisms have been studied equally: mammals (particularly lemurs), birds, amphibians and reptiles attract most of the research focus (Goodman and Ganzhorn, 2004, Yoder *et al.*, 2005), and very little is known about the invertebrate alpha diversity of the island. The most recent estimation gives 5808 as the total number of invertebrate species, showing 86% of endemism (Goodman and Benstead, 2005), but given the imbalance in Malagasy target groups found in the literature, this latter value has to be considered with scepticism. Moreover, within the described invertebrates, arthropod species are underrepresented since most of them remain to be discovered, but also because many specious groups are poorly described, especially within most of beetle families. Within insects, biological research covered so far an insubstantial number of taxa, mainly dung beetles (Monaghan *et al.*, 2005a, 2007, Hanski *et al.*, 2007, Orsini *et al.*, 2007), mayflies (Monaghan *et al.*, 2005b), butterflies (Lees, 1996, Lees *et al.*, 1999, Zakharov *et al.*, 2004, Linares *et al.*, 2009) and ants (Fisher, 2005, Smith *et al.*, 2005). The diversity of aquatic coleoptera on the island has also been tackled, both at the molecular and taxonomic levels (Rocchi, 1991, Nilsson *et al.*, 1997, Shaverdo *et al.*, 2008, Monaghan *et al.*, 2009) and is represented by three suborders: Myxophaga (two species), Hydradephaga (approximately 210 species), and Polyphaga (70 to 80 species). In this study, we will focus on the Hydradephagan water beetle fauna and its four representative families: Gyrinidae, Haliplidae, Noteridae and Dytiscidae.

1.3.2. Tackling insect endemism in Madagascar using the lentic/lotic model

Gene flow plays a central role in the mechanisms leading to speciation and endemism. The way it is affected determines how populations become reproductively isolated and eventually lead to species divergence (Rundle and Nosil, 2005). There are several determinant factors for the variation in gene flow but two remain fundamental: 1) the way organisms disperse, 2) the physical and non-physical barriers isolating populations. A complete comprehension of insects' dispersal patterns and the identification of the components of isolation in Madagascar would constitute the key to understanding the diversification of insect species on the island and to unravelling the causes of their endemism. Unfortunately, targeting the entire insect fauna of Madagascar is far too ambitious, and a sub-selection of taxa must be chosen as a model system for this objective. An adequate target group would gather a good predictability in terms of dispersal and isolation.

Water beetles (Coleoptera, Hydradephaga) live in aquatic habitats that can be classified in two categories (Dobson and Frid, 2008):

1) lotic habitats: referring to all running water habitats (e.g. streams, rivers, waterfalls, etc.)
2) lentic habitats: referring to all standing water habitats (e.g. pools, ponds, lakes, etc.)

Within both categories, water bodies present definite boundaries and do not form a continuous habitat, which is synonymous of potential source of isolation for populations. However, these beetles show exceptional dispersal capabilities, uncommon among aquatic organisms: they swim within their habitat and can walk or fly between habitat patches, which will have major consequences depending on the habitat type considered. Indeed, lentic water bodies are not stable in space and time:

ponds submit to climate fluctuation and possible alterations of the soil, and therefore can dry up, become filled up with vegetation or be formed relatively fast, whereas lotic habitats like streams and rivers show more temporal and spatial continuity due to the persistence of hydrographical networks on the evolutionary scale (Harper, 1992, Dobson and Frid, 2008). Thus, lentic species' survival relies on their effective dispersal capacities (Hof *et al.*, 2006), whereas lotic species' survival is facilitated by their habitat stability. Consequently lentic species are expected to show larger distributional ranges and higher gene flow than lotic species. Ribera and Vogler (2000) investigated the lentic/lotic model for aquatic Coleoptera and tested the hypothetical differences in dispersal strategies: based on a dataset of 490 species from the Iberian Peninsula and their associated range sizes, they found that lotic species had much smaller distributional ranges than lentic species. In their study, this strong association between habitat type and range size was found in four distinct monophyletic lineages (Hydradephaga, Hydrophiloidea, Hydraenidae and Byrrhoidea), and the authors explained the pattern by the greater geographic mobility required for species living in less permanent habitats (lentic). One year later, Ribera *et al.* (2001) explored the eventual effects of these differences in dispersal strategies between lotic and lentic species on gene flow and clade evolution. They compared species-level phylogenies based on two mitochondrial genes for the lentic genus *Ilybius* and the lotic genus *Deronectes*. The expected higher species turnover in lotic lineages due to their lower dispersal patterns compared to lentic species was not strongly supported by their results, and the frequency in recent splits in the *Deronectes* phylogeny was found higher than in *Ilybius*. However these results were not significant compared to expected results under a constant speciation rate null model. The authors failed to detect stronger differences between the two clades, but

explained it by the potential taxonomic and sampling artefacts and their consequences on the species-level phylogenies. Additionally, they discussed the fact that levels of species turnover can be found very similar, but hiding different habitats and completely different spatial scales. In 2003, Ribera *et al.* tested this time the effect of habitat use on species richness patterns of aquatic beetles in Europe. Based on the distribution of more than 800 species across 15 regions within Europe, they found that the number of species in standing waters was correlated with the geographic connectedness and the size of the areas considered, whereas the number of species in running waters was mostly correlated with latitude. With these three studies, aquatic beetles were proved to provide an excellent predictive system in terms of habitat stability and dispersal, and allow the anticipation of potential isolation factors. For these reasons, they constitute an ideal group for exploring insect endemism in Madagascar.

1.4 CLIMATE, SPECIATION AND ENDEMISM IN MADAGASCAR

The high degree of endemism across several taxonomic levels in Madagascar is partially explained on one hand by its long isolation from the African continent and India (Yoder and Nowak, 2006), and on the other hand by multiple colonisation events and subsequent radiations (Poux *et al.*, 2005; Yoder *et al.*, 2005). However, these hypotheses describe the processes responsible for global endemism patterns on the island, and fail to explain the uniqueness of taxa on very small geographic scales. The understanding of the evolution of Madagascar's microendemic biota remains a considerable scientific challenge, and so far, only a few attempts have been made to address this question. Specifically, two major biogeographic hypotheses have recently

been proposed, emphasizing the role of temporal and spatial climatic variation in generating local endemism across taxa: the "watershed hypothesis" (Wilme *et al.*, 2006) and the "current climate hypothesis" (Dewar and Richard, 2007; Pearson and Raxworthy, 2009).

1.4.1 The watershed hypothesis

Based on a comprehensive database of rivers and watersheds, and on the analysis of several thousands of vertebrate species' records across the island, Wilme *et al* (2006) investigated the effects of paleoclimatic shifts on dispersal patterns and vicariance within Madagascar in recent geological time. During the Quaternary climatic shifts, periods of glaciation resulted in a cooler and drier climate in Madagascar, but orographic precipitations enabled the river systems to persist. Hence, humid habitats could only be found nearby the hydrological network, creating buffer zones in riverine habitats, and corridors used by organisms to reach retreat areas at high elevation, towards the sources, with subsequent dispersal. The main statement of Wilme's model is that the climatic shifts would have created unequal environmental conditions among watersheds, depending on the elevation of their respective sources: lower watersheds would have submitted to more-pronounced aridity, and thus greater levels of habitat isolation and vicariant events, allowing for extensive allopatric speciation, whereas high elevation habitats, more stable, would have provided retreat areas allowing for future range expansion. Thus, river catchments with sources at low elevation would have led to the speciation of locally endemic, and high elevation zones would show lower microendemism levels.

1.4.2 The current climate hypothesis

The centres of endemism predicted by Wilme and colleagues were consistent with actual microendemism patterns of diurnal and nocturnal species of lemurs (Wilme *et al.*, 2006), however it failed to explain some differential patterns of local endemism across taxa (Kremen *et al.*, 2008). As an alternative, several authors underlined the potential role of environmental gradients and Madagascar's climate diversity (described in section ***1.1.3***) in generating local endemism (Dewar and Richards, 2007; Harcourt, 2008). In particular, Raxworthy *et al.* (2007) discussed the role of climatic gradients in promoting parapatric speciation of *Phelsuma* geckos, and the general idea of climate patterns engendering local endemism was refered afterward as the current climate hypothesis by Pearson and Raxworhty (2009). In this last study, they tested the coincidence between actual species' distributions and areas of endemism predicted by both the watershed and the current climate hypotheses. Their results showed that both hypotheses explained distribution patterns of lemurs, geckos, and chameleons, which strongly suggested that several processes are responsible for local endemism of other taxa on the island. With these two recent hypotheses, climate is now considered (either for its spatial or temporal variablility) to play an active role in the different processes leading to insular speciation and microendemism of Malagasy vertebrates. This study investigates its role in generating insect endemism.

1.5 AIMS AND OBJECTIVES

This project aimed at understanding the causes of insect endemism in Madagascar that remain to date uncertain. We use a multidisciplinary approach and the example of aquatic beetles in an evolutionary and ecological context, in order to improve our understanding of the mechanisms promoting insect species diversification and uniqueness, and explain the exceptional present diversity patterns in the number-one biodiversity hotspot. The main objectives were to:

1) Conduct a first thorough DNA inventory of the water beetle fauna across the island, embracing its macrohabitat and microhabitat diversity. Delimit evolutionary species, quantify the species diversity and evaluate its congruence with Linnaean taxonomy. Assess the level of endemism from both molecular and taxonomic data. Obtain a robust phylogeny of all Malagasy species from mitochondrial and nuclear markers, and conduct ancestral state character reconstructions to test for a correlation between endemism and habitat stability. (Chapter 2).

2) Test for the lotic-lentic differentiation in beta diversity patterns, spatial turnover, environmental constraints, and dispersal predicted by Ribera and Vogler (2000), Ribera *et al.* (2001, 2003) and Ribera (2008), by means of a cluster dendrogram analysis, a constrained analysis of proximities, and multivariate Mantel correlograms. Infer the role of habitat stability in structuring local endemism patterns in Madagascar. (Chapter 3).

3) Model the ecological niche of multiple endemic species at the large scale to investigate if niche uniqueness is responsible for the confinement of species on the island, or if dispersal is the main hindrance to reaching suitable habitats available in Africa, India or other Indian Ocean islands. Model the ecological niche within Madagascar of multiple endemic species, genera and intermediate clades so as to assess the level of niche conservatism among phylogenetic levels, and understand the role of climate variability in species diversification and local endemism on the island. (Chapter 4).

Overall conclusions are discussed in Chapter 5.

CHAPTER 2

Using mitochondrial DNA for inventorying and understanding highly endemic faunas in biodiversity hotspots: the case of the water beetles in Madagascar[1]

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2.1 ABSTRACT

Studying tropical biodiversity and its evolutionary history is considerably slowed down by the remaining non-described species. In this study we targeted the entire fauna of aquatic beetles of Madagascar and used a molecular species delimitation method to compensate for this taxonomic impediment. We obtain a mitochondrial DNA level inventory of 169 species out of 2043 individuals, representing close to three quarters of the estimated total species richness based on three alpha diversity estimators. The DNA delimited groups showed a high congruence with the existing morphological taxonomy, demonstrating the profitability of such a molecular inventory. The second part of the analysis emphasises a complex genetic structure of the fauna and the different communities, showing a very high spatial turnover both at the species and haplotype levels. Two additional markers (16S and 28S) were used to reconstruct ancestral state characters, suggesting that whether or not a species is endemic depends on the type of water bodies considered (lotic or lentic). In addition to a very first species quantification of the Malagasy aquatic beetle fauna, these results put forward a fast and accurate method for inventorying highly endemic groups with numerous rare and poorly described species, proving the potential of DNA taxonomy in a conservation context.

2.2 INTRODUCTION

2.2.1 Madagascar: a biodiversity hotspot

In a global context of race against species extinctions, conservationists came up with the concept of biodiversity hotspots: the most important reservoirs of rare and threatened diversity on earth (Myers, 1988, Myers *et al.*, 2000). Madagascar figures today at the top of the list of the biodiversity hotspots (Ganzhorn *et al.*, 2001, Mittermeier *et al.*, 2005, Brooks *et al.*, 2006). Originally part of the supercontinent Gondwana, Madagascar began to separate from Africa 165 millions years ago, and remained attached to India until its complete insularisation 88 millions years ago (de Wit, 2003, Yoder and Nowak, 2006). Presently, the Indian Ocean and the Mozambique Channel surround Madagascar; this long period of isolation combined with a history of contacts with other landmasses resulted in outstanding biodiversity richness and imbalance, as well as in very high levels of endemism across the different taxa. Unfortunately, this reservoir of biodiversity is presently at risk, mainly due to the destruction of the natural habitats by human activities: logging, mining, and slash-and-burn agriculture are responsible for the reduction of the primary humid and subhumid forests, and consequently for soil erosion (Green and Sussman, 1990, Consiglio *et al.*, 2006). In these conditions, very little is known about what is being lost. Besides, high levels of endemism have been identified mainly for well-studied vertebrates, it is less well quantified in invertebrates, especially insects. Moreover, the notion of endemism is entirely dependent on the hierarchical level of the entity of interest: one must define the biological subject in order to evaluate its uniqueness, or geographical range restrictions. Assessing endemism requires reliable taxonomic information, which is not available for numerous small organisms (Hawksworth and

Kalin-Arroyo, 1995, Woese, 1996). In the case of Madagascar, around 5800 invertebrate species have been described so far (Goodman and Benstead, 2003, 2005) but the actual taxonomic impediment is such that endemism studies of these groups remain hindered.

2.2.2 DNA taxonomy and the delimitation of species

It is well accepted now that the real amount of biodiversity present on earth exceeds by far the number of species described to date (Hammond, 1992, Hawksworth and Kalin-Arroyo, 1995, Blaxter, 2003, Wilson, 2003). The same system has been used for more than 250 years in order to complete the identification and the classification of species, based on the morphology of organisms (Linnaeus, 1758), but this system relies almost exclusively on the knowledge of a decreasing number of specialists, facing a growing estimate of the total amount of biodiversity. In the case of biodiversity hotspots, there is even a stronger need for a fast and accurate method to identify and delimit species, so as to overcome the issues encountered by traditional taxonomy. The use of DNA for taxonomical purposes has recently emerged (Tautz *et al.*, 2002, 2003) and consisted in a first time in identifying species by closest match within an existing database (Hebert and Gregory, 2005, Savolainen *et al.*, 2005). Then molecular data started to be used to delimit taxonomic entities directly from sequence divergence among samples, but the entities were not clearly defined and could represent any taxonomic level (Blaxter, 2004). The efficacy of the DNA barcode *cox1* to distinguish species was used to build the first catalogues of biodiversity of a given geographic region (Janzen *et al.*, 2005), and the emergence of the GMYC approach (Pons *et al.*, 2006, Fontaneto *et al.*, 2007, Papadopoulou *et al.*, 2008, 2009a, 2009b) facilitated these species inventories within entire faunas

(Monaghan *et al.*, 2009). Unfortunately, DNA inventories often remain catalogues per se, having for main objective to list reproductive entities from sampling across a region. This study aims at building reliable DNA inventories linked to traditional taxonomy so as to understand present patterns of spatial turnover, both at inter and intra-specific levels. We use here the molecular approach to test the effects of habitat diversity on species and haplotype turnover.

2.2.3 Aquatic beetles and the lotic-lentic model

A major concern for conservationists is the combination of endemism and small distributional ranges that leads to global extinctions. The most threatened and endangered species belong to the biodiversity hotspots and are known to be restricted to these geographic regions. However within the hotspots, how endemism relates to species range size is left poorly understood. To remedy it, identifying the potential predictors of species range size is fundamental. Aquatic beetles (Coleoptera, Hydradephaga) move by walking, swimming and flying, which confer them a considerable potential for dispersal, as well as heterogeneity in their dispersal capacities. Moreover, running water lasts longer in time than standing water; as a result, beetles have to disperse in order to survive when ponds are drying up, whereas rivers and streams provide a more stable habitat over time. Thus standing water species tend to be widespread and are expected to show a broader distributional range than running water species (Ribera and Vogler, 2000, Ribera *et al.*, 2001, 2003, Ribera, 2008), whose distribution would be more consistent with the hydrographical network itself. As a consequence, gene flow is expected to be higher for lentic species in comparison to lotic species. One should expect lotic species to occupy smaller

distributional ranges and show higher levels of endemism, whereas lentic species are expected to be widespread.

In Madagascar, water beetles are represented by five main families: Hydrophilidae, Dytiscidae, Noteridae, Gyrinidae and Haliplidae. More than 200 species have been described in the last four families (Rocchi, 1991, Nilsson, 2001, Nilsson and Van Vondel, 2005, Shaverdo *et al.*, 2008) based on morphological characters and more recently DNA sequence data (Balke *et al.*, 2004, Miller *et al.*, 2007). Given the similarities in morphology and the increasing availability of molecular data, several groups are still under revision. Moreover, recent studies put forward relatively recent extinctions, going up to 50% of known species in the case of dung beetles (Hanski *et al.*, 2007, 2009), which complicates estimations of species diversity. As a result, it is unclear what the aquatic beetle alpha diversity really is, but it has been proved that insects in Madagascar present high levels of species turnover and local endemism whatever the taxa considered (Smith *et al.*, 2005, Monaghan *et al.*, 2009). Two possible explanations for these patterns are the heterogeneous distribution of the remaining natural habitat (Consiglio *et al.*, 2006) and the high diversity of macro habitats represented (seven WWF ecoregions in total) (Smith *et al.*, 1997, Wilme *et al.*, 2006). In the case of aquatic beetles, another degree of habitat diversity to consider is the lotic-lentic system (Ribera and Vogler, 2000). From the literature and the personal information on sampling sites, species can arbitrarily be classified as lotic, lentic, or both (if found in running and standing waters), which linked to the haplotype data allows us to test the differential-dispersal scenario described above. Aquatic beetles constitute a valuable target group, providing a solid framework for understanding species diversification and endemism on the island.

2.3 MATERIAL & METHODS

2.3.1 Sampling

In collaboration with the department of Entomology, University of Antananarivo, and the Madagascar Institute for the Conservation of Tropical Ecosystems, water beetles (Coleoptera: Dytiscidae, Noteridae, Haliplidae, Gyrinidae) were collected between 2004 and 2007, following a sampling strategy designed to cover a maximum of land surface area, as well as a maximum of the habitat and microhabitat diversity of the island. To do so, several localities inside and outside protected areas were chosen within each of the seven WWF ecoregions encountered in Madagascar (http://www.panda.org/about_our_earth/ecoregions/ecoregion_list/): humid/lowland forests, subhumid forests, mangroves, succulent woodlands, dry deciduous forests, ericoid thickets, and spiny thickets (A summary map for these localities and the corresponding habitats is provided in Figure 2.1). In total, 153 sites were visited, covering 13 national parks (NP) and special reserves (SR): Andringintra NP, Isalo NP, Zombitse NP, Masoala NP, Mantadia-Andasibe NP, Marojejy NP, Zahamena NP, Montagne d'Ambre NP, Ranomafana NP, Kirindy Forest NP, Foret d'Ambre (SR), Ankarana (SR), and Analamazaotra (SR). Beetles were also collected in unprotected areas, mainly in the scrublands and spiny forest between Tulear and Ambovombe, and in the rainforest in the surrounding area of Fort Dauphin.

The collecting method per se consisted in active sampling, with hand nets and kitchen sieves, depending on the size of the water bodies and the type of microhabitat considered (e.g. streams, pools, rivers, water falls, hygropetric rocks). The GPS coordinates were taken if possible, and each site was assigned to a habitat category: lotic, lentic, or hygropetric. The insects were stored in 95% ethanol immediately after

collection and returned to the laboratory, where they were identified as Linnaean species, or, if not possible, sorted to morphospecies by an experienced taxonomist of aquatic beetles, Dr Johannes Bergsten. As a sample of the genetic variation of the fauna, a maximum of five individuals per Linnean species/morphospecies per locality were prepared for the molecular analysis. Specimens are kept in the research collection of the department of Entomology, Natural History Museum, London.

2.3.2 DNA extractions & Sequencing

In order to allow subsequent taxonomic identification, the DNA was extracted from the insects using non-destructive methods: depending on the size of the beetles, a leg, the whole individual, or the head together with the pro-thorax were placed overnight in a lysis buffer containing a solution of proteinase. The WIZARD SV 96 Genomic Kit (Promega) was used, allowing processing 96 individuals at a time. The mitochondrial DNA was amplified for the Cytochrome Oxydase I region (*cox1*) by PCR, using the set of primers Ron Inosine/Ron Dyt and Patty/Pat Dyt (Ron Dyt and Pat Dyt were designed by Dr Johannes Bergsten after a compilation of sequences from several genera of the Dytiscidae family). The resulting fragments are the same size: 1300 bp. The same PCR master mix was used for every reaction (quantities given per sample): 12µl of Trehalose 10% (see Spies et al., 2004), 4.65µl of DNA grade water, 2.5µl of Reaction Buffer, 1.25µl of $MgCl_2$, 0.5µl of Ron Dyt (or Ron Inosine), 0.5µl of Pat Dyt (or Patty), 0.5µl of dNTPs ([40mM]) and 0.1µl of *Taq* DNA polymerase (Promega). The following cycling conditions were used, for both sets of primers: 2 minutes at 94°C; 30 seconds at 94°C, 1 minute at 53°C, and 2 minutes and 15 seconds at 70°C (repeated over 35 cycles); 10 minutes at 70°C. The PCR products were purified and sequenced at the DNA Sequencing Facility of the

Natural History Museum. The primers Jerry and Pat were used for the sequencing reactions, targeting a fragment of 800bp within the Ron Dyt-Pat Dyt (or Ron Inosine-Patty) fragment. After delimitation of the species from the *cox*1 data (see details below), two additional markers were amplified and sequenced (targeting one sequence per marker per GMYC entity): partial 16S mitochondrial rRNA with primers 16Sar & 16Sb2 (Cognato and Vogler, 2001) and 28S rRNA gene with 28SDD & 28SFF (Monaghan *et al.*, 2007).

Contigs were assembled from the forward and reverse sequences, when both directions were available. Single-direction sequences and contigs were edited in Sequencher 4.6 (Gene Codes Corp, Ann Harbor, MI USA). A total of 2043 sequences were aligned in MAFFT version 6 (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) following the FFT-NS-1 strategy, recommended for large datasets (i.e. >2000 sequences), with default gap penalties (gap opening penalty = 1.53, offset value = 0). The resulting 740 characters long alignment (gap free) was visualized in QuickAlign 1.6.0 (Müller and Müller, 2003).

2.3.3 Species delimitation and the GMYC model

Maximum parsimony searches were conducted in PAUP*4.0b10 (Swofford, 2002), using the TBR branch swapping algorithm on 100 random addition sequence starting trees, and in TNT (Goloboff *et al.*, 2008), using tree drifting and tree fusing approaches. The best scoring tree was kept and used as a framework for the analysis. The redundant identical haplotypes were removed from the matrix using Perl (script developed by Douglas Chester, Molecular Systematics Laboratory, NHM London). In cases where two haplotypes differed only by missing data, they were recognised as identical, in order to avoid an overestimation of the haplotype diversity. A new

parsimony search was conducted in TNT with the reduced matrix of 1006 haplotypes. From this topology, branch lengths were estimated under a maximum likelihood using a GTR model and a uniform clock in PAUP*4.0b10. The resulting ultrametric tree was resolved in R version 2.6.1 (http://cran.r-project.org/) with the multi2di function of the ape library (Paradis, 2004).

The General Mixed Yule Coalescent (GMYC) approach (Pons *et al.*, 2006, Fontaneto *et al.*, 2007, Papadopoulou *et al.*, 2008, 2009a, 2009b Monaghan *et al.*, 2009) was used to conduct the species delimitation. The script compiling all the functions is available at http://r-forge.r-project.org/projects/splits. The number of lineages as a function of time was plotted in order to visualize the transition branching rate between inter- and intraspecific events. The GMYC was used on the tree and its likelihood compared to the likelihood of the null model (single branching process). The likelihood values of the GMYC model were plotted against the number of lineages considered.

2.3.4 Multiple alignment & phylogenetic analysis

One representative individual per GMYC group was selected for the phylogenetic analysis including all three markers. A total of 169 *cox*1 sequences, 157 16S sequences and 114 28S sequences constituted the matrix. When the alignments of *cox*1 and 16S were unambiguous, different strategies were tested for the length-variable 28S region: five alignment-settings combinations (gap opening penalty: 5-6.66-10-15-20 and gap extension penalty: 6.66) in ClustalW (Thompson *et al.*, 1994) and three distinct strategies (E-INS-i, G-INS-I and L-INS-i) in MAFFT version 6 (Katoh *et al.*, 2002, 2009). Congruence between the resulting alignments and the unambiguous aligned *cox*1-16S region was tested with the ILD test (Farris *et al.*,

1994). Parsimony searches in PAUP*4.0b10 were used to calculate tree lengths (1000 random addition sequence replicates and gaps treated as 5th state). The alignment strategy that gave the lowest ratio ILD/length of combined-analysis tree (Wheeler and Hayashi, 1998) was selected (see Table S1).

For all tree reconstructions, the single representative of the family Haliplidae was chosen as outgroup. Nucleotide substitution models were tested on five partitions of the dataset in MrModeltest 2.2 (Nylander, 2004): first, second and third codon positions for *cox*1, 16S and 28S. The partitioned dataset was implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) for Bayesian analysis using the best model according to the hierarchical likelihood ratio test and the AIC values. Two parallel MCMC runs were set on 5 million generations with one tree saved every 1000 generations. Given the use of only three markers, the limited taxonomic sampling from outside Madagascar, and based on the strong support for tribe level monophyly obtained in previous phylogenetic studies (Ribera *et al.*, 2008), the topologies for the Dytiscidae, Gyrinidae and Noteridae were constrained as monophyletic from the start. The mean log-likelihoods and estimated sample sizes (ESS) for the two chains were compared in Tracer v1.4.1 (Rambaut and Drummond, 2008). Because of the presence of local optima in one run, only one MCMC chain was retained. The burnin was set at 800 000 generations in order to get ESS values above 100 for the different parameters. Trees were summarized using an "all-compatible" consensus in MrBayes, storing both branch lengths and posterior probabilities.

2.3.5 Alpha diversity estimation and endemism

In order to visualize the quality of the sampling, the presences and absences of each GMYC species were reported for the 153 sites, and used in EstimateS (Gotelli and Colwell, 2001) to build a species accumulation curve with the Mao Tau (given with 95% confidence). Additionally, three different indices were used to estimate the total species richness: the Incidence-based Coverage Estimator (ICE), the Chao 2, and the Michaelis Menten MMRuns. The final estimation was given as an interval between the lowest and the highest estimation among the three indices.

For each GMYC species, the congruence with Linnaean species was assessed with the following rule: when GMYC clusters contained individuals all identified as same Linnaean species (from the morphological identification), the congruence was considered complete and the Linnaean name was associated to its corresponding GMYC entity (see Table S2.2, column 7). In cases where GMYC clusters included individuals identified as different Linnaean species ($n \geq 2$), the congruence was judged incomplete and none of the Linnaean names were associated to the GMYC entity. Only the congruent Linnaean species were taken into account for each genus in Table 2.1 (column 5). In order to avoid an overestimation of the level of endemism, only GMYC species associated to an endemic species of the Linnaean system (Rocchi, 1991, Nilsson, 2001, Nilsson and Van Vondel, 2005) were considered endemic.

2.3.6 Species and haplotype turnover

After estimating the alpha diversity, the objective was to compare the genetic structure of the fauna and its different communities by looking at the species and haplotype turnover: is there a correlation between the number of species and the

number of haplotypes among sites? If yes, does this correlation differ among the lotic and lentic habitats? To do so a first scatterplot was built, where each point represents a collection site, for which we reported the number of GMYC species found in the site as well as the number of haplotypes found in the same site. A non-parametric test, Spearman's rank correlation test, was used in R to calculate the rank-based correlation between the two discrete variables among the different sites. The Spearman rho (ρ) was also calculated for the different categories of sites for comparison: lotic (RW) and lentic (SW).

The second question was to know if the number of haplotypes per species was directly correlated to the number of sites where the species lives: a second scatterplot was build representing this time one point per GMYC species and reporting the number of sites where the GMYC species was found, as well as the number of haplotypes it corresponds to. Again, a Spearman test allowed calculating the rank-based degree of assiociation between number of sites and number of haplotypes among the different GMYC species, for the complete fauna, and at the community level.

For both scatterplots, Generalized Linear Models (GLMs) were fit to the discrete dataset in R (stats package), in order to evaluate the slope of each correlation presented. Given that we were dealing with count data, the Poisson family was used as a description of the error distribution and link function in the model. In the first scatterplot, three GLMs were ran: one for the complete dataset, one for the lotic sites (RW) only and one for the lentic sites (SW) (the number of points corresponding to hygropetric habitats was too low for this analysis). In each case, the number of GMYC species was used as response variable and the number of haplotypes as explanatory variable. In the second scatterplot, four GLMs were ran, one for the

complete dataset, one for the lotic-lentic species (RSW), one for the lotic species (RW) and one for the lentic species (SW). In each case, the number of sites was considered as response variable and the number of haplotypes as explanatory variable. For each GLM, the significance of the predictor (response variable) was obtained using the summary function on the corresponding model, given together with the intercept and slope values.

2.3.7 Ancestral state reconstructions

Ancestral state reconstructions were conducted in Mesquite (Maddison and Maddison, 2009) on the phylogenetic tree based on the three markers described above. First, two multi-state characters were considered for the terminal taxa: the endemism status (state 0: endemic, state 1: widespread) and the aquatic habitat (state 0: lotic, state 1: lentic, state 2: both). Using the likelihood method, we estimated the ancestral state for all nodes for both characters. Results were displayed as likelihood-ratios among states, according to the Likelihood Decision Threshold in Mesquite T = 2 (if the log likelihood of two characters differ by two or more, the one with lower likelihood was rejected). In the second place, we tested whether the evolution of endemism and habitat preferences were independent. To do so, we used the states of the aquatic habitat character to create three new binary traits: lotic character (state 0: yes, state 1: no), lentic character (state 0: yes, state 1: no), and lotic-lentic character (state 0: yes, state 1: no). Each of these three was paired with the binary endemic character described above, and Pagel's correlation test (Pagel, 1994) was conducted on the three pairs of traits as implemented in BayesDiscrete (part of BayesTraits, available at www.evolution.rdg.ac.uk) (Pagel *et al.*, 2004).

2.4 RESULTS

2.4.1 Species delimitation

We analyzed 2043 individuals from 153 collection sites (the parsimony reconstruction is shown in Figure S2.1), which produced a total of 1006 mtDNA haplotypes. The GMYC approach was applied to this new parsimony search (conducted on the 1006 haplotypes) for which branch lengths were estimated under a uniform clock. The transition between speciation and coalescent branching events was estimated to 0.014 substitutions per site (Figure 2.2), delimiting a total of 169 entities (referred here as GMYC species), 129 as distinct clusters of haplotypes and 40 as singletons. The maximum likelihood of the GMYC model was significantly higher than the likelihood of the null model: 9785.035 against 9567.816, $2\Delta L = 434.4380$, χ^2 test: $p < 0.001$ *** (Figure 2.3).

A three genera subset (Gyrinidae) of the species delimitation results is presented in Figure 2.4, showing the GMYC clusters in grey and the GMYC species represented by a unique haplotype as black terminal branches. The congruence with Linnaean species identified beforehand is shown on the same figure. The complete mtDNA cluster delimitation is presented in Figure S2.2. The number of congruent Linnaean species, number of haplotypes, number of sampling localities and number of GMYC species and clusters is summarized per genus in Table 2.1. The complete table containing all relevant information per GMYC species is presented in Table S2.2.

2.4.2 Alpha diversity estimation and endemism

The sample-based rarefaction curve was built from the incidences of the 169 GMYC species across the 153 sites (Figure 2.5). When an exhaustive sampling would

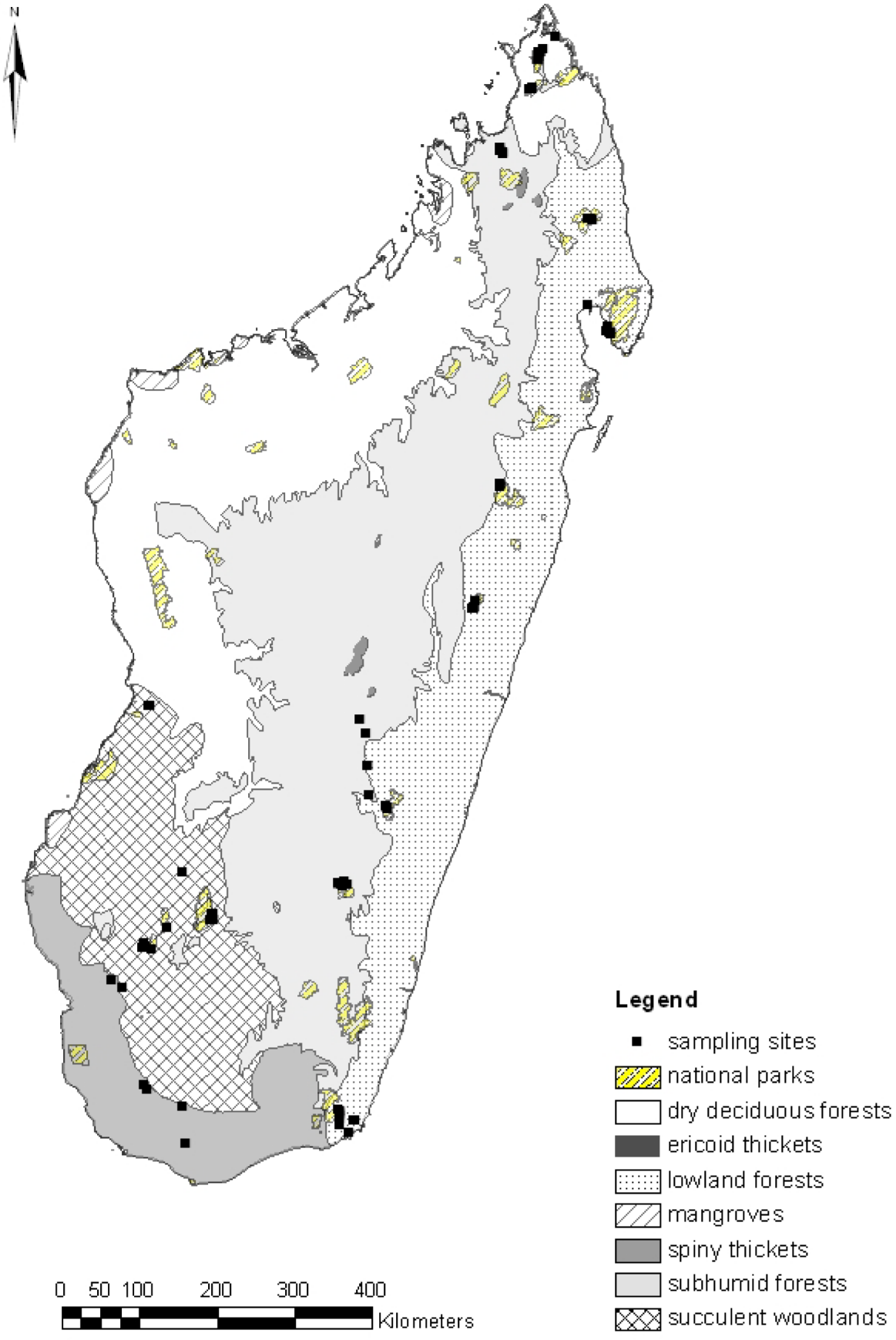


Figure 2.1: Distribution of the sampling localities in Madagascar across the seven ecoregions.

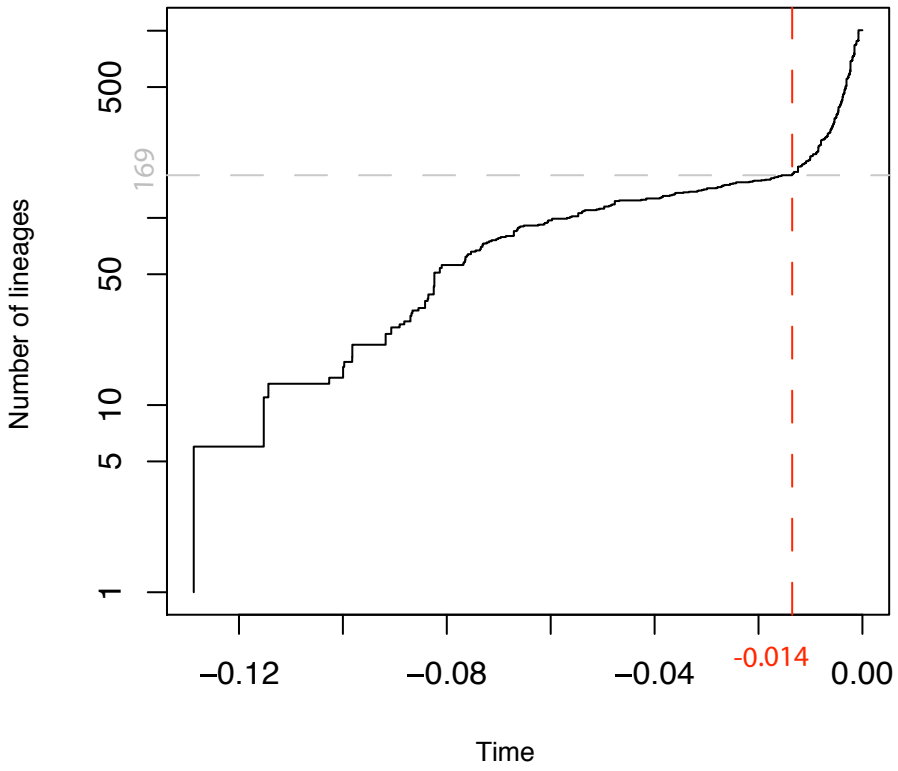


Figure 2.2: Plot of the number of lineages through time. The red line represents the threshold between speciation events and coalescent events.

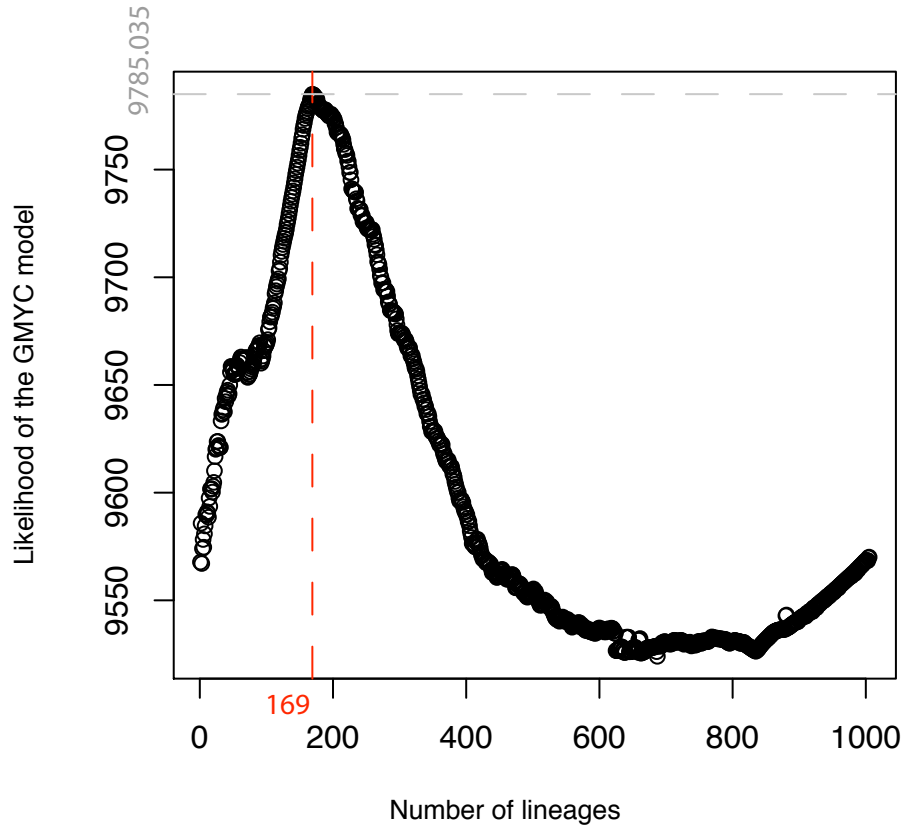


Figure 2.3: Likelihood values of the GMYC model obtained as a function of the number of lineages considered.

Figure 2.4: Parsimony subtree showing the GMYC species delimitation results for three genera of the family Gyrinidae: *Gyrinus*, *Aulonogyrus* and *Dineutus*. Linnaean names are indicated in cases of incongruence with the taxonomy.

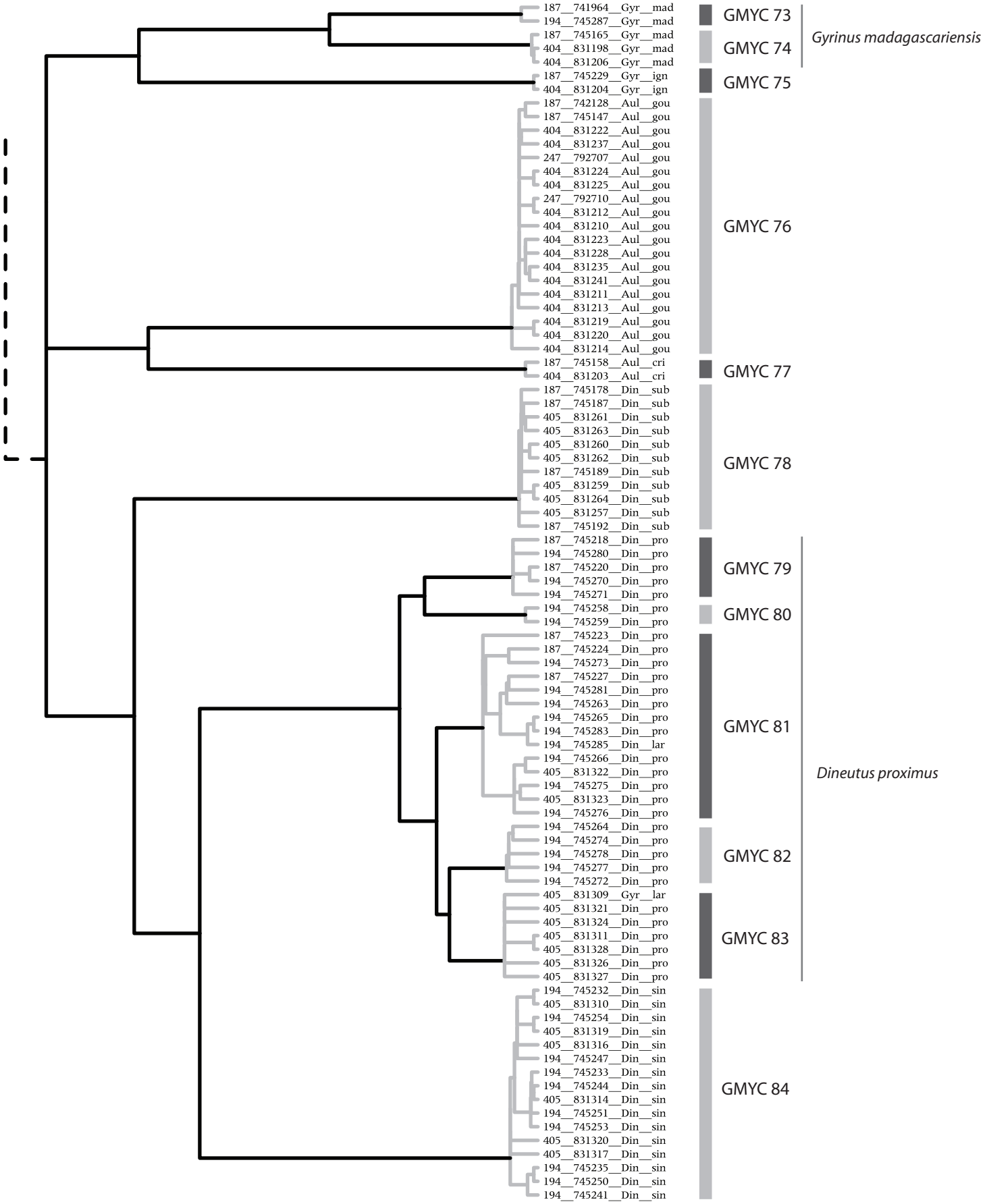


Table 2.1: Genera of aquatic beetles and their corresponding number of Linnaean species, GMYC species, geographic localities, haplotypes and *cox1* sequences. The percentage of endemism (% end) is given for the Linnaean species.

Genus	family	sub-family	tribe	Linn. sp	% end	MYC sp	MYC clusters	sites	cox1	haps
Aethionectes	Dytiscidae	Dytiscinae	Aciliini	1	100	1	1	2	6	2
Africophilus	Dytiscidae	Laccophilinae	Laccophilini	2	50	7	5	2	28	19
Bidessus	Dytiscidae	Hydroporinae	Bidessini	2	100	4	2	9	22	21
Clypeodytes	Dytiscidae	Hydroporinae	Bidessini	1	100	2	1	5	9	7
Copelatus	Dytiscidae	Copelatinae	Copelatini	-	-	17	9	49	267	93
Cybister	Dytiscidae	Dytiscinae	Cybistrini	7	29	9	7	18	71	49
Eretes	Dytiscidae	Dytiscinae	Aciliini	2	0	2	1	1	9	3
Herophydrus	Dytiscidae	Hydroporinae	Hygrotini	1	100	3	3	5	15	13
Hovahydrus	Dytiscidae	Hydroporinae	Hyphydrini	2	100	4	3	13	46	14
Prodaticus	Dytiscidae	Dytiscinae	Hydaticini	7	29	9	8	31	129	106
Hydroglyphus	Dytiscidae	Hydroporinae	Bidessini	4	25	5	5	22	121	72
Hydrovatus	Dytiscidae	Hydroporinae	Hydrovatini	7	29	9	6	6	93	26
Hyphydrus	Dytiscidae	Hydroporinae	Hyphydrini	3	33	4	4	16	39	21
Laccophilus	Dytiscidae	Laccophilinae	Laccophilini	7	57	12	10	65	300	104
Liodessus	Dytiscidae	Hydroporinae	Bidessini	1	0	1	1	7	16	5
Madaglymbus *	Dytiscidae	Copelatinae	Copelatini	-	100	23	14	50	259	113
Methles	Dytiscidae	Hydroporinae	Methlini	1	0	1	0	1	1	1
Neptosternus	Dytiscidae	Laccophilinae	Laccophilini	1	100	1	0	1	5	1
Pachynectes	Dytiscidae	Hydroporinae	Bidessini	2	100	5	4	11	34	28
Philaccolus	Dytiscidae	Laccophilinae	Laccophilini	1	100	3	3	9	17	9
Pseuduvarus	Dytiscidae	Hydroporinae	Bidessini	1	0	2	2	13	29	5
Rhantaticus	Dytiscidae	Dytiscinae	Aciliini	1	0	1	1	4	4	4
Rhantus	Dytiscidae	Colymbetinae	Colymbetini	1	0	1	0	1	1	1
Uvarus	Dytiscidae	Hydroporinae	Bidessini	5	100	9	7	18	44	25
Yola	Dytiscidae	Hydroporinae	Bidessini	1	100	1	1	14	64	33
Aulonogyrus	Gyrinidae	Gyrininae	Gyrinini	2	100	2	2	16	32	21
Dineutus	Gyrinidae	Gyrininae	Enhydrini	3	33	7	7	28	77	60
Gyrinus	Gyrinidae	Gyrininae	Gyrinini	2	100	4	3	8	20	8
Orectogyrus	Gyrinidae	Gyrininae	Orectochilini	6	100	9	8	33	139	66
Peltodytes	Haliplidae	-	-	1	0	1	1	2	2	2
Canthydrus	Noteridae	Noterinae	Noterini	3	67	5	5	23	72	31
Hydrocanthus	Noteridae	Noterinae	Noterini	3	33	3	3	8	21	11
Neohydrocoptus	Noteridae	Noterinae	Neohydrocoptini	1	0	1	1	16	47	28
Synchortus	Noteridae	Noterinae	Noterini	1	100	1	1	2	4	4
			Total	83	57	169	129	153	2043	1006

Linn. sp : number of Linnaean species fitting with MYC entities without conflict (i.e. one Linnaean species only per MYC entity).

% end : percentage of endemic species based on the number of Linnaean species described as endemic in the literature.

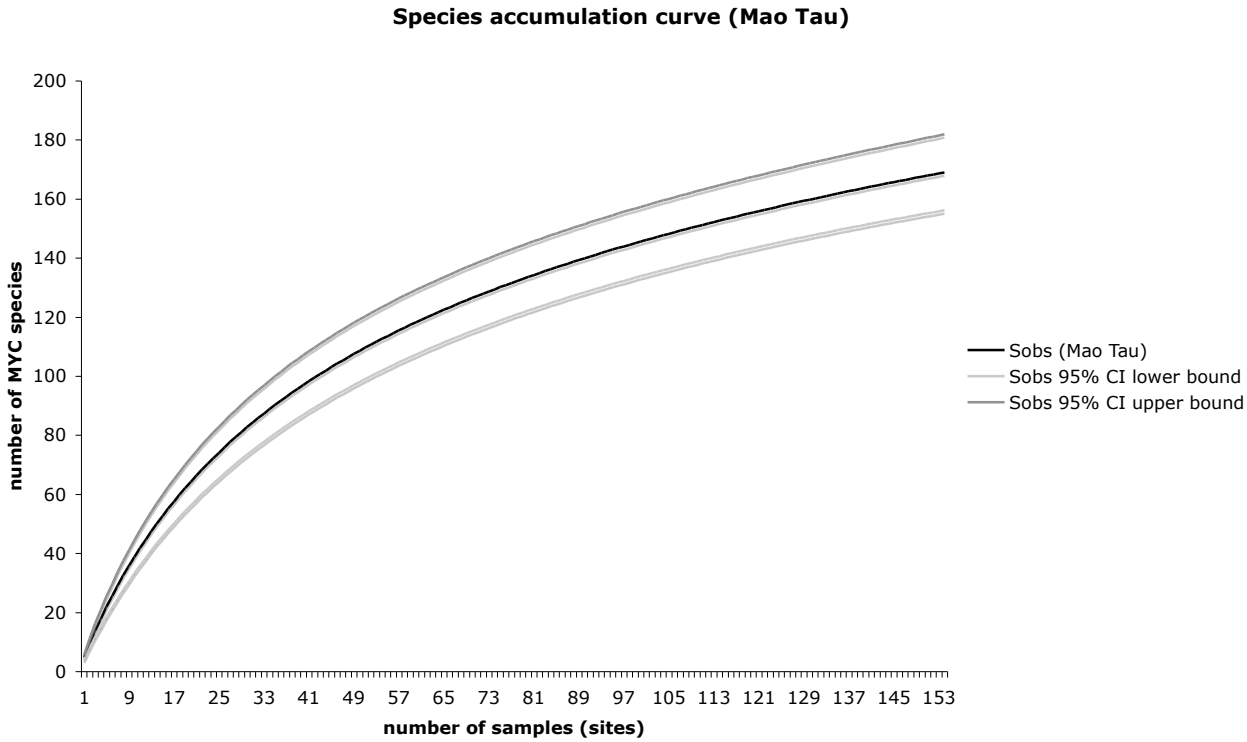
MYC sp : number of entities delimited by the GMYC model.

sites : number sites where the genus was found.

cox1 : number of cox1 sequences used per genus.

haps: number of haplotypes per genus.

Figure 2.5: Species accumulation curve of the water beetle fauna (Mao Tau) with the 95% confidence interval limits and three species richness estimations: ICE, Chao2 and MMRuns estimators (95% confidence limits are given when available).



	ICE	Chao2	MM Runs
mean	216.25	227.17	210.31
95% lower bound	-	198.44	-
95% upper bound	-	283.96	-

result in a curvilinear function ending with a plateau passed the total number of sites, we can see here that the curve approaches, but does not reach, the asymptote. Therefore three different total species richness estimators were used (Figure 2.5): the ICE, the Chao2, and the MMRuns respectively assessed the total species richness at 216, 227 and 210 species. Thus the sampling in this study was estimated between 74.4% and 80.5% of the actual fauna.

Based only on the congruent GMYC species with Linnaean species, we estimated 51% of the 169 GMYC species as endemics, with a lot of variability depending on the genus considered: from 25% for *Hydroglyphus* to 100% for *Orectogyrus* (Table 2.1), *Madaglymbus* being the only endemic genus described. The lotic community showed 62% of endemism against 37% for the lentic species and 49% for the lotic-lentic group.

2.4.3 Genetic structure and spatial turnover

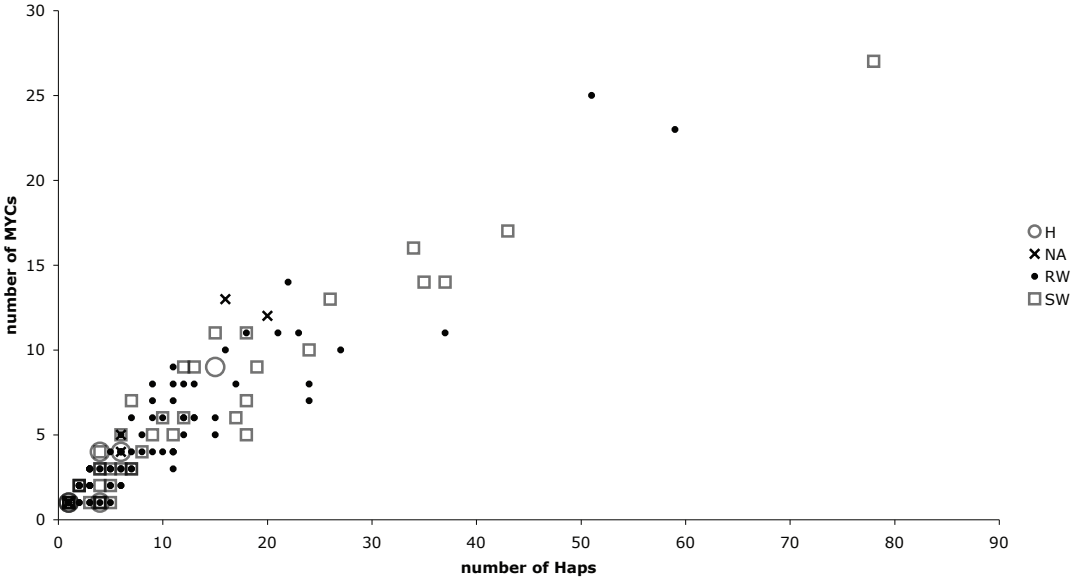
Figure 2.6 shows a strong correlation between the number of species and haplotypes among the different sites: Spearman test, $\rho = 0.92$, $p < 0.001$. It was also the case when considering only the lotic sites: $\rho = 0.92$, $p < 0.001$, or only the lentic sites: $\rho = 0.93$, $p < 0.001$ (Spearman's test was not run on the hygropetric sites because of the small number of paired samples). For all GLMs, the response variable was found as a linear function of the explanatory variable (no quadratic or polynomial terms). The intercept and slope values obtained with the GLM for the complete dataset were 1.067 and 0.038 respectively ($p < 0.001$). When running the GLMs separately for the running and standing water sites, we obtained a higher slope for the lotic group in comparison to the lentic group: 0.044 (intercept: 0.994, $p < 0.001$) against 0.032 (intercept: 1.199, $p < 0.001$). Figure 2.7 shows the correlation between

the number of sites in which we found the different GMYC species and the number of haplotypes per species: $\rho = 0.85$, $p < 0.001$. When separating the different species regarding the type of habitat they live in, we found the same correlation than within the whole fauna: RSW, $\rho = 0.86$, $p < 0.001$; RW, $\rho = 0.84$, $p < 0.001$; SW, $\rho = 0.83$, $p < 0.001$. The GLM for the complete dataset indicated a value of 0.060 for the slope of the relationship (intercept: 0.967, $p < 0.001$). When comparing the GLMs for the different communities, the SW species showed the highest slope value, 0.088 (intercept: 0.608, $p < 0.001$), followed by the RW species (slope: 0.073, intercept: 0.849, $p < 0.001$) and the RSW species (slope: 0.039, intercept: 1.522, $p < 0.001$). Out of 169 species and 1006 haplotypes, more than 94% of the GMYC species were represented by less than 25 haplotypes, and were found in less than 15 sites. More than 96% of the 153 sites counted no more than 15 GMYC species and 40 haplotypes.

2.4.4 Ancestral aquatic habitat and endemism

The aim of this analysis was to test whether the evolution of endemism and lotic/lentic preferences is independent. The ancestral character state reconstruction was based on the Bayesian tree presented in Figure 2.8 (both posterior probabilities and branch lengths are shown). Figure 2.9 shows a subset of the character history obtained in Mesquite for endemism and aquatic habitat characters, with states judged best by the threshold marked with an asterisk. The complete ancestral character reconstruction (all clades for both characters) is available in Figure S2.3 and the likelihood values per node are summarized in Table S2.3. Pagel's test on the new binary traits resulted in a significant correlation between the lotic habitat character and endemism character, $2\Delta L = 9.61664$, χ^2 test: p = 0,0474, whereas neither the lentic habitat nor the lotic/lentic habitat showed significant correlation with the

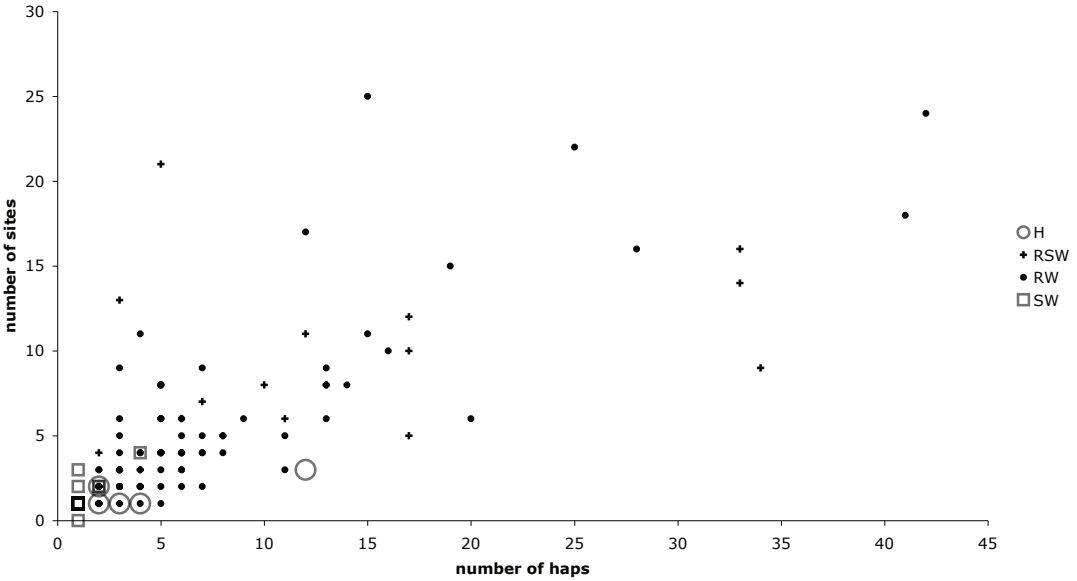
Figure 2.6: Correlation between the number of GMYC species and the number of haplotypes. Each symbol represents a collecting site (H = hygropetric sites, NA = unknown habitat, RW = lotic sites and SW = lentic sites). Rho and *p* values from the Spearman test are given.



Spearman:

rho = 0.93
$p < 2.2 * 10^{-16}$

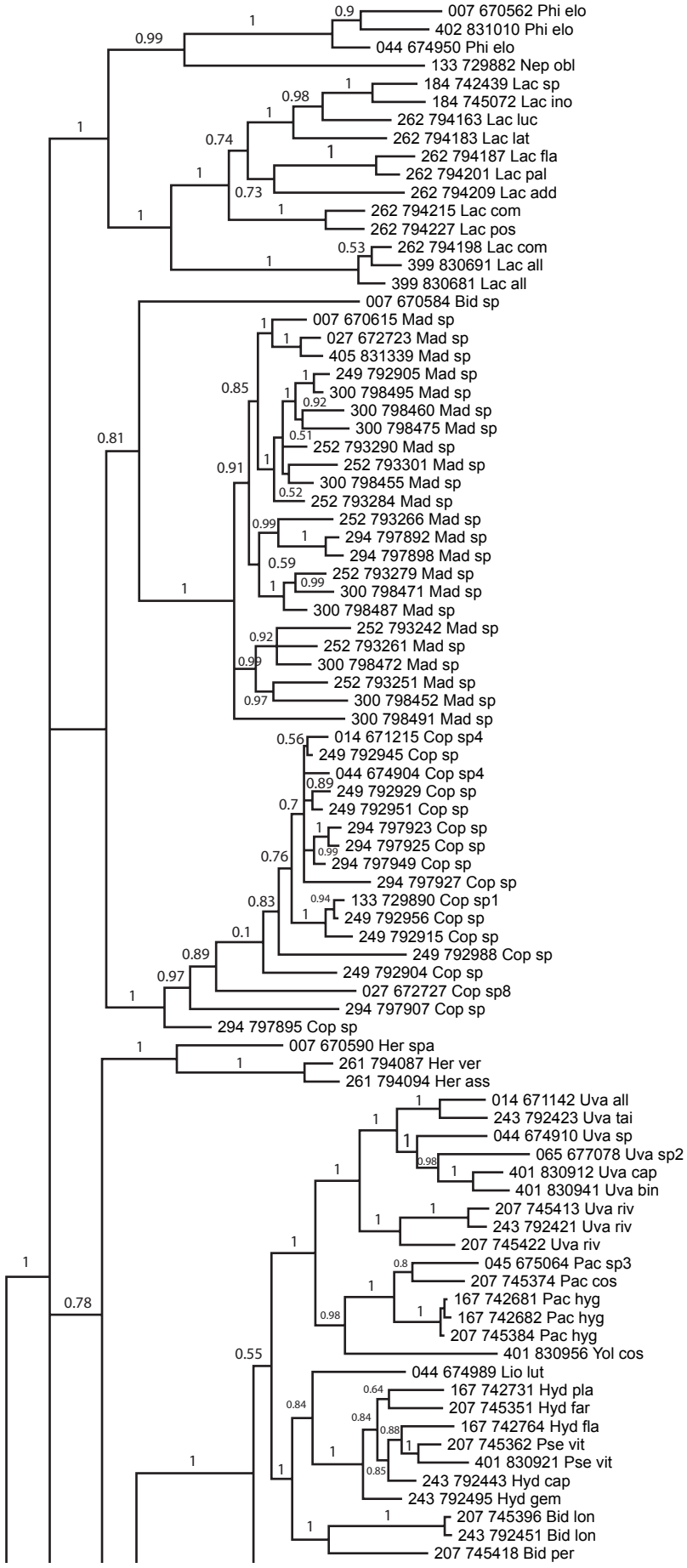
Figure 2.7: Correlation between the number of sites and the number of haplotypes. Each symbol represents a GMYC species (H = hygropetric species, RSW = lotic & lentic species, RW = lotic species, and SW = lentic species). Rho and *p* values from the Spearman test are given.

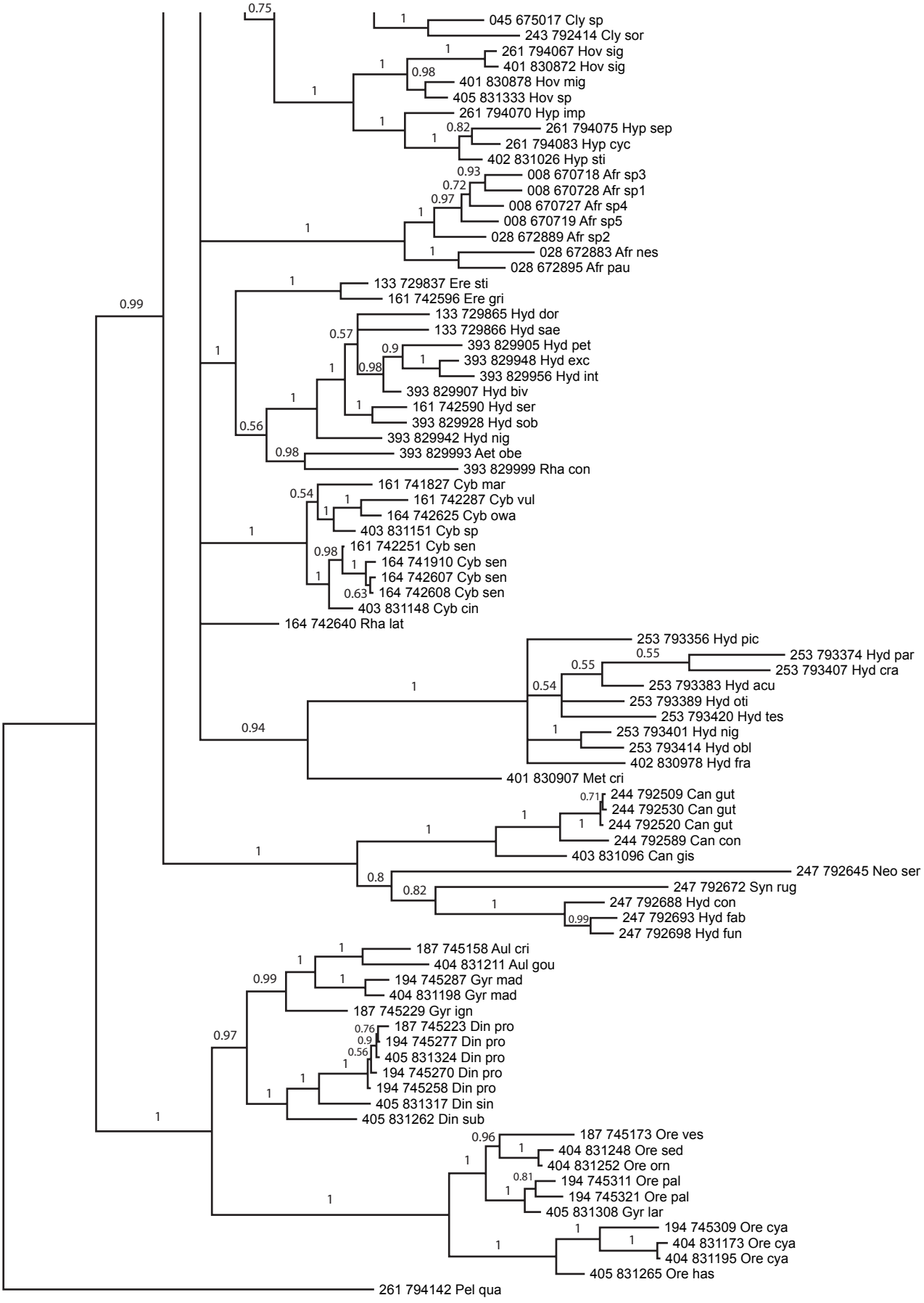


Spearman:

rho = 0.86
$p < 2.2 * 10^{-16}$

Figure 2.8: Bayesian tree reconstruction of the 169 GMYC species showing branch lengths and posterior probabilities.




045 675017 Cly sp
243 792414 Cly sor
261 794067 Hov sig
401 830872 Hov sig
401 830878 Hov mig
405 831333 Hov sp
261 794070 Hyp imp
261 794075 Hyp sep
261 794083 Hyp cyc
402 831026 Hyp sti
008 670718 Afr sp3
008 670728 Afr sp1
008 670727 Afr sp4
008 670719 Afr sp5
028 672889 Afr sp2
028 672883 Afr nes
028 672895 Afr pau
133 729837 Ere sti
161 742596 Ere gri
133 729865 Hyd dor
133 729866 Hyd sae
393 829905 Hyd pet
393 829948 Hyd exc
393 829956 Hyd int
393 829907 Hyd biv
161 742590 Hyd ser
393 829928 Hyd sob
393 829942 Hyd nig
393 829993 Aet obe
393 829999 Rha con
161 741827 Cyb mar
161 742287 Cyb vul
164 742625 Cyb owa
403 831151 Cyb sp
161 742251 Cyb sen
164 741910 Cyb sen
164 742607 Cyb sen
164 742608 Cyb sen
403 831148 Cyb cin
164 742640 Rha lat
253 793356 Hyd pic
253 793374 Hyd par
253 793407 Hyd cra
253 793383 Hyd acu
253 793389 Hyd oti
253 793420 Hyd tes
253 793401 Hyd nig
253 793414 Hyd obl
402 830978 Hyd fra
401 830907 Met cri
244 792509 Can gut
244 792530 Can gut
244 792520 Can gut
244 792589 Can con
403 831096 Can gis
247 792645 Neo ser
247 792672 Syn rug
247 792688 Hyd con
247 792693 Hyd fab
247 792698 Hyd fun
187 745158 Aul cri
404 831211 Aul gou
194 745287 Gyr mad
404 831198 Gyr mad
187 745229 Gyr ign
187 745223 Din pro
194 745277 Din pro
405 831324 Din pro
194 745270 Din pro
194 745258 Din pro
405 831317 Din sin
405 831262 Din sub
187 745173 Ore ves
404 831248 Ore sed
404 831252 Ore orn
194 745311 Ore pal
194 745321 Ore pal
405 831308 Gyr lar
194 745309 Ore cya
404 831173 Ore cya
404 831195 Ore cya
405 831265 Ore has
261 794142 Pel qua
0.1 substitutions/site

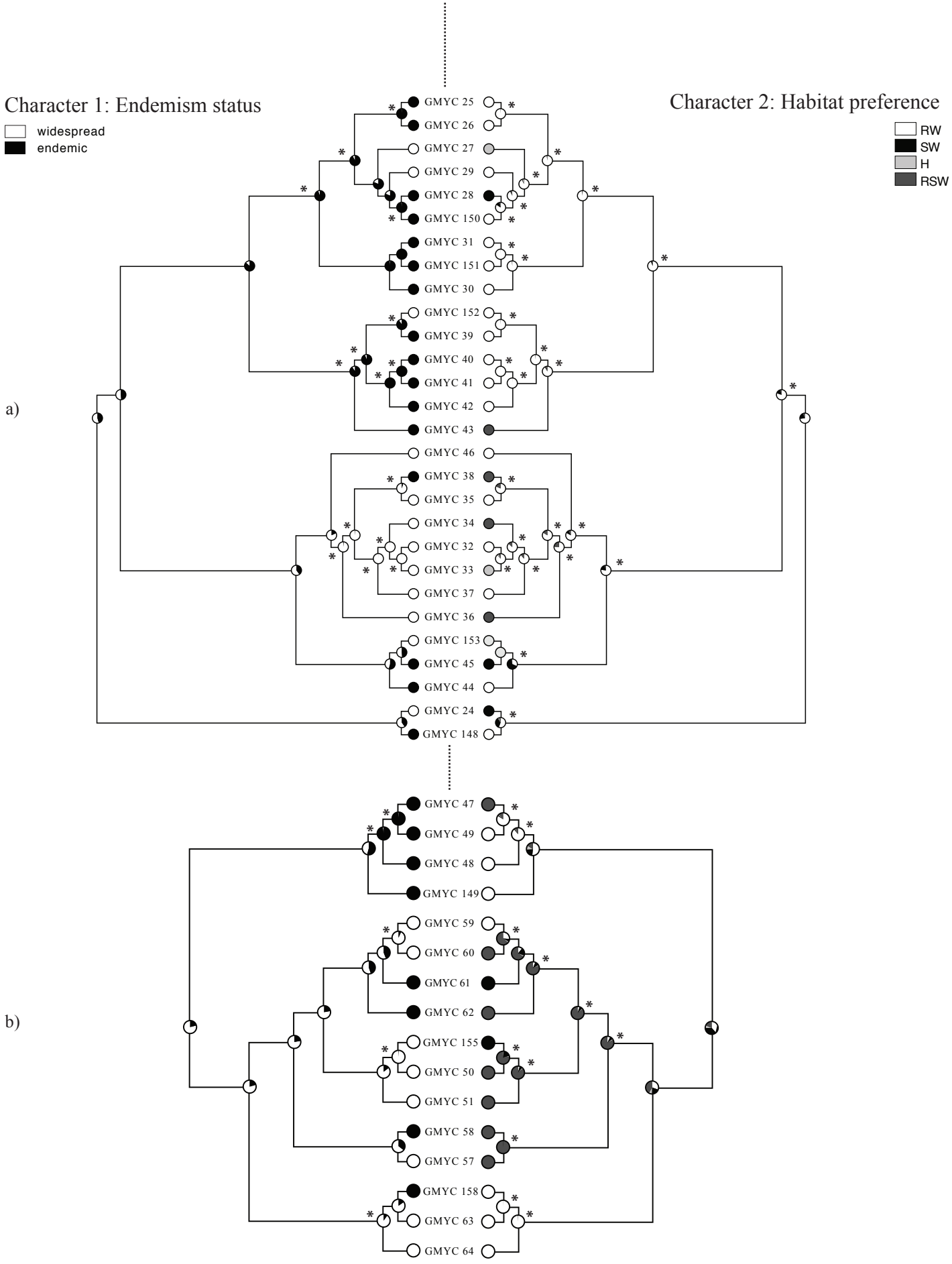


Figure 2.9: Ancestral character states reconstructions (likelihood approach) for two clades a) the tribe Bidessini, b) part of the sub-family Laccophilinae of the phylogenetic tree based on three molecular markers. Two characters are represented: endemism status and habitat preference. The states judged best by the likelihood decision threshold are marked with an asterisk.

endemism trait: $2\Delta L = 8.89240$, χ^2 test: $p = 0.0639$, $2\Delta L = 7.06588$, χ^2 test: $p = 0.1324$.

2.5 DISCUSSION

2.5.1 Species delimitation and endemism

Studying biodiversity on earth is not an easy task, slowed down mainly by the difficulty of putting living things into categories. Traditional taxonomy is time-consuming and it is often impossible to distinguish between entities based on morphological or ecological characters (Blaxter, 2004). From an evolutionary model, the GMYC approach offers the possibility to distinguish clusters evolving independently (species level), keeping at the same time a certain congruence with morphology based species. The GMYC has recently been used on Malagasy insects (Monaghan *et al.*, 2009) and has been proved to be a fast and efficient way of delimiting species within these highly endemic and poorly described faunas. In this study, we targeted the entire fauna of aquatic beetles, based on four years of sampling, and identified 169 GMYC entities or species. It is probable that additional sampling would result in a higher total number of species, but according to the alpha diversity estimators, we captured a considerable portion of the total species richness (>70%) from more than 2000 individuals. In addition to these estimations, the accuracy of the GMYC results could be verified on one hand by the clear peak of the likelihood curve (and the shape of the lineage through time plot), and on the other hand by the level of congruence with the morphological species. Close to 50% of the GMYC entities were congruent with the Linnaean species described to date, but considering that several groups of the Linnaean system need or are already under revisions (Miller *et al.*,

2009) (Johannes Bergsten, personal communication), it is likely to be the case that several clusters excluded because of the presence of at least two Linnaean names, would change to clusters perfectly congruent with one taxonomic species (within the genus *Laccophilus* for example). Thus the 50% congruence between GMYC species and taxonomic species would be an underestimation of the real match between the two species delimitation methods.

Similarly, the percentage of endemism (51%) calculated from the congruence of the GMYC clusters with endemic Linnaean species is probably far below the real level of uniqueness of the water beetle fauna. It is reasonable to think that any GMYC species is endemic when associated to an endemic Linnaean name. But for practical reasons, in cases when one non-endemic Linnaean species was represented by several GMYC species, all of them were considered non-endemic. The reality is that some of these GMYC species might very well be unique to the island, but it is impossible to state from the existing literature and the absence of samples from the African continent. So when a rough estimation of endemism overpasses half of the species, we can expect a very high percentage of the fauna to be unique to Madagascar. Actually, this molecular species quantification could not only change the endemism figures at the species level but could also change the "scale" of endemism: when one endemic Linnaean species corresponds to several GMYC species, do their geographic ranges overlap, or do they belong to distinct regions of the island? Does the molecular species identification bring endemism to a regional scale? Are the molecular species micro-endemic, in opposition to Linnaean species described as endemic at the scale of the island? This is one of the remaining objectives, but in order to answer these questions, the geographic distributions of both Linnaean and GMYC species must be compared. Part of these issues will be tackled in Chapter 4.

2.5.2 Genetic structure and spatial turnover

A striking preliminary result in this study was the great haplotype diversity given the total number of individuals: almost 50% of the 2043 individuals were unique haplotypes. When related to the number of species delimited, this figure underlines an important genetic diversification at the inter- and intraspecific levels. Secondly, the number of GMYC species and haplotypes per site were very low compared to the totals. These results suggest a strong spatial turnover both at the species and haplotype levels, the latter being confirmed by the correlation between the number of sites and the number of haplotypes given per species.

Given the differential dispersal patterns between running water and standing water species and the potential for a higher gene flow within the lentic species, one could expect a higher correlation between the number of haplotypes and the number of species among the lotic sites in comparison to the lentic sites. This was confirmed by our results, when looking at the slopes of the different relationships portrayed. Similarly, when considering the correlation between the number of sites and the number of haplotypes per species, a higher degree of association could be expected for the RW and RSW communities. Surprisingly, our results showed that the lentic community had the highest correlation slope. The combination of these results suggests a higher species turnover for the lotic community, and a higher haplotype turnover for the lentic species. With a more active dispersal, lentic species would present a higher genetic diversification at the intraspecific level (more distant sites engendering a higher haplotype diversity), but the important gene flow associated to the dispersal pattern would prevent the genetic diversification at the species level. This part of the analysis drew attention to a very diverse and strongly structured fauna, genetically and geographically speaking. The relevant question is now to

understand what is driving such a high turnover and this will be possible with a prospective spatial analysis of the data, investigating which variables predict best the actual beta diversity patterns (Chapter 3). Geographic distance and habitat diversity will be considered as the main spatial and environmental predictors respectively.

2.5.3 Ancestral aquatic habitat and endemism

In this analysis, we tested if habitat preferences of the different GMYC species were responsible for present endemism patterns based on the differential dispersal patterns of lotic and lentic species. We found that the evolution of endemism and lotic preference were dependent, with a *p* value just below the significant threshold; when looking at lentic and lotic/lentic characters, no dependence was found with the endemism trait, which tends to confirm a higher level of endemism for running water species. However, for multiple reasons, these results should be taken with caution: some GMYC species were defined as non-endemic by default when corresponding to one common widespread Linnaean species, or when belonging to a clade showing incomplete Linnaean identification, as it is the case for the genus *Copelatus*. Therefore several "widespread" states could be erroneous and it is probable that missing information on the endemism status of these specific taxa would affect the results of the correlation test for the complete tree. Additionally, this dataset includes so far species known from Africa and Madagascar, but all specimens sampled in Madagascar. A complete taxonomic sampling and supplementary markers would notably increase the reliability of the phylogeny, allowing drawing major conclusions on the ancestral character states reconstruction, particularly when considering the deeper nodes in the phylogeny.

2.5.4 Conclusions

In the present conservation context, where time and resources are the limiting factors, it remains difficult to quantify biodiversity and tackle macroecological problems. This is particularly true in biodiversity hotspots where the number of rare or non-described species is higher than everywhere else. This study targeted the water beetle fauna of Madagascar and aimed at identifying and understanding its diversity. From 2043 individuals, the GMYC model allowed delimiting 169 species, which might represent an incomplete sampling, but the different alpha diversity estimators gave us confidence that at least 74% of the whole fauna was sampled. Within this fauna, we found a complex genetic structure with a high species and haplotype turnover, and a strong correlation between species diversity and haplotype diversity among the different communities. The last part of the analysis confirmed our hypothesis that endemism was dependent on the type of water bodies considered, and that lotic communities were subject to higher levels of endemicity. By combining here a molecular species delimitation method and an ancestral character state analysis, we made a step further towards a better understanding of the causes of beetle endemism in the number one biodiversity hotspot. These results bring the first inventory of aquatic beetles in Madagascar, and confirm the efficacy of the GMYC approach at delimiting species. As a further matter, this study emphasizes the role of ecological factors like habitat diversity in determining endemism patterns in Madagascar.

CHAPTER 3

The effect of dispersal and environmental variability on local endemism patterns in Madagascar[1]

[1]To be published as: Isambert, B., Baselga, A. and Vogler, A. P.

3.1 ABSTRACT

Biodiversity hotspots are demarcated from the rest of the world by their outstanding levels of endemism and imbalance across taxa. These 34 regions are a recurrent target for large-scale endemism studies, but the processes leading to microendemism patterns within the hotspots remain poorly understood. In this study, we target Madagascar and one of its highly endemic fauna: aquatic beetles (Dytiscidae, Gyrinidae, Noteridae, Haliplidae). Based on a molecular species delimitation of the fauna, we investigate how the lotic/lentic preferences and their associated dispersal patterns affect its spatial turnover: we test if biotic dissimilarities among lotic and lentic communities is best explained by differential bioclimatic niche constraints or spatial correlation, and explore the biological consequences for microendemism in Madagascar. The results show a strong correlation between bioclimatic predictors and species composition within the lotic community, but very little effect of the same climatic constraints on the lentic fauna. The second part of the analysis puts forward a wider distribution range for the standing water fauna compared to the running water species. For the former, biotic dissimilarities at the species level appeared spatially correlated only at a local scale, when considering distance below 100km. We discussed the opposite effects of niche restrictions and strong dispersal on beta diversity patterns and suggest that the lotic fauna is more likely to present a strong microendemism component on the island. A possible explanation for the absence of niche restriction and spatial structure of the standing water fauna is the high dispersal of individuals leading to short, temporary beta diversity patterns, suggesting a poor candidacy of this community for microendemic structuring.

3.2 INTRODUCTION

3.2.1 Tackling molecular beta diversity in Madagascar

Beta diversity is defined as the variation in species composition among sites in a geographic area (Whittaker, 1972). It is a key concept in ecology and evolution that relies almost entirely on 1) the definition of the entity studied (one has to know what a species is in order to describe the species composition within a geographic region), and 2) the choice of the geographic region itself. Multiple studies across taxa have looked at correlations between Linnaean species beta diversity patterns and environmental gradients (Williams *et al.*, 1999, Tuomisto *et al.*, 2003, Rodriguez and Arita, 2004, Baselga, 2008), as well as the impact of geographic distance on biotic dissimilarities (Baselga, 2007, Soininen and Hillebrand, 2007, Soininen *et al.*, 2007) using a standard set of well-tested methodologies (Legendre and Fortin, 1989, Tuomisto and Ruokolainen, 2006). The present study follows a similar approach, but based on the molecular inventory (GMYC species) produced in the previous chapter to understand the molecular beta diversity patterns of the water beetle fauna within the Malagasy biodiversity hotspot. Given the fact that we are dealing with aquatic organisms, and that climate heterogeneities of the island have recently been put forward as responsible for the evolution of fauna's distinctive characteristics and the formation of local endemism (Dewar and Richard, 2007, Pearson and Raxworthy, 2009), we will consider climatic constraints as main environmental variables in this study. Additionally, Madagascar's area representing almost six times the size of England (1600km long, 600 km wide), and extending across 14° latitude (de Wit, 2003), spatial constraints will be taken into account in the beta diversity analysis, and

the impact of geographic distance on the decline in biotic similarity will be tested separately.

3.2.2 Differential dispersal in freshwater biodiversity

Fundamentally, freshwater habitats can be divided in two categories presenting distinct environmental and spatio-temporal dynamics: the lotic (running water) and the lentic (standing water) type. Lotic habitats (e.g. streams, rivers, waterfalls, etc.) are anchored to hydrographical networks, and therefore remain relatively stable at the evolutionary scale (Dobson and Frid, 2008). Oppositely, lentic habitats (e.g. ponds, pools, lakes, etc.), particularly small sized ones, are at risk from drying up and disappearing rapidly, but can also be formed on a very short time scale (Harper, 1992). Most aquatic organisms can be classified as lotic or lentic with respect to their habitat preferences (during their adult life or their larval stage in the case of insects) (Hof *et al.*, 2006, Ribera *et al.*, 2008). The consequences of these differences in habitat longevity can be considerable for species range sizes, species turnover and beta diversity patterns: in least predictable habitats like the lentic sites, species need a minimum dispersal in order to find new sources of water for survival, whereas lotic sites, providing a much more stable habitat, reduce this need for dispersal. As a result, lentic species are expected to show broader distributional ranges, higher gene flow, and little species turnover among sites whereas lotic species would have smaller range sizes, reduced gene flow and greater differences in species composition among sites (Ribera and Vogler, 2000, Bilton *et al.*, 2001, Ribera *et al.*, 2001). These differential dispersal patterns among lotic and lentic species and their consequences are well documented for invertebrates, especially for aquatic beetles (Ribera and Vogler, 2000, Ribera *et al.*, 2001, 2003, Ribera, 2008, Abellan *et al.*,

2009) which therefore constitute an ideal target group in this domain of biological research. This study aims at understanding the relationship between beta diversity patterns, habitat preferences, environmental variability and spatial constraints within the exceptionally endemic fauna of Malagasy aquatic beetles. We compare the pure effects of spatial position and climatic variability on the biotic dissimilarity level of both lotic and lentic communities. We further assessed the correlation between geographic distance and species turnover on these same communities across and within different ecoregions and discuss the implications for insect microendemism in Madagascar.

3.3 MATERIAL & METHODS

3.3.1 Biological data and predictor variables

Water beetles were collected over four years in Madagascar, with a total of 153 sampling sites, spread on more than 1000 km across seven WWF ecoregions: lowland forest, subhumid forest, ericoid thickets, spiny thickets, dry deciduous forest, succulent woodland and mangroves. Species were delimited from the mitochondrial DNA dataset (*cox1*) as described in chapter 2, using the General Mixed Yule Coalescent (GMYC) approach (Pons *et al.*, 2006, Fontaneto *et al.*, 2007, Papadopoulou *et al.*, 2008, 2009a, 2009b, Monaghan *et al.*, 2009). The GMYC method allowed us to distinguish 169 species. Only the sampling sites with exact geographic positions were retained for the spatial analysis, leaving 142 out of 153 sites, and 168 species out of 169 (one singleton with unknown GPS coordinates was excluded). From the literature (Rocchi, 1991, Nilsson, 2001, Nilsson and Van Vondel, 2005) and personal information noted directly on the collecting sites, the fauna was

divided in three different communities: the lotic one (RW) for the species living in majority in running waters, the lentic one (SW) for the species living mainly in standing waters, and the lotic-lentic community (RSW) for the species living equally in both lotic and lentic habitats. Four incidence matrices were built: one for the complete fauna, based on the presence/absence of the 168 species in the 142 sites, and one for each of the communities previously described. These matrices were used as primary data for the rest of the analysis.

Two sets of variables were used as predictors: 1) spatial position: Longitude and Latitude (L_{on}, L_{at}) and 2) climatic variables: annual mean temperature (T_{ann}), temperature seasonality (T_{sea}), maximum temperature of the warmest month (T_{max}), minimum temperature of the coldest month (T_{min}), annual precipitation (P_{ann}), precipitation seasonality (P_{sea}), precipitation of the wettest month (P_{wm}) and precipitation of the driest month (P_{dm}). The spatial position consisted of the geographic coordinates in decimal degrees measured in the field for each of the 142 sites. The climatic predictors were obtained from the WorldClim layers version 1.4 (Hijmans *et al.*, 2005) available at http://www.worldclim.org under the 30 seconds spatial resolution. The climatic values at the 142 sites were extracted using the spatial analyst tool of ArcGIS 9.2 (Environmental Systems Research Institute, Inc., Redlands, California, USA). The eight climatic variables were chosen among a list of 19, commonly used for their hypothetical biological relevance (classified as "bioclimatic" variables in WorldClim). All 19 variables were tested for auto-correlation with a Spearman test (see Table S3.1) and the number of predictors was reduced according to two criteria: 1) minimizing autocorrelations between predictors 2) keeping the most relevant predictors for our aquatic target group among average and extreme variables within the precipitation and temperature sets of predictors.

3.3.2 Beta diversity and spatial analysis

The beta diversity aspect of the analysis was done in R version 2.6.1 (http://cran.r-project.org/), under the vegan package (Oksanen *et al.*, 2007). To investigate the faunal dissimilarities between running and standing water sites, differences in terms of species composition among all sites were analyzed with a dissimilarity index. In order to have a measure of the species turnover between sites and not the species richness variation, the Simpson index of beta diversity (β_{sim}) was preferred to the Sorensen index (Baselga, 2007). The resulting Simpson matrix (simba package: Jurasinski, 2007) was split into two different matrices: one including the lotic sites only and one including the lentic sites only. These two faunal dissimilarity matrices were submitted to a nonparametric multidimensional scaling analysis (NMDS) (Legendre and Legendre, 1998) in R using the ecodist package (Goslee and Urban, 2008). The number of dimensions (seven in both cases) was obtained using a scree-plot procedure. The axes scores of the two NMDS were submitted to a cluster analysis to compare the similarity between sites in terms of species composition for both the lotic and lentic habitats. Two dendrograms were built in R, using Euclidean distance as distance measure and following an average linkage procedure for the agglomeration method. Since the number of sites differed for running and standing waters, we calculated the number of clusters for five different levels of fusion on both dendrograms: $h = 0.5$, $h = 0.6$, $h = 0.7$, $h = 0.8$, $h = 0.9$. The significance of the groupings obtained was tested with an analysis of similarities (ANOSIM).

The second part of the beta diversity analysis consisted of a constrained analysis of principal coordinates (CAP), so as to explore the correlation between the molecular species composition among sites and the aforementioned sets of spatial and climatic predictors. The resulting models were labelled as S for spatial, E for

environmental (climate), and S + E for the complete model. The CAP was run on four different communities: 1) the whole water beetle fauna, 2) only the RSW (lotic-lentic) species, 3) only the RW (lotic) species and 4) only the SW (lentic) species. Since the order of entry of the predictors in the model has an effect on the results of the permutation test in vegan, we tested the independent contribution of each variable to the total variation explained by the model. To do so, one variable at a time was removed from the model and the reduction of explained variation was re-calculated every time. The variables were ranked in the significance test from the greatest independent contributor to the least. Only significant variables were included in the models. Last, partial CAPs were performed on each variable independently and on the models S, E, (S + E), in order to obtain the pure effects of each predictor separately and each combination of predictors (the three models).

3.3.3 Geographic distance and biotic dissimilarity

Multivariate Mantel correlograms (Oden and Sokal, 1986, Sokal, 1986, Legendre and Fortin, 1989) were computed in R using the ecodist package to examine the relationship between geographic distance and biotic dissimilarities among sites. Species composition similarities were calculated with the Simpson index. Eight or six classes of distance were used for the correlogram and the results were interpreted only for the lower half of the maximum distance (600km to 800km). The plot.mgram function (ecodist) was modified in R so as to apply a Bonferroni correction on all points (Legendre and Legendre, 1998). Four cases were distinguished: 1) biotic dissimilarities at the species level against geographic distance (eight classes), 2) biotic dissimilarities at the haplotype level against geographic distance (eight classes), 3) biotic dissimilarities at the species level against geographic distance but only within

the lowland forest ecoregion (six classes), and 4) biotic dissimilarities at the species level against geographic distance but only within the subhumid forest ecoregion (six classes). Each of these four cases was analysed at the community level like previously described: for the complete fauna, the RSW group, the RW group and the SW group.

3.4 RESULTS

3.4.1 Turnover within lotic and lentic sites

The two cluster dendrograms showing the degree of similarity between sites in terms of species composition (β_{sim}) based on the scores of the NMDS are presented in Figure 3.1, together with the geographic distribution of the running and standing water sampling sites. The lentic and lotic hierarchical cluster analyses appear respectively on the left and right hand side of the distribution map. For a level of fusion (h) of 0.8 (which amounts to very low species composition similarity values), the 83 RW sites clustered into 14 groups (ANOSIM, R = 0.8036, $p < 0.001$) against 11 groups for the 43 SW sites (ANOSIM, R = 0.7086, $p < 0.001$). We explored the level of clustering by redoing the analysis with additional height thresholds: at h = 0.9, we obtained 9 RW clusters (ANOSIM, R = 0.5307, $p < 0.001$) and 7 SW clusters (ANOSIM, R = 0.5708, $p < 0.001$). For a higher degree of similarity, equal to $h = 0.7$, lotic sites clustered into 18 groups (ANOSIM, R = 0.8585, $p < 0.001$) against 14 for the lentic ones (ANOSIM, R = 0.8736, $p < 0.001$). The number of faunal groups and their corresponding ANOSIM R values increased with the decreasing values of h, passing from 9 to 27 (lotic sites) and from 7 to 18 (lentic sites). The results for all h values are presented in Table 3.1.

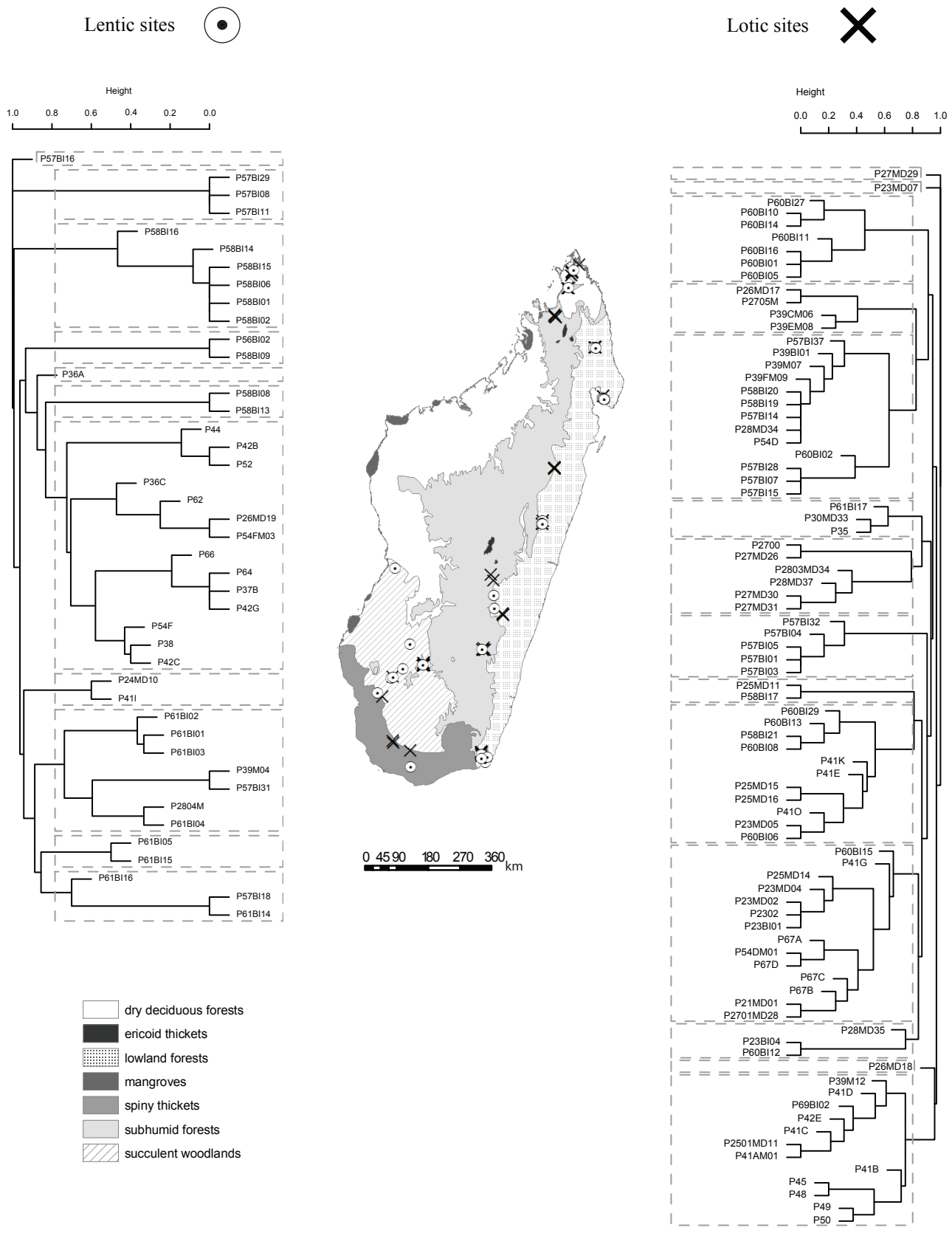


Figure 3.1: Cluster dendrograms of the lotic and lentic sites based on their water beetle species composition. The distance measure is Euclidean distance based on the score of the NMDS at seven dimensions (linkage rule = average).The number of clusters represented here were obtained for a level of fusion of 0.8 (significance tested with ANOSIM). The position of the sites is shown on the map together with the ecoregions' boundaries.

	Lotic sites			Lentic sites		
h	no of clusters	R	*p* value	no of clusters	R	*p* value
0.9	9	0.5307	< 0.001	7	0.5708	< 0.001
0.8	14	0.8036	< 0.001	11	0.7086	< 0.001
0.7	18	0.8585	< 0.001	14	0.8736	< 0.001
0.6	23	0.9191	< 0.001	15	0.8882	< 0.001
0.5	27	0.9326	< 0.001	18	0.9613	< 0.001

Table 3.1: Number of clusters obtained for different levels of fusion on the lentic and lotic dendrograms. The significance of the groups in each case was tested with an ANOSIM (*p* and R values are reported).

Clusters made of sites coming from the same ecoregion were not common: 3 out of 11 within the lentic category and 2 out of 14 within the lotic one (see Figure 3.1 and Table S3.1). In both habitat types, one cluster could include sites from up to 5 ecoregions (for example [Spiny thicket/Succulent woodland/Lowland forest/Dry decidious forest/Subhumid forest] and [Ericoid thickets/Subhumid forest/Lowland forest/Succulent woodland/Spiny thicket] in the case of the lentic and lotic sites respectively), which reflects the absence of macroecological structuring on the dendrogram. The fact that several clusters were made only of sites with the same "P" labels (e.g. P57, P58, P60, etc…) indicates similar species compositions between close sites and suggests an effect of the geographic distance on the clustering. This will be tested thoroughly by the means of multivariate Mantel correlograms in the last part of this study.

3.4.2 Constrained analysis of proximities

When considering the entire fauna, temperature seasonality, latitude, longitude, annual precipitation, precipitation of the wettest month, precipitation of the driest month, maximum temperature and minimum temperature were proved to be significantly related to beta diversity (Table 3.2). Among them, T_{sea}, L_{at}, L_{on}, P_{ann}, P_{dm}

Community	Variable	Variation (%)	Pseudo-F	*p*
Fauna	T_{sea}	1.7	3.27	0.005 **
	Lat	1.7	5.14	0.005 **
	Lon	1.7	4.32	0.005 **
	P_{ann}	1.7	4.29	0.005 **
	P_{wm}	1.0	2.28	0.005 **
	P_{dm}	2.4	3.65	0.005 **
	P_{sea}	-	1.40	0.055
	T_{max}	1.5	3.41	0.005 **
	T_{min}	1.4	2.29	0.010 *
	T_{ann}	-	0.91	0.610
	Model (S)	4.1	3.34	0.005 **
	Model (E)	12.0	3.20	0.005 **
	Model (S+E)	17.7	3.58	0.005 **
RSW species	T_{sea}	2.7	3.49	0.005 **
	Lat	2.7	8.58	0.005 **
	Lon	2.3	4.15	0.005 **
	P_{sea}	2.0	4.19	0.005 **
	P_{wm}	2.0	3.23	0.005 **
	P_{dm}	-	1.70	0.065
	P_{ann}	2.6	2.87	0.005 **
	T_{min}	3	3.05	0.005 **
	T_{ann}	3.3	3.95	0.005 **
	T_{max}	-	0.74	0.775
	Model (S)	9	5.12	0.005 **
	Model (E)	18.2	3.44	0.005 **
	Model (S+E)	29.5	4.18	0.005 **
RW species	T_{sea}	3.2	4.21	0.005 **
	Lat	2.2	2.90	0.005 **
	Lon	3.1	5.22	0.005 **
	P_{ann}	1.2	2.22	0.015 *
	P_{wm}	1.5	2.09	0.015 *
	T_{min}	2.6	2.90	0.005 **
	T_{ann}	-	1.21	0.265
	T_{max}	-	1.42	0.125
	P_{sea}	2.0	2.40	0.015 *
	P_{dm}	-	0.76	0.735
	Model (S)	5.2	2.96	0.005 **
	Model (E)	11.9	2.74	0.005 **
	Model (S+E)	19.0	3.11	0.005 **
SW species	P_{dm}	-	1.61	0.060
	P_{sea}	-	1.23	0.280
	P_{wm}	-	0.67	0.865
	P_{ann}	2.9	1.75	0.040 *
	Lat	2.9	1.79	0.025 *
	T_{sea}	-	1.28	0.180
	T_{min}	-	1.37	0.175
	Lon	-	1.58	0.070
	T_{ann}	-	1.50	0.090
	T_{max}	-	0.72	0.840
	Model (S)	-	1.47	0.15
	Model (E)	-	1.42	0.14
	Model (S+E)	6.4	1.55	0.030 *

Table 3.2: Results of the constrained analysis of proximities, showing the correlation between predictors and molecular beta diversity (Simpson index) for the complete fauna, the species living in both running and standing waters, the lotic species and the lentic species. The models for the different groups of variables are: space (S), environment (E), and space + environment (S + E). Percentages of variation explained by the individual variables and by the models are shown (inertia).

explained individually a greater portion of the variation in species composition. Partial CAPs showed that the pure effects of all three models (S), (E), and (S + E) were significant and model (S + E), explaining the highest variation (17.7%, Pseudo-F = 3.58, p < 0.005), was considered the best model. When focusing on the RSW community, most of the predictors were significant: only precipitation of the driest month and maximum temperature yielded in *p* values above 0.05. T_{ann}, T_{sea}, L_{at}, P_{ann} and L_{on} showed the highest values of explained individual variation in species composition. Similarly, all three models were significant and model (S + E) was the best model, with 29.5% of explained variation (Pseudo-F = 4.18, $p < 0.005$). Seven predictors were significant in the case of the RW community, with T_{ann}, T_{max} and P_{dm} excluded from the partial CAP. Individually, the most important predictors appeared to be T_{sea}, L_{on}, T_{min}, L_{at} and P_{sea}. Model (S) and (E) explained respectively 5.2% and 11.9% of the variation in species composition (Pseudo-F = 2.96, $p < 0.005$, Pseudo-F = 2.74, $p < 0.005$) but (S + E) was once again the best model with 19.0% of explained variation (Pseudo-F = 3.11, $p < 0.005$). Regarding the SW species, only two predictors out of 10 were significant, one per set of predictors: P_{ann} (E) and L_{at} (S), both at 2.9% of explained variation. Consequently the results for the two predictors constituted also the results for model (S) and model (E) (Pseudo-F = 1.75, $p = 0.040$, Pseudo-F = 1.79, $p = 0.025$). Model (S + E) explained 6.4% of the variation with a pseudo-F value at 1.55, $p = 0.030$. A complete list of the sampling sites is provided in Table S3.2.

3.4.3 Biotic dissimilarities and geographic distance

For all tests in Figure 3.2, positive Mantel statistics correspond to positive autocorrelations, whereas negative significant values indicate distances between peaks

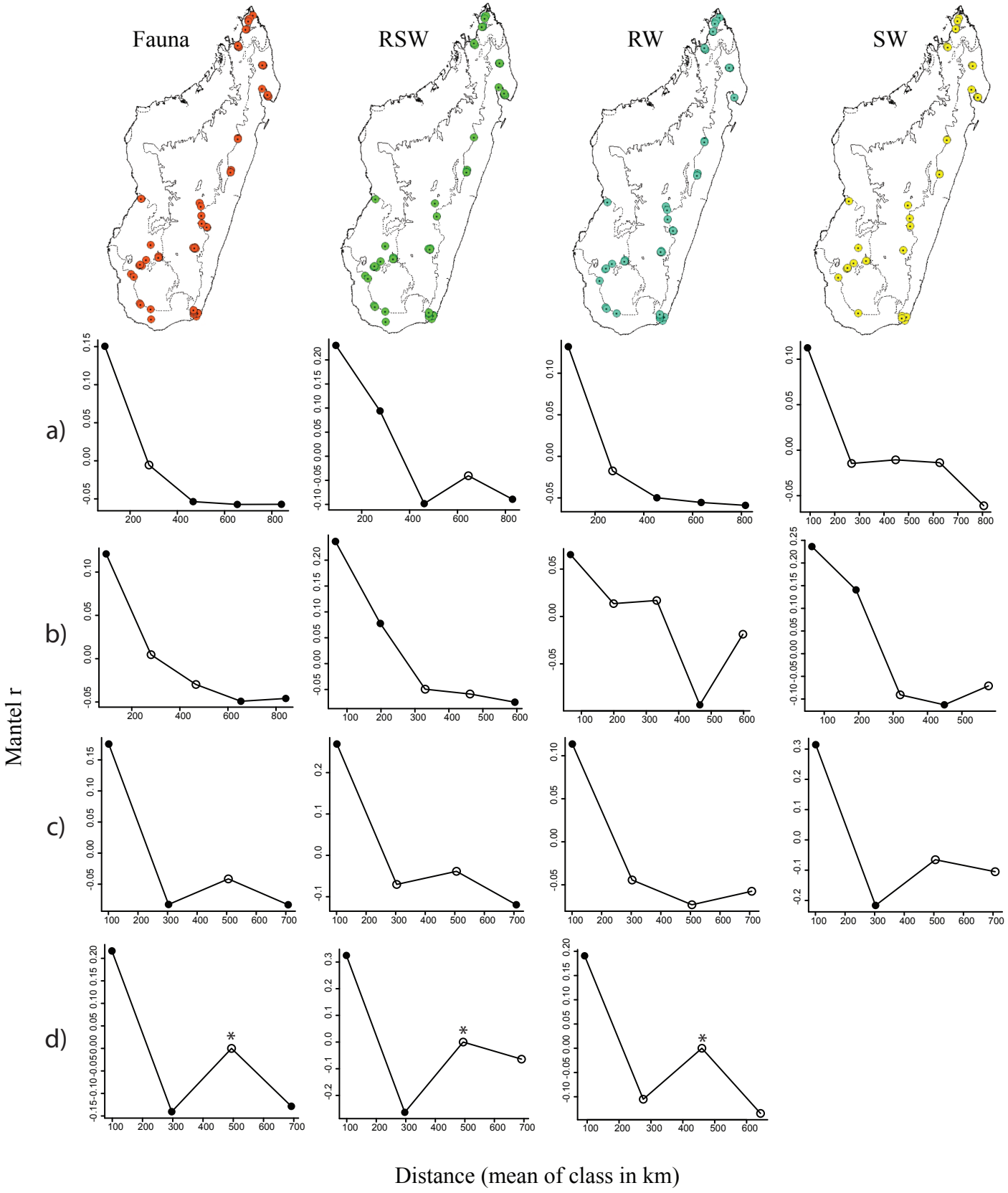


Figure 3.2: Distribution of the sampling sites for the different communities (Fauna, Running and Standing Water, Running Water, Standing Water) and multivariate Mantel correlograms showing the correlation between biotic similarity and classes of geographic distance. Four cases are represented: **a)** biotic similarity at the species level, **b)** biotic similarity at the haplotype level, **c)** biotic similarity at the species level within the lowland forest ecoregion only, **d)** biotic similarity at the species level within the subhumid forest ecoregion only. In the correlograms, black points and white points represent respectively Mantel statistics that are significant and not significant after the Bonferroni correction ($\alpha = 0.05$). Asterisks indicate insufficient numbers of distances obtained within a given class.

and troughs of the spatial structure. The detailed results of the mantel correlograms are available in Table S3.2. At the species level (Figure 3.2a), a significant correlation between the Simpson similarity index and the geographic distance was obtained for the Fauna, the RSW and the RW communities for all classes of distance except for the second neighbours (class 2), fourth neighbours (class 4) and second neighbours respectively. With four autocorrelations out of five classes of distance, these three presented an overall significant correlation after the Bonferroni correction. The SW group though produced a significant correlation only for the first neighbouring sites that is to say for distances below 100 km, which means that the geographic distance doesn't have any effect on the species composition for distances between 100 and 800km. When considering the biotic similarities at the haplotype level (Figure 3.2b), we found similar results than for the species level at least for the Fauna, the RSW and the RW communities, with multiple autocorrelations (≥ 2) over the different classes of distance, implicating an overall effect of geographic distance on the haplotype beta diversity patterns. The SW community, that globally showed no correlation between distance and biotic similarities, presented significant autocorrelations at the haplotype level for the first, second, and fourth neighbour distances (up to 450 km). With significance for three out of five classes of distance, the geographic distance was proved to have a strong general effect on the haplotype diversity patterns of the lentic community. In order to assess the pure effect of the geographic distance and eliminate a potential effect of the macrohabitat diversity on beta diversity patterns, we examined the same relationships than in Figure 3.2a (species level) but within the lowland humid forest and the subhumid forest (two distinct ecoregions) (Figure 3.2c and 3.2d). Note that the absence of mantel correlograms for the SW group in Figure 3.2d is due to the fact that the minimum number of pairs of sites required to run the

analysis was not reached. The results we obtained in Figures 3.2c and 3.2d for the fauna and the RSW community were similar to the ones in Figures 3.2a and 3.2b (significant correlation for at least two classes of distance). However, the most striking results concern the lotic and lentic communities in the lowland humid forest: we found in both ecoregions for the RW group a significant correlation only for the nearest neighbours (< 100km) which means that the geographic distance within this ecoregion doesn't have any major effect on the species composition similarities between sites. These results are at variance with Figure 3.2a but it has to be taken into account that Figure 3.2c deals with the lowland ecoregion only (which is longitudinal and along the east cost). Concerning the SW community this time, for which we found no major effect of the geographic distance on biotic similarities at the species level, we obtained a significant correlation for the first and second neighbours. With more than one significant correlation among the classes of distance, the overall effect of geographic distance on biotic similarities is significant. From the shape of all correlograms (Figures 3.2a, 3.2b, 3.2c, 3.2d), there are no obvious spatial patterns to observe (as described in Legendre and Legendre, 1998), except maybe for a potential gradient in the first correlogram (Figure 3.2a: the fauna at the species level). However, when analysing field data, it is difficult to see clearly the correspondence between individual significant spatial autocorrelation coefficients and the main elements of the spatial structure (Legendre and Legendre, 1998); for these reasons we will not interpret further the spatial structure of the fauna from the shape of the correlograms.

3.5 DISCUSSION

3.5.1 Differential dispersal and beta diversity patterns

It has been proven that water beetle species occurring in standing water bodies have a much larger distributional range than those living in running water (Ribera and Vogler, 2000, Ribera *et al.*, 2001): lentic sites are more likely to disappear over time, increasing the need for its species to disperse in order to find new suitable habitats whereas lotic habitats tend to be more continuous both in terms of time and space, allowing its populations to remain local. The link between habitat use and species richness patterns or speciation rates has been investigated before, from taxonomic data (Ribera *et al.*, 2003). Here we used a fauna delimited at the species level exclusively from molecular data and we aimed at understanding what were the consequences of these differential habitat uses for the molecular beta diversity patterns. The results showed that for a given level of biotic similarity, the lotic sites are strongly clustered, presenting up to 27 significant clusters for the highest degree of similarity. The lentic sites, for which we had a smaller number of localities but collected over the same range as the lotic ones (see the overlap on the map Figure 3.1), appeared to be less clustered for the same similarity thresholds. Thus these results suggest a higher species turnover within the lotic sites than among the lentic sites, which is in accordance with Ribera *et al.* (2001). However this analysis is not a direct comparison of lotic and lentic faunas: it was based on the species composition of lentic and lotic sites, which means that lotic species, lentic species and species found in both types of habitats (both categories of sites) were included. As a result, this is an interpretation in terms of turnover on the sites only, and not directly on the lentic/lotic communities.

3.5.2 Environmental variability and local endemism

The constraint analysis of proximities showed that three of the four communities defined in this study had their species composition strongly dependent on environmental and spatial variables (the fauna, the lotic-lentic group and the lotic community). The combined models of climate and space explained up to one third of the variation in the molecular species composition, and we saw that most of the individual predictors contributed to these results. Thus, it appears that the present diversity patterns of the lotic species are partly structured by the climate variability throughout the island. Given the strong climate heterogeneity of Madagascar (Thompson, 1965, Jury, 2003, Dewar and Richard, 2007) and the fact that lotic species disperse less and tend to remain local (Ribera and Vogler, 2000, Ribera *et al.*, 2001, 2003, Ribera, 2008), the percentage of variation explained by the different climate predictors separately and as a whole could reflect the existence of strong climatic niches, where the species would have differential climatic preferences. We suggest that the lotic fauna respond to important climatic constraints and that these environmental niches structure the species composition in space.

The lentic community showed a striking difference with the previous results in the sense that only one spatial and one climate predictor had a significant effect on the species composition of this group. Not only the complete model explained a very small percentage of variation, but the *p* values obtained for the model and the individual predictors were the highest in the whole analysis (up to 0.04), meaning that the significance of the two variables should be taken with caution. The fact that climate predictors don't affect the species composition of the lotic fauna as well as the lentic fauna could be surprising: both groups are strongly water-dependent and it would be reasonable to think that each habitat use implicates specific needs in terms

of temperature and precipitation for the populations. This is not verified here, where only the annual precipitations seem to play a weak role in structuring the lentic fauna. A possible explanation could be that with the standing water bodies drying up quickly, the lentic species disperse a lot, more than the lotic species (as described above). Consequently, the lentic species composition among the different sites at a time t could be completely different at the time $t - 1$, or $t + 1$. Then the predictors' values for the collecting sites at the time t might not reflect the climatic niche constraints of this group but may just correspond to climate data associated to a random position of the species at this time, resulting from the high dispersal activity of the group. Thus it would be very difficult to correlate fixed environmental and spatial variables to a permanently changing species composition in time and space.

We showed that within the highly endemic fauna of Malagasy water beetles (Rocchi, 1991), the consequences of environmental variability could differ greatly depending on which community we consider: lotic or lentic. The potential implication of these results in terms of endemism is not negligible: the lotic fauna, who tends to be localised, and who obeys to considerable climatic restrictions, would present a high level of local endemism patterned on the region's heterogeneity in terms of rainfall and temperatures. Oppositely, endemic lentic species, being less constrained by environmental fluctuations and dispersing more, are expected to be found throughout the island. The response to environmental variability from both communities would create endemism at the scale of the island for the standing water fauna and micro-endemism within Madagascar for the running water communities. Endemism range variation will be studied thoroughly in the next chapter by means of a species distribution modelling approach.

3.5.3 Geographic distance, macrohabitat diversity and biotic dissimilarities

Knowing how climatic and spatial predictors correlate to the species composition of the water beetle fauna and its different communities explains part of the present biodiversity patterns on the island. But Madagascar is an extremely large territory (de Wit, 2003), and this last analysis emphasized the role of such an important surface in structuring the actual beta diversity of a fauna with two differential dispersal patterns. It is difficult to explain why at the species level, the fauna's biotic dissimilarity increased considerably with the geographic distance even on very long distances, because the entire fauna hides three distinct communities; but the fact that we found a similar case for the lotic community is concordant with its low dispersal activity. If lotic species have a restricted range, passed this range, the distance between two sites will increase the species composition dissimilarity betweens sites. And the phenomenon can continue until the geographic distance overpasses the species' largest range sizes.

For a highly dispersive group like the lentic species community, we expect much larger distribution range sizes, and consequently a weaker or null effect of the geographic distance on the biotic similarity between sites. In our case, the distance seemed to increase species dissimilarity only below 100 km, which could be due to the presence of some poor dispersers among the lentic community. Once the range of these particular species is passed, the effect of geographic distance wouldn't be visible. The lentic fauna at the haplotype level though, behaved like the lotic group at the species level, which means that the geographic distance increases the haplotype dissimilarities but not species dissimilarities within this group. Distant populations within a given lentic species might present a high haplotype diversity, responding to the effect of geographic distance, whereas this given species will still be present at the

same classes of distance, resulting in an unchanged biotic similarity value between sites.

We saw in these two series of correlograms what could be the impact of geographic distance on biotic dissimilarity for the whole island. But in the case of Madagascar, we face a very large area, extremely heterogeneous in terms of macrohabitats: seven ecoregions are defined in this study area, from the mangroves to the dry deciduous forest, passing by humid and subhumid forests. Consequently, even short distances can hide a transition between two different macrohabitats, and multiple ecozones can be encountered over longer distances. By isolating the communities in separate habitats, we evaluated the pure effect of geographic distance. Doing so with the lowland humid forest and the subhumid forest, the lotic fauna presented this time an increase in biotic dissimilarity only for short distances, whereas the effect of the distance for the lentic fauna was notable at larger classes of distance. It is possible that lotic species disperse more actively within the same ecoregion, remaining in their climatic niche when responding to environmental variability. Thus two very distant points in the same habitat could have a higher similarity index than two neighbouring sites across two ecoregions. A possible explanation for the lentic case is that the lowland forest ecoregion is the one receiving the highest rainfalls and providing the most stable habitat over time, limiting the need for dispersal for the lentic species in comparison to other ecoregions. Moreover, it is important to consider here that the two ecoregions constitute an almost unidirectional space, where physical barriers might have a bigger impact on dispersal, which could affect the results of the mantel correlograms. Though this remains difficult to assess without knowing the limits of the different species' dispersal capacities.

CHAPTER 4

Exploring the causes of global and local insect endemism in Madagascar: an approach using molecular phylogenetics and ecological niche modelling[1]

[1]To be published as: Isambert, B., Guisan, A, and Vogler, A. P.

4.1 ABSTRACT

The outstanding level of endemism characterising Madagascar is unrivalled even among other biodiversity hotspots. The evolutionary processes that engendered such unique biodiversity patterns at the global scale have been investigated intensively across taxa, and the long history of isolation and contacts with other landmasses has been identified as the primary cause of evolution of distinctive Malagasy faunas and floras. Nevertheless, the vicariance and dispersal hypotheses fail to explain some of the exceptional local endemism patterns. Recent studies brought environmental variability to the fore as a potential initiator of species diversification on the island. In particular, Madagascar's extreme climate was indicated as a driving force for speciation. In this study, we use a combination of molecular phylogenetics and species distribution modelling to identify the causes of insect endemism both at the global and the local scale. Based on molecular delimitation of the water beetle fauna, our results suggest that the uniqueness of Malagasy climatic niches is responsible for the confinement of numerous species to the island. We also identify the existence of potential suitable habitat on the mainland and interpret the absence of Malagasy species as a consequence of dispersal limitations. Second, distribution models at three distinct phylogenetic levels demonstrate a high divergence of the climatic niche among closely related taxa, and highlight the role of climate variability for species diversification and the structuration of local endemism in Madagascar.

4.2 INTRODUCTION

4.2.1 Molecular phylogenetics and Species distribution modelling

When high species endemism and threatened biodiversity are well defined globally by biodiversity hotspots (Myers *et al.*, 2000), the sparseness of available biological data within the hotspots hampers the understanding of local endemism and prevents effective conservation (Carnaval *et al.*, 2009). In this context, predicting species distribution is becoming indispensable; it can provide invaluable sources of biological information, helping decision making for conservation priorities in fast changing tropical habitats (Kremen *et al.*, 2008). Despite the difficulty to obtain numerous records for rare or endemic species, recent modelling approaches have been successful at mapping their potential distributions (Engler *et al.*, 2004, Pearson *et al.*, 2007). In Madagascar specifically, Raxworthy *et al.* (2003) used historical and recent datasets to predict the presence of known reptiles species, and proved that niche models offered a great potential for discovering unknown distributional areas as well as unknown species. More recently, Allnutt *et al.* (2008) used Generalized Dissimilarity Models to quantify the impact of deforestation on Malagasy species diversity, and Kremen *et al.* (2008) put forward high-resolution multitaxonomic modelling approaches to identify areas optimising the persistence of biodiversity. But the possibilities offered by the use of species distribution modelling are not restricted to depicting areas of endemism or suitable habitat distribution. The recent advances in molecular biology and phylogenetics combined to distribution modelling offer a new range of applications, starting with the delimitation of species within taxonomically problematic groups (Rissler and Apodaca, 2007, Leache *et al.*, 2009). For example, Raxworthy *et al.* (2007) successfully evaluated the species limits of *Phelsuma* geckos

in Madagascar by integrating ecological niche modelling, morphological and mitochondrial data. Beyond helping the delimitation of species, predicted distributions can now be interpreted in the face of reliable phylogenies to improve our comprehension of biogeographic patterns and evolutionary processes, especially mechanisms of speciation (Jakob *et al.*, 2007, Moussalli *et al.*, 2009, Murienne *et al.*, 2009). This multi-disciplinary approach is particularly suitable for investigating the roles of niche divergence and conservatism in promoting lineage diversification (Graham *et al.*, 2004, Pyron and Burbrink, 2009).

4.2.2 Endemism and Environmental constraints in Madagascar

The outstanding levels of endemism and imbalance across Malagasy taxa (Goodman and Benstead, 2003, 2005) have long been explained only by the island's history of isolation and contacts with other landmasses (Storey *et al.*, 1995, de Wit and Masters, 2004, Yoder and Nowak, 2006). Nevertheless, the processes leading to some remarkable levels of local endemism remain undetermined (Yoder *et al.*, 2005, Wollenberg *et al.*, 2008). Several hypotheses have been proposed, notably allopatric speciation by isolation caused by rivers (Pastorini *et al.*, 2003, Goodman and Ganzhorn, 2004) and watersheds (Wilme *et al.*, 2006), and more recently parapatric speciation along climatic gradients (Raxworthy *et al.*, 2007) refered also as the "current climate hypothesis" (Pearson and Raxworthy, 2009). Dewar and Richard (2007) and Harcourt (2008) also put forward Madagascar's climate and its unpredictability as potential explanations for the evolution of distinctive characteristics in organisms In fact, Madagascar gathers exceptional climatic conditions (Jury, 2003): substantial rainfalls come from the east, with the Indian Ocean's tropical cyclones and trade winds (Thompson, 1965, Ganzhorn, 1995), and

the steep slope of the longitudinal range of mountains along the east coast prevents these precipitations from reaching the western side of the island (de Wit, 2003). Consequently, temperatures and precipitations vary considerably across the island (Nassor and Jury, 1997, 1998) and present a significant seasonality (Pearson and Raxworthy, 2009), which combined to the geology of the island, results in a high macrohabitat diversity and a complex network of ecotones. If climatic gradients constitute the main driver of species diversification on the island, one should expect even closely related taxa to show distinct climatic niches, and consequently a poor degree of niche conservatism across the deeper phylogenetic levels. Our previous results emphasized how climate variability correlates to beta diversity patterns within the aquatic beetle fauna of Madagascar and suggested a strong dependence of species' occurrences to such environmental factors. In this particular study, we first investigate the effects of climate predictors on individual GMYC species by reconstructing their ecological niche using a distribution modelling approach. We test whether species endemism is best explained by habitat uniqueness on the global scale or by species' incapability to colonise potentially suitable habitats in mainland Africa, India or other Indian Ocean islands. Second we calculate the degree of ecological niche conservatism within Madagascar across three different phylogenetic levels to evaluate the role of climatic gradients in species diversification.

4.3 MATERIAL & METHODS

4.3.1 Biological data and predictor variables

This study is based on the exact same water beetle incidence data as described in the previous chapter: presences and absences were compiled for the 168 species among the 142 sites for which GPS coordinates were available. The resulting matrix was used in the two following species distribution modelling approaches, together with the set of climate predictors used in the beta diversity analysis: annual mean temperature (T_{ann}), temperature seasonality (T_{sea}), maximum temperature of the warmest month (T_{max}), minimum temperature of the coldest month (T_{min}), annual precipitation (P_{ann}), precipitation seasonality (P_{sea}), precipitation of the wettest month (P_{wm}) and precipitation of the driest month (P_{dm}). The climatic predictors were obtained from the WorldClim layers version 1.4 (Hijmans *et al.*, 2005), available at http://www.worldclim.org, under the 30 seconds spatial resolution. In ArcGIS 9.2 (Environmental Systems Research Institute, Inc., Redlands, California, USA), predictor values were extracted for all pixels of Madagascar, but also for all pixels in the geographic area defined between 38°N, -35°S, -25°W and 92°E, which comprises Madagascar, Africa, India, and the main Indian Ocean islands. This last set of predictor values was used for large-scale maximum entropy modelling whereas the climate predictors of Madagascar only were used for local scale ecological niche modelling.

4.3.2 Maximum entropy modelling

We conducted maximum entropy modelling of species' geographic distributions in Maxent (Phillips *et al.*, 2006, Phillips and Dudik, 2008) for all GMYC

species presenting at least 10 presence records (absences are not integrated in Maxent models) using the climate predictors extracted for Africa, Madagascar, India and the Indian Ocean islands. Each GMYC species dataset was split in two partitions: one for training data and one for testing the models. The random test percentage was set to 20, leaving 80% of the presence data for training. The regularization multiplier was kept at its default value (1) and the number of maximum iterations was set to 500. A maximum of 10 000 background points was chosen and the convergence threshold was fixed at 0.0001. The models' outputs were presented under the logistic format, as a picture of the predictions together with different response curves. The accuracy of the statistical models is usually assessed by the agreement between observations and predictions from which four fractions can be extracted: sensitivity, specificity, false positive fraction and false negative fraction. Sensitivity is described as the ratio of positive sites (presences) correctly predicted over the number of positive sites in the samples. Specificity is the ratio of negative sites (absences) correctly predicted on the number of negative sites in the sample. False positive fraction equals 1 – specificity, and false negative fraction equals 1 – sensitivity. The Receiver Operating Characteristic curve (ROC curve) is a graphical method showing the relationship between sensitivity and (1 – specificity). When predictions expected by chance would produce a 45 degrees line, a good model performance would result in a curve passing close to the upper left corner of the graph (i. e. maximising sensitivity for low values of (1-specificity)). We used the area between the curve and the 45 degrees line, called Area Under the Curve or AUC statistic (varying from 0 to 1), to give the performance score of the model (Fielding and Bell, 1997). According to Swets, (1988), predictions with an AUC value of 0.7 can be considered useful, and good models would have an AUC above 0.9. Because Maxent only uses occurrence data (no absences), the

fraction of the total study area predicted as present (fractional predicted area) is used for the AUC calculation instead of the standard (1- specificity) (Phillips *et al.*, 2006).

4.3.3 Modelling the climatic niches of Malagasy aquatic coleoptera

In order to assess the degree of conservatism of water beetles' climatic niche, we first built distribution models at three distinct phylogenetic levels based on the climate predictors: the GMYC species level, the genus level (all the GMYC species within the same genus), and an intermediate clade level between the last two (all the GMYC species within the clade). For all three levels, we used only the entities present in at least ten sites, but unlike the previous approach, both presences and absences were considered in the statistical models. In total, 11 GMYC species, 7 genera and 6 intermediate clades were selected for the species, genus and clade level respectively. All distribution models were run in R version 2.8.1 (http://cran.r-project.org/) using the BIOMOD package (BIOdiversity MODelling: Thuiller, 2003, Thuiller *et al.*, 2009) available at http://r-forge.r-project.org/projects/biomod/. Three different models were tested for each GMYC species, genus and clade: a Generalised Additive Model (GAM), a Generalised Boosting Model (GBM) and a Generalised Linear Model (GLM). GAMs have recently been used in ecology and biogeography (Guisan and Zimmermann, 2000, Guisan and Thuiller, 2005, Elith *et al.*, 2006) and are particularly useful when the relationship between the different variables is too complex to be fitted easily in linear or non-linear models. It uses a set of equations to generalise data into smooth curves by local fitting to subsections of the dataset (Hastie and Tibshirani, 1990). GBMs, or boosting regression trees (Friedman, 2001) as implemented in BIOMOD, combine predictions from numerous simple models to give more robust estimates of the response. Each model is a classification or

regression tree, built by repeated splits of the data according to one single explanatory variable into two groups as homogenous as possible in terms of response. A recent example of the use of GBMs to predict species richness patterns is given by Thuiller *et al.* (2006). GLMs attempt to fit the most parsimonious model that best explains the distribution of species with respect to the environmental predictors provided. GLMs can be run with linear terms only, quadratic terms (linear, 2^{nd} and 3^{rd} order) or polynomial terms. Within BIOMOD, a stepwise procedure using the Akaike Information Criterion (AIC) or the Bayesian Information Criterion (BIC) allows to remove redundant predictor variables from the model so as to reduce multicolinearity. GAMs were run using a spline function with a degree of smoothing of 3, and GBMs used a maximum of 2000 trees. GLMs were set to use linear, quadratic or polynomial terms, and the stepwise procedure chosen was the AIC. For all models, a repeated split-sample was run 100 times, using 80% of the data for training and 20% for testing (prevalence was conserved in every calibration and evaluation datasets.). Three different methods were used to evaluate the predictive performance of the models: the ROC/AUC statistic (previously described), Cohen's Kappa statistic (Kappa), and the Hanssen-Kuiper Skill Score (KSS) also known as the "True Skill Statistic" (TSS). Kappa is a measure of inter-raters agreement (not randomly obtained) for categorical variables, and varies between -1 and 1; it is calculated for all probabilistic thresholds between 0 and 1 and its greatest score is kept as best Kappa value. TSS is usually employed to calculate the accuracy of weather forecasts and is defined in our case as sensitivity + specificity -1. For each species, genus and clade, the model retained was the one for which at least two of the three statistics agreed. The predictions of the best models were projected on all pixels in Madagascar and mapped in ArcGIS using the highest binary threshold among the agreeing statistics.

4.3.4 Niche conservatism indices

The degree of conservatism of the niche was estimated by comparing the predicted distributions of the different phylogenetic levels two by two: *species-clade*, *clade-genus* and *species-genus*. For each comparison, we distinguish the "lowest phylogenetic entity" (that is to say the species, when looking at *species-clade* and *species-genus* comparisons, and the clade, when looking at the *clade-genus* comparison) from the "highest phylogenetic entity" (the clade, when looking at the *species-clade* comparison, and the genus, when looking at the *clade-genus* and *species-genus* comparisons). We refer to them hereafter as LPE and HPE respectively. We measured the extent of the climatic niche conservatism by calculating a first conservatism index (*NC1*), defined as the number of pixels (≈ 1km^2 each) where the LPE was predicted as present but not the HPE ($HPE_{predictions} - LPE_{predictions} = -1$) divided by the total number of LPE's presences. A second conservatism index (*NC2*) was given by the number of pixels where the HPE was predicted as present but not the LPE ($HPE_{predictions} - LPE_{predictions} = 1$) divided by the total number of HPE's presences. For both *NC1* and *NC2*, complete niche divergence would correspond to a value of 100%, whereas 0% would represent a perfect conservatism of the niche between the two phylogenetic levels considered. All pixels counts were extracted directly from the BIOMOD outputs in R.

4.4 RESULTS

4.4.1 Large-scale distribution models

The Maxent models were run on five GMYC species considered as endemic (like described in Chapter 2), two GMYC species within the highly endemic genus *Copelatus* (hardly definable as endemic or non-endemic), and five GMYC species considered non-endemic. All models presented AUC values above 0.9, both for the training and the test data, and the 10 percentile threshold values obtained were between 0.330 and 0.824 (Table 4.1). Heuristic estimates of the eight climate variables' contribution to the models gave P_{dm}, T_{sea} and T_{max} as greatest contributors, with contributions varying from 30% to 79.5%. The complete list of individual contribution of climate predictors to each GMYC species model is available in Appendix III, Table S4.1.

GMYC Species	AUC Training data	AUC test data	logistic threshold	Greatest Contribution
11*	0.97	0.98	0.330	P_{dm} (68.5%)
15*	0.99	0.98	0.714	P_{dm} (50.9%)
20*	0.99	0.99	0.824	T_{max} (30%)
43*	0.94	0.96	0.587	T_{sea} (79.5%)
58*	0.97	0.97	0.263	T_{sea} (52.8%)
65*	0.99	1.00	0.624	P_{dm} (61.6%)
76*	0.99	0.99	0.572	T_{max} (33.9%)
32	0.99	0.87	0.700	T_{max} (41.9%)
57	0.99	0.98	0.568	P_{dm} (54.7%)
63	0.99	0.99	0.489	P_{dm} (38.9%)
101	0.97	0.88	0.398	T_{sea} (56.1%)
124	0.96	0.98	0.478	T_{sea} (66.9%)

Table 4.1: Summary of the Maxent results obtained for the different GMYC species. The AUC values are given for the training data and the test data, together with the 10 percentile training presence threshold and the variable that contributed the most to each model.

Predicted niche distributions were obtained for five endemic GMYC species together with the two *Copelatus* species mentioned above, namely *sp11* (*Madaglymbus sp*), *sp15* (*Copelatus sp*), *sp20* (*Copelatus sp*), *sp43* (*Yola costipennis*), *sp58* (*Laccophilus complicatus*), *sp65* (*Orectogyrus ornaticolis*) and *sp76* (*Aulonogyrus goudoti*) (see Table S4.2 in Appendix I for details on each GMYC species). If considering only the highest presence probabilities, between 0.77 and 1 (yellow to red pixels on the maps), only three species showed large homogenous predicted niche distributions outside Madagascar (Figure 4.1): *sp11* (10 percentile threshold t = 0.330), predicted present in central Africa, south of Ivory Coast and Liberia, as well as on the west coast of India and Sri Lanka, *sp43* (t = 0.587), also predicted present in central Africa, south of Ivory Coast and Liberia, but mainly in southern Africa (from Angola to Mozambique, and in Namibia and eastern South Africa), and finally *sp58* (t = 0.263), mostly along the South African coast. The remaining species for the same probability levels presented very scattered predicted niche distribution outside Madagascar: *Sp15* (t = 0.714) in La Réunion, Mauritius, the Comoro islands, sparsely in east Africa (within Mozambique, Tanzania and Kenya), Southern India and Sri Lanka, *sp20* (t =0.824) in Mozambique, Tanzania and Equatorial Guinea, *sp65* (t = 0.624) almost exclusively in La Réunion and Mauritius, and *sp76* (t = 0.572) in La Réunion, Mauritius, the Comoro islands, and over distant patches along the eastern African coast from South Africa to the Ethiopian region.

For the distribution models of the non-endemic species, we reported the known distributions of the corresponding Linnaean species from the literature (Rocchi, 1991, Nilsson, 2001, Nilsson and Van Vondel, 2005). Despite the fact that we only used occurrence data from Madagascar to model the distribution of species known to be widespread, the areas defined above the 10 percentile training presence

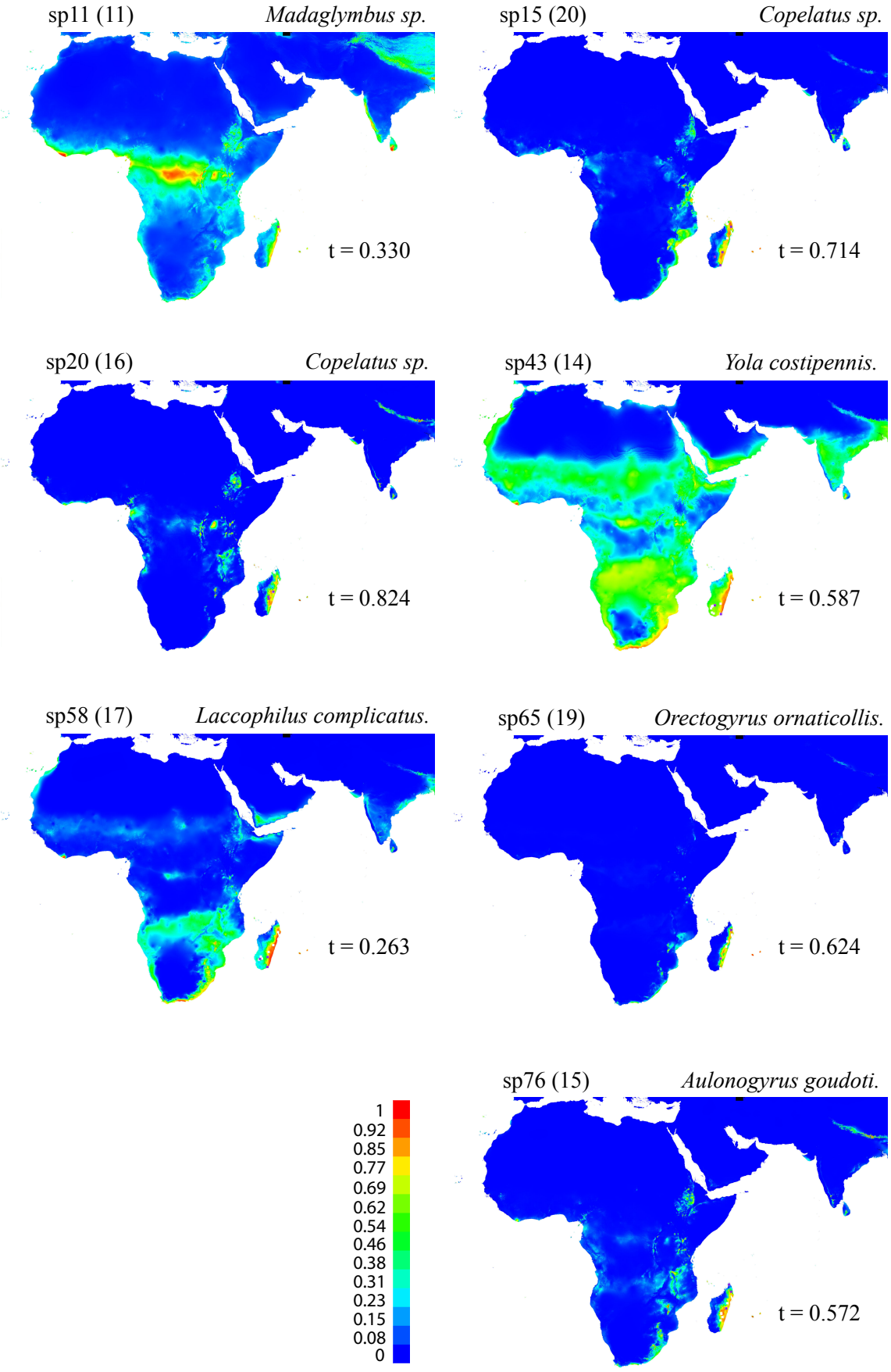


Figure 4.1: Maximum entropy distribution models of seven endemic molecular species (>10 presence occurrences). The GMYC label is shown above each prediction map together with the name of the corresponding Linnaean species. The number of respective presence data is given in parentheses and the 10 percentile training presence threshold t figures on the lower right corner of each map.

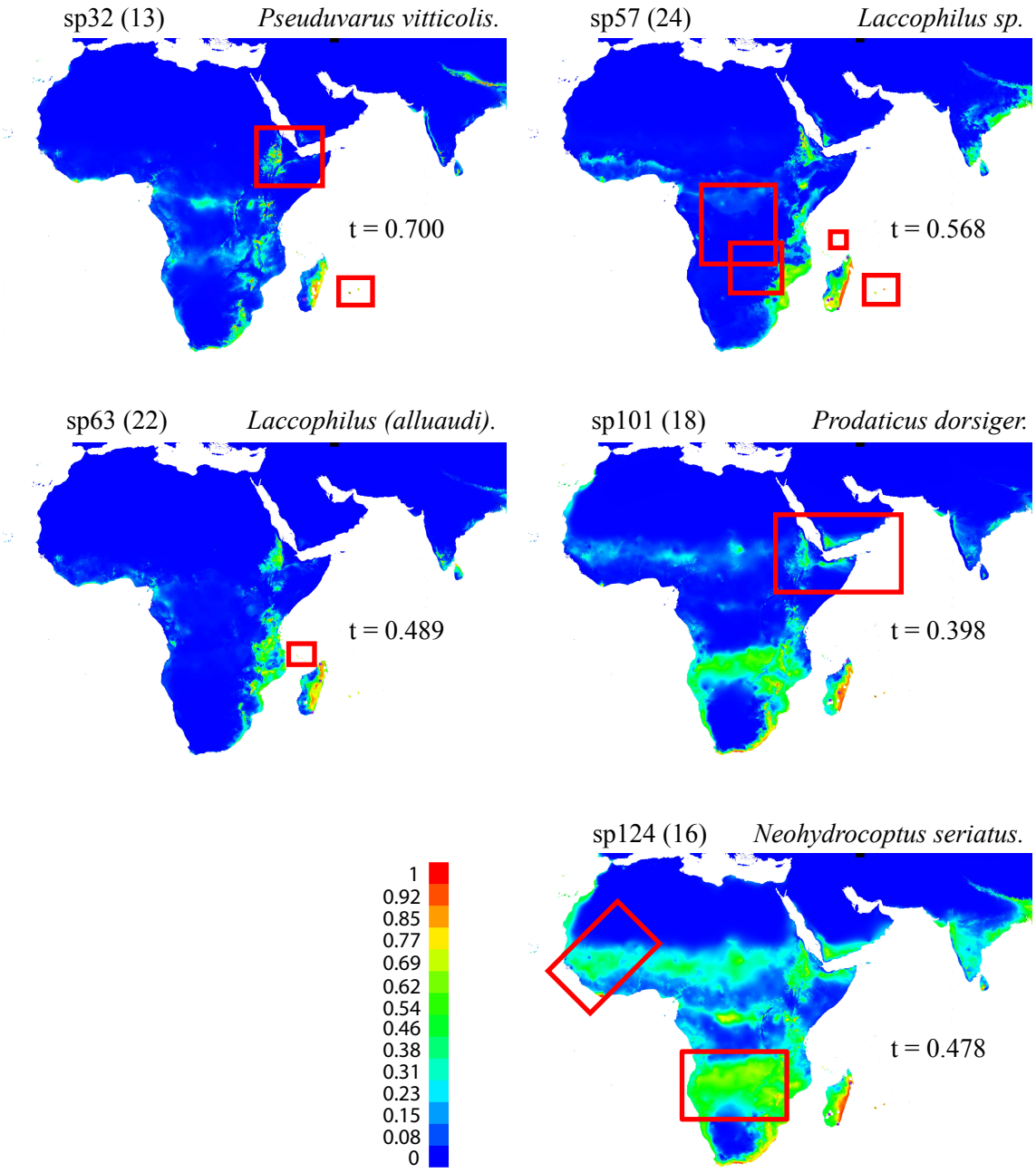


Figure 4.2: Maximum entropy distribution models of five molecular species defined as non-endemic (>10 presence occurences). The GMYC label is shown above each prediction map together with the name of the corresponding Linnaean species. The number of respective presence data is given in parentheses and the 10 percentile training presence threshold t figures on the right half of each map. Red rectangles show the known distribution of the corresponding Linnaean species.

threshold correctly predicted positive sites for all five GMYC species (Figure 4.2). *Sp32* (*Pseuduvarus vitticolis*) was correctly predicted in all the regions outside Madagascar where it is known to occur, that is to say La Réunion, Mauritius, and the Ethiopian region. *Sp57* (*Laccophilus sp*, possibly *addendus*, *posticus*, or *complicatus*) was predicted present in the Seychelles (where *addendus* and *posticus* occur), La Réunion (*addendus* only) and Mauritius (*posticus* only), but not in Congo and Zambia (where *addendus* is found). *Sp63* (*Laccophilus sp*, possibly *alluaudi*) was correctly predicted in the Comoro islands and *sp101* (*Hydaticus dorsiger*), which is known outside Madagascar only from the Ethiopian region and the Arabian peninsula, was predicted present in both these areas. Finally, *sp124* (*Neohydrocoptus seriatus*), was correctly predicted in the region of Mali, Guinea and Guinea Bissau, in Angola, and in the Mascarene islands.

4.4.2 Niche conservatism at the species, clade and genus levels

Based on a 10 minimum presence records rule, niche models were obtained for the following GMYC species: *sp11*, *sp15*, *sp20*, *sp36*, *sp38*, *sp57*, *sp58*, *sp63*, *sp101*, *sp107*, *sp115* (details on each GMYC species are listed in Appendix I, Table S2.2). Additional models were built for their respective Linnaean genera (*Madaglymbus*, *Copelatus*, *Hydroglyphus*, *Laccophilus*, *Prodaticus*, *Cybister* and *Hydrovatus*) as well as for additional clades within these genera (each clade including at least one endemic species). Since the genus *Pseuduvarus* was nested within *Hydroglyphus* on the phylogeny obtained in Figure 2.8, the model run for *Hydroglyphus* included also the *Pseuduvarus* occurrence data (this complex is referred hereafter as *Hydroglyphus-Pseuduvarus*). The best models for each species, genus and clade were chosen among GAMs, GBMs and GLMs according to the

Kappa, ROC and TSS statistic values (Table 4.2) and the resulting predicted distributions were placed in the face of the phylogenetic relationships characterising the taxa (Figures 4.3 to 4.6). The degree of conservatism for the predicted niches across the three phylogenetic levels (*species-clade*, *clade-genus* and *species-genus*) was first assessed graphically (Figures 4.7 to 4.10). With green pixels representing the LPE only predicted as present, grey pixels representing the HPE only predicted as present, and black pixels representing co-occurrence of the LPE and HPE, a high degree of niche conservatism generated large black areas and very few green and grey pixels, whereas divergent niches resulted in a dominance of green and grey regions on the maps.

The pixel numbers of the individual distribution maps and the values for the two niche conservatism indices (Table 4.3) show that in only one case we found a high degree of climatic niche conservatism across the three phylogenetic levels: the *sp107* group (Figure 4.9) shows *NC1* and *NC2* values all under 15%, and in particular null *NC1* results for the *species-clade* and the *species-genus* levels. Oppositely, the *sp101*, *sp15* and *sp20* groups showed very high niche divergence for all levels (Figures 4.3, 4.4, 4.7 and 4.8) with *NC1* values up to 99.02%, 85.58%, and 68.13% respectively, and *NC2* values between 62.06% and 97.07%. In comparison, the *sp11*, *sp115*, *sp57*, *sp58* and *sp63* groups were very heterogeneous and presented different degrees of climatic niche divergence depending on the phylogenetic level considered (Figures 4.3, and 4.5 to 4.9). Their predicted distributions at the clade and genus levels overlapped almost completely but the *species-clade* and *species-genus* comparisons resulted in high values for at least one niche conservatism index. For example, the *sp115* group (Figures 4.5 and 4.8) showed a high niche similarity at the *clade-genus* level (*NC1* = 2.47%, *NC2* = 15.40%) but indices values over 50% at the

Table 4.2: Evaluation results for the different models (GAM, GBM, GLM) per GMYC species, per intermediate clade and per genus. For each individual species, genus and clade used in the models, the list of GMYC species is given. The score of each model is given according to the three statistics Kappa, ROC and TSS. The best model according to at least two statistics was kept and is shown in bold characters. The number of statistics agreeing on the best models is given in parentheses beside it.

	GMYC	Model evaluated	Kappa		ROC		TSS	
			Cross val.	Final mod.	Cross val.	Final mod.	Cross val.	Final mod.
Madaglymbus sp	11*	GAM	0.770	0.947	0.924	0.981	0.875	0.947
		GBM (3)	0.782	0.924	0.933	0.980	0.878	0.924
		GLM	0.614	0.969	0.866	0.990	0.768	0.969
Copelatus sp	15	**GAM (3)**	0.522	0.794	0.832	0.916	0.679	0.794
		GBM	0.460	0.761	0.748	0.923	0.581	0.761
		GLM	0.480	0.670	0.797	0.888	0.610	0.670
Copelatus sp	20	GAM	0.619	0.802	0.875	0.938	0.797	0.802
		GBM (2)	0.676	0.811	0.883	0.967	0.771	0.811
		GLM	0.592	0.796	0.846	0.935	0.734	0.796
Hydroglyphus gemoides	36	GAM	0.712	0.947	0.921	0.988	0.836	0.947
		GBM (3)	0.763	0.909	0.958	0.979	0.925	0.909
		GLM	0.608	0.924	0.839	0.991	0.693	0.924
Hydroglyphus plagiatus	38*	GAM	0.748	0.817	0.873	0.943	0.787	0.817
		GBM (2)	0.725	0.847	0.915	0.973	0.839	0.847
		GLM	0.643	0.833	0.821	0.973	0.675	0.833
Laccophilus sp	57	**GAM (3)**	0.659	0.806	0.892	0.943	0.789	0.806
		GBM	0.624	0.764	0.884	0.939	0.775	0.764
		GLM	0.586	0.857	0.843	0.968	0.691	0.857
Laccophilus complicatus	58*	**GAM (3)**	0.344	0.568	0.665	0.859	0.475	0.568
		GBM	0.282	0.632	0.648	0.872	0.428	0.632
		GLM	0.328	0.722	0.660	0.926	0.440	0.722
Laccophilus sp	63	**GAM (2)**	0.492	0.672	0.809	0.889	0.647	0.672
		GBM	0.529	0.656	0.784	0.908	0.624	0.656
		GLM	0.472	0.642	0.795	0.885	0.630	0.642
Prodaticus dorsiger	101	GAM	0.394	0.455	0.663	0.756	0.493	0.455
		GBM (3)	0.412	0.694	0.741	0.901	0.567	0.694
		GLM	0.342	0.570	0.659	0.859	0.464	0.570
Cybister cinctus	107*	GAM	0.598	0.870	0.748	0.958	0.593	0.870
		GBM (3)	0.644	0.847	0.864	0.958	0.777	0.847
		GLM	0.435	0.810	0.711	0.942	0.498	0.810
Hydrovatus pictulus	115*	GAM	0.605	0.817	0.868	0.943	0.774	0.817
		GBM (3)	0.616	0.878	0.880	0.957	0.805	0.878
		GLM	0.537	0.749	0.821	0.923	0.687	0.749
Madaglymbus*	1 to 14 & 130 to 138	GAM	0.362	0.481	0.680	0.797	0.413	0.481
		GBM (2)	0.399	0.546	0.707	0.841	0.455	0.546
		GLM	0.400	0.482	0.702	0.807	0.441	0.482
Copelatus	15 to 23 & 139 to 146	GAM	0.270	0.311	0.584	0.717	0.293	0.311
		GBM (3)	0.361	0.567	0.645	0.857	0.376	0.567
		GLM	0.207	0.262	0.529	0.620	0.229	0.262
Laccophilus	50, 51, 57 to 64, 155 & 158	GAM	0.579	0.567	0.763	0.815	0.582	0.567
		GBM	0.527	0.542	0.777	0.856	0.531	0.542
		GLM (3)	0.599	0.612	0.782	0.830	0.600	0.612
Hydroglyphus (Pse)	32 to 38	GAM	0.547	0.649	0.783	0.891	0.583	0.649
		GBM (2)	0.538	0.709	0.802	0.918	0.619	0.709
		GLM	0.545	0.651	0.777	0.881	0.587	0.651

Prodaticus	95 to 102 & 163	GAM	0.232	0.402	0.511	0.715	0.242	0.402
		GBM (3)	0.296	0.520	0.602	0.811	0.327	0.520
		GLM	0.233	0.399	0.513	0.722	0.257	0.399
Cybister	106 to 112, 165 & 166	GAM	0.546	0.701	0.742	0.922	0.577	0.701
		GBM (3)	0.557	0.696	0.765	0.919	0.600	0.696
		GLM	0.504	0.712	0.721	0.913	0.533	0.712
Hydrovatus	113 to 118 & 167 to 169	GAM	0.455	0.621	0.693	0.869	0.538	0.621
		GBM (3)	0.497	0.709	0.786	0.913	0.618	0.709
		GLM	0.468	0.576	0.672	0.872	0.491	0.576
Clade Madaglymbus*	8 to 14 & 132 to 135	GAM	0.296	0.446	0.632	0.774	0.355	0.446
		GBM (3)	0.358	0.484	0.665	0.820	0.383	0.484
		GLM	0.318	0.415	0.647	0.741	0.363	0.415
Clade Copelatus	16 to 19 & 139 to 143	GAM	0.078	0.466	0.475	0.783	0.160	0.466
		GBM	0.159	0.564	0.527	0.840	0.262	0.564
		GLM (3)	0.198	0.509	0.550	0.817	0.327	0.509
Clade Laccophilus	50, 51, 59 to 62 & 155	**GAM (2)**	0.452	0.497	0.721	0.780	0.543	0.497
		GBM	0.451	0.690	0.758	0.875	0.539	0.690
		GLM	0.428	0.605	0.705	0.798	0.526	0.605
Clade Prodaticus	97 to 100	GAM	0.161	0.519	0.552	0.809	0.341	0.519
		GBM (2)	0.294	0.718	0.623	0.914	0.464	0.718
		GLM	0.268	0.650	0.641	0.845	0.458	0.650
Clade Cybister	106 & 110 to 112	GAM	0.322	0.528	0.631	0.757	0.447	0.528
		GBM (2)	0.332	0.672	0.662	0.881	0.496	0.672
		GLM	0.343	0.596	0.642	0.867	0.475	0.596
Clade Hydrovatus	113, 114, 116, 117 & 167	GAM	0.466	0.665	0.720	0.910	0.590	0.665
		GBM (2)	0.426	0.749	0.781	0.917	0.670	0.749
		GLM	0.460	0.672	0.695	0.912	0.511	0.672

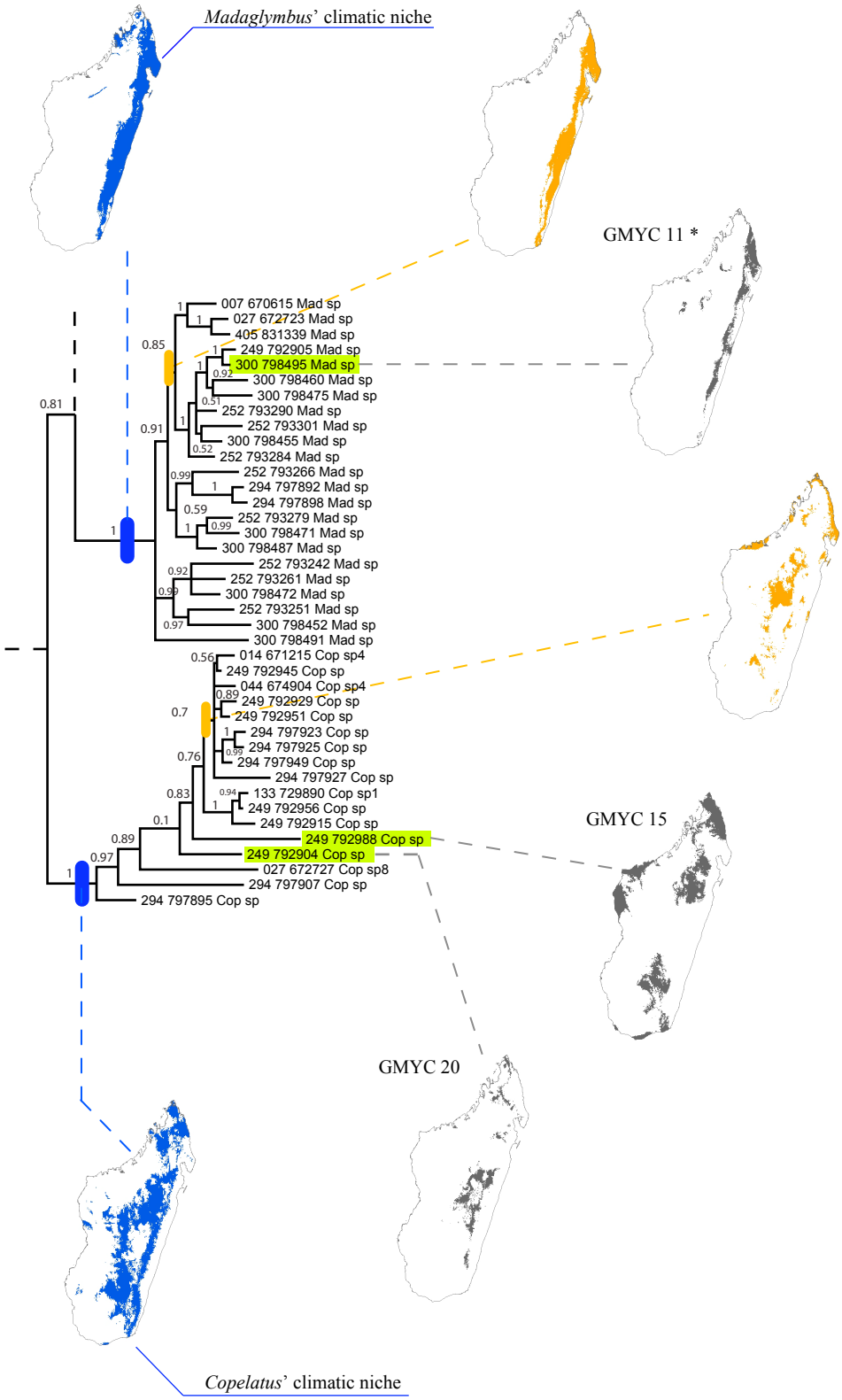


Figure 4.3: Climatic niche models for two genera of water beetles: *Madaglymbus* and *Copelatus*. Blue maps represent the biomod distribution models at the genus level, orange maps show the intermediate clades distribution models and grey maps show the GMYC species level distributions (species labels figure on the upper left corner of each map, with an asterisk for endemic species). The phylogenetic relationships for these taxa are represented by a sub-sample of the Bayesian tree shown in Chapter 2 Figure 2.8.

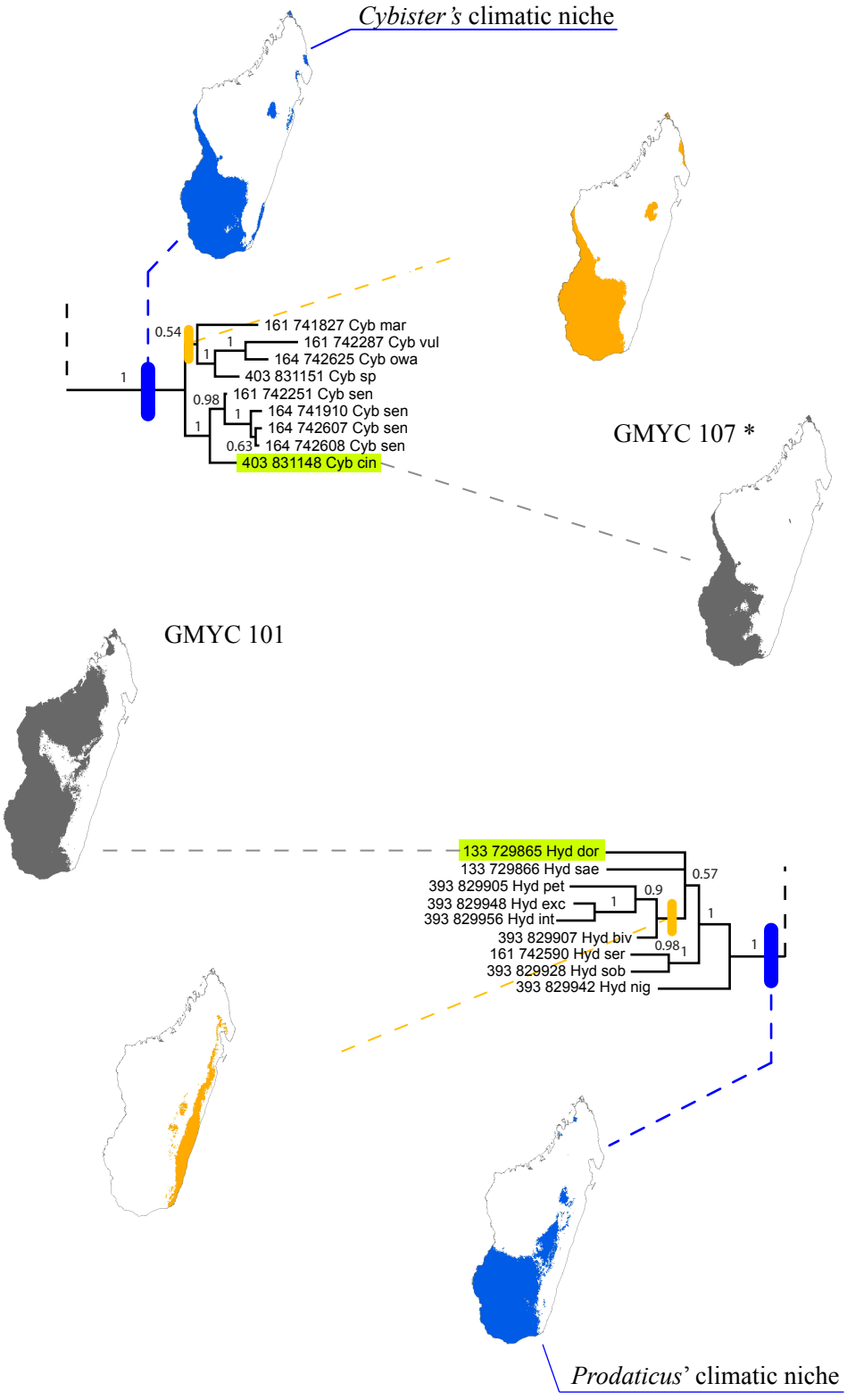


Figure 4.4: Climatic niche models for two genera of water beetles: *Cybister* and *Prodaticus*. Blue maps represent the biomod distribution models at the genus level, orange maps show the intermediate clades distribution models and grey maps show the GMYC species level distributions (species labels figure on the upper left corner of each map, with an asterisk for endemic species). The phylogenetic relationships for these taxa are represented by a sub-sample of the Bayesian tree shown in Chapter 2 Figure 2.8.

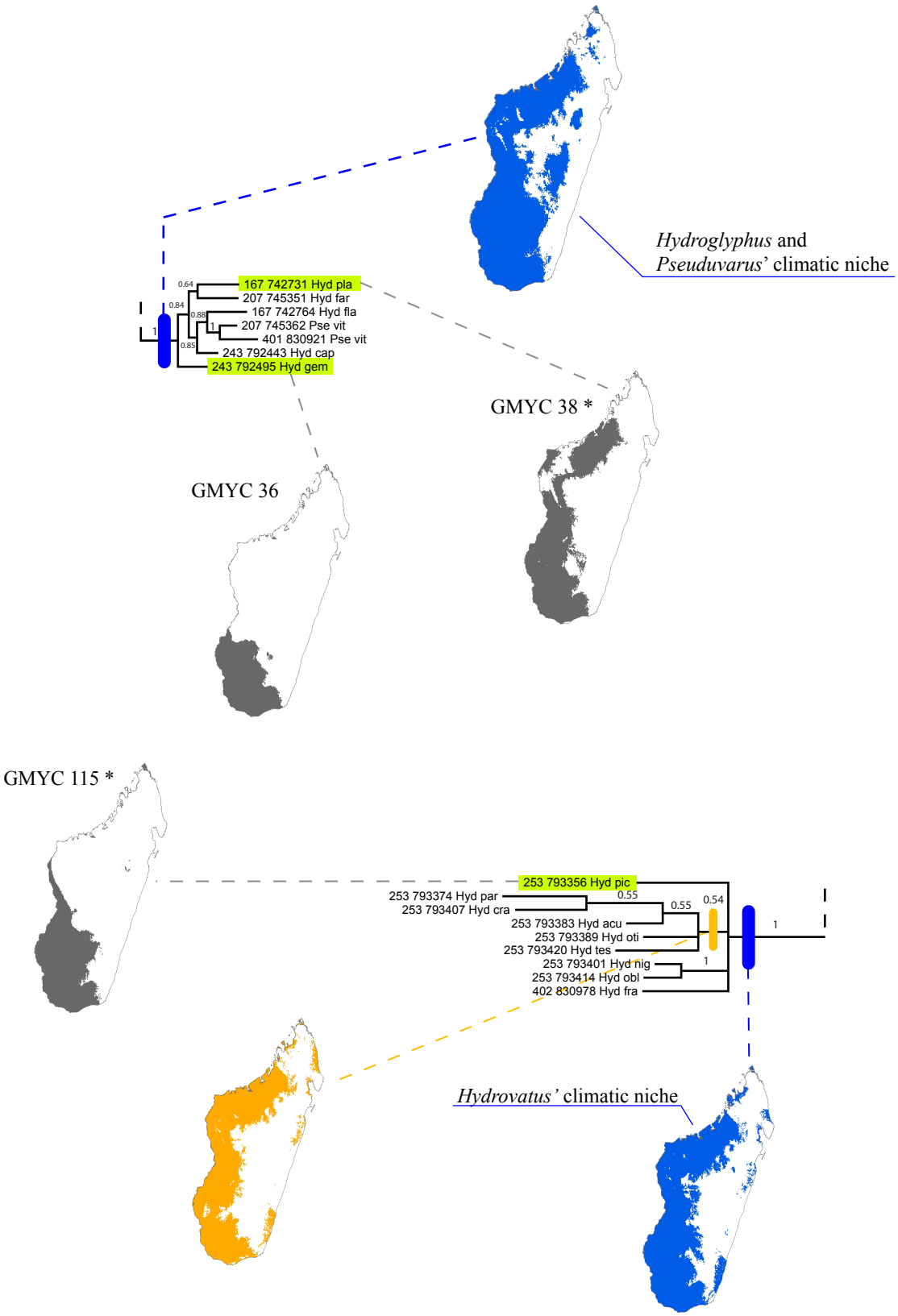


Figure 4.5: Climatic niche models for two genera of water beetles: *Hydroglyphus* and *Hydrovatus*. Blue maps represent the biomod distribution models at the genus level, orange maps show the intermediate clades distribution models and grey maps show the GMYC species level distributions (species labels figure on the upper left corner of each map, with an asterisk for endemic species). The phylogenetic relationships for these taxa are represented by a sub-sample of the Bayesian tree shown in Chapter 2 Figure 2.8.

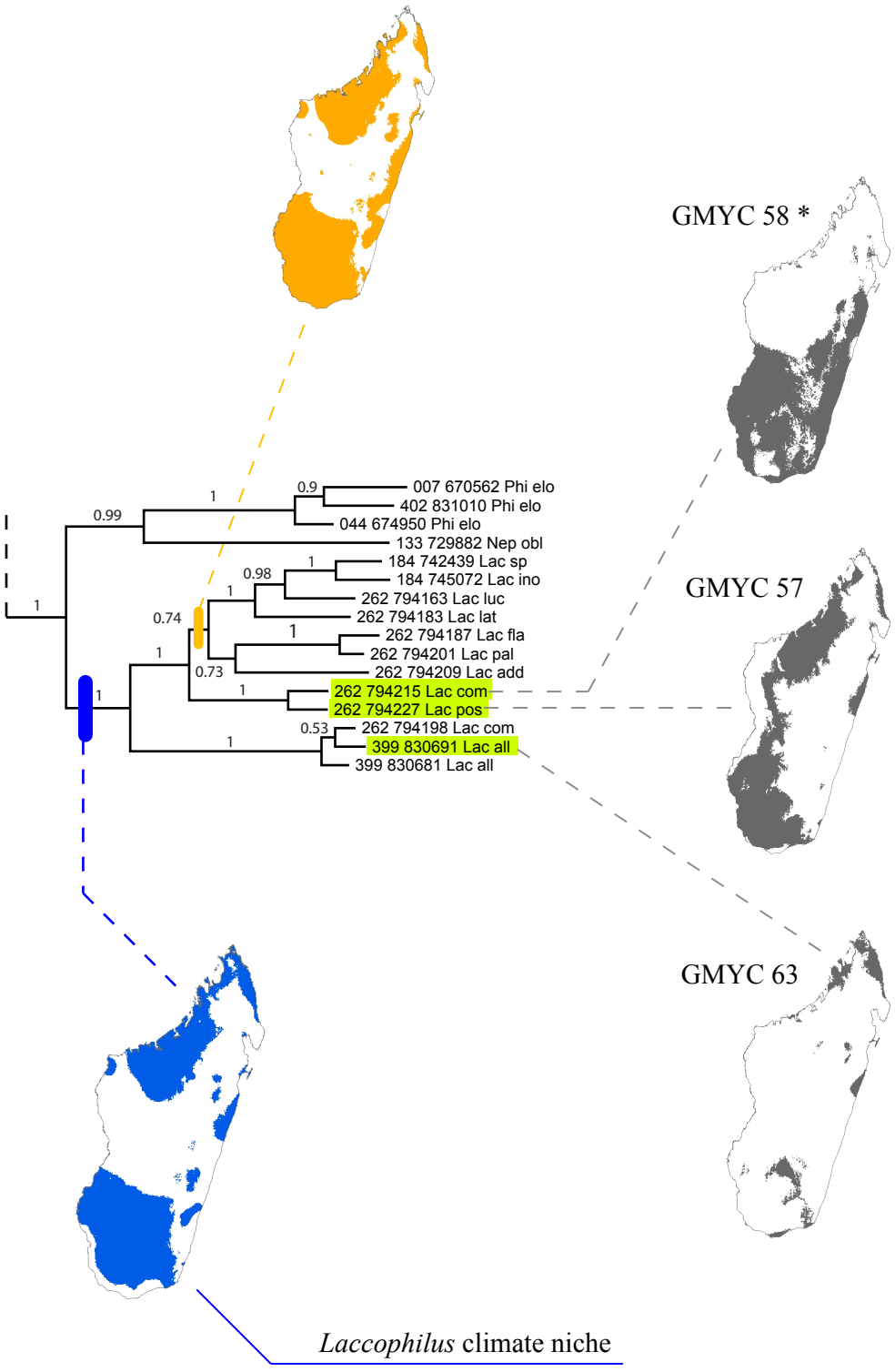


Figure 4.6: Climatic niche models for the water beetle genus *Laccophilus*. Blue maps represent the biomod distribution models at the genus level, orange maps show the intermediate clades distribution models and grey maps show the GMYC species level distributions (species labels figure on the upper left corner of each map, with an asterisk for endemic species). The phylogenetic relationships for these taxa are represented by a sub-sample of the Bayesian tree shown in Chapter 2 Figure 2.8.

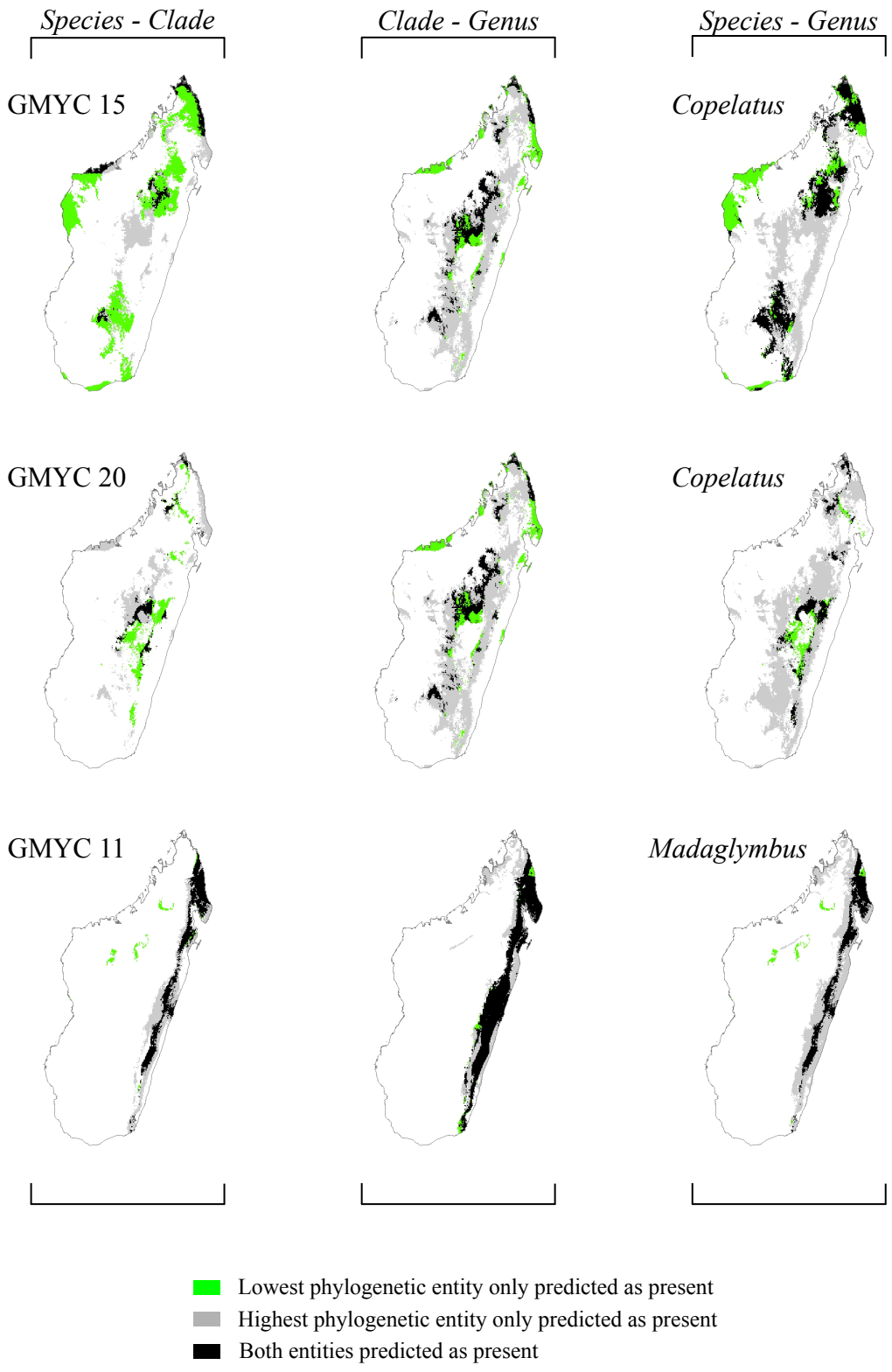


Figure 4.7: Conservatism of the climatic niche of three aquatic beetle species across three phylogenetic levels: GMYC species-clade (column 1), clade-genus (column 2) and GMYC species-genus (column 3). The GMYC label and the corresponding genus name are given for each line on the first and last maps respectively.

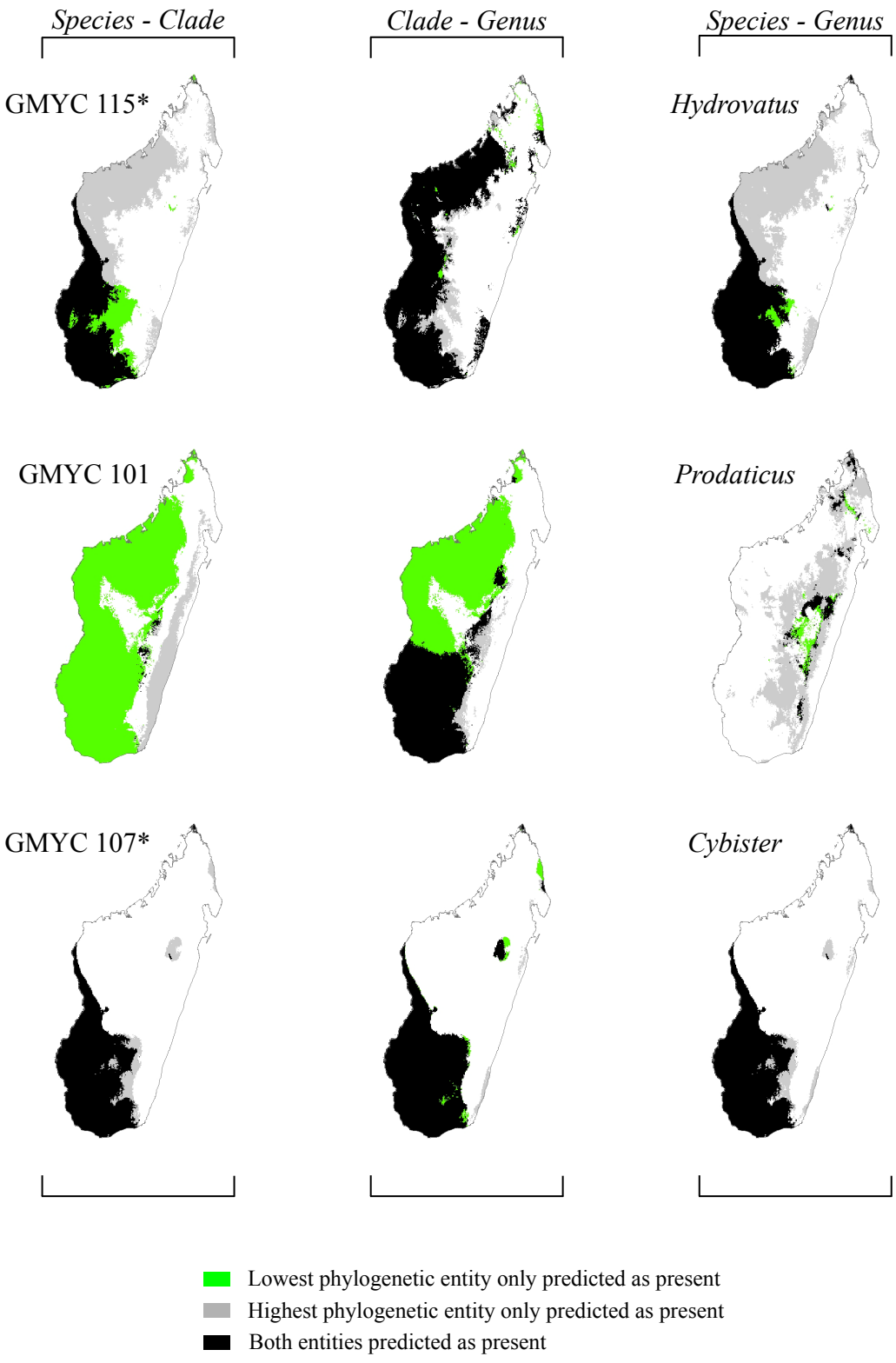


Figure 4.8: Conservatism of the climatic niche of three aquatic beetle species across three phylogenetic levels: GMYC species-clade (column 1), clade-genus (column 2) and GMYC species-genus (column 3). The GMYC label and the corresponding genus name are given for each line on the first and last maps respectively.

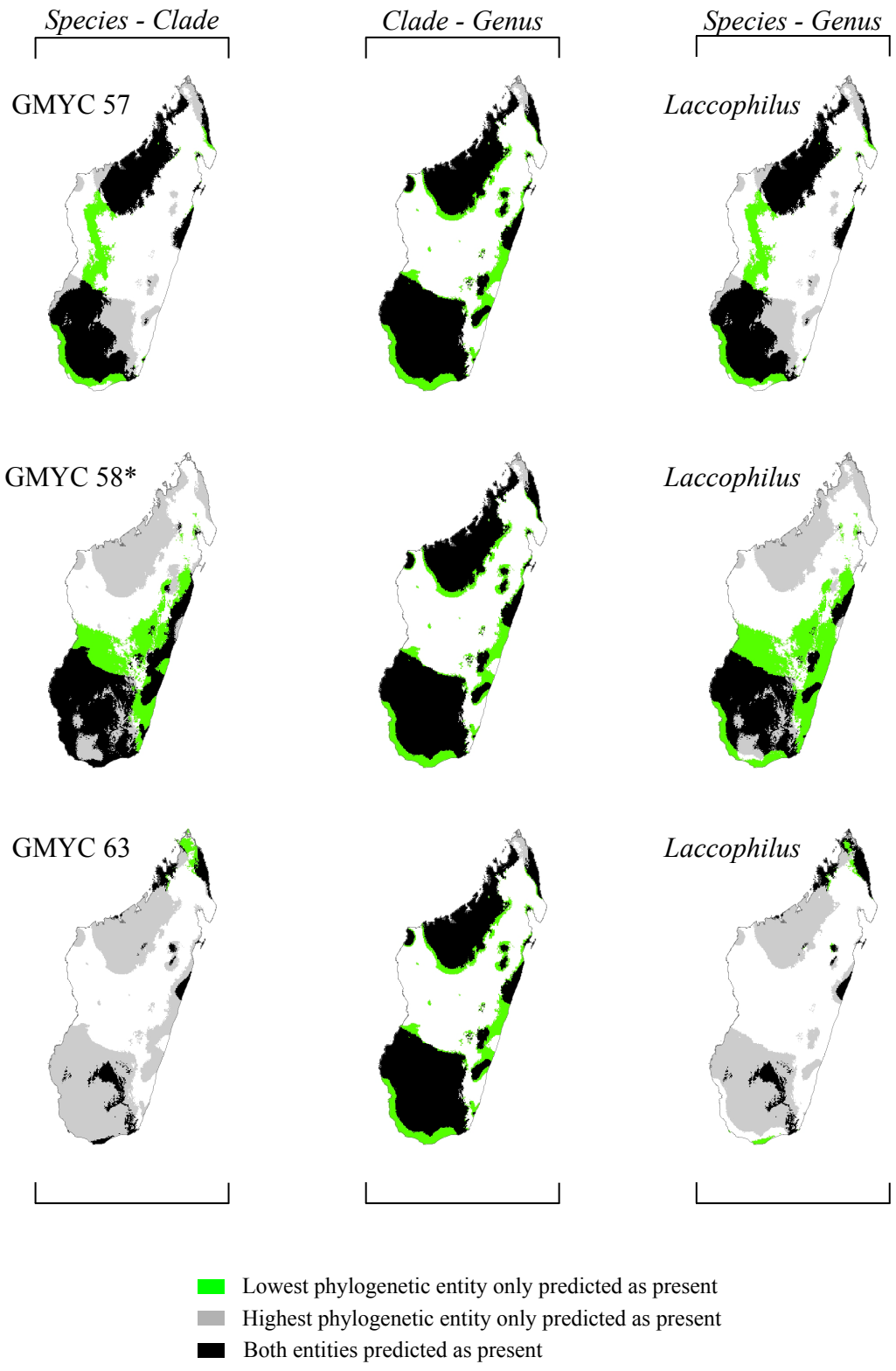


Figure 4.9: Conservatism of the climatic niche of three aquatic beetle species across three phylogenetic levels: GMYC species-clade (column 1), clade-genus (column 2) and GMYC species-genus (column 3). The GMYC label and the corresponding genus name are given for each line on the first and last maps respectively.

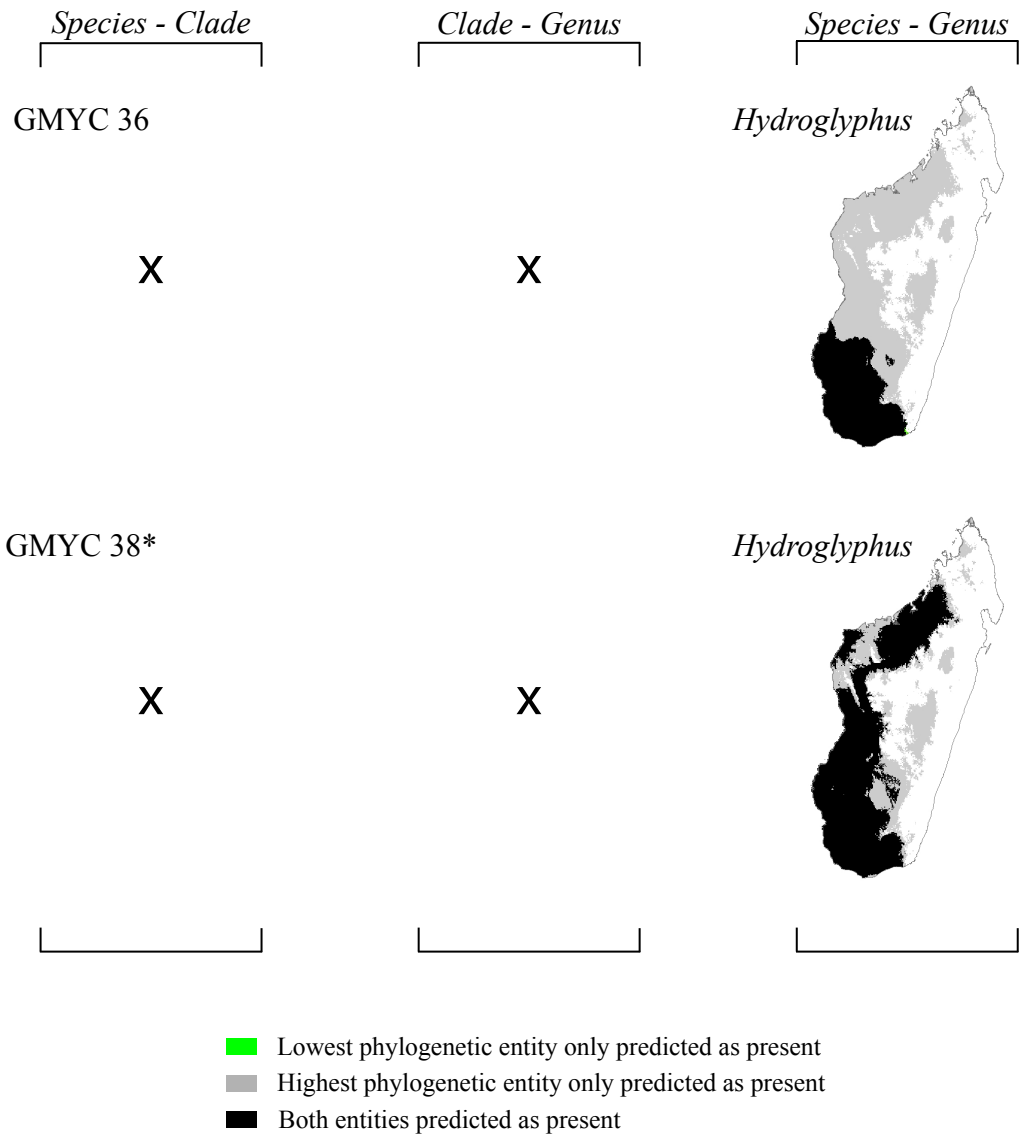


Figure 4.10: Conservatism of the climatic niche of two aquatic beetle species across the phylogenetic level GMYC species-genus (column 3). The GMYC label and the corresponding genus name are given for each line on the first and last columns.

species-clade and *species-genus* levels (*NC2* = 53.37% and *NC2* = 50.9% respectively). In the case of *sp36* and *sp38* (Figures 4.5 and 4.10), the minimum number of 10 occurences was not reached at any clade level, therefore only the *species-genus* level results are available. The two species showed an important niche divergence with their genus according to the *NC2* index only (68.08% and 31.97% respectively).

LPE	Species presences	Clade presences	Genus presences	*Species-Clade*		*Clade-Genus*		*Species-Genus*	
				NC 1	*NC 2*	*NC 1*	*NC 2*	*NC 1*	*NC 2*
sp11*	46554	89638	135744	11.12	53.84	4.18	36.73	10.54	69.32
sp15	130228	73376	211074	85.58	74.41	44.79	80.81	38.51	62.06
sp20	41236	73376	211074	68.13	82.09	44.79	80.81	37.08	87.71
sp36	140171	-	438259	-	-	-	-	0.19	68.08
sp38*	298140	-	438259	-	-	-	-	0.00	31.97
sp57	292960	396977	329103	12.04	35.08	19.15	2.47	20.44	29.18
sp58*	319958	396977	329103	33.98	46.78	19.15	2.47	48.93	50.35
sp63	56348	396977	329103	14.77	87.90	19.15	2.47	11.35	84.82
sp101*	479545	66612	253247	99.02	92.95	88.86	97.07	53.02	11.03
sp107*	205012	240018	238113	0.00	14.58	3.75	2.98	0.00	13.90
sp115	209686	354349	408491	21.20	53.37	2.47	15.40	4.34	50.90

Table 4.3: Presence pixels counts for the species, clade and genus distributions given per LPE and niche conservation indices at the three phylogenetic levels (given in %).

All GBM response curves to the individual climate variables are presented in Appendix III, Figures S4.1 to S4.17 and the relative influence of the predictors in Figures S4.18 to S4.20. The response curves for the GAMs are available in Figure S.4.21 and the summary statistics for the GLMs are given in Table S4.2.

4.5 DISCUSSION

4.5.1 Niche uniqueness vs dispersal limitations

Madagascar's climate variability has recently been considered as one of the main parameters influencing local endemism patterns (Pearson and Raxworthy, 2009) but also as an important contributor to insular speciation that led to the exceptional level of global endemism of the island (Dewar and Richard, 2007). Modelling the distribution of endemic GMYC species on the global scale offered here the possibility to investigate the extent of the respective climatic niche, and assess the range of habitat suitability on the mainland or other islands. We found that three endemic GMYC species presented high probabilities to find substantial suitable climatic environment in Africa and eventually India, whereas the remaining four endemic species showed environmental niches mainly confined to Madagascar. The high AUC values obtained for all models could be explained partly by the narrow range of the species relative to the total study area (which is an artefact of the AUC statistic), and might not represent the actual predictive performance of the model. But the models run on the non-endemic GMYC species only based on Malagasy occurrences lead to positive predictions of most known distributions for the corresponding Linnaean species, which adds to the reliability of the models if we consider the accuracy of the taxonomic identifications performed. We are aware though that the interpretation of these results depends also on the sampling effort both at the local and global scale: on one hand, insufficient sampling within Madagascar could lead directly to erroneous predictions, on the other hand, insufficient taxonomic sampling in Africa or other mainland would result in a misclassification of the Linnaean species as endemic, in which case the predictions of endemic GMYC species outside Madagascar could be

the actual distribution of a true non-endemic species. The large-scale taxonomic sampling bias can hardly be controlled for, and thus we assume that Linnaean species described only from Madagascar are genuinely endemic. Regarding the local sampling issue, the low number of presence records in this study was obtained from a total of 142 collecting sites across the island and its ecoregions and is not the result of a poor sampling effort: it comes together with a high number of absences and reflects the true rarity of the species in the field. We conclude that Malagasy water beetle endemism at the global scale is caused for some species by the uniqueness of their climatic niche and its confinement to the island, and otherwise that dispersal capacities remain the foremost obstacle to colonising suitable habitats present on the mainland and surrounding Indian Ocean islands.

4.5.2 Niche conservatism across phylogenetic levels

Madagascar's global endemism features are well recognized but the mechanisms structuring its biodiversity at the local scale remain poorly understood (Pearson and Raxworthy, 2009). In fact, few studies were successful at exploring it, mainly because the scarceness of micro-endemic organisms inhibits the collection of sufficient biological data for robust ecological investigation. In this study, we illustrate the advantages of using a novel approach combining molecular species delimitation, subsequent multi-markers phylogenetic reconstructions and ecological niche models across several phylogenetic levels to quantify the impact of climate variability on species diversification on the island. Similar integrative methods are still being developed but proved their efficacy already at delimiting species (Rissler and Apodaca, 2007) and understanding speciation mechanisms (Graham *et al.*, 2004, Jakob *et al.*, 2007, Pyron and Burbrink, 2009). Though, most of these examples focus

on a limited number of taxa: either one species complex or a group of species within the same genus. Here, we targeted all taxa with sufficient occurrence data within a fauna comprising 169 GMYC species, and assessed the general role of niche divergence for water beetle species diversification. We were able to conduct this analysis on 11 species, 7 genera and 6 clades. When comparing the predicted climatic niche of GMYC species to those of their closely related clade and respective genus, we observed a propensity for high levels of divergence: only one GMYC species out of 11 showed conservation indices below 35%, and more than half of the species' index values went beyond 60%. In particular, within the entire endemic radiation of *Madaglymbus*, the *species-genus* comparison level reached a niche conservation index of 69%. For the cases where the lowest phylogenetic entity was not endemic, it has to be considered that each clade contained at least one endemic GMYC species. As a result, all three-level comparisons gave indications on the degree of niche divergence between endemic and widespread species. It is arguable that the list of widespread species included in our dataset is not exhaustive, and that additional sampling in mainland Africa would modify both the phylogeny used as a framework for this analysis and the niche predictions at the clade and genus levels. However, given the uniqueness of Madagascar's climate with respect to mainland Africa (de Wit, 2003, Dewar and Richard, 2007), we anticipate that the incorporation of external climatic variables in the models would result in even higher niche divergence between endemic and non-endemic species. In accordance with Pearson and Raxworthy (2009), we conclude that climate variability plays an active role in the species diversification of the aquatic beetle fauna, and that local endemism is patterned up on climatic niche differentiation.

CHAPTER 5

General discussion

This chapter presents a summary of the main findings of the study regarding the quantification of the insect fauna and its endemism, the consequences of microhabitat differential stability on local endemism patterns, and the role of climatic variability in generating global and local endemism. Potential issues related to the different approaches or the results themselves are discussed within each part. The final point of this discussion deals with the achievement of the project and the resulting prospects.

5.1 INSECT DIVERSITY AND ENDEMISM IN MADAGASCAR

The GMYC approach has recently been successful at delimiting species within poorly known groups (Pons *et al.*, 2006, Fontaneto *et al.*, 2007, Papadopoulou *et al.*, 2008, 2009a, 2009b). In particular, it has been applied to quantify insect diversity in Madagascar (Monaghan *et al.*, 2009), using a sub-sample of the termite, butterfly, water beetle and mayfly faunas. Here we present the first comprehensive inventory of the Malagasy water beetle fauna, by the means of thorough sampling across the island, targeting both the diversity of macrohabitats (ecoregions) and freshwater microhabitats. Based on more than four years of intensive fieldwork and laboratory work, we identified 169 species with the GMYC approach, estimated to represent more than 74% of the total species richness of the island, and showing very high spatial turnover both at the species and haplotype levels. The taxonomic identifications conducted on the dataset only distinguished 83 species, and allowed us to assess the level of endemism at 51% at least. Though this latter value was calculated with the exclusion of all species of the highly endemic genus *Copelatus* (because of the lack of taxonomic expertise for this group) and was based only on

GMYC species congruent with Linnaean endemic species. Without the incongruent species, endemism reaches approximately 61%, and when removing the unidentified *Copelatus* species, the value goes up to 70%. Taxonomic identifications within the genus *Copelatus* and accurate knowledge of the endemism status of the 31 incongruent GMYC species would bring the level of endemism to at least 75%. Moreover, this DNA-based inventory revealed the presence of approximately 30 non-described species. We concluded that today, water beetles, and very probably numerous other beetle families and insect faunas in Madagascar, are strongly underestimated both in terms of species diversity and endemism. We put forward for consideration that insects in Madagascar not only would exceed the combined alpha diversity of mammals, reptiles, amphibians and birds, but also could equal the outstanding percentages of endemism characterising the flagship vertebrates and plants. Insects on the number-one biodiversity hotspot are completely left aside in comparison to floras and other faunas, but a comprehensive inventory of their diversity and endemism could raise Madagascar to a new status of uniqueness.

5.2 HABITAT STABILITY AND LOCAL ENDEMISM

Lotic and lentic species showed divergent patterns of beta diversity on the island, in agreement with the documented differential habitat stability and its associated dispersal patterns in freshwater biodiversity (Ribera and Vogler, 2000, Bilton *et al.*, 2001, Ribera *et al.*, 2001, 2003, Ribera, 2008): spatial turnover was higher among lotic sites than among lentic sites, and biotic similarities were strongly constrained by the climate variability for the lotic fauna only. Geographic distance correlated to the species composition of both communities but over longer distances

for the lotic species. Additionally, the ancestral character state analysis suggested a dependent evolution of endemism and habitat types. We concluded that lotic habitat stability, engendering strong niche restriction and low dispersal activities, offers propitious conditions for a high microendemic structuration of the fauna. However, it has to be considered that classifying species as lotic or lentic is both a critical and delicate task, often left implicit within the literature. While dealing with Linnaean species, it can be straightforward to obtain this type of information from previous descriptions of the taxa and their ecology. In this study, the species were delimited from DNA sequence divergence, and despite the partial congruence with the Linnaean species, the classification as lotic or lentic relied essentially on the number of lotic and lentic sites where they were collected. In the field, GMYC species could be encountered in equal numbers in running and standing waters, and therefore were placed in a third category: the lotic-lentic (or RSW). We argue that with supplementary ecological knowledge or additional sampling, some of the GMYC species within this third category could be reclassified as lotic only or lentic only, changing the quantitative results of the analysis but not the qualitative conclusions.

5.3 THE ROLE OF MADAGASCAR'S CLIMATE IN GENERATING LOCAL AND GLOBAL ENDEMISM

Madagascar's unpredictable climate (Dewar and Richard, 2007) has recently been put forth as a potential promoter of the substantial insular speciation responsible for the extreme local and global endemism patterns (Wilme *et al.*, 2006, Dewar and Richard, 2007, Pearson and Raxworthy, 2009). The climatic niche modelling approach on the large scale allowed distinguishing between species for which

endemism is best explained by the uniqueness of the niche and endemic species for which potentially suitable habitat is widely distributed on the mainland. In the latter case, we concluded that dispersal was the main hindrance to occupying the full extent of the niche, and thus responsible for the confinement of species on the island. Locally in Madagascar, niche conservatism across phylogenetic levels was found very poor, stressing the potential role of climate in ecological speciation, and suggesting that local endemism is patterned on the distribution of individual climatic niches. We concluded that climate variability and uniqueness in Madagascar could play a major role in both the diversification of species, and their isolation, resulting in high levels of endemism on the global and local scales.

From the methodological point of view, the combination of a fast DNA inventory and ecological niche modelling across an entire fauna constituted a relatively new approach with an exploratory component. The use of ecological niche modelling to answer fundamental questions on the evolutionary history of lineages, niche evolution and ecological speciation has recently emerged in the literature (Graham *et al.*, 2004, Rissler and Apodaca, 2007, Murienne *et al.*, 2009), but has been limited so far to the study of single species complexes (Jakob *et al.*, 2007, Pyron and Burbrink, 2009), sister species comparisons (Warren *et al.*, 2008), or multiple species within the same genus (Murienne *et al.*, 2009). To our knowledge, this study is a first attempt to investigate the role of niche conservatism and divergence in promoting speciation on an entire fauna, in a geographic region renown for its exceptional endemism across taxa. The main challenges in this approach resided first in the difficulty of cataloguing the complete species diversity of a large and complex area. The accelerated DNA-based inventory provided an efficient solution to this problem, but engendered a new issue in the face of distribution modelling: when sufficient

occurrence data can usually be obtained at the Linnaean species level, the subdivision of Linnaean species into multiple GMYC species reduced considerably the sample size for each molecular entity. As a result, the minimum number of incidences required for modelling the ecological niche of species was reached only for a subset of the 169 taxa. In these conditions, the recent quantitative approaches to niche conservatism between sister taxa (Kozak and Wiens, 2006, Warren *et al.*, 2008) were unsuitable throughout the water beetle phylogeny. Our alternative approach consisted in testing for niche conservatism across phylogenetic levels instead of between sister species. We insist on the fact that this method doesn't aim to predict distributions of entire genera or clades (for which the biological meaning is questionable) but uses their hypothetical niche models for the quantification of niche conservatism across a given phylogeny. This method presents the additional advantage to include rare endemic taxa within the chosen clades, for which occurrence data are often too sparse to be used in this context.

5.4 PROMISES OF MULTIDISCIPLINARY APPROACHES

Species being one of the most fundamental units in biology (De Queiroz, 2007), advances in ecology and evolutionary biology suffer greatly from the current taxonomic impediment (Tautz *et al.*, 2003). The recent craze for DNA taxonomy and species delimitation methods improved considerably our appreciation of the number of living things, and brought new hopes to understand the macroecological and evolutionary processes responsible for the global biodiversity patterns. Yet, the integration of the latest developments in the fields of molecular phylogenetics, ecology, and environmental niche modelling have just began (Graham *et al.*, 2004,

Graham and Fine, 2008, Peterson and Nyari, 2008, Carnaval *et al.*, 2009, Moussalli *et al.*, 2009) and its full potential to address these issues remains unexploited.

Over a four-year timescale, we brought an accelerated DNA-based inventorying method to an evolutionary perspective, and proved its efficacy to evaluate the alpha diversity and the level of endemism of one of Madagascar's faunas and assess its number of non-described species. We reconstructed the phylogenetic relationships within the group to suggest a dependent evolution of endemism and habitat types. We further combined these results to a traditional beta diversity approach and were successful at explaining molecular species turnover with environmental variability, and proved the role of habitat stability and dispersal in patterning microendemism at the genetic level. We finally integrated these findings in a mixed ecological niche modelling approach at the species level on the global scale, and at three distinct phylogenetic levels on the local scale, to emphasize the importance of Madagascar's unique climate in promoting the diversification and the isolation of lineages within insect faunas.

This research project provides an instance of the possibilities offered by the combination of molecular phylogenetics, traditional ecology and ecological niche modelling, by leading to concrete answers on the causes of insect endemism in the most exceptional reservoir of biodiversity.

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Appendices

Appendix I: Supplementary material for Chapter 2

Figure S2.1 **134**
Parsimony tree reconstruction of the complete water beetle dataset (2043 sequences, 4 families).

Figure S2.2 **149**
Water beetle *cox1* parsimony tree – complete mtDNA cluster delimitation.

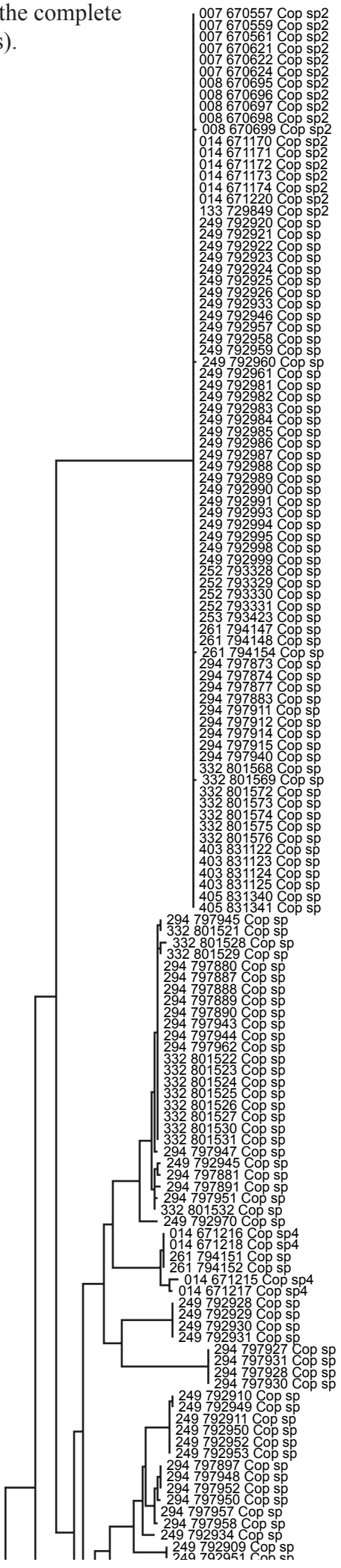
Figure S2.3 **159**
Ancestral state reconstructions of the water beetle fauna for two characters: endemism status (left: endemic in black, widespread in white) and aquatic habitat (right: RSW in black, SW in grey, RW in white and H in light grey). The two clades detailed in Figure 2.9 are shown with vertical grey bars.

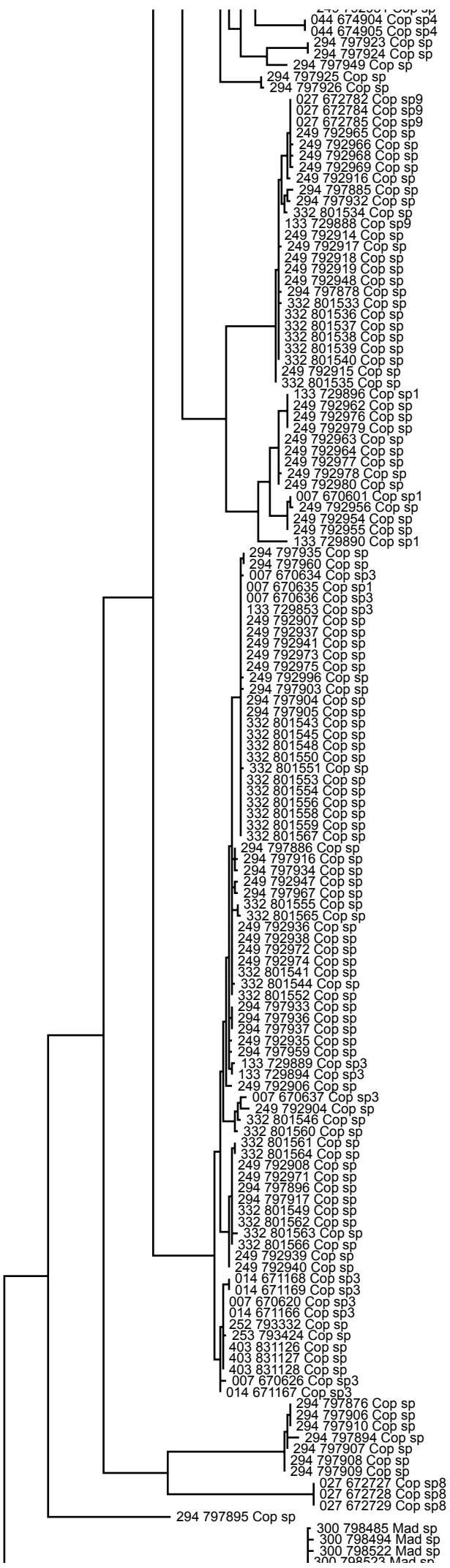
Table S2.1 **160**
Description of alignments for the length variable gene region (28S). The alignment that gave the lowest incongruence with the protein coding region was selected.

Table S2.2 **161**
Taxonomy, habitat specificities and molecular characteristics of the different entities delimited by the GMYC model.

Table S2.3 **164**
Proportional likelihood values obtained for both Habitat and Endemism characters during the ancestral state reconstruction (states judged best according to the Mesquite threshold are marked with an asterisk).

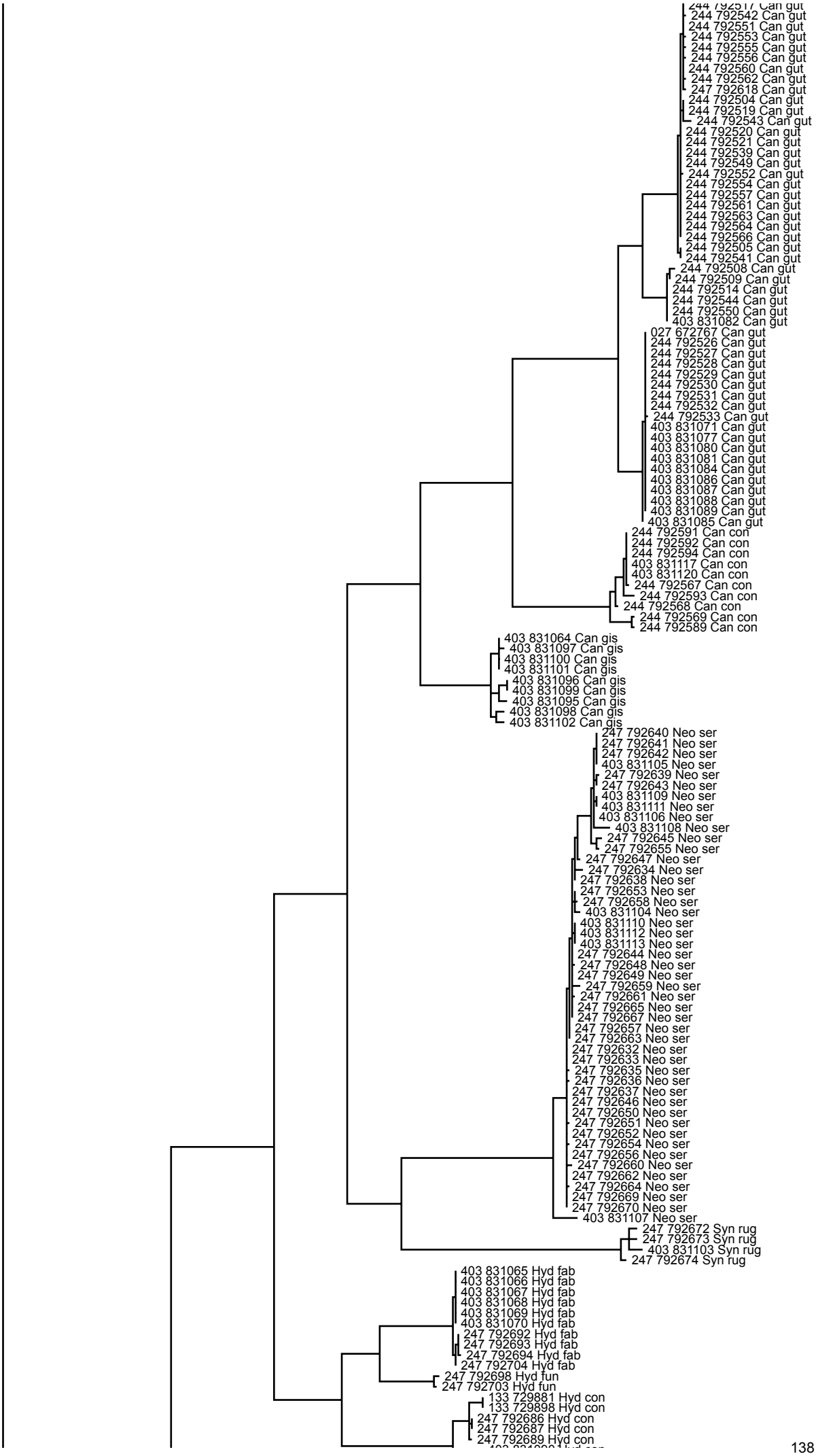
Figure S2.1: Parsimony tree reconstruction of the complete water beetle dataset (2043 sequences, 4 families).

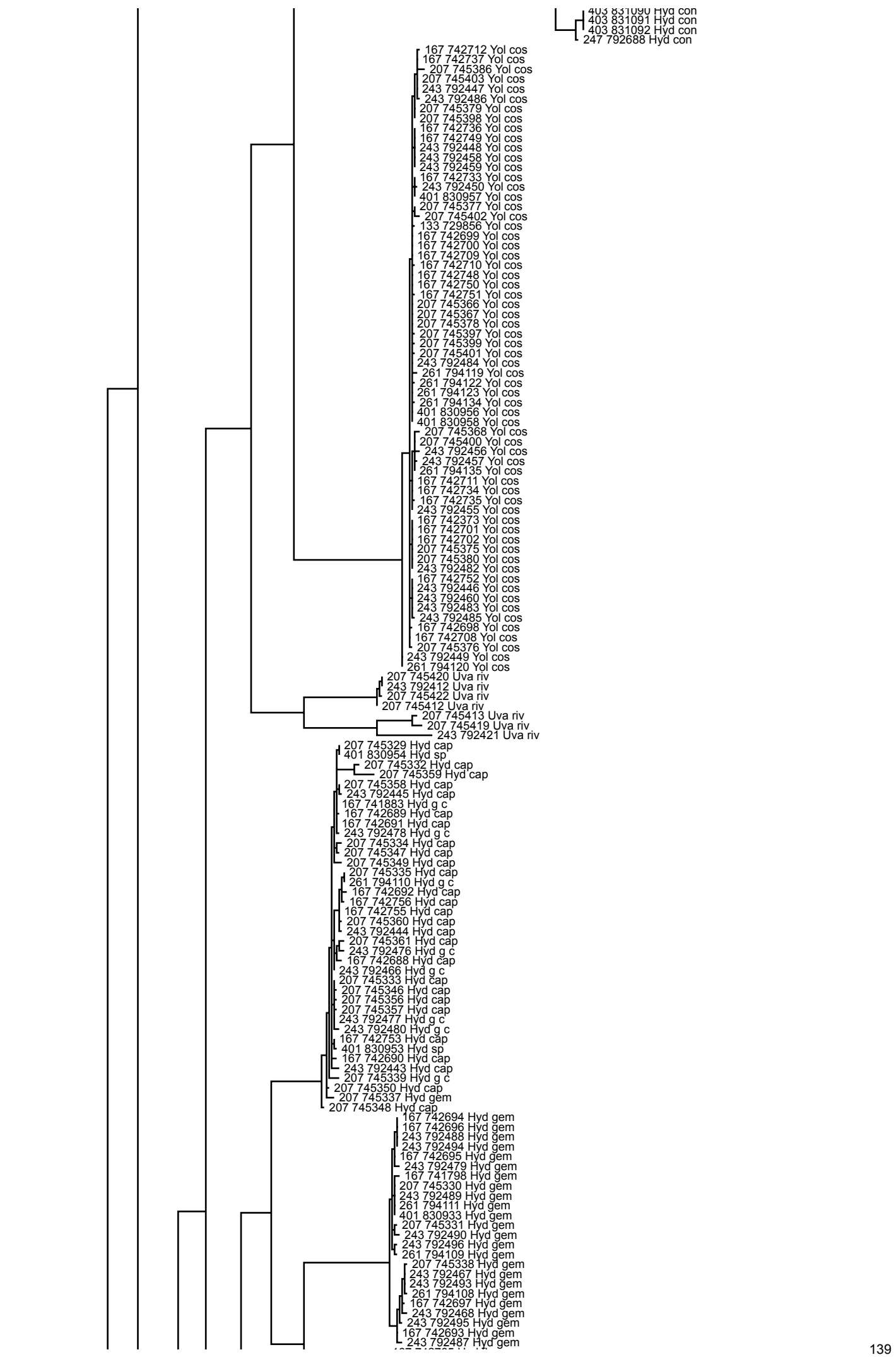



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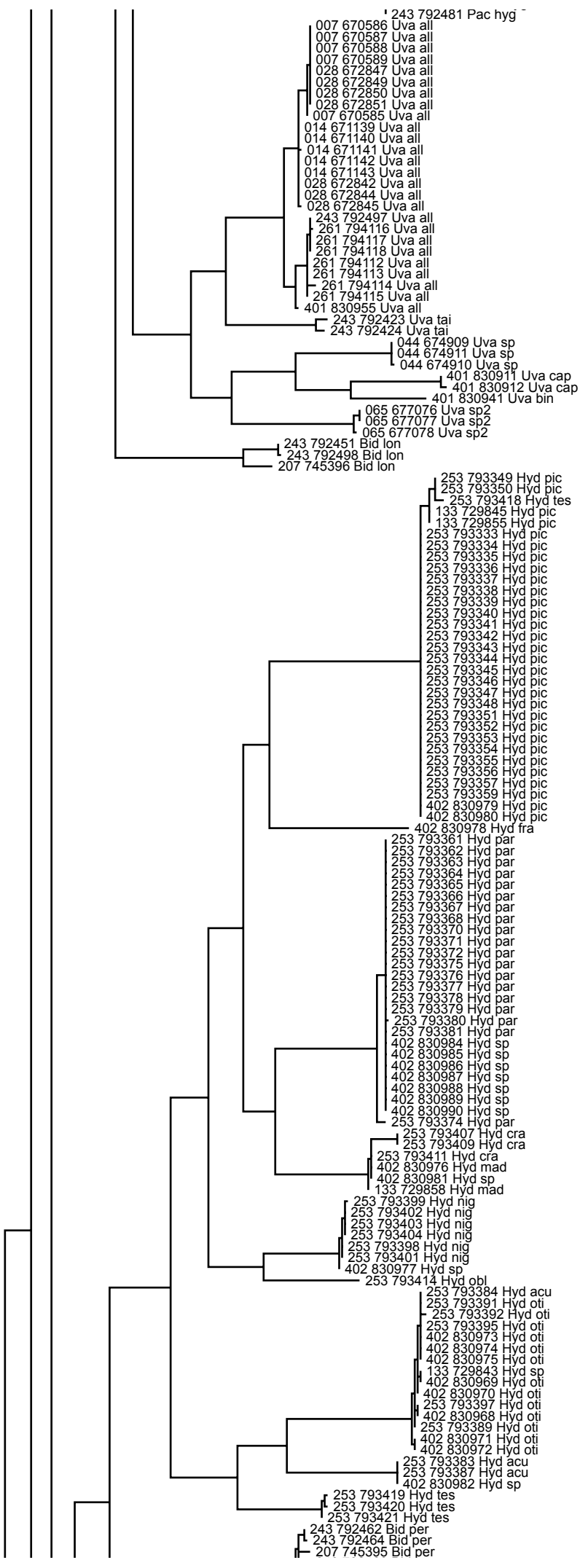

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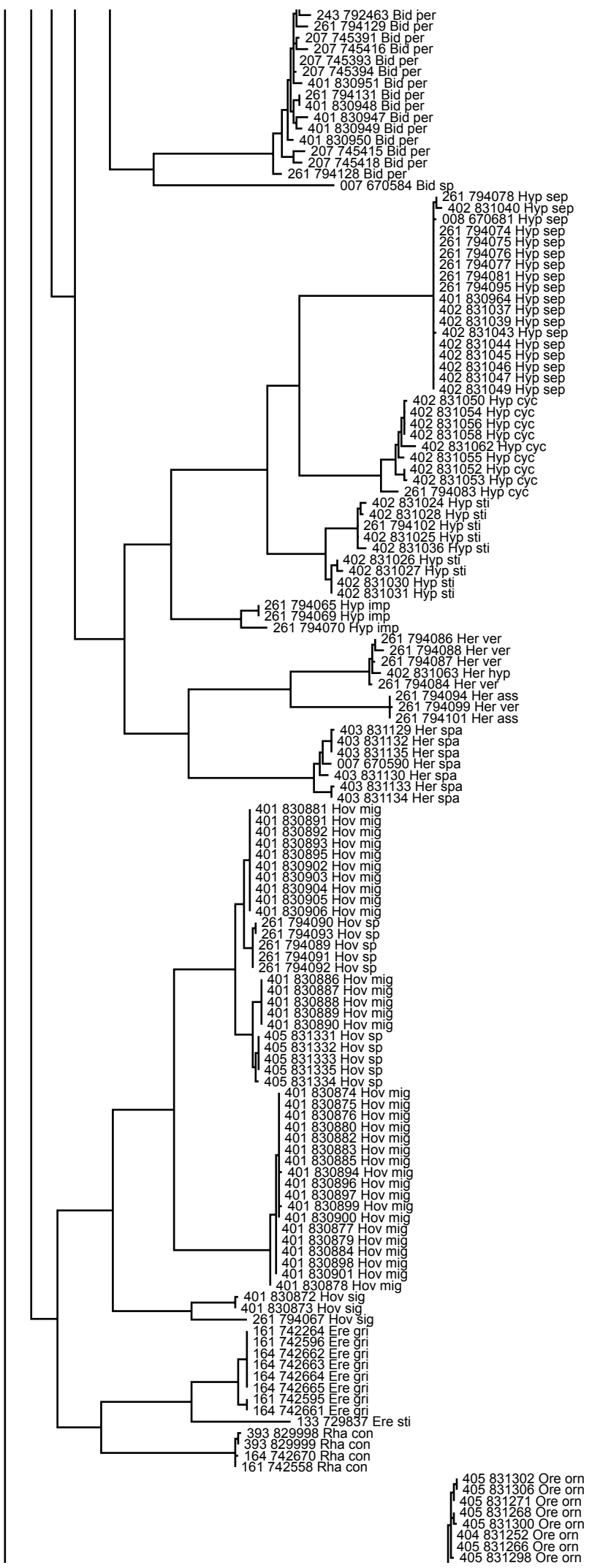

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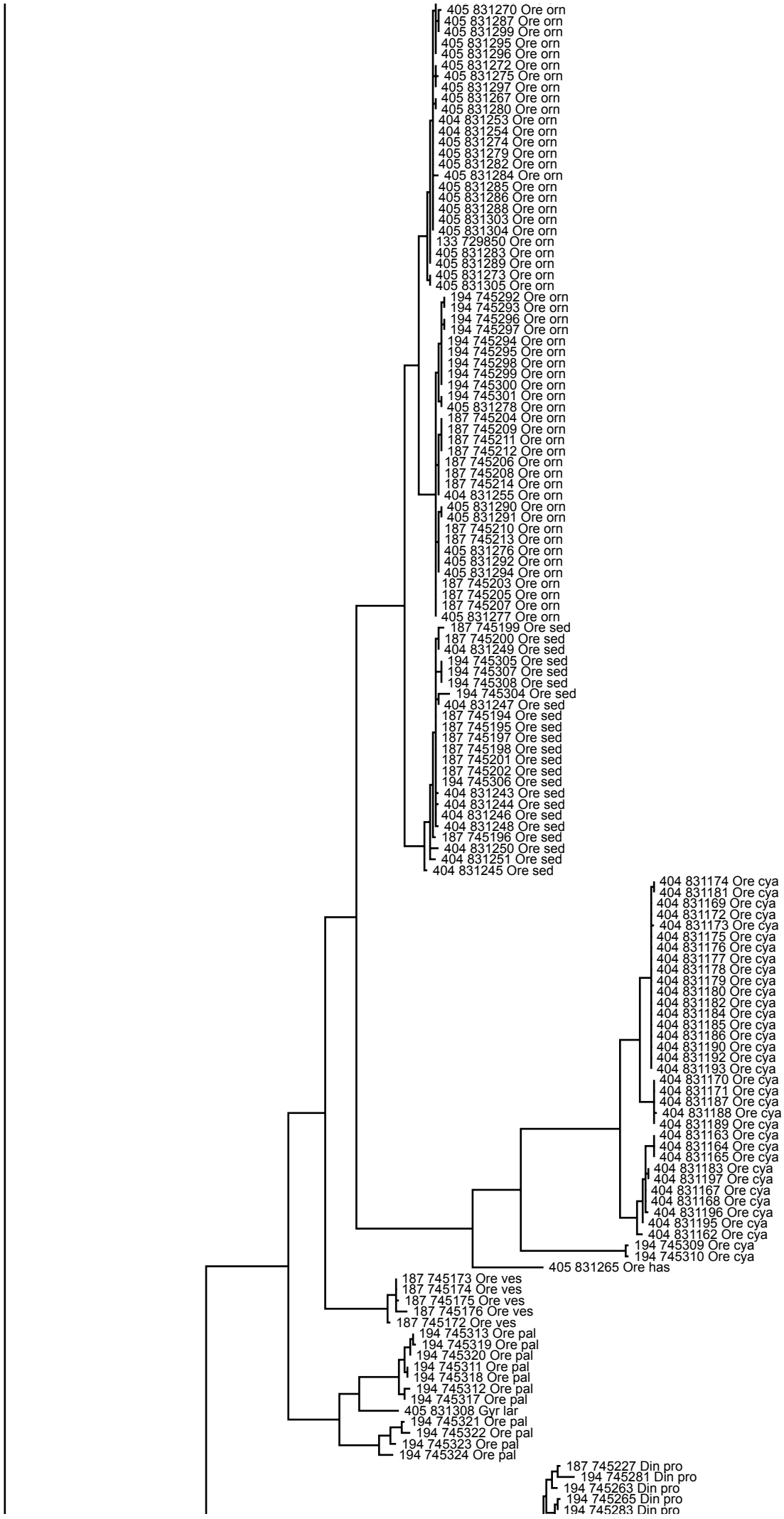

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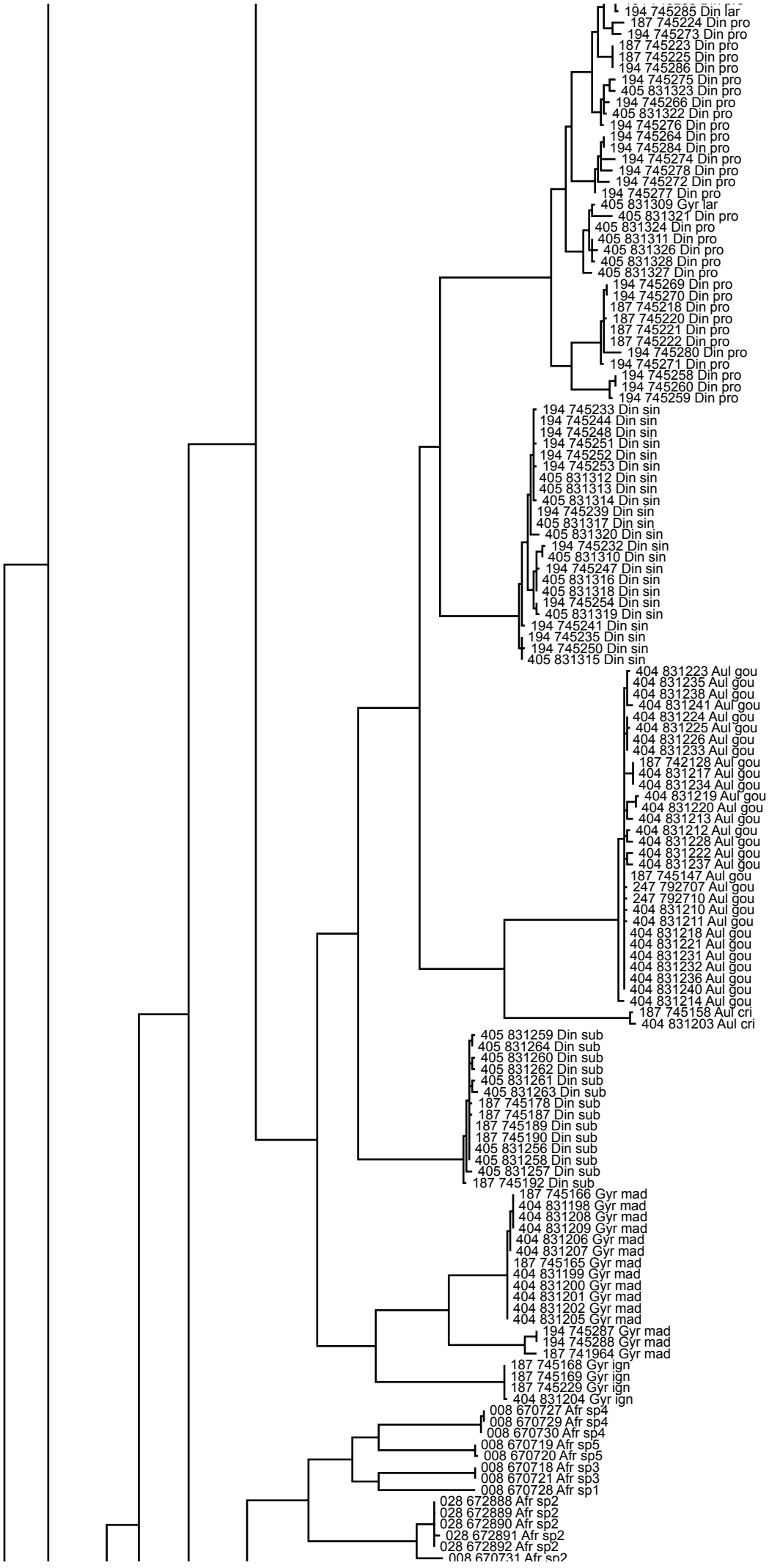

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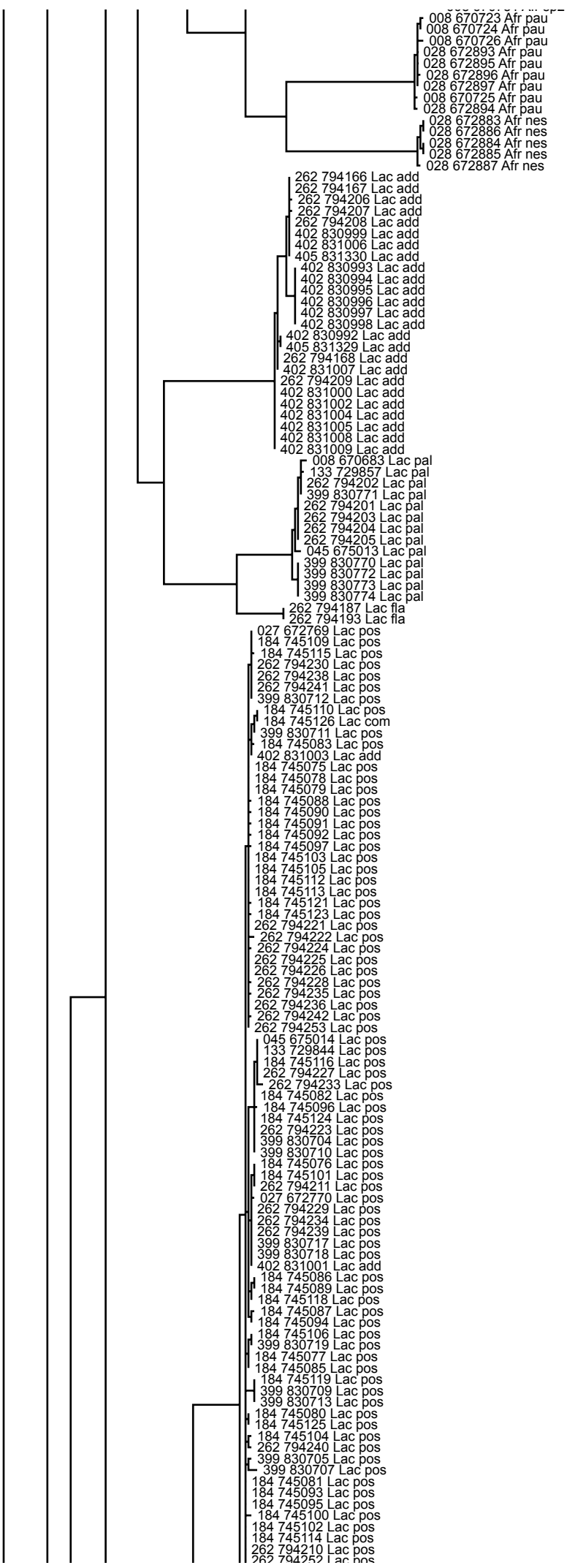

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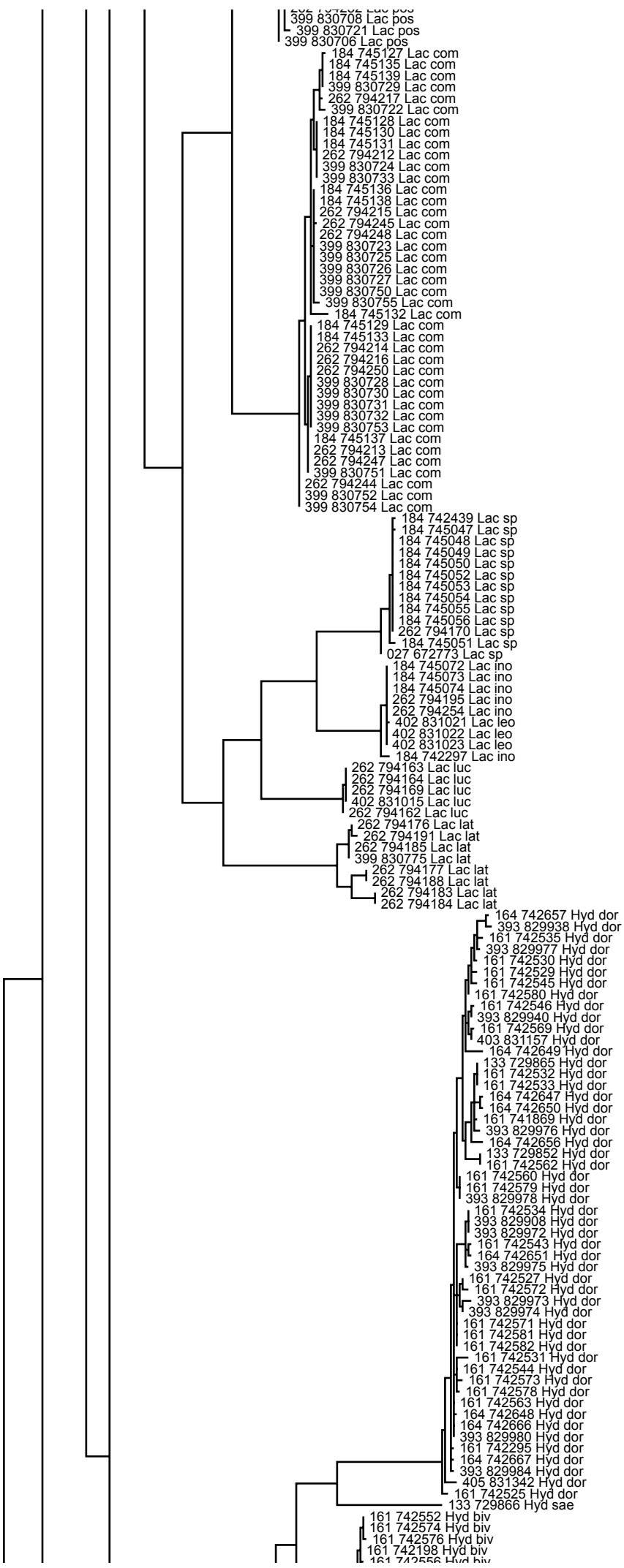

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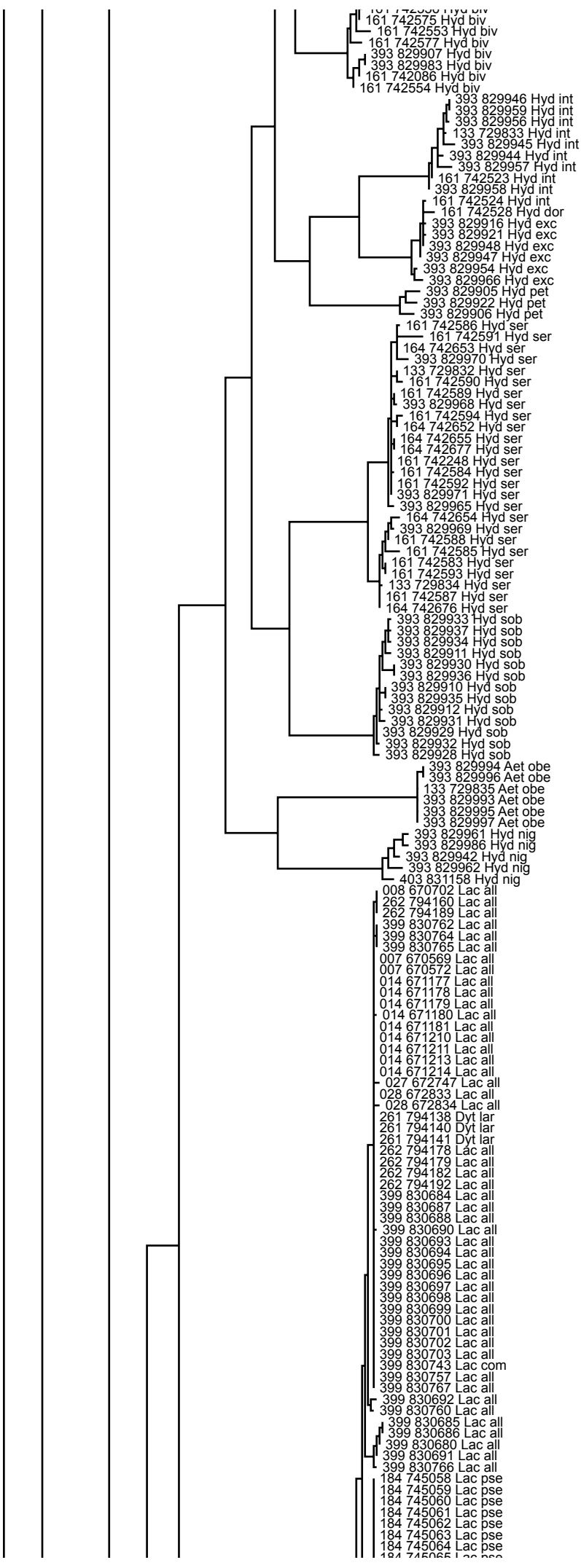

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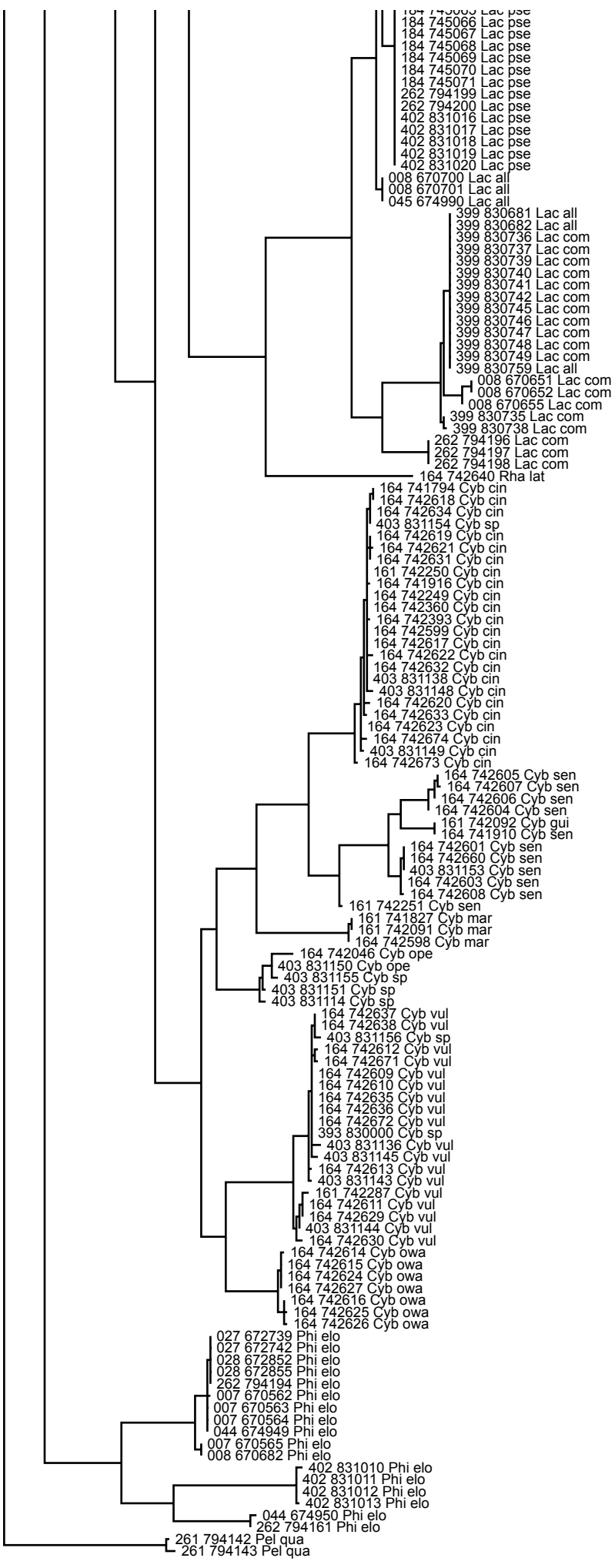
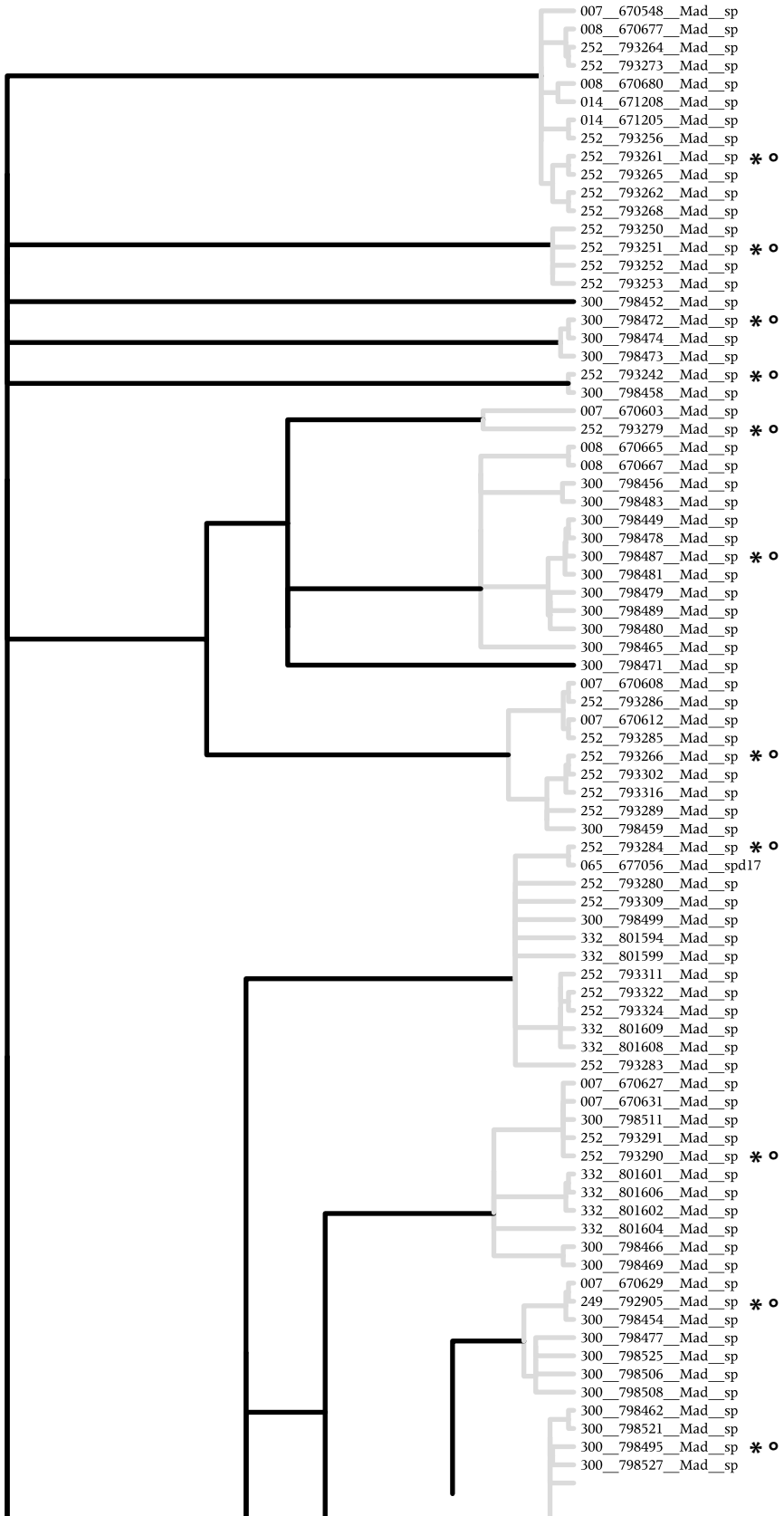
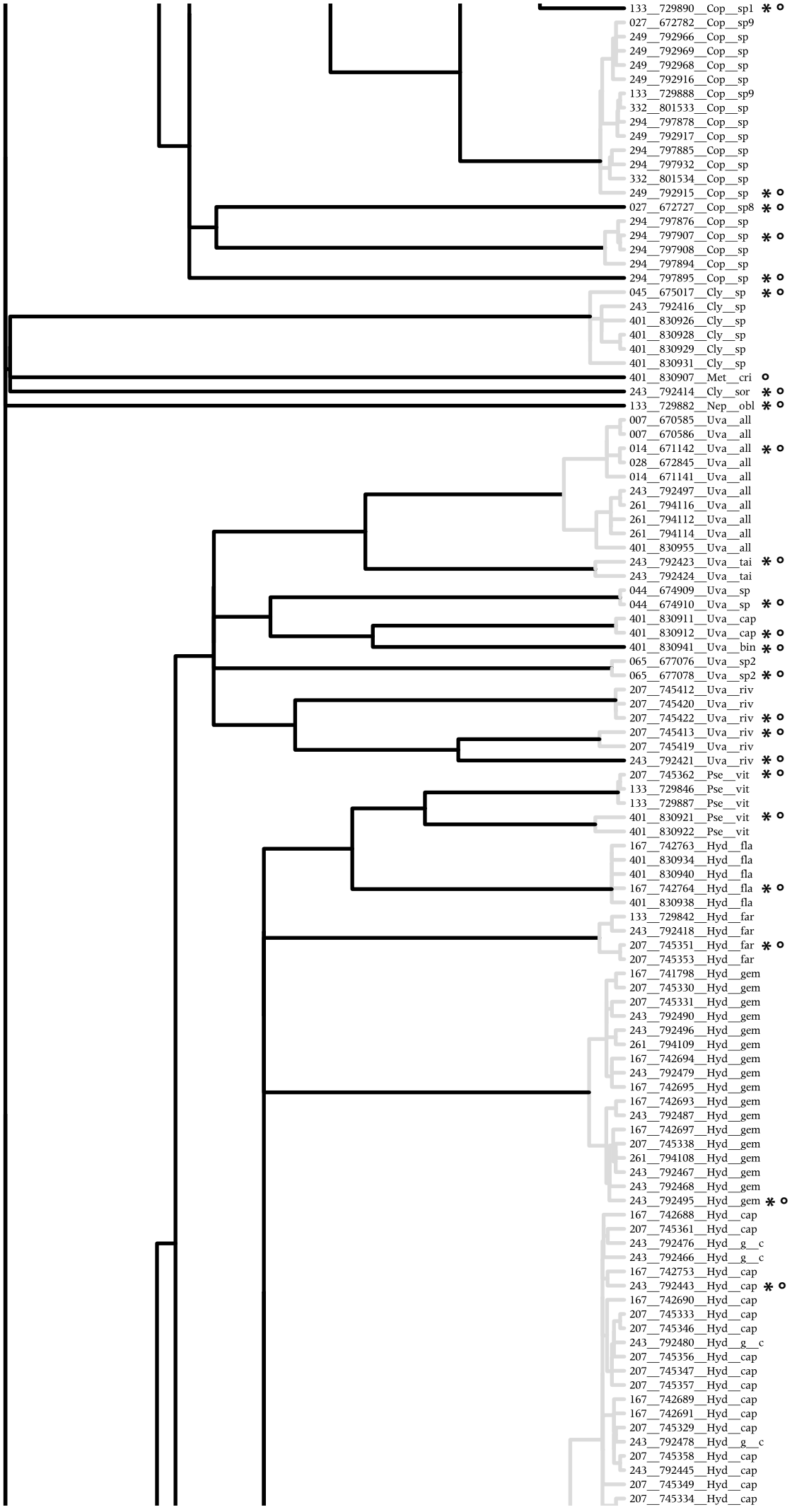
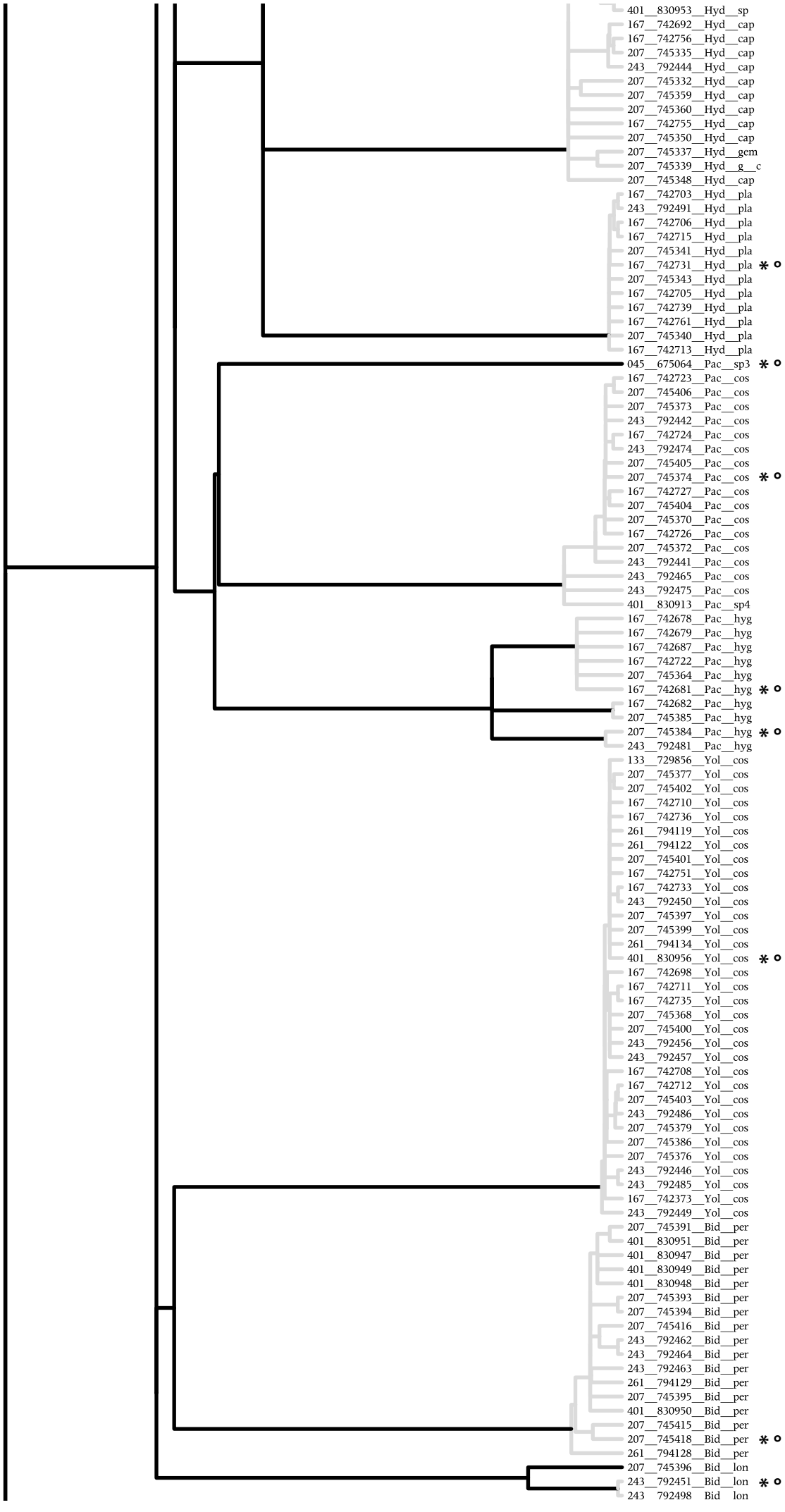

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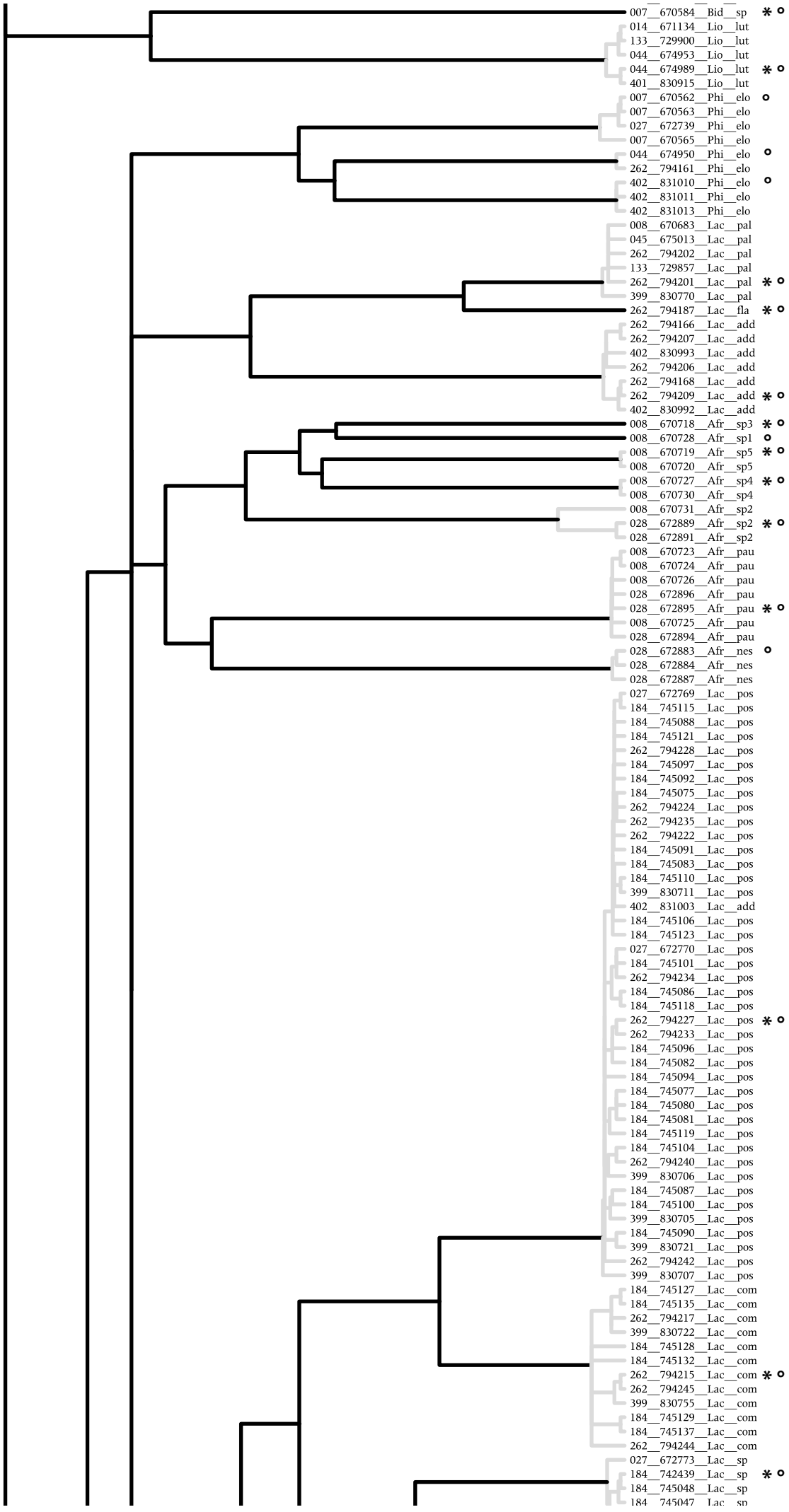
Figure S2.2: Water beetles *cox*1 parsimony tree - complete mtDNA cluster delimitation.

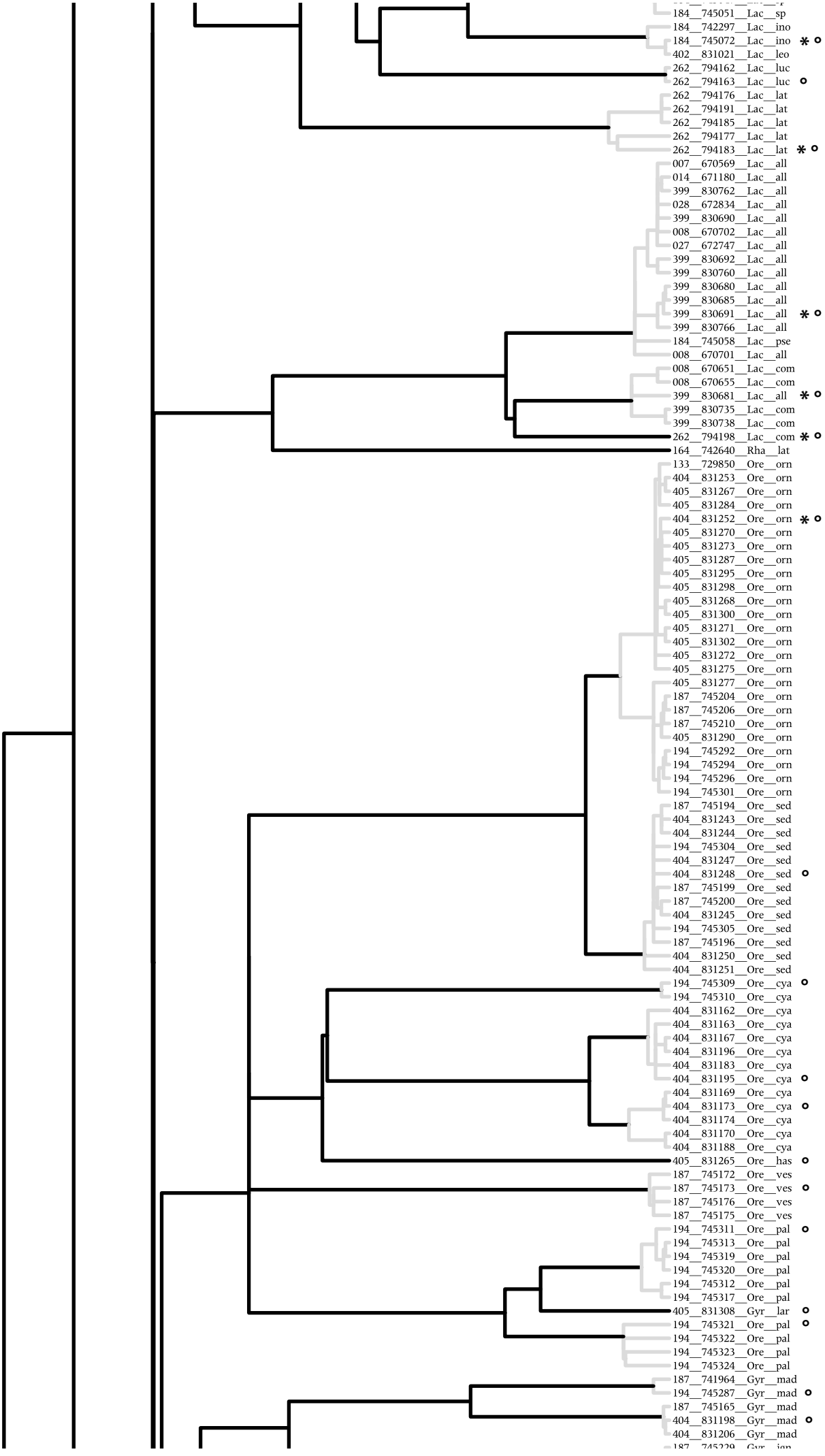


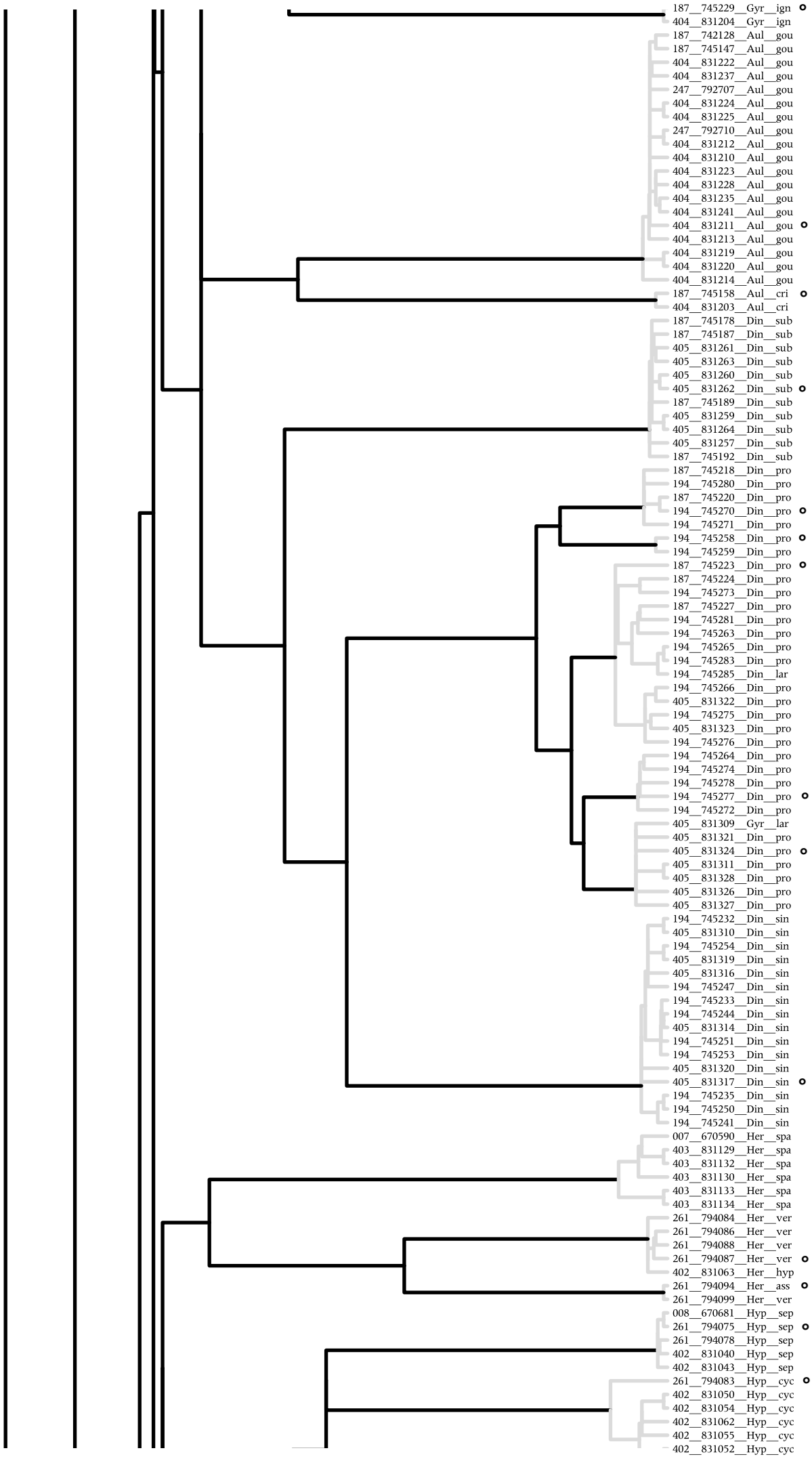

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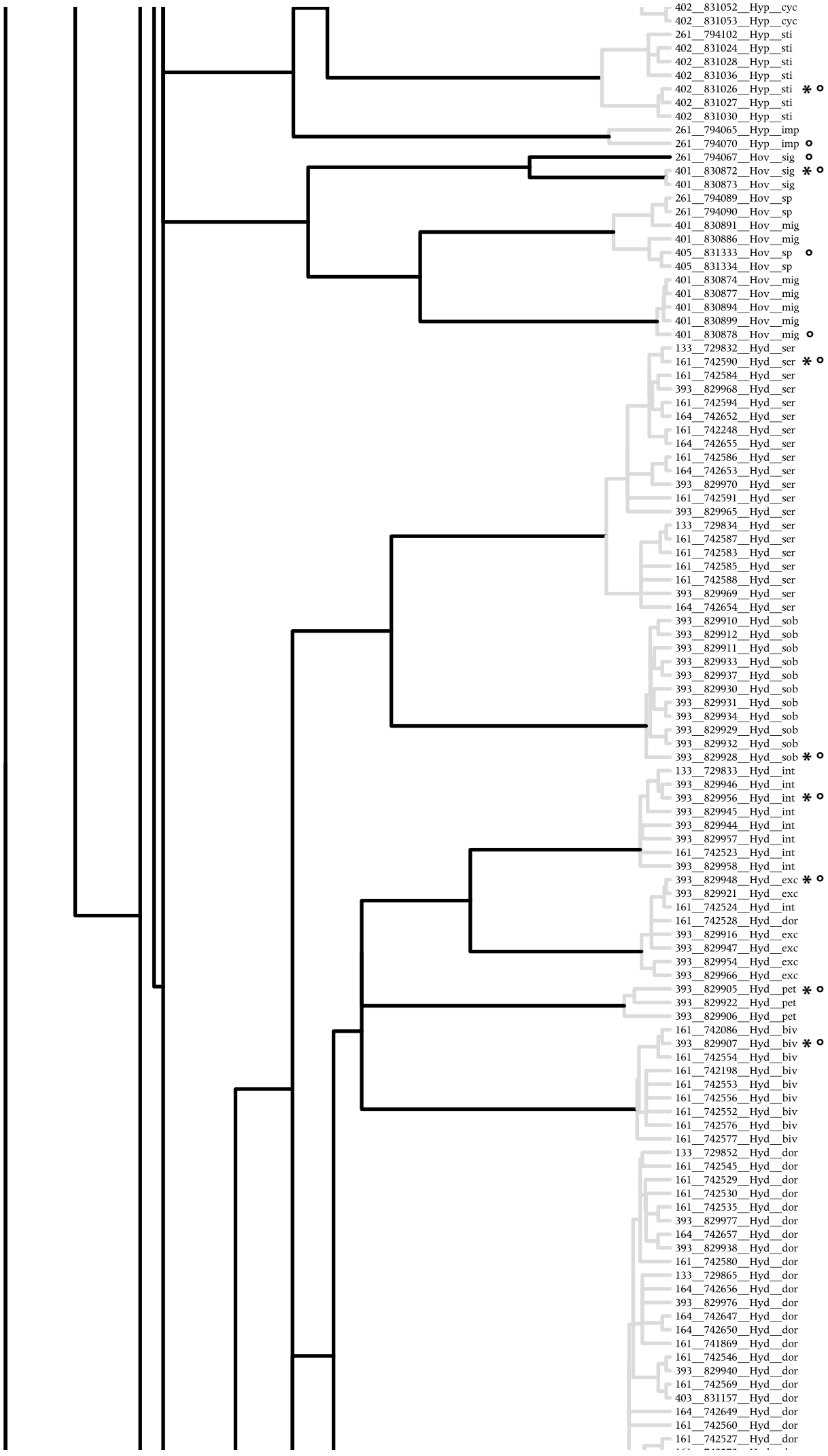

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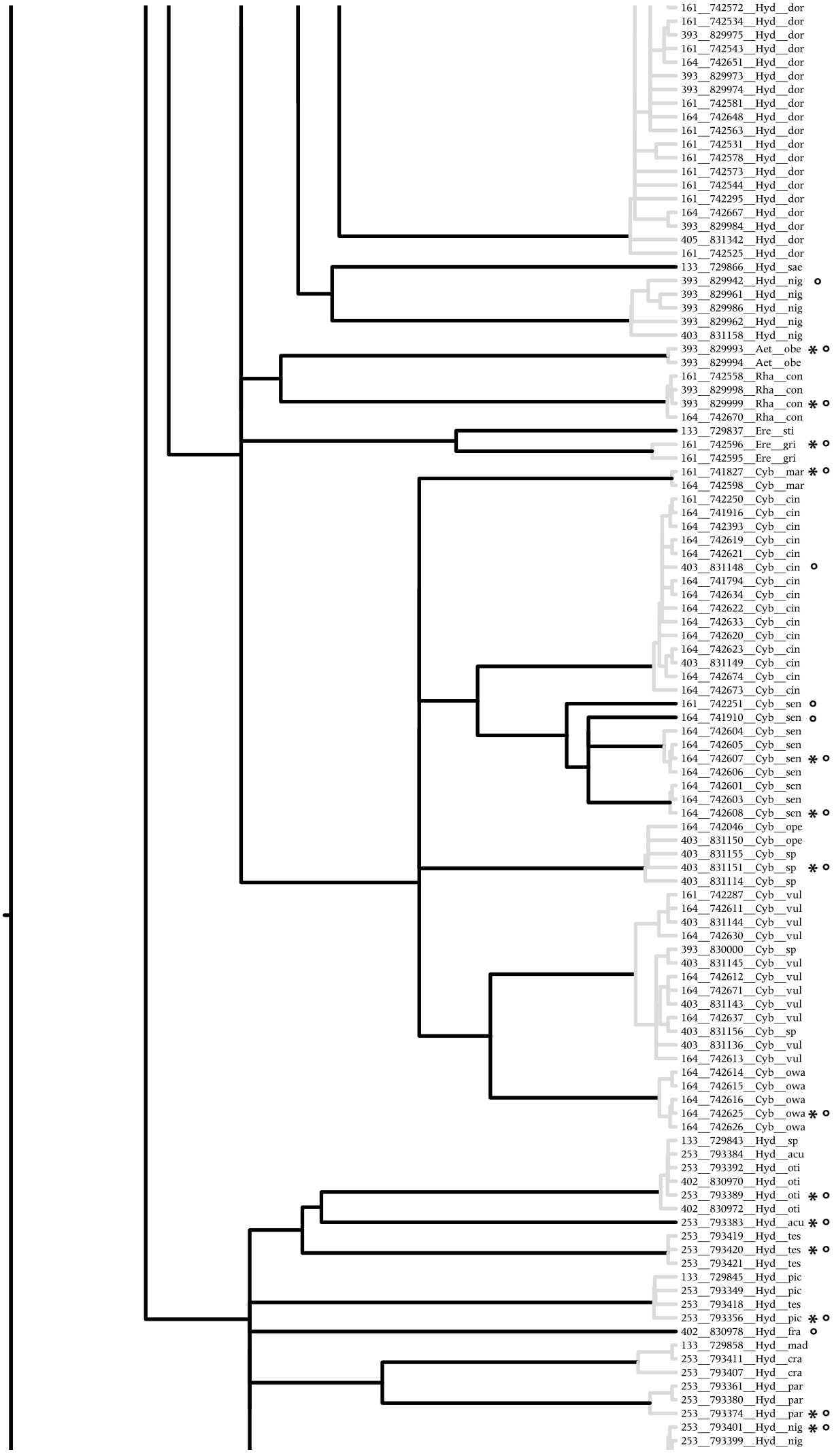

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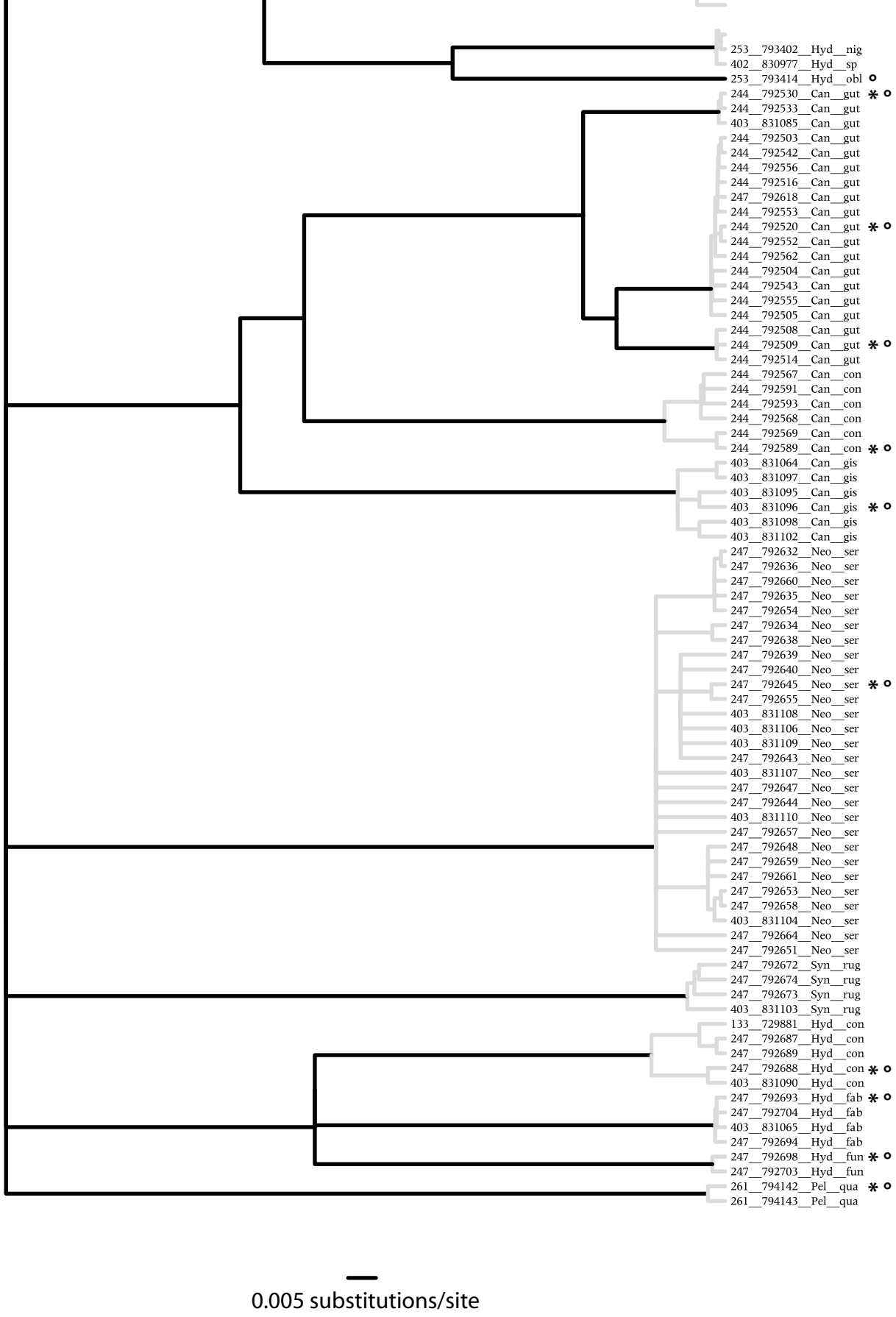

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253__793399__Hyd__nig



Grey shading indicates the different clusters delimited by the GMYC model (branches allocated to coalescence). Individuals subsequently sequenced for 16S and 28S are marked with ° and * respectively.

Figure S2.3: Ancestral state reconstructions of the water beetle fauna for two characters: endemism status (left: endemic in black, widespread in white) and aquatic habitat (right: RSW in black, SW in grey, RW in white and H in light grey). The two clades detailed in Figure 2.9 are shown with vertical grey bars.

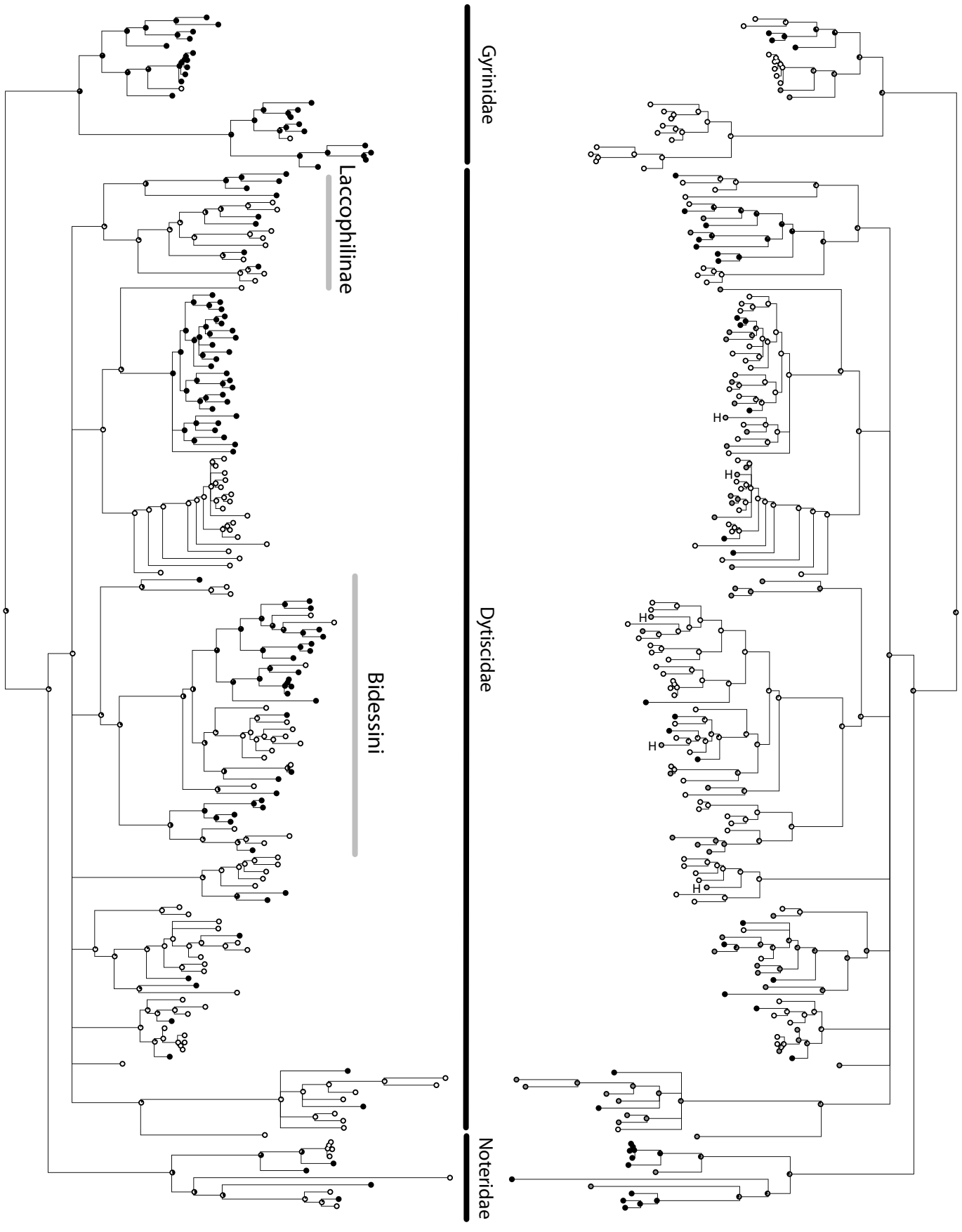


Table S2.1: Description of alignments for the length variable gene region (28S). The alignment that gave the lowest incongruence with the protein coding region was selected (bold).

28S alignment	chars	inform chars	gapped chars	L	CI	RI	Lcomb	Lsum	ILD	ratio
ClustalW 5-6.66	807	221	344	1352	0.491	0.847	13207	11800	1407	0,106534414
ClustalW 6.66-6.66	802	223	341	1362	0.488	0.845	13216	11810	1406	0,106386199
ClustalW 10-6.66	**796**	**224**	**337**	**1363**	**0.487**	**0.850**	**13183**	**11811**	**1372**	**0,104073428**
ClustalW 15-6.66	785	217	340	1445	0.458	0.839	13276	11893	1383	0,104172944
ClustalW 20-6.66	789	219	336	1473	0.446	0.840	13329	11921	1408	0,105634331
Mafft E-INS-i	803	263	371	1576	0.451	0.802	13531	12024	1507	0,111373882
Mafft G-INS-i	789	246	364	1497	0.464	0.806	13371	11945	1426	0,106648717
Mafft L-INS-i	804	257	372	1567	0.450	0.802	13514	12015	1499	0,110922007

Table S2.2: Taxonomy, habitat specificities and molecular characteristics of the different entities delimited by the GMYC model.

GMYC	voucher	*cox1*	16S	28S	*Genus*	*species*	hap	π	sites	habitat	WWF ecoregion
1	252_793261_Mad_sp	♦	♦	♦	*Madaglymbus*	-	12	0.00682	3	RW	DDF / SHF
2	252_793251_Mad_sp	♦	♦	♦	*Madaglymbus*	-	4	0.00338	1	RW	SHF
3	300_798472_Mad_sp	♦	♦	♦	*Madaglymbus*	-	3	0.00270	1	SW	LLF
4	252_793242_Mad_sp	♦	♦	♦	*Madaglymbus*	-	2	0.00136	2	H	LLF
5	252_793279_Mad_sp	♦	♦	♦	*Madaglymbus*	-	2	0.02297	1	RW	SHF
6	300_798487_Mad_sp	♦	♦	♦	*Madaglymbus*	-	12	0.01467	9	RSW	LLF / SHF
7	252_793266_Mad_sp	♦	♦	♦	*Madaglymbus*	-	9	0.01108	6	RW	LLF / SHF
8	252_793284_Mad_sp	♦	♦	♦	*Madaglymbus*	-	13	0.00346	9	RW	LLF / SHF
9	252_793290_Mad_sp	♦	♦	♦	*Madaglymbus*	-	11	0.01243	7	RW	LLF
10	249_792905_Mad_sp	♦	♦	♦	*Madaglymbus*	-	7	0.01019	7	RSW	LLF
11	300_798495_Mad_sp	♦	♦	♦	*Madaglymbus*	-	17	0.00469	12	RSW	DDF / LLF / SHF
12	300_798475_Mad_sp	♦	-	-	*Madaglymbus*	-	2	0.00270	4	SW	LLF
13	252_793301_Mad_sp	♦	♦	♦	*Madaglymbus*	-	4	0.00518	2	RW	LLF
14	007_670615_Mad_sp	♦	♦	♦	*Madaglymbus*	-	6	0.01246	4	RW	LLF / SHF
15	249_792988_Cop_sp	♦	♦	♦	*Copelatus*	-	5	0.00208	21	RW	DDF / LLF / SHF / SW
16	014_671215_Cop_sp4	♦	♦	♦	*Copelatus*	-	3	0.00721	1	RW	DDF
17	249_792945_Cop_sp	♦	♦	♦	*Copelatus*	-	11	0.00721	6	SW	LLF / SHF
18	249_792951_Cop_sp	♦	♦	♦	*Copelatus*	-	8	0.01423	5	RW	ET / LLF
19	294_797925_Cop_sp	♦	♦	♦	*Copelatus*	-	2	0.00135	1	SW	LLF
20	249_792904_Cop_sp	♦	♦	♦	*Copelatus*	-	33	0.00808	16	RSW	LLF / SHF
21	249_792956_Cop_sp	♦	♦	♦	*Copelatus*	-	6	0.00865	4	RW	LLF / SHF
22	249_792915_Cop_sp	♦	♦	♦	*Copelatus*	-	13	0.00611	8	RSW	LLF / SHF / SW
23	294_797907_Cop_sp	♦	♦	♦	*Copelatus*	-	4	0.00405	3	SW	LLF
24	045_675017_Cly_sp	♦	♦	♦	*Clypeodytes*	-	6	0.00910	4	SW	DDF / LLF / SW
25	014_671142_Uva_all	♦	♦	♦	*Uvarus*	*alluaudi* *	10	0.01267	8	RW	ET / SHF / SW
26	243_792423_Uva_tai	♦	♦	♦	*Uvarus*	*taitii* *	2	0.00946	1	RW	LLF
27	044_674910_Uva_sp	♦	♦	♦	*Uvarus*	-	2	0.00135	1	H	LLF
28	401_830912_Uva_cap	♦	♦	♦	*Uvarus*	*caprai* *	2	0.00270	2	SW	LLF
29	065_677078_Uva_sp2	♦	♦	♦	*Uvarus*	-	2	0.00405	1	RW	SHF
30	207_745422_Uva_riv	♦	♦	♦	*Uvarus*	*rivulorum* *	3	0.00284	3	RW	DDF / SHF / SW
31	207_745413_Uva_riv	♦	♦	♦	*Uvarus*	*rivulorum* *	2	0.00811	2	RW	DDF / SHF
32	207_745362_Pse_vit	♦	♦	♦	*Pseuduvarus*	*vitticollis*	3	0.00180	13	RW	DDF / ET / LLF / SHF / SW
33	401_830921_Pse_vit	♦	♦	♦	*Pseuduvarus*	*vitticollis*	2	0.00946	1	H	LLF
34	167_742764_Hyd_fla	♦	♦	♦	*Hydroglyphus*	*flavoguttatus*	5	0.00327	4	RSW	LLF / SHF
35	207_745351_Hyd_far	♦	♦	♦	*Hydroglyphus*	*farquharensis*	4	0.00631	3	RW	DDF / SHF / ST
36	243_792495_Hyd_gem	♦	♦	♦	*Hydroglyphus*	*geminoides*	17	0.00835	10	RSW	LLF / ST / SW
37	243_792443_Hyd_cap	♦	♦	♦	*Hydroglyphus*	-	34	0.00812	8	RW	DDF / SHF / ST / SW
38	167_742731_Hyd_pla	♦	♦	♦	*Hydroglyphus*	*plagiatus* *	12	0.00348	11	RSW	DDF / LLF / ST / SW
39	207_745374_Pac_cos	♦	♦	♦	*Pachynectes*	*costulifer* *	17	0.00911	5	RW	SHF / ST / SW
40	167_742681_Pac_hyg	♦	♦	♦	*Pachynectes*	*hygrotoides* *	6	0.01021	6	RW	ST / SW
41	167_742682_Pac_hyg	♦	-	-	*Pachynectes*	*hygrotoides* *	2	0.00292	2	RW	SW
42	207_745384_Pac_hyg	♦	♦	♦	*Pachynectes*	*hygrotoides* *	2	0.00542	2	RW	ST / SW
43	401_830956_Yol_cos	♦	♦	♦	*Yola*	*costipennis* *	33	0.00503	14	RSW	LLF / SHF / ST / SW
44	207_745418_Bid_per	♦	♦	♦	*Bidessus*	*perexiguus* *	17	0.01115	5	RW	DDF / ST / SW
45	243_792451_Bid_lon	♦	♦	♦	*Bidessus*	*longistriga* *	2	0.00135	2	SW	SW
46	044_674989_Lio_lut	♦	♦	♦	*Liodessus*	*luteopictus*	5	0.00432	8	RW	DDF / LLF / SHF / SW
47	007_670562_Phi_elo	♦	♦	-	*Philaccolus*	*elongatus* *	4	0.00495	4	RSW	DDF / SHF
48	044_674950_Phi_elo	♦	♦	-	*Philaccolus*	*elongatus* *	2	0.00270	2	RW	SHF
49	402_831010_Phi_elo	♦	♦	-	*Philaccolus*	*elongatus* *	3	0.00270	4	RW	LLF
50	262_794201_Lac_pal	♦	♦	♦	*Laccophilus*	*pallescens*	6	0.00459	4	RSW	DDF / LLF / ST
51	262_794209_Lac_add	♦	♦	♦	*Laccophilus*	*addendus*	7	0.00541	9	RSW	LLF / ST / SW
52	008_670719_Afr_sp5	♦	♦	♦	*Africophilus*	-	2	0.00135	1	RW	SHF
53	008_670727_Afr_sp4	♦	♦	♦	*Africophilus*	-	2	0.00135	1	RW	SHF
54	028_672889_Afr_sp2	♦	♦	♦	*Africophilus*	-	3	0.01532	2	H	LLF / SHF
55	028_672895_Afr_pau	♦	♦	♦	*Africophilus*	*pauliani* *	7	0.00347	2	RW	LLF / SHF
56	028_672883_Afr_nes	♦	♦	-	*Africophilus*	*nesiotes* *	3	0.00360	1	RW	LLF
57	262_794227_Lac_pos	♦	♦	♦	*Laccophilus*	-	42	0.00536	24	RSW	DDF / LLF / SHF / ST / SW
58	262_794215_Lac_com	♦	♦	♦	*Laccophilus*	*complicatus* *	12	0.00753	17	RSW	ET / LLF / SW
59	184_742439_Lac_sp	♦	♦	♦	*Laccophilus*	-	5	0.00432	4	RW	SHF / SW
60	184_745072_Lac_ino	♦	♦	♦	*Laccophilus*	-	3	0.00541	3	RSW	LLF / SHF / SW
61	262_794163_Lac_luc	♦	♦	-	*Laccophilus*	*luctuosus* *	2	0.00135	3	SW	LLF / SHF
62	262_794183_Lac_lat	♦	♦	♦	*Laccophilus*	*lateralis* *	5	0.01486	6	RSW	LLF / SHF / SW
63	399_830691_Lac_all	♦	♦	♦	*Laccophilus*	-	15	0.00857	25	RW	DDF / ET / LLF / SHF / SW
64	399_830681_Lac_all	♦	♦	♦	*Laccophilus*	-	5	0.00919	6	RW	LLF
65	404_831252_Ore_orn	♦	♦	♦	*Orectogyrus*	*ornaticollis* *	25	0.00999	22	RW	ET / LLF / SHF
66	404_831248_Ore_sed	♦	♦	-	*Orectogyrus*	*sedilloti* *	13	0.00566	6	RW	ET / LLF / SHF / SW
67	194_745309_Ore_cya	♦	♦	-	*Orectogyrus*	*cyanicollis* *	2	0.00271	1	RW	SHF
68	404_831195_Ore_cya	♦	♦	-	*Orectogyrus*	*cyanicollis* *	6	0.00459	4	RW	LLF / SHF
69	404_831173_Ore_cya	♦	♦	-	*Orectogyrus*	*cyanicollis* *	5	0.00892	8	RW	LLF
70	187_745173_Ore_ves	♦	♦	-	*Orectogyrus*	*vestitus* *	4	0.00563	1	RW	SHF
71	194_745311_Ore_pal	♦	♦	-	*Orectogyrus*	*pallidocinctus* *	6	0.00649	2	RW	SHF
72	194_745321_Ore_pal	♦	♦	-	*Orectogyrus*	*pallidocinctus* *	4	0.01329	2	RW	LLF
73	194_745287_Gyr_mad	♦	♦	-	*Gyrinus*	*madagascarensis* *	2	0.00543	2	RSW	LLF / SHF

Table S2.2 (cont.)

MYC	voucher	*cox1*	16S	28S	*Genus*	*species*	hap	π	sites	habitat	WWF ecoregion
74	404_831198_Gyr_mad	♦	♦	-	*Gyrinus*	*madagascarensis* *	3	0.00180	3	RSW	LLF
75	187_745229_Gyr_ign	♦	♦	-	*Gyrinus*	*ignitus* *	2	0.00135	3	RSW	LLF
76	404_831211_Aul_gou	♦	♦	-	*Aulonogyrus*	*goudoti* *	19	0.00582	15	RW	ET / LLF / SHF
77	187_745158_Aul_cri	♦	♦	-	*Aulonogyrus*	*cristatus* *	2	0.00405	2	RW	ET / SHF
78	405_831262_Din_sub	♦	♦	-	*Dineutus*	*subspinosus*	11	0.00462	5	SW	LLF / SHF / SW
79	194_745270_Din_pro	♦	♦	-	*Dineutus*	*proximus* *	5	0.00514	3	RW	ET / LLF / SW
80	194_745258_Din_pro	♦	♦	-	*Dineutus*	*proximus* *	2	0.00405	1	RW	SHF
81	187_745223_Din_pro	♦	♦	-	*Dineutus*	*proximus* *	14	0.01476	8	RW	ET / LLF / SHF
82	194_745277_Din_pro	♦	♦	-	*Dineutus*	*proximus* *	5	0.00865	4	RW	LLF / SHF
83	405_831324_Din_pro	♦	♦	-	*Dineutus*	*proximus* *	7	0.00727	4	RW	LLF / SHF
84	405_831317_Din_sin	♦	♦	-	*Dineutus*	*sinuosipennis*	16	0.00646	10	SW	LLF / SHF / SW
85	007_670590_Her_spa	♦	-	-	*Herophydrus*	*spadiaceus* *	6	0.01226	3	SW	LLF / SHF
86	261_794087_Her_ver	♦	♦	-	*Herophydrus*	-	5	0.00568	2	SW	LLF
87	261_794094_Her_ass	♦	♦	-	*Herophydrus*	-	2	0.00135	1	SW	SW
88	261_794075_Hyp_sep	♦	♦	-	*Hyphydrus*	*separandus*	5	0.00297	8	SW	DDF / LLF / SW
89	261_794083_Hyp_cyc	♦	♦	-	*Hyphydrus*	-	7	0.01076	4	SW	LLF / SW
90	402_831026_Hyp_sti	♦	♦	♦	*Hyphydrus*	*stipes* *	7	0.01506	5	SW	LLF / SHF
91	261_794070_Hyp_imp	♦	♦	-	*Hyphydrus*	*impressus*	2	0.02058	2	RW	ST
92	401_830872_Hov_sig	♦	♦	♦	*Hovahydrus*	*sinapi* *	2	0.00135	2	RW	LLF
93	405_831333_Hov_sp	♦	♦	-	*Hovahydrus*	*minutissimus* *	6	0.01306	6	RW	LLF / SHF
94	401_830878_Hov_mig	♦	♦	-	*Hovahydrus*	*minutissimus* *	5	0.00298	8	RW	LLF
95	161_742590_Hyd_ser	♦	♦	♦	*Prodaticus*	*servilianus*	20	0.01416	6	SW	LLF / ST / SW
96	393_829928_Hyd_sob	♦	♦	♦	*Prodaticus*	*sobrinus*	11	0.00598	3	SW	LLF
97	393_829956_Hyd_int	♦	♦	♦	*Prodaticus*	*intermedius*	8	0.00829	4	SW	LLF / SHF
98	393_829948_Hyd_exc	♦	♦	♦	*Prodaticus*	-	8	0.00636	5	RSW	LLF / SHF / SW
99	393_829905_Hyd_pet	♦	♦	♦	*Prodaticus*	*petitii* *	3	0.01441	2	SW	LLF
100	393_829907_Hyd_biv	♦	♦	♦	*Prodaticus*	*bivittatus*	9	0.00851	6	RW	ET / LLF / SHF / SW
101	133_729865_Hyd_dor	♦	-	-	*Prodaticus*	*dorsiger*	41	0.01095	18	RSW	ET / LLF / SHF / SW
102	393_829942_Hyd_nig	♦	♦	-	*Prodaticus*	*nigrotaeniatus* *	5	0.01297	4	RSW	LLF / SHF
103	393_829993_Aet_obe	♦	♦	♦	*Aethionectes*	*oberthuri* *	2	0.00270	2	SW	LLF
104	393_829999_Rha_con	♦	♦	♦	*Rhantaticus*	*congestus*	4	0.00222	4	RSW	LLF / SHF / ST / SW
105	161_742596_Ere_gri	♦	♦	♦	*Eretes*	*griseus*	2	0.00811	1	SW	ST
106	161_741827_Cyb_mar	♦	♦	♦	*Cybister*	*marginicollis*	2	0.00140	2	RW	SHF / SW
107	403_831148_Cyb_cin	♦	♦	-	*Cybister*	*cinctus* *	15	0.00497	11	RSW	LLF / SHF / ST / SW
108	164_742607_Cyb_sen	♦	♦	♦	*Cybister*	*senegalensis*	4	0.00293	2	RW	SW
109	164_742608_Cyb_sen	♦	♦	♦	*Cybister*	*senegalensis*	3	0.00180	3	SW	LLF / SW
110	403_831151_Cyb_sp	♦	♦	♦	*Cybister*	*operosus* *	5	0.00946	4	RW	LLF / SW
111	161_742287_Cyb_vul	♦	-	-	*Cybister*	*vulneratus*	13	0.00884	8	RSW	LLF / SHF / SW
112	164_742625_Cyb_owa	♦	♦	♦	*Cybister*	*owas*	5	0.00419	1	RW	SHF
113	253_793389_Hyd_oti	♦	♦	♦	*Hydrovatus*	-	6	0.00364	5	SW	LLF / SW
114	253_793420_Hyd_tes	♦	♦	♦	*Hydrovatus*	*testudinarius* *	3	0.00271	2	RSW	SW
115	253_793356_Hyd_pic	♦	♦	♦	*Hydrovatus*	*pictulus* *	4	0.00642	11	RSW	LLF / SHF / ST / SW
116	253_793407_Hyd_cra	♦	-	-	*Hydrovatus*	-	3	0.00902	2	SW	LLF / SW
117	253_793374_Hyd_par	♦	♦	♦	*Hydrovatus*	*parvulus*	3	0.00636	9	SW	LLF / SHF / SW
118	253_793401_Hyd_nig	♦	♦	♦	*Hydrovatus*	*nigrotaeniatus*	4	0.00235	3	SW	LLF / SW
119	244_792530_Can_gut	♦	♦	♦	*Canthydrus*	*guttula*	3	0.00180	6	RSW	LLF / SHF
120	244_792520_Can_gut	♦	♦	♦	*Canthydrus*	*guttula*	13	0.00340	9	RSW	ST / SW
121	244_792509_Can_gut	♦	♦	♦	*Canthydrus*	*guttula*	3	0.00270	5	RSW	LLF / SHF / SW
122	244_792589_Can_con	♦	♦	♦	*Canthydrus*	*concolor* *	6	0.01290	3	RSW	LLF / SW
123	403_831096_Can_gis	♦	♦	♦	*Canthydrus*	*gibberosus* *	6	0.01207	4	SW	DDF / LLF / SW
124	247_792645_Neo_ser	♦	♦	♦	*Neohydrocoptus*	*seriatus*	28	0.01041	16	RSW	LLF / SHF / SW
125	247_792672_Syn_rug	♦	-	-	*Synchortus*	*rugosopunctatus* *	4	0.01059	2	SW	LLF
126	247_792688_Hyd_con	♦	♦	♦	*Hydrocanthus*	*constrictus*	5	0.01622	6	RSW	LLF / SHF
127	247_792693_Hyd_fab	♦	♦	♦	*Hydrocanthus*	*fabiennae* *	4	0.00293	4	RSW	LLF / SW
128	247_792698_Hyd_fun	♦	♦	♦	*Hydrocanthus*	*funebris*	2	0.00405	2	RSW	SHF / SW
129	261_794142_Pel_qua	♦	♦	♦	*Peltodytes*	*quadratus*	2	0.00541	2	RW	ET / SW
130	300_798452_Mad_sp	♦	♦	♦	*Madaglymbus*	-	1	-	1	SW	SHF
131	300_798471_Mad_sp	♦	♦	♦	*Madaglymbus*	-	1	-	1	SW	LLF
132	300_798460_Mad_sp	♦	♦	♦	*Madaglymbus*	-	1	-	1	SW	SHF
133	300_798455_Mad_sp	♦	♦	♦	*Madaglymbus*	-	1	-	1	RW	LLF
134	027_672723_Mad_sp	♦	♦	♦	*Madaglymbus*	-	1	-	1	NA	ET
135	405_831339_Mad_sp	♦	♦	♦	*Madaglymbus*	-	1	-	1	RW	SHF
136	300_798491_Mad_sp	♦	♦	♦	*Madaglymbus*	-	1	-	1	RW	DDF
137	294_797892_Mad_sp	♦	♦	♦	*Madaglymbus*	-	1	-	1	SW	LLF
138	294_797898_Mad_sp	♦	-	-	*Madaglymbus*	-	1	-	2	RW	LLF
139	044_674904_Cop_sp4	♦	♦	♦	*Copelatus*	-	1	-	1	H	LLF
140	294_797923_Cop_sp	♦	♦	♦	*Copelatus*	-	1	-	1	SW	LLF
141	294_797949_Cop_sp	♦	♦	♦	*Copelatus*	-	1	-	1	RW	LLF
142	249_792929_Cop_sp	♦	♦	-	*Copelatus*	-	1	-	1	RW	SW
143	294_797927_Cop_sp	♦	♦	♦	*Copelatus*	-	1	-	1	SW	SHF
144	133_729890_Cop_sp1	♦	♦	♦	*Copelatus*	-	1	-	1	RW	SHF
145	027_672727_Cop_sp8	♦	♦	♦	*Copelatus*	-	1	-	1	NA	ET
146	294_797895_Cop_sp	♦	♦	♦	*Copelatus*	-	1	-	1	RW	LLF
147	401_830907_Met_cri	♦	♦	-	*Methles*	*cribratellus*	1	-	1	SW	LLF
148	243_792414_Cly_sor	♦	♦	♦	*Clypeodytes*	*sordidipennis* *	1	-	1	RW	SW

Table S2.2 (cont.)

MYC	voucher	*cox1*	16S	28S	*Genus*	*species*	hap	π	sites	habitat	WWF ecoregion
149	133_729882_Nep_obl	♦	♦	♦	*Neptosternus*	*oblongus* *	1	-	1	RW	SHF
150	401_830941_Uva_bin	♦	♦	♦	*Uvarus*	*binaghii* *	1	-	1	RW	LLF
151	243_792421_Uva_riv	♦	♦	♦	*Uvarus*	*rivulorum* *	1	-	1	RW	ST
152	045_675064_Pac_sp3	♦	♦	♦	*Pachynectes*	-	1	-	1	RW	LLF
153	207_745396_Bid_lon	♦	-	-	*Bidessus*	*longistriga*	1	-	1	NA	SHF
154	007_670584_Bid_sp	♦	♦	♦	*Bidessus*	-	1	-	1	SW	SHF
155	262_794187_Lac_fla	♦	♦	♦	*Laccophilus*	*flaveolus*	1	-	1	SW	SW
156	008_670718_Afr_sp3	♦	♦	♦	*Africophilus*	-	1	-	1	RW	SHF
157	008_670728_Afr_sp1	♦	♦	-	*Africophilus*	-	1	-	1	RW	SHF
158	262_794198_Lac_com	♦	♦	♦	*Laccophilus*	*comes* *	1	-	1	RW	SHF
159	164_742640_Rha_lat	♦	-	-	*Rhantus*	*latus*	1	-	1	SW	SW
160	405_831265_Ore_has	♦	♦	-	*Orectogyrus*	*hastatus* *	1	-	1	RW	LLF
161	405_831308_Gyr_lar	♦	♦	-	*Gyrinus*	-	1	-	1	RW	LLF
162	261_794067_Hov_sig	♦	♦	-	*Hovahydrus*	*sinapi* *	1	-	1	RW	SW
163	133_729866_Hyd_sae	♦	-	-	*Prodaticus*	*saecularis*	1	-	1	RW	SHF
164	133_729837_Ere_sti	♦	-	-	*Eretes*	*sticticus*	1	-	1	NA	NA
165	161_742251_Cyb_sen	♦	♦	-	*Cybister*	*senegalensis*	1	-	1	SW	ST
166	164_741910_Cyb_sen	♦	♦	-	*Cybister*	-	1	-	1	RW	SHF
167	253_793383_Hyd_acu	♦	♦	♦	*Hydrovatus*	*acuminatus*	1	-	3	SW	LLF / SHF / SW
168	402_830978_Hyd_fra	♦	♦	-	*Hydrovatus*	*frater*	1	-	1	RW	LLF
169	253_793414_Hyd_obl	♦	♦	-	*Hydrovatus*	*oblongipennis*	1	-	1	SW	SW

♦ : entities for which cox1, 16S and/or 28S sequences are available

* : endemic species

hap : number of haplotypes within each entity

π : nucleotide diversity with Jukes and Cantor correction, Pi(JC)

sites : number of collection localities where the entity was found

habitat : habitat preferences (RW = running water, SW = standing water, RSW = both running and standing waters, NA = non attributed)

WWF ecoregion : ecoregions (as defined by the WWF) where the entities were found

LLF = lowland forest
SHF = subhumid forest
DDF = dry decidious forest
ST = spiny thickets
ET = ericoid thickets
SW = succulent woodland

Table S2.3: Proportional likelihood values obtained for both Habitat and Endemism characters during the ancestral state reconstruction (states judged best according to the Mesquite threshold are marked with an asterisk).

	Endemism		Habitat			
	widespread	**endemic**	**RW**	**SW**	**H**	**RSW**
node 2	0.57643115	0.42356885	0.27421932	0.39841779	0.13966393	0.18769896
node 3	0.19210419	0.80789581	0.25428134	0.34220976	0.10807858	0.29543032
node 4	0.07814519	0.92185481*	0.21483935	0.36758679	0.06597695	0.35159691
node 5	0.02169897	0.97830103*	0.17208071	0.11328900	0.03738079	0.67724949
node 6	0.01190222	0.98809778*	0.23204233	0.06562175	0.03246052	0.669875399
node 7	0.01172089	0.98827911*	0.83535571*	0.02796587	0.02327721	0.11340121*
node 8	0	1.0	1.0	0	0	0
node 9	0	1.0	1.0	0	0	0
node 10	0.00186382	0.99813618*	0.00609091	0.00280226	0.00214696	0.98895987*
node 11	0	1.0	0	0	0	1.0
node 12	0	1.0	0	0	0	1.0
node 13	0	1.0	0	0	0	1.0
node 14	0.08559669	0.91440331*	0.19545695*	0.64425143*	0.04098509	0.11930653*
node 15	0.17353248	0.82646752	0.27549323*	0.62806315*	0.03285467	0.06358895
node 16	1.81377322E-4	0.99981862*	0.99950578*	3.36472766E-4	7.20481831E-5	8.57020503E-5
node 17	1.35966432E-5	0.99998640*	0.99998113*	1.05902298E-5	3.96783206E-6	4.30978717E-6
node 18	2.03786833E-6	0.99999796*	0.99999822*	6.04994935E-7	5.86945015E-7	5.87877043E-7
node 19	2.61262687E-6	0.99999739*	0.99999769*	7.71556084E-7	7.70849381E-7	7.70885872E-7
node 20	0	1.0	1.0	0	0	0
node 21	0	1.0	1.0	0	0	0
node 22	0	1.0	1.0	0	0	0
node 23	0	1.0	1.0	0	0	0
node 24	0	1.0	1.0	0	0	0
node 25	1.0	0	0	1.0	0	0
node 26	0	1.0	0	1.0	0	0
node 27	0.09327101	0.90672899*	0.88626944*	0.03912099	0.03609348	0.03851609
node 28	0.06439759	0.93560241*	0.98884172*	0.00379014	0.00361332	0.00375482
node 29	0.03146229	0.96853771*	0.99223541*	0.00261538	0.00254741	0.00260180
node 30	0	1.0	1.0	0	0	0
node 31	3.49845737E-4	0.99965015*	0.99980803*	6.40862434E-5	6.38447096E-5	6.40379849E-5
node 32	0	1.0	1.0	0	0	0
node 33	0	1.0	1.0	0	0	0
node 34	0.24569552	0.75430448	0.99965653*	1.14901811E-4	1.13869571E-4	1.14695569E-4
node 35	0.04182426	0.95817574*	0.99956436*	1.45263073E-4	1.45134965E-4	1.45237477E-4
node 36	0	1.0	1.0	0	0	0
node 37	0	1.0	1.0	0	0	0
node 38	0	1.0	1.0	0	0	0
node 39	0.01488544	0.98511456*	0.98076526*	0.00644046	0.00636825	0.00642603
node 40	0.01246277	0.98753723*	0.98388793*	0.00537537	0.00536367	0.00537303
node 41	0	1.0	1.0	0	0	0
node 42	1.468844899E-4	0.99985312*	0.99984830*	5.057086098E-5	5.05576099E-5	5.05682134E-5
node 43	0	1.0	1.0	0	0	0
node 44	0	1.0	1.0	0	0	0
node 45	0	1.0	1.0	0	0	0
node 46	0.79307580	0.206924196	0.29437625	0.52970669	0.07514298	0.10077408
node 47	0.95104193*	0.04895807	0.32300848*	0.65363213*	0.00577074	0.01758866
node 48	0.79009123	0.20990877	0.38338391	0.36645247	0.06847922	0.18168439
node 49	0.46047913	0.53952087	0.517649695	0.22073640	0.10501604	0.15659786

node 53	0	1.0	1.0	0	0	0
node 54	0	1.0	1.0	0	0	0
node 55	0	1.0	1.0	0	0	0
node 56	0.79481306	0.20518694	0.30482073	0.20302474	0.07816785	0.41398668
node 57	0.77679805	0.22320195	0.07269778	0.08046552	0.02775399	0.81908271*
node 58	0.77998088	0.22001912	0.05220667	0.07546101	0.02030932	0.852022995*
node 59	0.55532433	0.44467567	0.06222328	0.10298191	0.02326920	0.81152561*
node 60	0.55912781	0.44087219	0.11080256	0.19867499	0.031604498	0.65891796
node 61	0.93551935*	0.06448065	0.26237294*	0.07454444*	0.02846086	0.63462176*
node 62	1.0	0	1.0	0	0	0
node 63	1.0	0	0	0	0	1.0
node 64	0	1.0	0	1.0	0	0
node 65	0	1.0	0	0	0	1.0
node 66	0.85431919	0.14568081	0.04386814	0.07711803	0.02501310	0.85400072*
node 67	0.99231723*	0.00768277	0.01819488	0.18966409*	0.01667025	0.77547078*
node 68	1.0	0	0	1.0	0	0
node 69	1.0	0	0	0	0	1.0
node 70	1.0	0	0	0	0	1.0
node 71	0.64520316	0.35479684	0.00534764	0.00554145	0.00422629	0.98488462*
node 72	0	1.0	0	0	0	1.0
node 73	1.0	0	0	0	0	1.0
node 74	0.90486145*	0.09513855	0.99285098*	0.00232524	0.00207394	0.00274984
node 75	0.84962698	0.15037302	0.99893195*	3.50491349E-4	3.26453991E-4	3.91105590E-4
node 76	0	1.0	1.0	0	0	0
node 77	1.0	0	1.0	0	0	0
node 78	1.0	0	1.0	0	0	0
node 79	0.90169789*	0.09830211	0.66048447*	0.27443973*	0.02971642	0.03535939
node 80	0.73997359	0.26002641	0.66697158*	0.24100176*	0.044337396	0.04768926
node 81	1.0	0	1.0	0	0	0
node 82	0.00375234	0.99624766*	0.985513599*	0.01206323	0.00139196	0.00103121
node 83	1.82123891E-4	0.99981788*	0.99793914*	0.00161834	1.11259739E-4	3.31262631E-4
node 84	1.29186836E-4	0.99987081*	0.99975401*	1.46628352E-4	4.18232713E-5	5.75376249E-5
node 85	6.21991979E-4	0.99937801*	0.99951857*	1.67758513E-4	1.55951994E-4	1.57722250E-4
node 86	0	1.0	1.0	0	0	0
node 87	5.459881795E-4	0.99945401*	-	-	-	-
node 88	0	1.0	-	-	-	-
node 89	0	1.0	1.0	0	0	0
node 90	1.81245521E-4	0.99981875*	0.99972952*	1.14231905E-4	6.37517657E-5	9.24996486E-5
node 91	4.94797026E-6	0.99999505*	0.99933389*	3.83405299E-4	2.92244152E-5	2.534801098E-4
node 92	1.33359572E-4	0.99986664*	0.36646377*	0.37781247*	0.01086490	0.24485886*
node 93	1.16722822E-4	0.99988328*	0.00715108	0.00735332	8.14103733E-4	0.98468150*
node 94	0	1.0	0	0	0	1.0
node 95	0	1.0	0	0	0	1.0
node 96	6.16327305E-4	0.99938367*	0.15545193*	0.73043219*	0.00881105	0.10530483*
node 97	0	1.0	0	1.0	0	0
node 98	0	1.0	0	1.0	0	0
node 99	0	1.0	1.0	0	0	0
node 100	3.00465455E-4	0.99969953*	0.99946276*	2.32343966E-4	1.15438569E-4	1.89459224E-4
node 101	0	1.0	1.0	0	0	0
node 102	0	1.0	1.0	0	0	0
node 103	0	1.0	1.0	0	0	0
node 104	3.34009635E-4	0.99966599*	0.98903637*	0.00574682	7.08692371E-4	0.00450812

node 105	0.002113099	0.99788690*	0.98158575*	0.01265829	0.00213003	0.00362593
node 106	0	1.0	1.0	0	0	0
node 107	5.00420096E-4	0.99949958*	0.84375044*	0.14688568*	0.00459537	0.00476851
node 108	0	1.0	0	1.0	0	0
node 109	0	1.0	1.0	0	0	0
node 110	3.79591771E-4	0.99962041*	0.83606580*	0.04437907	0.00632540	0.11322973*
node 111	6.52103508E-4	0.999347896*	0.84258191*	0.08024167	0.00736805	0.06980838
node 112	0	1.0	1.0	0	0	0
node 113	0	1.0	0	1.0	0	0
node 114	0	1.0	0	0	0	1.0
node 115	9.67390703E-4	0.99903261*	0.93805680*	0.05279547	0.00664894	0.00249879
node 116	3.47001118E-4	0.999652998*	0.863347797*	0.10183539	0.02917168	0.00564513
node 117	0	1.0	0	0	1.0	0
node 118	0	1.0	1.0	0	0	0
node 119	0	1.0	0	1.0	0	0
node 120	0.00320674	0.99679326*	0.90594513*	0.07683838	0.00988771	0.00732879
node 121	0	1.0	1.0	0	0	0
node 122	0	1.0	0	1.0	0	0
node 123	0	1.0	1.0	0	0	0
node 124	0.98368643*	0.01631357	0.86968586*	0.08969921	0.01550520	0.02510973
node 125	0.98553593*	0.01446407	0.82990946*	0.10368549	0.02217800	0.04422705
node 126	0.98625606*	0.01374394	-	-	-	-
node 127	0.99573437*	0.00426563	0.87288069*	0.03829598	0.01357213	0.07525120
node 128	0.997748898*	0.00225110	0.95235783*	0.01903883	0.00483035	0.02377299
node 129	0.99954941*	4.50594484E-4	0.97683261*	0.01408914	9.29135447E-4	0.00814912
node 130	0.99999998*	1.555537796E-8	0.97418156*	0.02567728	1.12653572E-4	2.85062959E-5
node 131	0.99998425*	1.57478428E-5	0.94393545*	0.05524436	4.33387664E-4	3.86803141E-4
node 132	1.0	0	1.0	0	0	0
node 133	1.0	0	0	1.0	0	0
node 134	1.0	0	0	0	1.0	0
node 135	0.99993510*	6.48951917E-5	0.99857647*	0.00132582	5.09544993E-5	4.67582073E-5
node 136	1.0	0	1.0	0	0	0
node 137	1.0	0	1.0	0	0	0
node 138	0.99992948*	7.05217155E-5	0.95637799*	0.04232082	6.66708196E-4	6.34486898E-4
node 139	0.99991503*	8.49665030E-5	0.02118964	0.97734507*	7.32990076E-4	7.32300391E-4
node 140	1.0	0	0	1.0	0	0
node 141	1.0	0	0	1.0	0	0
node 142	1.0	0	1.0	0	0	0
node 143	1.0	0	0	1.0	0	0
node 144	0.99966881*	3.31190407E-4	0.96124447*	0.00377324	0.00191495	0.03306734
node 145	0.99997824*	2.17571872E-5	0.99949284*	5.92222526E-5	3.69050051E-5	4.11030841E-4
node 146	1.0	0	1.0	0	0	0
node 147	1.0	0	1.0	0	0	0
node 148	1.0	0	0	0	0	1.0
node 149	1.0	0	1.0	0	0	0
node 150	1.0	0	0	0	0	1.0
node 151	1.0	0	-	-	-	-
node 152	1.0	0	0	1.0	0	0
node 153	1.0	0	1.0	0	0	0
node 154	0.76479461	0.23520539	0.34773966*	0.58629322*	0.03047469	0.03549244
node 155	0.62765641	0.37234359	0.12165889*	0.79029652*	0.04340347	0.04464112
node 156	0	1.0	0	1.0	0	0
node 157	0.98662204*	0.01337796	0.00413387	0.98924052*	0.00330626	0.00331935

node 158	1.0	0	0	1.0	0	0
node 159	1.0	0	0	1.0	0	0
node 160	0.67844871	0.32155129	0.50241528*	0.40611251*	0.04408690	0.04738530
node 161	0.54706507	0.45293493	0.73108895*	0.21926985*	0.02145196	0.02818924
node 162	0.51131796	0.48868204	0.80183419*	0.16821664*	0.01032149	0.01962768
node 163	0.13871783	0.86128217	0.93682564*	0.03733769	0.00902681	0.01680986
node 164	0.05710588	0.94289412*	0.98278713*	0.00773635	0.00505089	0.00442563
node 165	0.07767206	0.92232794*	0.97850471*	0.00715636	0.01097341	0.00336552
node 166	0.01493217	0.98506783*	0.99287085*	0.00237551	0.00275576	0.00199788
node 167	0	1.0	1.0	0	0	0
node 168	0	1.0	1.0	0	0	0
node 169	0.19004183	0.80995817	0.92925467*	0.02112218	0.04189955	0.00772360
node 170	1.0	0	0	0	1.0	0
node 171	0.18739537	0.81260463	0.93244198*	0.03646238	0.02333753	0.00775810
node 172	1.0	0	1.0	0	0	0
node 173	0.01957330	0.980426698*	0.84434017*	0.13201700*	0.01405040	0.00959243
node 174	0	1.0	0	1.0	0	0
node 175	0	1.0	1.0	0	0	0
node 176	0.01934839	0.98065161*	0.98878307*	0.00408362	0.00362056	0.00351275
node 177	0.00140185	0.99859815*	0.99868396*	4.42138253E-4	4.374937499E-4	4.36412375E-4
node 178	0	1.0	1.0	0	0	0
node 179	0	1.0	1.0	0	0	0
node 180	0	1.0	1.0	0	0	0
node 181	0.09239933	0.90760067*	0.91701647*	0.03026964	0.01547143	0.03724246
node 182	0.05950782	0.94049218*	0.99134788*	0.00301753	0.00226021	0.00337438
node 183	0.09973725	0.90026275*	0.99399158*	0.00204559	0.00180289	0.00215994
node 184	1.0	0	1.0	0	0	0
node 185	0	1.0	1.0	0	0	0
node 186	9.54224431E-5	0.99990458*	0.99996513*	1.17068256E-5	1.12418625E-5	1.19259131E-5
node 187	5.14111791E-6	0.99999486*	0.99999655*	1.15162448E-6	1.14588529E-6	1.15432875E-6
node 188	0	1.0	1.0	0	0	0
node 189	0	1.0	1.0	0	0	0
node 190	0	1.0	1.0	0	0	0
node 191	0	1.0	0	0	0	1.0
node 192	0.60134010	0.398659897	0.77141106*	0.192857495*	0.00976999	0.02596146
node 193	0.81879716	0.18120284	0.83123295*	0.10304737	0.012802597	0.05291708
node 194	1.0	0	1.0	0	0	0
node 195	0.98625087*	0.01374913	0.76361854*	0.01816485	0.00829220	0.20992441*
node 196	0.99157237*	0.00842763	0.84137633*	0.00371962	0.00311423	0.15178982*
node 197	0.95463806*	0.04536194	0.81887267*	0.00622423	0.00580868	0.16909442*
node 198	0	1.0	0	0	0	1.0
node 199	1.0	0	1.0	0	0	0
node 200	0.99870261*	0.00129739	0.88907146*	0.00191546	0.00410043	0.10491264
node 201	0.999143199*	8.56800705E-4	0.85606572*	0.00452505	0.01778815	0.12162107*
node 202	1.0	0	0	0	0	1.0
node 203	0.99906448*	9.35517632E-4	0.88354855*	0.006379795	0.04921352	0.06085813
node 204	1.0	0	1.0	0	0	0
node 205	1.0	0	0	0	0	0
node 206	1.0	0	1.0	0	0	0
node 207	1.0	0	0	0	0	1.0
node 208	0.46037747	0.53962253	0.31702442*	0.66156257*	0.00960425	0.01180876
node 209	0.51169753	0.48830247	-	-	-	-
node 210	1.0	0	-	-	-	-

node 211	0	1.0	0	1.0	0	0
node 212	0	1.0	1.0	0	0	0
node 213	0.59761642	0.40238358	0.55959772*	0.35798221*	0.04015665	0.04226343
node 214	1.0	0	0	1.0	0	0
node 215	0	1.0	1.0	0	0	0
node 216	0.51141708	0.48858292	0.75432402*	0.17717587*	0.03402049	0.03447961
node 217	0.05710605	0.94289395*	0.97019440*	0.01603700	0.006869599	0.00689900
node 218	0.00123172	0.99876828*	0.99892106*	3.82641728E-4	3.48093081E-4	3.48203883E-4
node 219	0	1.0	1.0	0	0	0
node 220	0	1.0	1.0	0	0	0
node 221	0.00794543	0.99205457*	0.99615589*	0.001823896	0.00100880	0.00101142
node 222	0	1.0	1.0	0	0	0
node 223	0	1.0	1.0	0	0	0
node 224	0.75360577	0.24639423	0.68769407*	0.25069632*	0.03074261	0.03086700
node 225	1.0	0	1.0	0	0	0
node 226	0.68323893	0.31676107	0.01474123	0.97878055*	0.00323802	0.00324019
node 227	0.87931215	0.12068785	0.00544772	0.99086288*	0.00184436	0.00184504
node 228	1.0	0	0	1.0	0	0
node 229	1.0	0	0	1.0	0	0
node 230	0	1.0	0	1.0	0	0
node 231	0.72651901	0.27348099	0.83903951*	0.04204426	0.08796505	0.03095118
node 232	0.95193435*	0.04806565	0.83394955*	0.02313507	0.12359053*	0.01932485
node 233	0.99651677*	0.00348323	0.99210695*	0.00150038	0.00502601	0.00136666
node 234	0.99874996*	0.00125004	0.99808613*	4.30563993E-4	0.00107727	4.06034865E-4
node 235	0.99832679*	0.00167321	0.99809306*	5.93173426E-4	7.25616418E-4	5.88149933E-4
node 236	1.0	0	1.0	0	0	0
node 237	1.0	0	1.0	0	0	0
node 238	1.0	0	1.0	0	0	0
node 239	1.0	0	1.0	0	0	0
node 240	1.0	0	0	0	1.0	0
node 241	0.15284048	0.84715952	0.95257174*	0.01424783	0.02042475	0.01275567
node 242	0	1.0	1.0	0	0	0
node 243	0	1.0	1.0	0	0	0
node 244	0.91768461*	0.08231539	0.11060682	0.84087984*	0.013049199	0.03546414
node 245	0.98967846*	0.01032154	-	-	-	-
node 246	1.0	0	-	-	-	-
node 247	1.0	0	0	1.0	0	0
node 248	0.84045430	0.159545697	0.09825149	0.77455049*	0.02519212	0.10200590
node 249	0.81642864	0.18357136	0.132670796*	0.60592556*	0.02694768	0.23445596*
node 250	0.98687009*	0.01312991	0.19057386*	0.65505672*	0.01310685	0.14126257*
node 251	0.99686270*	0.003137297	0.37266091*	0.45665991*	0.01186601	0.15881317*
node 252	1.0	0	0	0	0	1.0
node 253	1.0	0	1.0	0	0	0
node 254	0.99369237*	0.00630763	0.48444231*	0.40208316*	0.01105039	0.10242414*
node 255	0.945319996*	0.05468000	0.22608171*	0.60474262*	0.01936016	0.14981552*
node 256	0	1.0	0	1.0	0	0
node 257	0.99479450*	0.005205495	0.06070075	0.56484850*	0.01661469	0.35783606*
node 258	1.0	0	0	0	0	1.0
node 259	1.0	0	0	1.0	0	0
node 260	1.0	0	1.0	0	0	0
node 261	0.99598993*	0.00401007	0.02308241	0.95404765*	0.00485283	0.01801711
node 262	1.0	0	0	1.0	0	0
node 263	1.0	0	0	1.0	0	0

node 264	0	1.0	0	0	0	1.0
node 265	0.68970473	0.31029527	0.08471024	0.73173603*	0.04709715	0.13645658*
node 266	0	1.0	0	1.0	0	0
node 267	1.0	0	0	0	0	1.0
node 268	0.90981472*	0.09018528	0.65700549*	0.25231872*	0.01481729	0.07585850
node 269	0.90993425*	0.09006575	0.91035327*	0.05931561	0.00585182	0.02447929
node 270	1.0	0	1.0	0	0	0
node 271	0.83813530	0.161864696	0.97417927*	0.01135591	0.00208945	0.01237538
node 272	0.97864269*	0.02135731	0.94395855*	0.00813743	0.00527971	0.04262431
node 273	1.0	0	0	0	0	1.0
node 274	1.0	0	1.0	0	0	0
node 275	0	1.0	1.0	0	0	0
node 276	0.87854600	0.121453997	0.30082345*	0.470011995*	0.01418541	0.21497914*
node 277	0.99818807*	0.00181193	0.18292654*	0.79648102*	0.00232299	0.01826945
node 278	1.0	0	0	1.0	0	0
node 279	0.99991975*	8.02523077E-5	0.66573059*	0.33267416*	6.64137307E-4	9.311096595E-4
node 280	1.0	0	1.0	0	0	0
node 281	0.99999356*	6.44125597E-6	0.65488301*	0.34430333*	3.64452067E-4	4.49206059E-4
node 282	1.0	0	1.0	0	0	0
node 283	1.0	0	0	1.0	0	0
node 284	0	1.0	0	0	0	1.0
node 285	1.0	0	0	1.0	0	0
node 286	0.81768244	0.18231756	0.20961349	0.54038118	0.12160346	0.12840187
node 287	0.95013251*	0.04986749	0.06130019	0.83926558*	0.01851728	0.08091694
node 288	0	1.0	0	0	0	1.0
node 289	0.95326587*	0.04673413	0.01819357	0.91630755*	0.00947191	0.05602697
node 290	0.97456800*	0.025431997	0.01161492	0.95847523*	0.00961364	0.02029621
node 291	0.95691185*	0.04308815	0.02329267	0.92916605*	0.02300629	0.02453499
node 292	1.0	0	0	1.0	0	0
node 293	1.0	0	0	1.0	0	0
node 294	1.0	0	0	1.0	0	0
node 295	1.0	0	0	1.0	0	0
node 296	0	1.0	0	0	0	1.0
node 297	0.99139078*	0.00860922	0.00629998	0.98231398*	0.00405813	0.00732792
node 298	1.0	0	0	1.0	0	0
node 299	1.0	0	0	1.0	0	0
node 300	1.0	0	1.0	0	0	0
node 301	1.0	0	0	1.0	0	0
node 302	0.52240857	0.47759143	0.18519626	0.31133924	0.16431573	0.33914878
node 303	0.26375121	0.73624879	0.08537321	0.40947673	0.08335782	0.42179223
node 304	0.39003484	0.60996516	0.01558895	0.035905296	0.01546262	0.93304314*
node 305	0.99956316*	4.36837618E-4	1.45917189E-5	2.21521232E-5	1.45447056E-5	0.99994871*
node 306	0.99998847*	1.15321167E-5	7.66554971E-7	8.78840403E-7	7.65856740E-7	0.99999759*
node 307	1.0	0	0	0	0	1.0
node 308	1.0	0	0	0	0	1.0
node 309	1.0	0	0	0	0	1.0
node 310	0	1.0	0	0	0	1.0
node 311	0	1.0	0	1.0	0	0
node 312	0.54293091	0.45706909	0.17967878	0.28578042	0.16620324	0.36833756
node 313	1.0	0	0	0	0	1.0
node 314	0.55967951	0.44032049	0.16214639	0.26537664	0.15482710	0.41764987
node 315	0	1.0	0	1.0	0	0

node 316	0.86335731	0.13664269	0.00868375	0.00992066	0.00859605	0.97279954*
node 317	1.0	0	0	0	0	1.0
node 318	0.80661551	0.19338449	9.88477619E-4	0.00106103	9.83333461E-4	0.99696716*
node 319	0	1.0	0	0	0	1.0
node 320	1.0	0	0	0	0	1.0
node 321	1.0	0	1.0	0	0	0
node 322	-	-	-	-	-	-

Appendix II: Supplementary material for Chapter 3

Table S3.1 **172**
Spearman correlation matrix of the 19 bioclimatic variables from WorldClim. The Spearman rho values and the corresponding *p* values are given respectively above and under the diagonal. The *p* values above 0.05 are highlighted in grey.

Table S3.2 **173**
List of the sampling sites with their geographic coordinates in decimal degrees (Longitude, Latitude), the ecoregions they belong to, and the type of water body they represent: running water (RW), standing water (SW), and hygropetric (H). In cases where sites correspond to terrestrial traps, the mention "unknown" was used to describe the habitat type.

Table S3.3 **176**
Results of the Mantel correlograms showing the different classes of distance, the number of pairs of sites per class, the Mantel statistic, and the *p* values obtained before and after the Bonferroni correction.

	P_{cq}	P_{waq}	P_{dq}	P_{weq}	P_{sea}	P_{dm}	P_{wm}	P_{ann}	T_{cq}	T_{waq}	T_{dq}	T_{weq}	T_{ran}	T_{min}	T_{max}	T_{sea}	I_{the}	D_{ran}	T_{ann}
P_{cq}	-	0.672	0.987	0.555	-0.938	0.962	0.473	0.812	0.031	0.029	0.167	0.026	-0.537	0.335	-0.173	-0.077	-0.511	-0.618	-0.012
P_{waq}	<0.001	-	0.663	0.911	-0.510	0.714	0.879	0.878	0.048	-0.075	0.139	-0.082	-0.725	0.339	-0.263	-0.332	-0.164	-0.684	-0.041
P_{dq}	<0.001	<0.001	-	0.542	-0.950	0.959	0.453	0.806	0.033	0.028	0.160	0.026	-0.535	0.339	-0.166	-0.095	-0.484	-0.601	-0.014
P_{weq}	<0.001	<0.001	<0.001	-	-0.359	0.630	0.976	0.901	0.239	0.093	0.318	0.086	-0.761	0.485	-0.082	-0.462	-0.047	-0.720	0.154
P_{sea}	<0.001	<0.001	<0.001	<0.001	-	-0.913	-0.264	-0.677	0.019	-0.011	-0.101	-0.010	0.370	-0.255	0.161	-0.031	0.559	0.445	0.052
P_{dm}	<0.001	<0.001	<0.001	<0.001	<0.001	-	0.547	0.866	0.083	0.067	0.223	0.065	-0.552	0.380	-0.137	-0.131	-0.502	-0.664	0.037
P_{wm}	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	-	0.844	0.282	0.128	0.364	0.121	-0.754	0.505	-0.058	-0.486	-0.039	-0.740	0.196
P_{ann}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	0.238	0.149	0.346	0.144	-0.713	0.520	-0.045	-0.346	-0.255	-0.716	0.170
T_{cq}	0.715	0.571	0.699	0.004	0.823	0.327	0.001	0.004	-	0.929	0.969	0.927	-0.363	0.895	0.863	-0.502	0.152	-0.303	0.970
T_{waq}	0.735	0.377	0.737	0.273	0.898	0.427	0.128	0.077	<0.001	-	0.930	1.000	-0.109	0.758	0.953	-0.190	-0.038	-0.138	0.983
T_{dq}	0.046	0.100	0.057	<0.001	0.234	0.008	<0.001	<0.001	<0.001	<0.001	-	0.929	-0.393	0.907	0.826	-0.457	0.016	-0.417	0.955
T_{weq}	0.757	0.332	0.760	0.308	0.907	0.443	0.151	0.087	<0.001	<0.001	<0.001	-	-0.105	0.756	0.953	-0.186	-0.036	-0.135	0.983
T_{ran}	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.196	<0.001	0.213	-	-0.663	0.097	0.777	-0.149	0.852	-0.199
T_{min}	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	0.613	-0.661	0.124	-0.590	0.813
T_{max}	0.039	0.002	0.048	0.330	0.056	0.104	0.495	0.594	<0.001	<0.001	<0.001	<0.001	0.253	<0.001	-	-0.100	0.078	0.104	0.937
T_{sea}	0.365	<0.001	0.260	<0.001	0.714	0.121	<0.001	<0.001	<0.001	0.023	<0.001	0.027	<0.001	<0.001	0.235	-	-0.433	0.592	-0.317
I_{the}	<0.001	0.051	<0.001	0.579	<0.001	<0.001	0.648	0.002	0.070	0.654	0.849	0.673	0.077	0.143	0.356	<0.001	-	0.228	0.071
D_{ran}	<0.001	0.000	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.100	<0.001	0.110	<0.001	<0.001	0.218	<0.001	0.006	-	-0.189
T_{ann}	0.888	0.624	0.866	0.067	0.538	0.664	0.019	0.044	<0.001	<0.001	<0.001	<0.001	0.018	<0.001	<0.001	<0.001	0.398	0.024	-

Table S3.1: Spearman correlation matrix of the 19 bioclimatic variables from WorldClim. The Spearman rho values and the corresponding *p* values are given respectively above and under the diagonal. The *p* values above 0.05 are highlighted in grey.

T_{ann} = Annual Mean Temperature
D_{ran} = Mean Diurnal Range
I_{the} = Isothermality
T_{sea} = Temperature Seasonality
T_{max}= Max Temperature of Warmest Month
T_{min} = Min Temperature of Coldest Month
T_{ran} = Temperature Annual Range
T_{weq} = Mean Temperature of Wettest Quarter
T_{dq} = Mean Temperature of Driest Quarter
T_{waq} = Mean Temperature of Warmest Quarter

T_{cq} = Mean Temperature of Coldest Quarter
P_{ann} = Annual Precipitation
P_{wm} = Precipitation of Wettest Month
P_{dm} = Precipitation of Driest Month
P_{sea} = Precipitation Seasonality
P_{weq} = Precipitation of Wettest Quarter
P_{dq} = Precipitation of Driest Quarter
P_{waq} = Precipitation of Warmest Quarter
P_{cq} = Precipitation of Coldest Quarter

Table S3.2: List of the sampling sites with their geographic coordinates in decimal degrees (Longitude, Latitude), the ecoregions they belong to, and the type of water body they represent: running water (RW), standing water (SW), and hygropetric (H). In cases where sites correspond to terrestrial traps, the mention "unknown" was used to describe the habitat type.

Site	Longitude	Latitude	Ecoregion *	Habitat
P21MD01	49.35350	-12.33360	DDF	RW
P2302	49.17253	-12.52830	SHF	RW
P23BI01	49.17344	-12.50146	SHF	RW
P23BI04	49.17220	-12.52075	SHF	RW
P23MD02	49.17253	-12.52830	SHF	RW
P23MD04	49.17400	-12.49940	SHF	RW
P23MD05	49.17600	-12.49920	SHF	RW
P23MD07	49.15990	-12.59610	SHF	RW
P24MD10	49.21730	-12.47540	SHF	SW
P2501MD11	48.72100	-13.64800	SHF	RW
P2506M	48.72120	-13.60930	SHF	H
P25MD11	48.72100	-13.64800	SHF	RW
P25MD12	48.72670	-13.65180	SHF	unknown
P25MD14	48.75670	-13.67840	SHF	RW
P25MD15	48.75370	-13.66820	SHF	RW
P25MD16	48.72120	-13.60930	SHF	RW
P25MT38	48.71310	-13.61260	SHF	unknown
P26MD17	49.05860	-12.93020	DDF	RW
P26MD18	49.09520	-12.92610	DDF	RW
P26MD19	49.08660	-12.92150	DDF	SW
P26MD24A	49.05620	-12.93110	DDF	unknown
P2700	47.42000	-21.26130	LLF	RW
P2701MD28	47.42000	-21.26130	LLF	RW
P2705M	47.39644	-21.23586	LLF	RW
P27MD26	47.42470	-21.26800	LLF	RW
P27MD29	47.42140	-21.25820	LLF	RW
P27MD30	47.41314	-21.26295	LLF	RW
P27MD31	49.15990	-12.59610	SHF	RW
P2803_LT63	48.42900	-18.85500	LLF	unknown
P2803MD34	48.42900	-18.85500	LLF	RW
P2804M	48.42900	-18.85500	LLF	SW
P28MD34	48.42900	-18.85500	LLF	RW
P28MD35	48.40630	-18.94300	LLF	RW
P28MD37	48.41700	-18.93300	LLF	RW
P2901MD32	47.17900	-20.77350	LLF	H
P30	47.10022	-20.23876	SHF	unknown
P30MD33	47.10022	-20.23876	SHF	RW
P35	47.16300	-20.39370	SHF	RW
P36A	47.18098	-20.77193	LLF	SW
P36B	47.18020	-20.77295	LLF	H
P36C	47.17915	-20.77293	LLF	SW
P37B	47.20240	-21.10338	SHF	SW
P38	45.04960	-22.00975	SW	SW
P39BI01	46.89951	-22.15120	ET	RW

Table S3.2 (cont.)

P39BI08	46.84596	-22.12944	SHF	unknown
P39CM06	46.90055	-22.15333	ET	RW
P39D	46.88932	-22.13653	ET	H
P39EM08	46.92073	-22.10425	SHF	RW
P39FM09	46.95033	-22.15080	SHF	RW
P39M04	46.89154	-22.14583	ET	SW
P39M07	46.88932	-22.13653	ET	RW
P39M12	46.88932	-22.13653	ET	RW
P41AM01	45.40123	-22.55002	SW	RW
P41B	45.39772	-22.54662	SW	RW
P41C	45.39175	-22.48932	SW	RW
P41D	45.38362	-22.48613	SHF	RW
P41E	45.40107	-22.54870	SW	RW
P41G	45.37940	-22.53922	SW	RW
P41I	45.37710	-22.53913	SW	SW
P41K	45.36815	-22.55343	SW	RW
P41O	45.37782	-22.54302	SW	RW
P42A	44.69240	-22.88647	SW	unknown
P42B	44.57693	-22.86853	SW	SW
P42C	44.61725	-22.86008	SW	SW
P42E	44.60035	-22.82940	SW	RW
P42G	44.86443	-22.64012	SW	SW
P44	44.22927	-23.24257	ST	SW
P45	44.33922	-23.34678	SW	RW
P48	44.59472	-24.46937	ST	RW
P49	44.62313	-24.52037	ST	RW
P50	45.04765	-24.71840	ST	RW
P52	45.07375	-25.13560	ST	SW
P54D	46.86368	-24.75972	LLF	RW
P54DM01	46.86368	-24.75972	LLF	RW
P54F	46.86623	-24.82453	LLF	SW
P54FM03	46.86623	-24.82453	LLF	SW
P56BI02	44.67150	-20.07250	DDF	SW
P56BI04	44.65433	-20.06683	DDF	unknown
P57BI01	49.77317	-14.43700	SHF	RW
P57BI03	49.77317	-14.43700	SHF	RW
P57BI04	49.77417	-14.43783	SHF	RW
P57BI05	49.77417	-14.43783	SHF	RW
P57BI07	49.77000	-14.43550	SHF	RW
P57BI08	49.76950	-14.43500	SHF	SW
P57BI11	49.75683	-14.43383	SHF	SW
P57BI12	49.75633	-14.43400	SHF	H
P57BI13	49.75650	-14.43417	SHF	unknown
P57BI14	49.75633	-14.43400	SHF	RW
P57BI15	49.75633	-14.43400	SHF	RW
P57BI16	49.75000	-14.43700	SHF	SW
P57BI18	49.73617	-14.43883	SHF	SW
P57BI28	49.77050	-14.43333	SHF	RW
P57BI29	49.78567	-14.44283	LLF	SW
P57BI31	49.79083	-14.45733	LLF	SW
P57BI32	49.77575	-14.43714	SHF	RW
P57BI37	49.76812	-14.43428	SHF	RW
P58BI01	49.95900	-15.72650	LLF	SW

Table S3.2 (cont.)

P58BI02	49.97000	-15.73483	LLF	SW
P58BI06	49.98900	-15.75717	LLF	SW
P58BI08	49.98750	-15.75550	LLF	SW
P58BI09	50.01000	-15.76700	LLF	SW
P58BI13	50.01000	-15.76800	LLF	SW
P58BI14	50.00200	-15.75867	LLF	SW
P58BI15	50.00200	-15.75867	LLF	SW
P58BI16	50.00200	-15.75867	LLF	SW
P58BI17	49.96500	-15.67550	LLF	RW
P58BI19	49.96500	-15.68700	LLF	RW
P58BI20	49.96500	-15.68517	LLF	RW
P58BI21	49.96500	-15.68483	LLF	RW
P58BI22	49.73800	-15.42467	LLF	unknown
P60BI01	48.72283	-17.50800	LLF	RW
P60BI02	48.72250	-17.50750	LLF	RW
P60BI05	48.72400	-17.50800	LLF	RW
P60BI06	48.72400	-17.50617	LLF	RW
P60BI08	48.72450	-17.50500	LLF	RW
P60BI10	48.72517	-17.50450	LLF	RW
P60BI11	48.72400	-17.50717	LLF	RW
P60BI12	48.72067	-17.51733	LLF	RW
P60BI13	48.72217	-17.51850	LLF	RW
P60BI14	48.72200	-17.51983	LLF	RW
P60BI15	48.72133	-17.52050	LLF	RW
P60BI16	48.72067	-17.52183	LLF	RW
P60BI27	48.72033	-17.54050	LLF	RW
P60BI29	48.72183	-17.54167	LLF	RW
P61BI01	48.41750	-18.93483	LLF	SW
P61BI02	48.41833	-18.93500	LLF	SW
P61BI03	48.41867	-18.93533	LLF	SW
P61BI04	48.41967	-18.93650	LLF	SW
P61BI05	48.40900	-18.92617	LLF	SW
P61BI14	48.41783	-18.93533	LLF	SW
P61BI15	48.41667	-18.93750	LLF	SW
P61BI16	48.42083	-18.94317	LLF	SW
P61BI17	48.41817	-18.94167	LLF	RW
P62	46.97319	-25.02119	LLF	SW
P63	47.04406	-24.87297	LLF	H
P64	47.02503	-24.88250	LLF	SW
P66	46.86317	-24.93056	LLF	SW
P67A	46.86331	-24.78611	LLF	RW
P67B	46.86286	-24.85944	LLF	RW
P67C	46.86244	-24.79944	LLF	RW
P67D	46.86253	-24.75972	LLF	RW
P69BI02	46.85385	-24.75879	LLF	RW

* the different ecoregions are represented by the following abbreviations:

LLF: LowLand Forest
SHF: SubHumid Forest
DDF: Dry Decidious Forest
ET: Ericoid Thickets
SW: Succulent Woodland
ST: Spiny Thickets.

Table S3.3: Results of the Mantel correlograms showing the different classes of distance, the number of pairs of sites per class, the Mantel statistic, and the *p* values obtained before and after the Bonferroni correction.

		class of distance (km)	pairs of sites per class	Mantel r_M	*p* value	*p* value corrected
Species level	Fauna	93	1883	0,150	0,001	0,008*
		280	1983	-0,006	0,599	4,792
		467	1697	-0,054	0,001	0,008*
		653	1234	-0,057	0,001	0,008*
		841	1141	-0,057	0,001	0,008*
		1027	1018	-0,013	0,276	2,208
		1214	774	0,001	0,948	7,584
		1401	256	-0,003	0,828	6,624
	RSW	92	736	0,230	0,001	0,008*
		277	654	0,094	0,001	0,008*
		461	582	-0,098	0,001	0,008*
		645	531	-0,041	0,091	0,728
		830	293	-0,089	0,001	0,008*
		1014	464	-0,072	0,001	0,008*
		1198	464	-0,069	0,001	0,008*
		1382	181	-0,059	0,011	0,088
	RW	91	918	0,132	0,001	0,008*
		272	985	-0,018	0,244	1,952
		454	812	-0,050	0,006	0,048*
		635	741	-0,055	0,001	0,008*
		816	542	-0,059	0,002	0,016*
		998	469	-0,001	0,955	7,64
		1179	399	0,023	0,161	1,288
		1361	170	0,014	0,428	3,424
	SW	89	183	0,112	0,003	0,024*
		268	186	-0,015	0,634	5,072
		447	219	-0,011	0,75	6
		626	185	-0,014	0,67	5,36
		805	82	-0,061	0,048	0,384
		984	130	-0,042	0,209	1,672
		1163	97	0,015	0,672	5,376
		1342	42	-0,019	0,588	4,704
Haplotype level	Fauna	93	1883	0,121	0,001	0,008*
		280	1983	0,005	0,661	5,288
		467	1697	-0,030	0,016	0,128
		654	1234	-0,049	0,001	0,008*
		841	1141	-0,046	0,002	0,016*
		1027	1018	-0,024	0,047	0,376
		1214	774	-0,030	0,016	0,128
		1401	256	0,020	0,101	0,808
	RSW	66	393	0,236	0,001	0,008*
		198	413	0,078	0,001	0,008*
		330	501	-0,049	0,027	0,216
		462	408	-0,059	0,017	0,136
		594	220	-0,075	0,001	0,008*
		726	239	-0,080	0,003	0,024*
		858	282	-0,074	0,002	0,016*
		991	93	-0,054	0,019	0,152

Table S3.3 (cont.)

Haplotype level	RW	66	429	0,065	0,003	0,024*
		199	420	0,014	0,648	5,184
		332	449	0,017	0,56	4,48
		465	240	-0,093	0,004	0,032*
		598	294	-0,019	0,443	3,544
		731	263	-0,057	0,034	0,272
		864	236	0,003	0,923	7,384
		996	145	0,045	0,148	1,184
	SW	64	179	0,236	0,001	0,008*
		192	121	0,141	0,001	0,008*
		321	202	-0,091	0,01	0,08
		449	169	-0,113	0,003	0,024*
		577	63	-0,071	0,031	0,248
		706	116	-0,054	0,159	1,272
		834	119	-0,065	0,075	0,6
		962	18	-0,038	0,315	2,52
Species level / Lowland forest	Fauna	101	637	0,175	0,001	0,006*
		303	681	-0,083	0,001	0,006*
		506	203	-0,042	0,073	0,438
		708	355	-0,083	0,002	0,012*
		910	182	-0,024	0,338	2,028
		1113	208	0,000	0,992	5,952
	RSW	101	187	0,269	0,001	0,006*
		303	157	-0,070	0,113	0,678
		506	57	-0,039	0,402	2,412
		708	141	-0,119	0,006	0,036*
		910	9	-0,057	0,312	1,872
		1112	108	-0,116	0,009	0,054
	RW	101	373	0,114	0,003	0,018*
		303	265	-0,044	0,199	1,194
		505	141	-0,073	0,032	0,192
		707	135	-0,058	0,083	0,498
		909	126	-0,038	0,28	1,68
		1111	36	0,101	0,007	0,042*
	SW	101	94	0,314	0,001	0,006*
		303	92	-0,216	0,001	0,006*
		505	22	-0,065	0,259	1,554
		707	45	-0,105	0,057	0,342
		909	8	-0,070	0,158	0,948
		1111	36	0,037	0,529	3,174
Species level / Subhumid forest	Fauna	99	392	0,216	0,001	0,006*
		296	154	-0,140	0,001	0,006*
		494	0	-	-	-
		692	88	-0,129	0,002	0,012*
		890	116	-0,022	0,609	3,654
		1087	60	-0,112	0,009	0,054
	RSW	99	158	0,325	0,001	0,006*
		297	75	-0,263	0,001	0,006*
		494	0	-	-	-
		692	14	-0,064	0,417	2,502
		890	36	0,002	0,98	5,88
		1087	38	-0,185	0,004	0,024*

Table S3.3 (cont.)

Species level / Subhumid forest	**RW**	92	181	0,191	0,001	0,006*
		276	76	-0,105	0,042	0,252
		460	0	-	-	-
		644	30	-0,134	0,023	0,138
		828	59	-0,030	0,6	3,6
		1012	26	-0,131	0,034	0,204

Appendix III: Supplementary material for Chapter 4

Figure S4.1 **181**
Response curves of the GMYC species *sp11** to the individual climate variables.

Figure S4.2 **182**
Response curves of the GMYC species *sp20* to the individual climate variables.

Figure S4.3 **183**
Response curves of the GMYC species *sp36* to the individual climate variables.

Figure S4.4 **184**
Response curves of the GMYC species *sp38** to the individual climate variables.

Figure S4.5 **185**
Response curves of the GMYC species *sp101* to the individual climate variables.

Figure S4.6 **186**
Response curves of the GMYC species *sp107** to the individual climate variables.

Figure S4.7 **187**
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Response curves of the genus *Copelatus* to the individual climate variables.

Figure S4.9 **189**
Response curves of the genus *Cybister* to the individual climate variables.

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Response curves of the genus *Hydroglyphus-Pseuduvarus* to the individual climate variables.

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Response curves of the genus *Madaglymbus* to the individual climate variables.

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Response curves of the *Cybister* clade to the individual climate variables.

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Response curves of the *Hydrovatus* clade to the individual climate variables.

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Response curves of the *Madaglymbus* clade to the individual climate variables.

Figure S4.17 **197**
Response curves of the *Prodaticus* clade to the individual climate variables.

Figure S4.18 **198**
Individual contribution of the climatic variables in the GBM models from BIOMOD (part 1). The x axis represents the relative influence in %.

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Individual contribution of the climatic variables in the GBM models from BIOMOD (part 2). The x axis represents the relative influence in %.

Figure S4.20 **200**
Individual contribution of the climatic variables in the GBM models from BIOMOD (part 3). The x axis represents the relative influence in %.

Figure S4.21 **201**
Response curves of the different Generalised Additive Models based on the predictions and the observed presences and absences.

Table S4.1 **202**
Individual contribution (in %) of the climatic variables in the Maxent models for the twelve GMYC species.

Table S4.2 **203**
Summary statistics for the Generalised Linear Models. Both the null and the residual deviances are given, together with the detailed dependent variables.

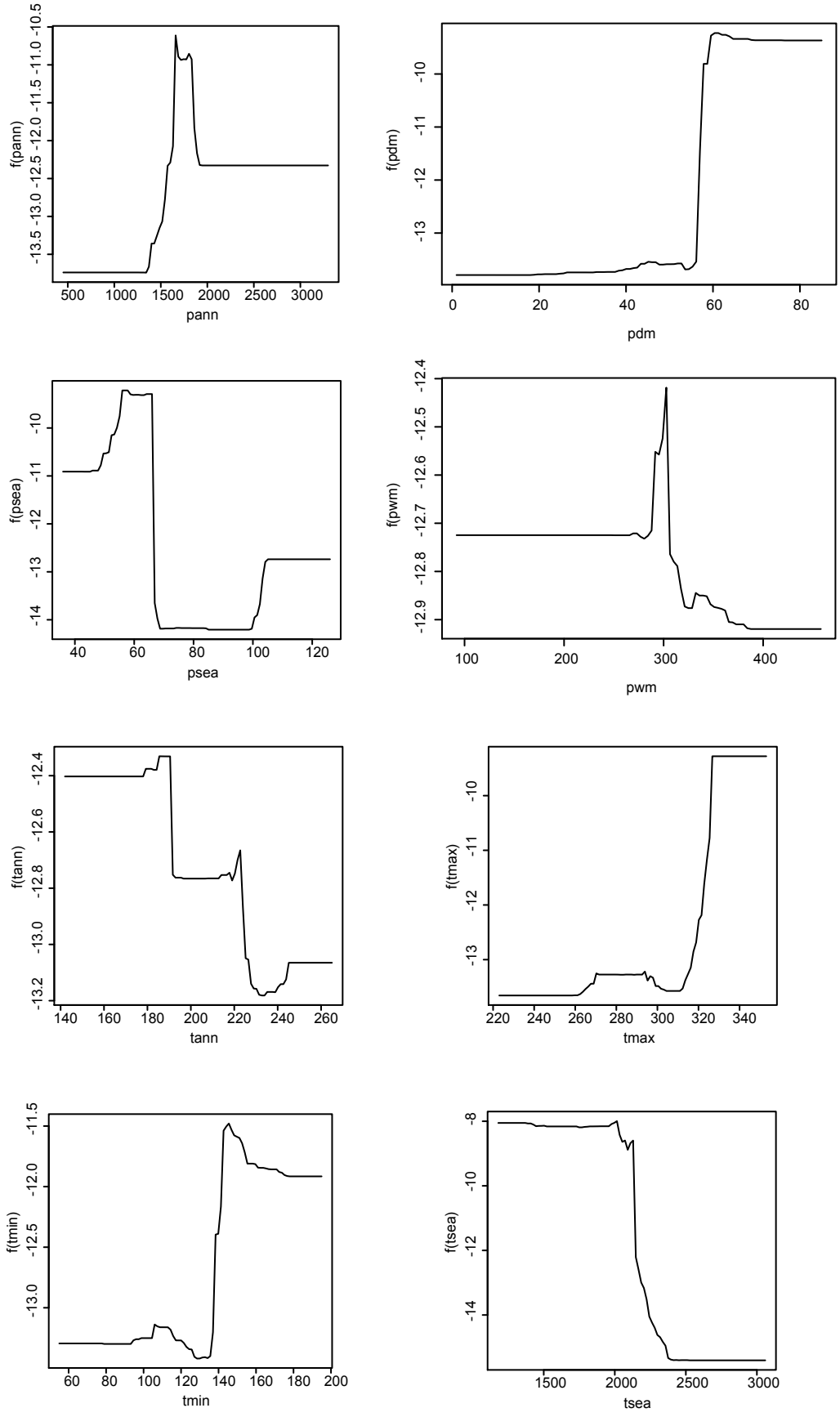


Figure S4.1: Response curves of the GMYC species *sp11** to the individual climate variables.

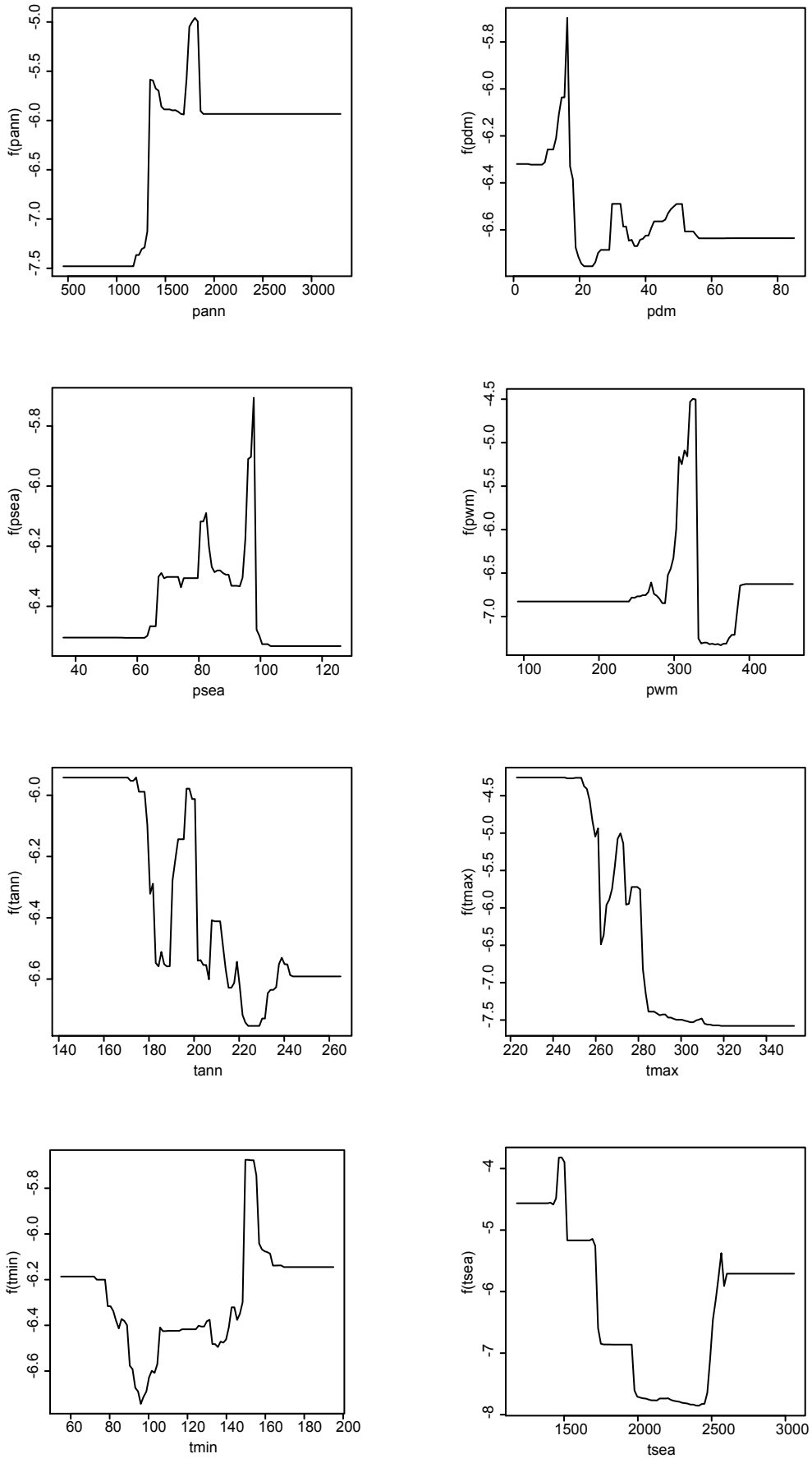


Figure S4.2: Response curves of the GMYC species *sp20* to the individual climate variables.

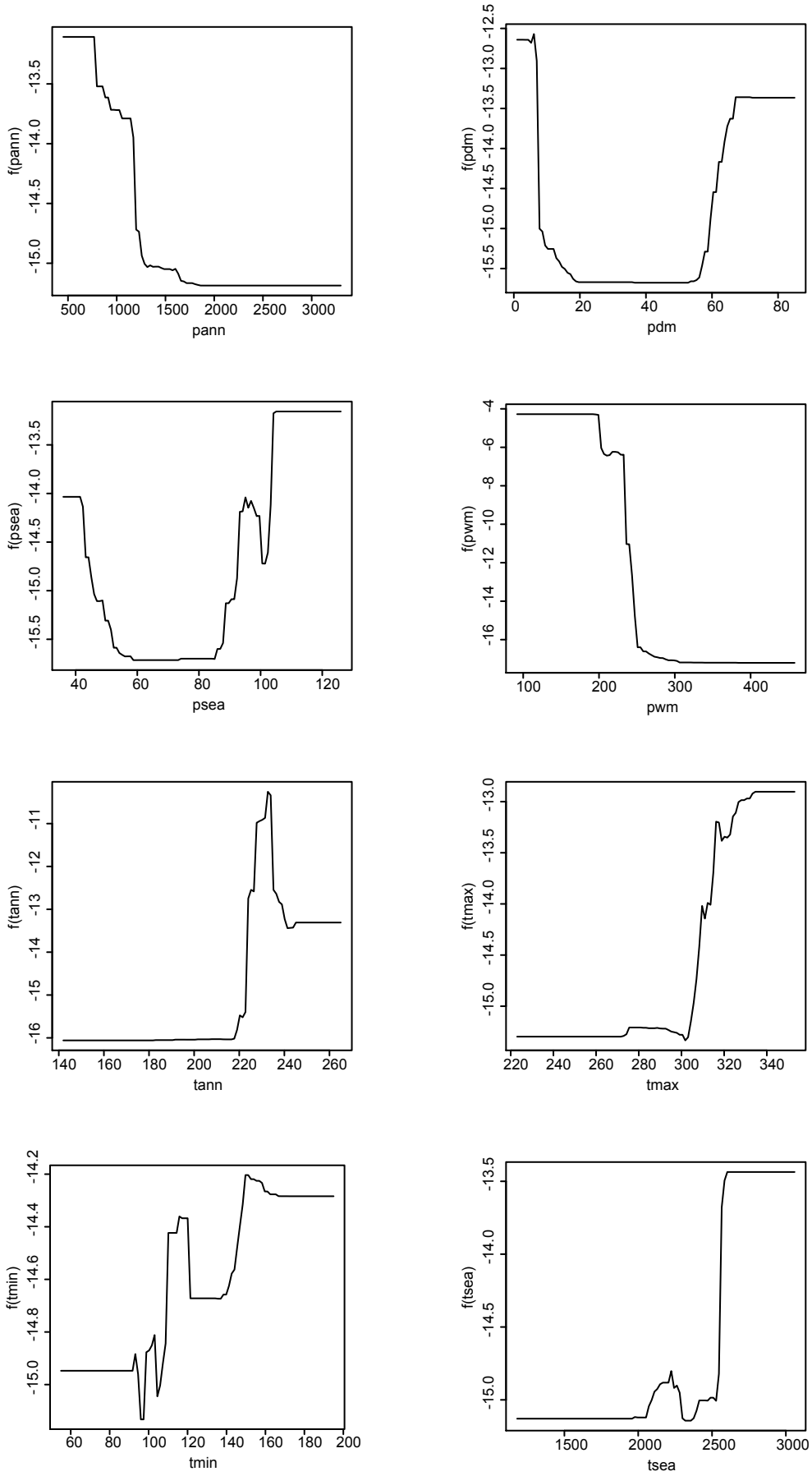


Figure S4.3: Response curves of the GMYC species *sp36* to the individual climate variables.

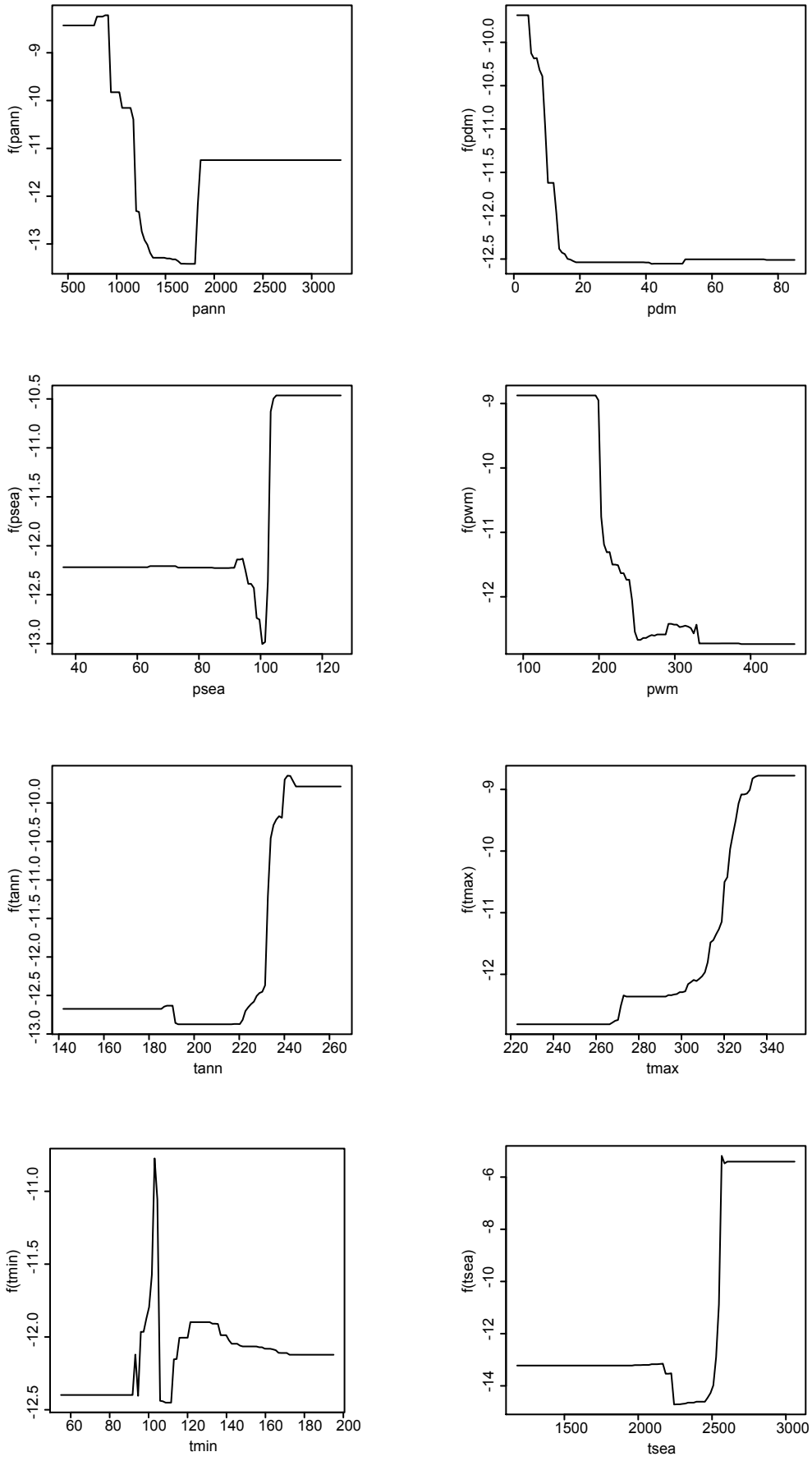


Figure S4.4: Response curves of the GMYC species *sp38** to the individual climate variables.

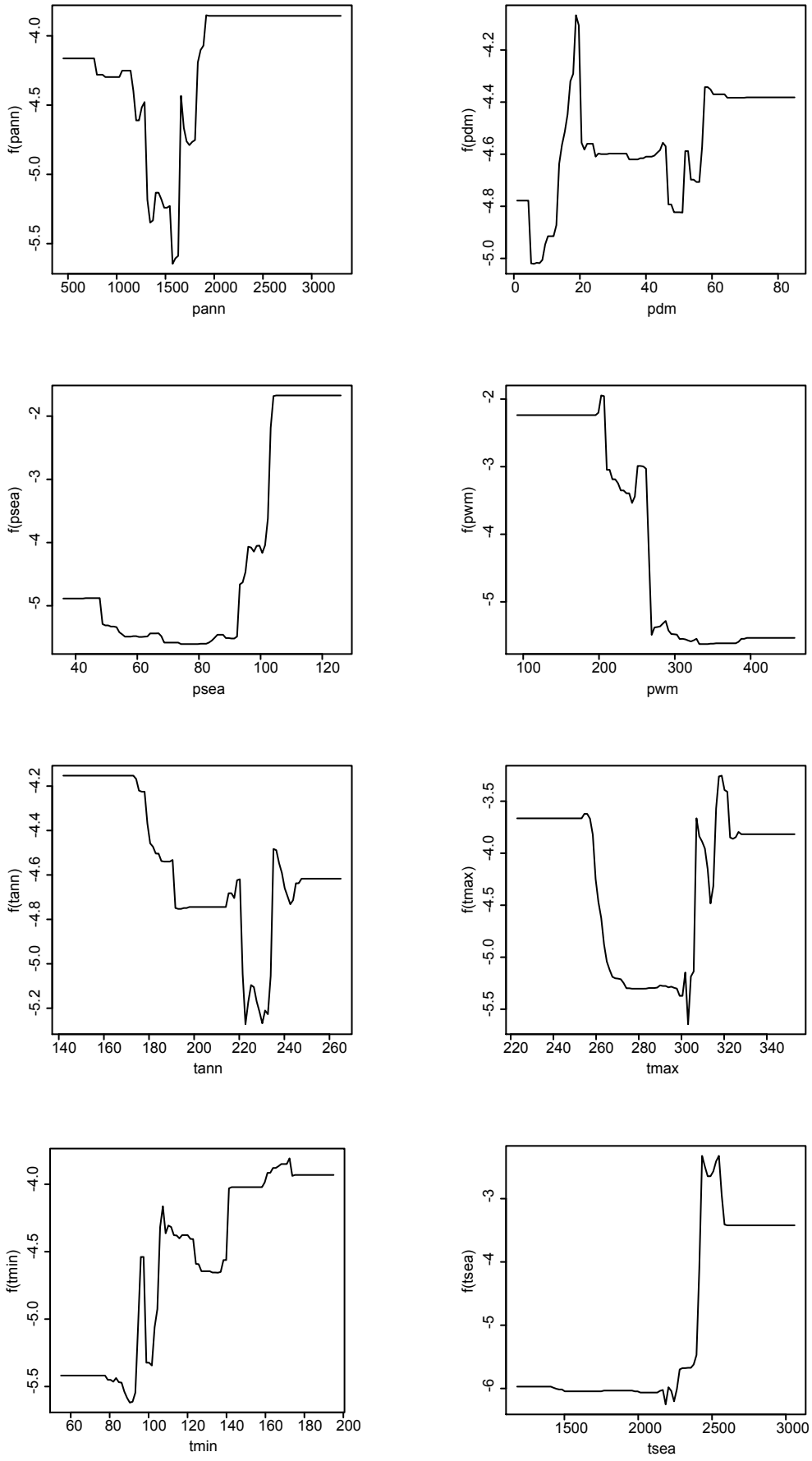


Figure S4.5: Response curves of the GMYC species *sp101* to the individual climate variables.

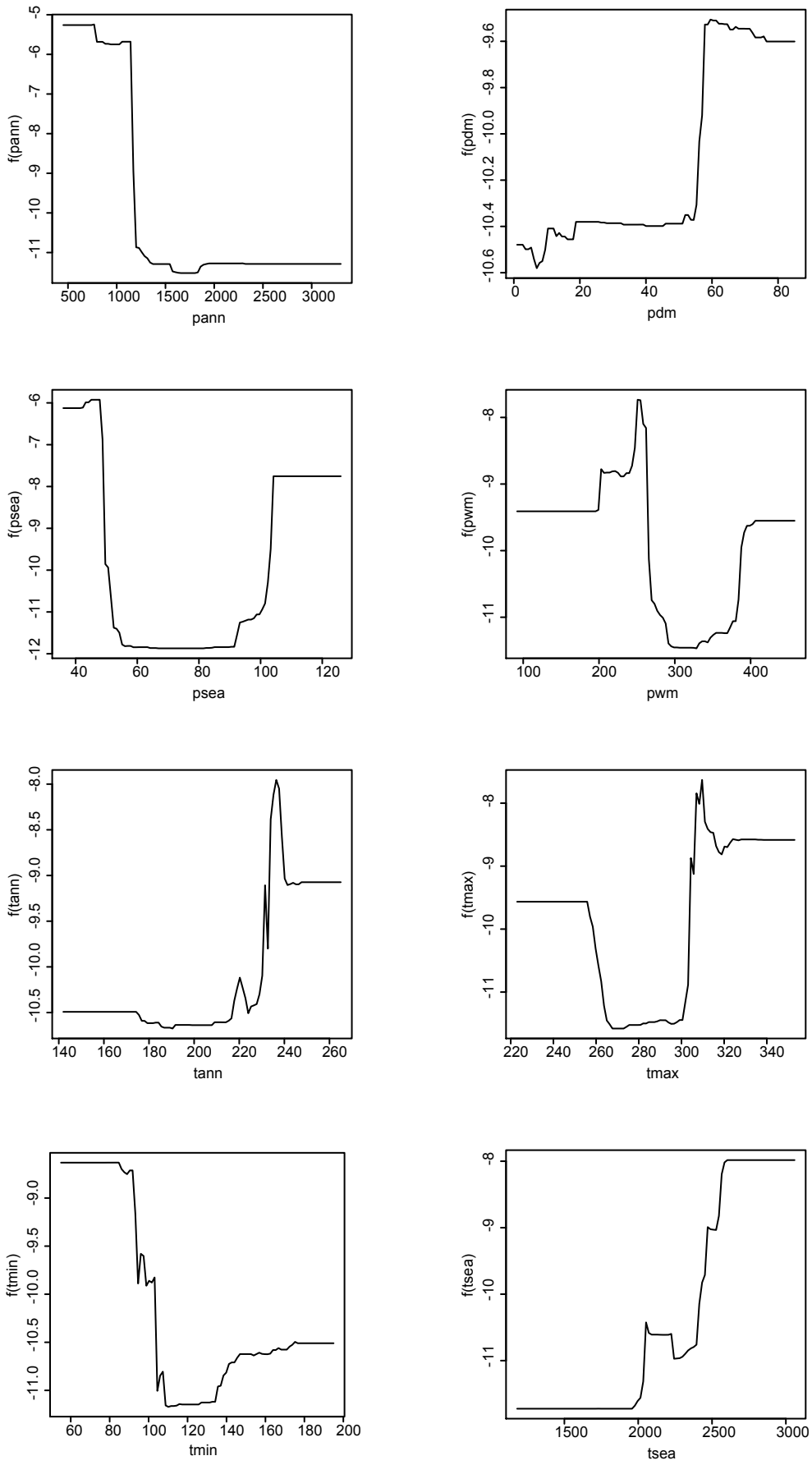


Figure S4.6: Response curves of the GMYC species *sp107** to the individual climate variables.

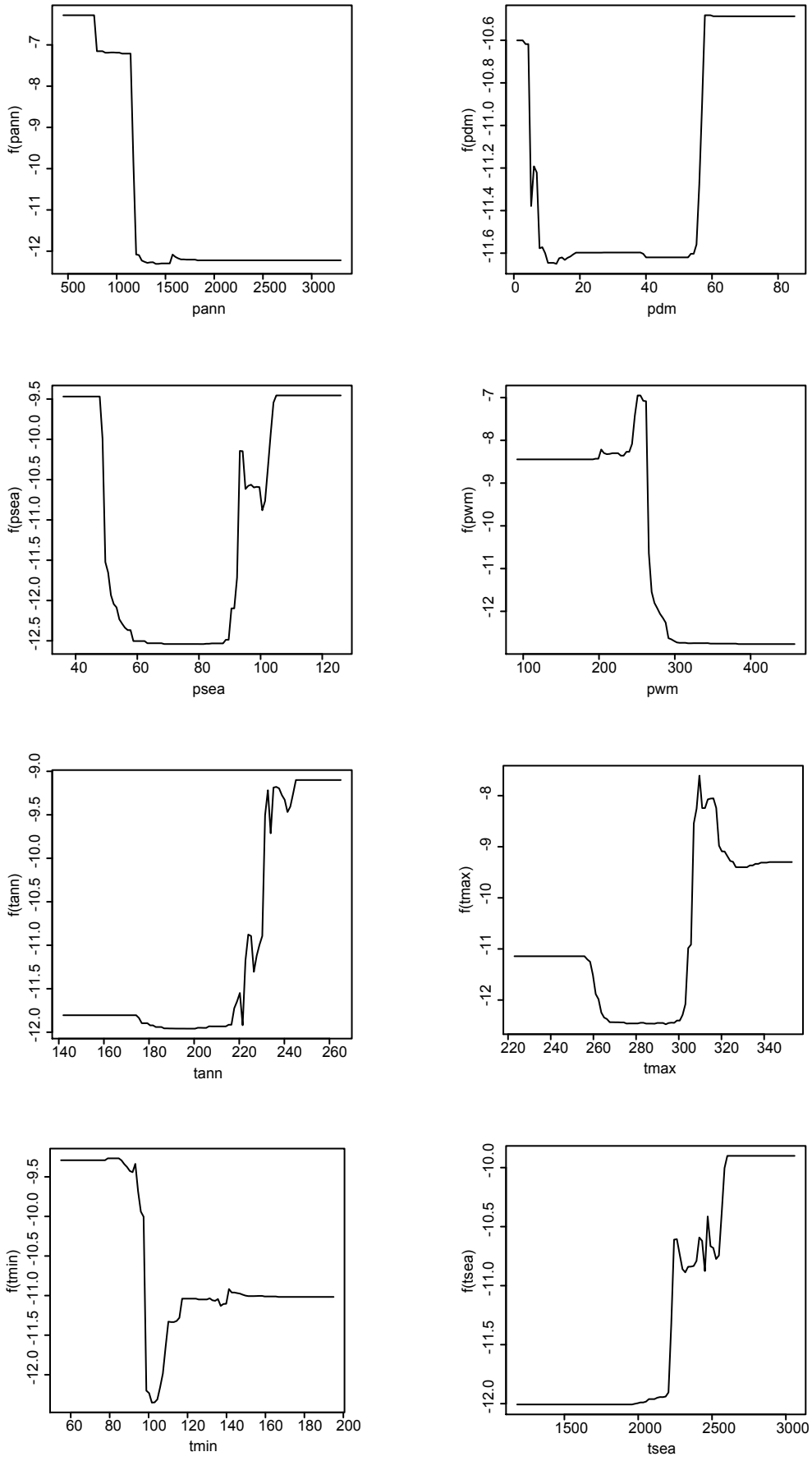


Figure S4.7: Response curves of the GMYC species *sp115** to the individual climate variables.

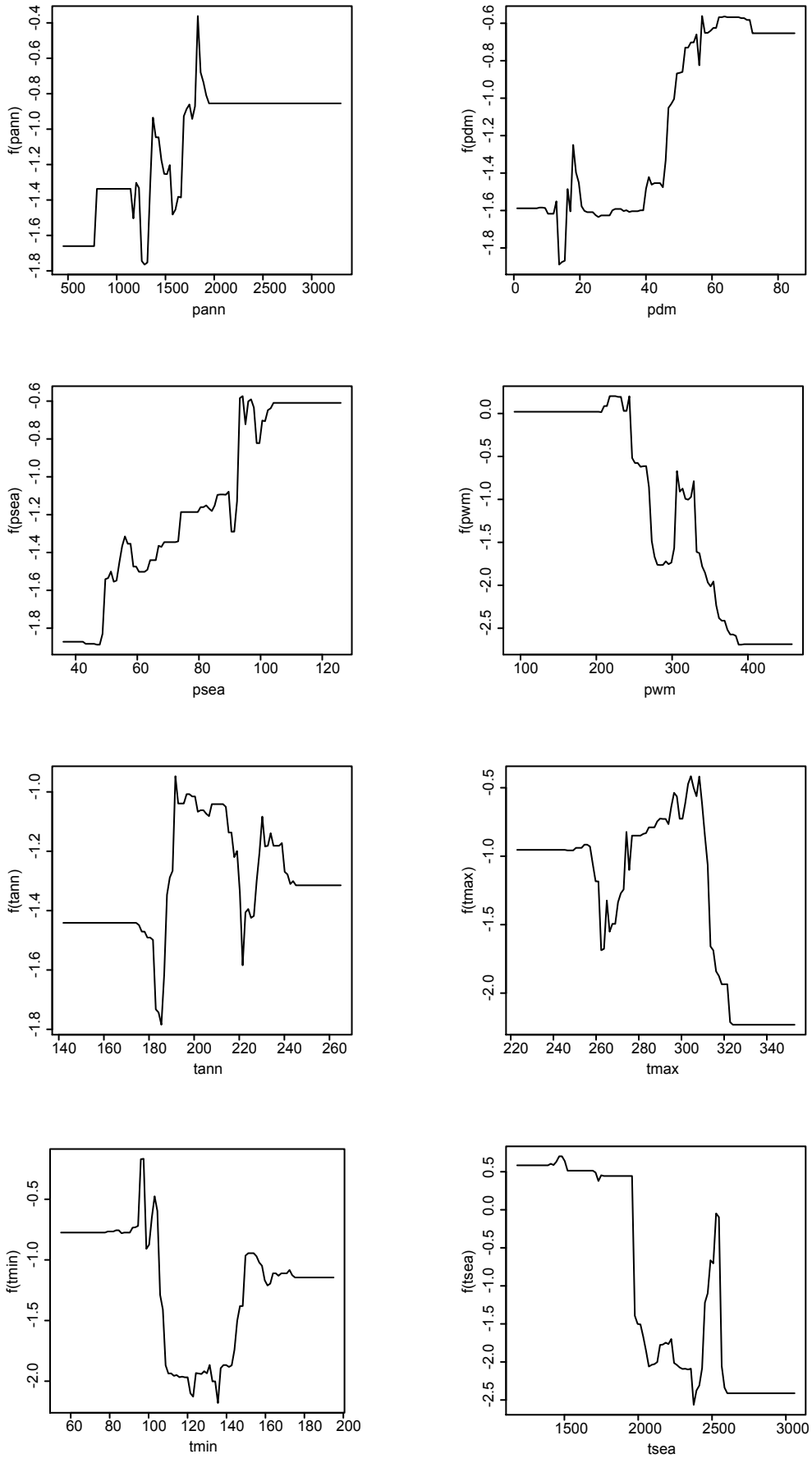


Figure S4.8: Response curves of the genus *Copelatus* to the individual climate variables.

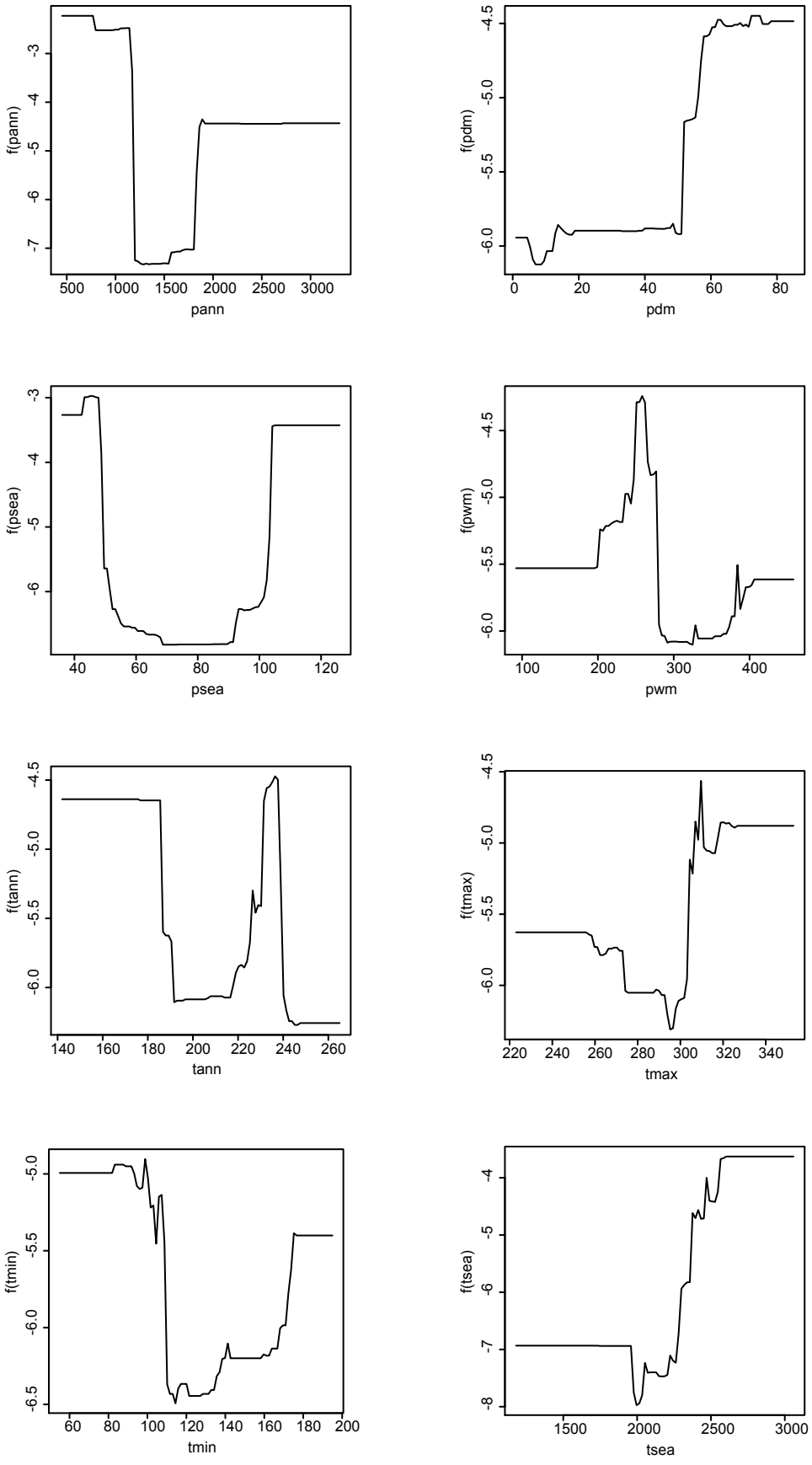


Figure S4.9: Response curves of the genus *Cybister* to the individual climate variables.

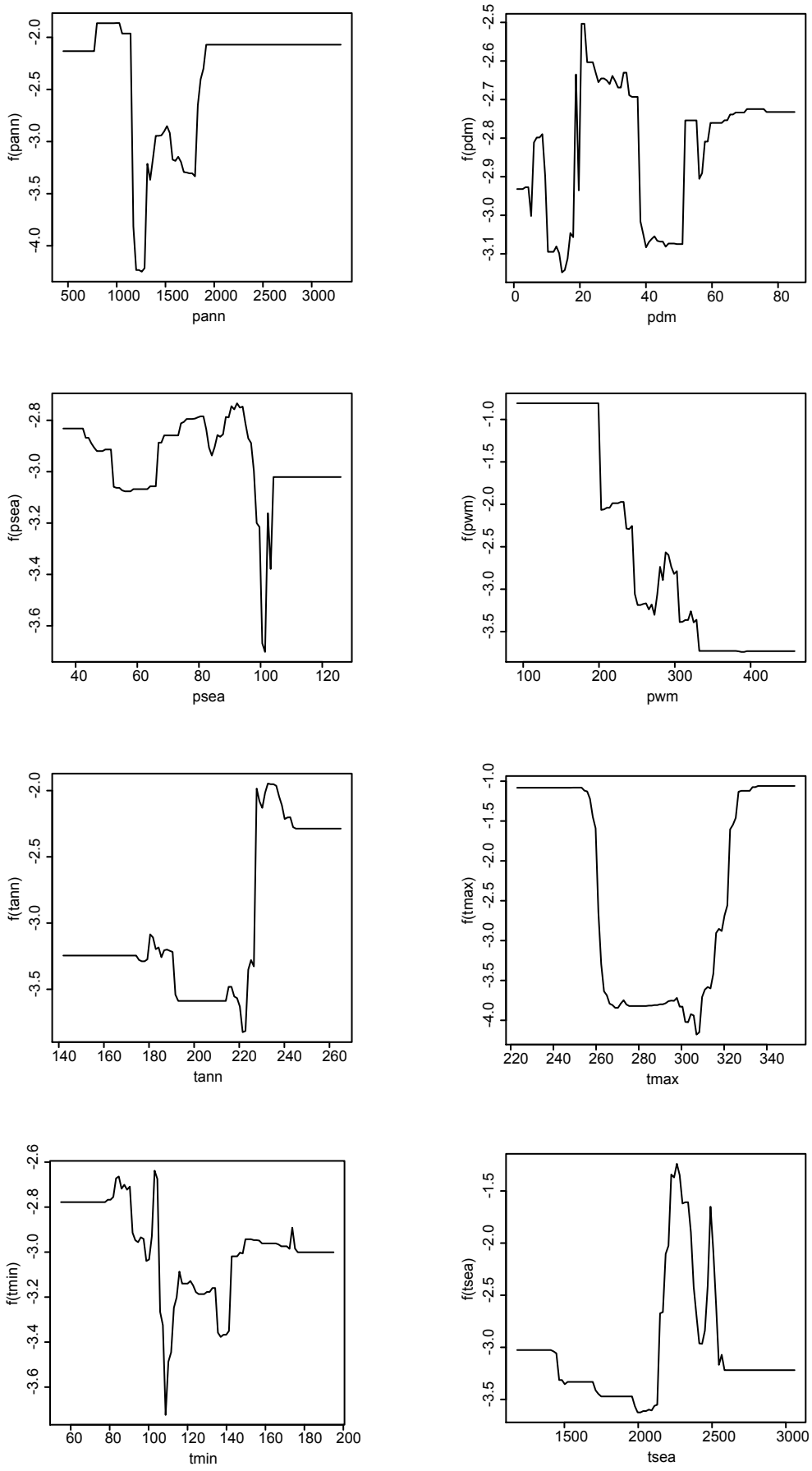


Figure S4.10: Response curves of the genus *Hydroglyphus-Pseuduvarus* to the individual climate variables.

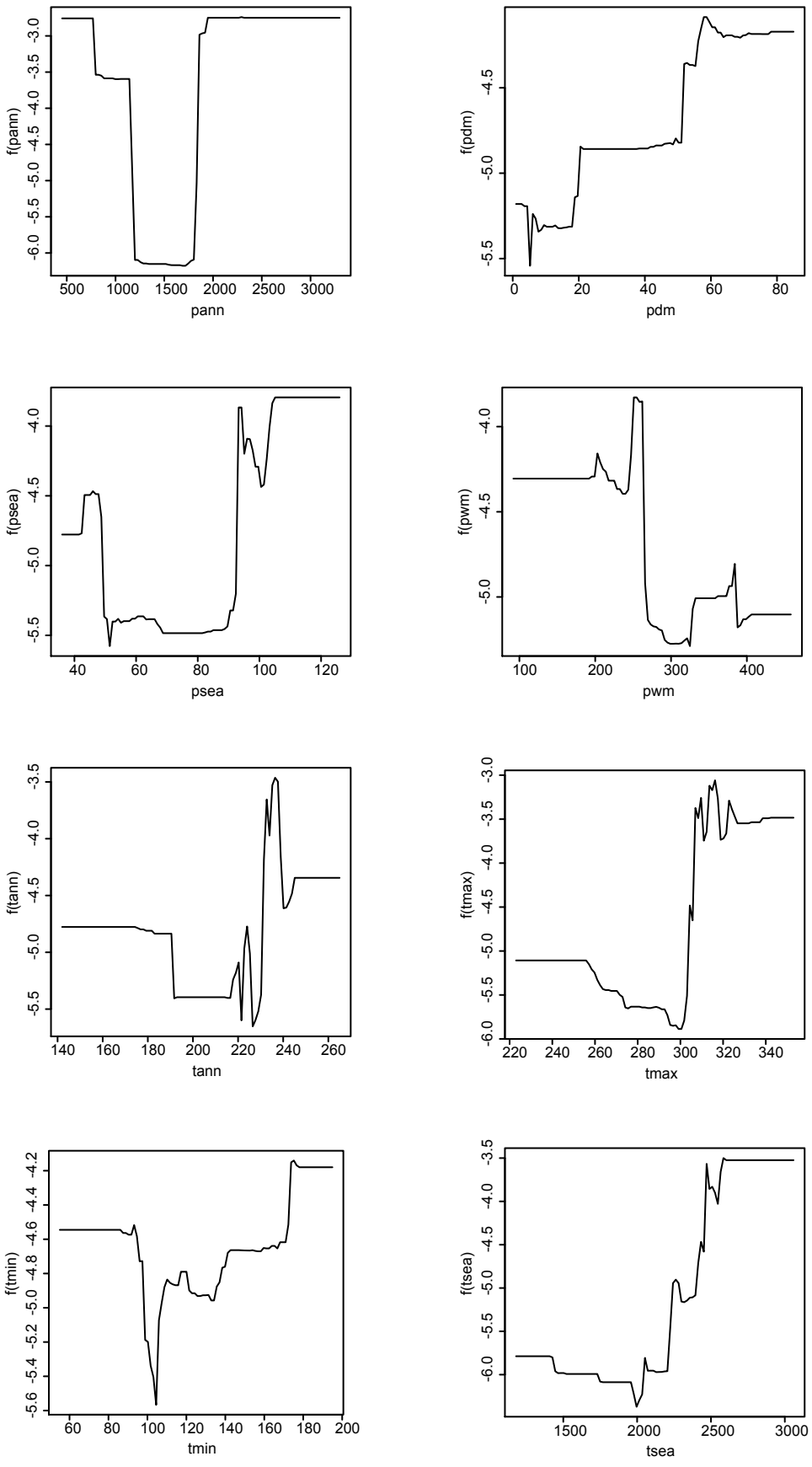


Figure S4.11: Response curves of the genus *Hydrovatus* to the individual climate variables.

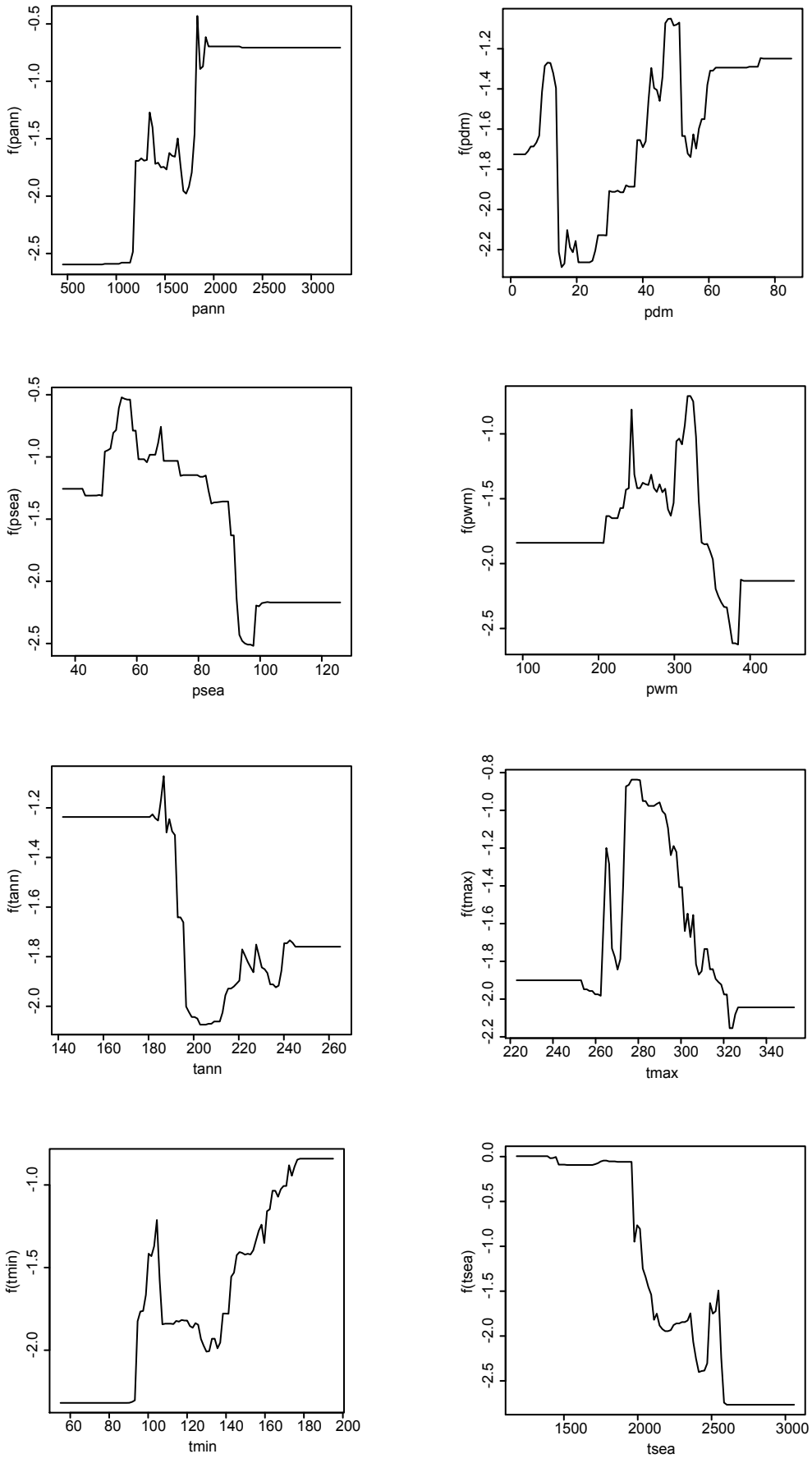


Figure S4.12: Response curves of the genus *Madaglymbus* to the individual climate variables.

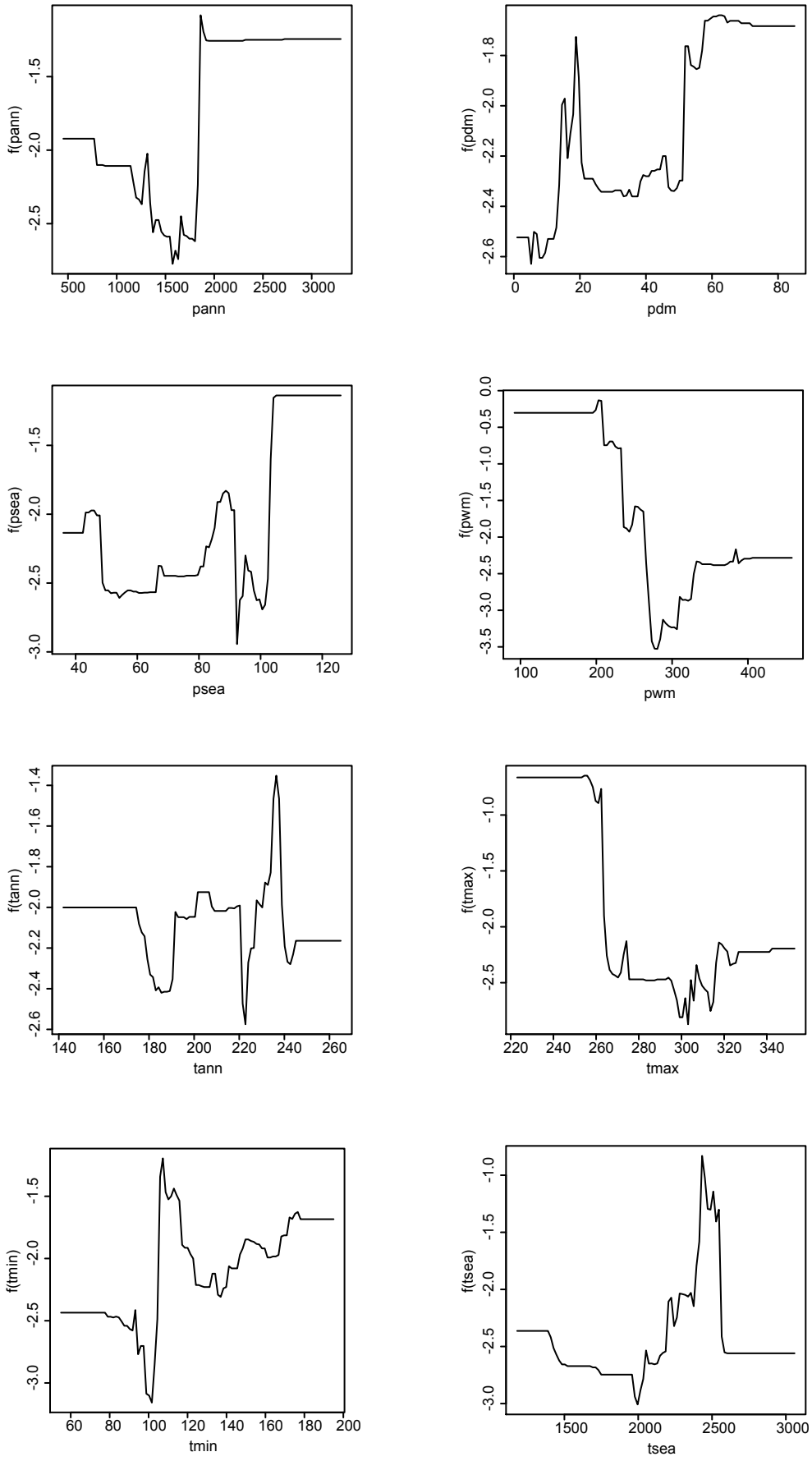


Figure S4.13: Response curves of the genus *Prodaticus* to the individual climate variables.

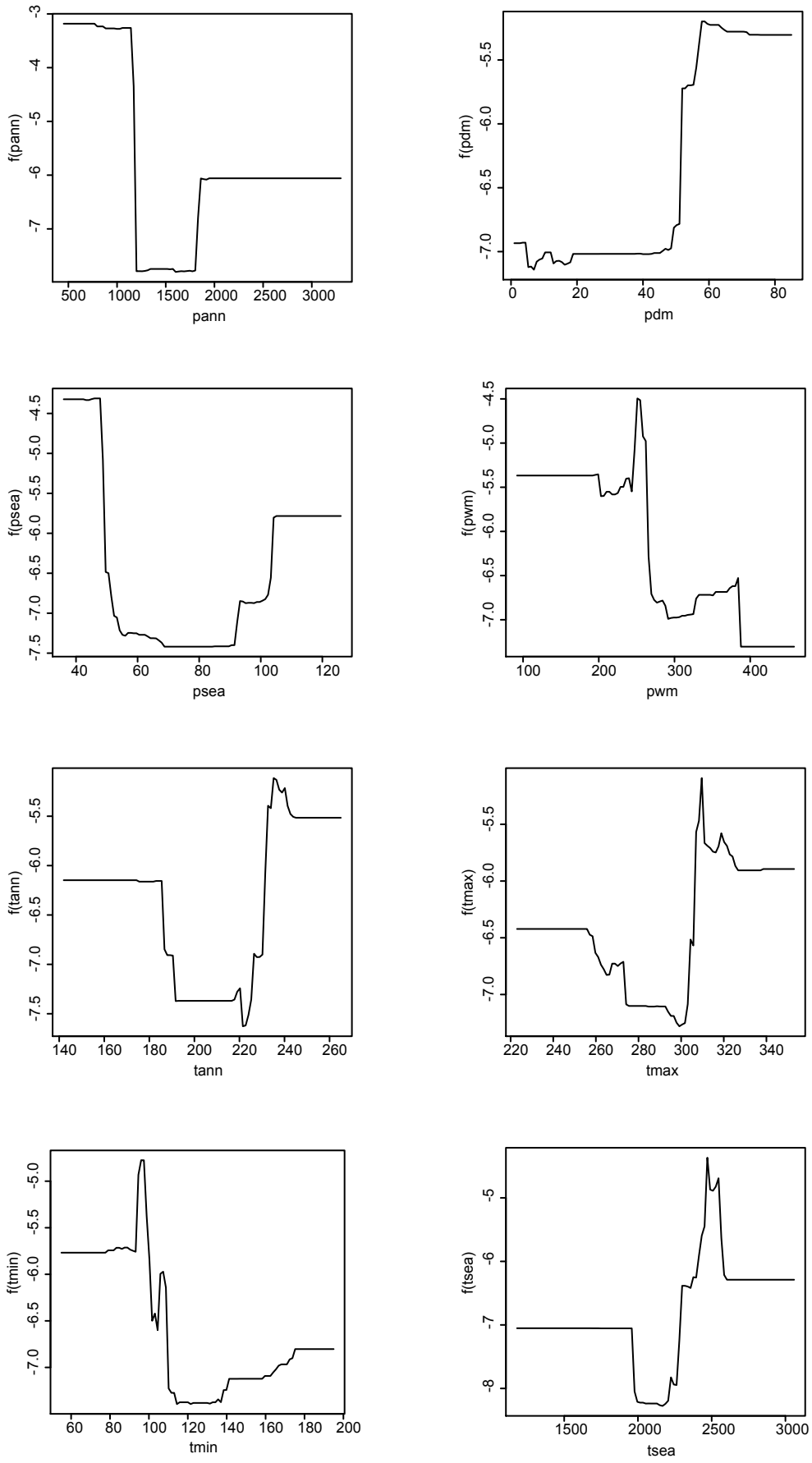


Figure S4.14: Response curves of the *Cybister* clade to the individual climate variables.

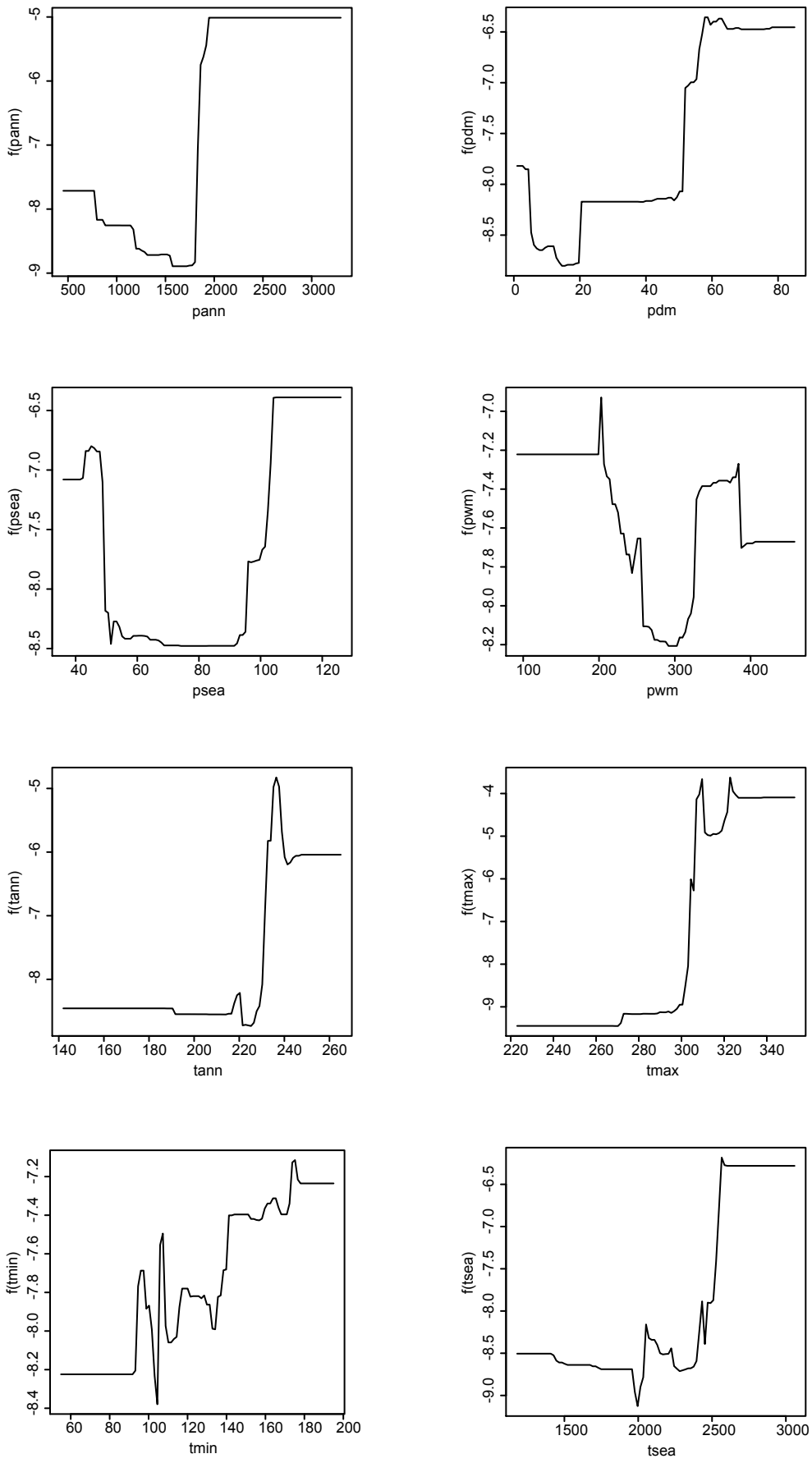


Figure S4.15: Response curves of the *Hydrovatus* clade to the individual climate variables.

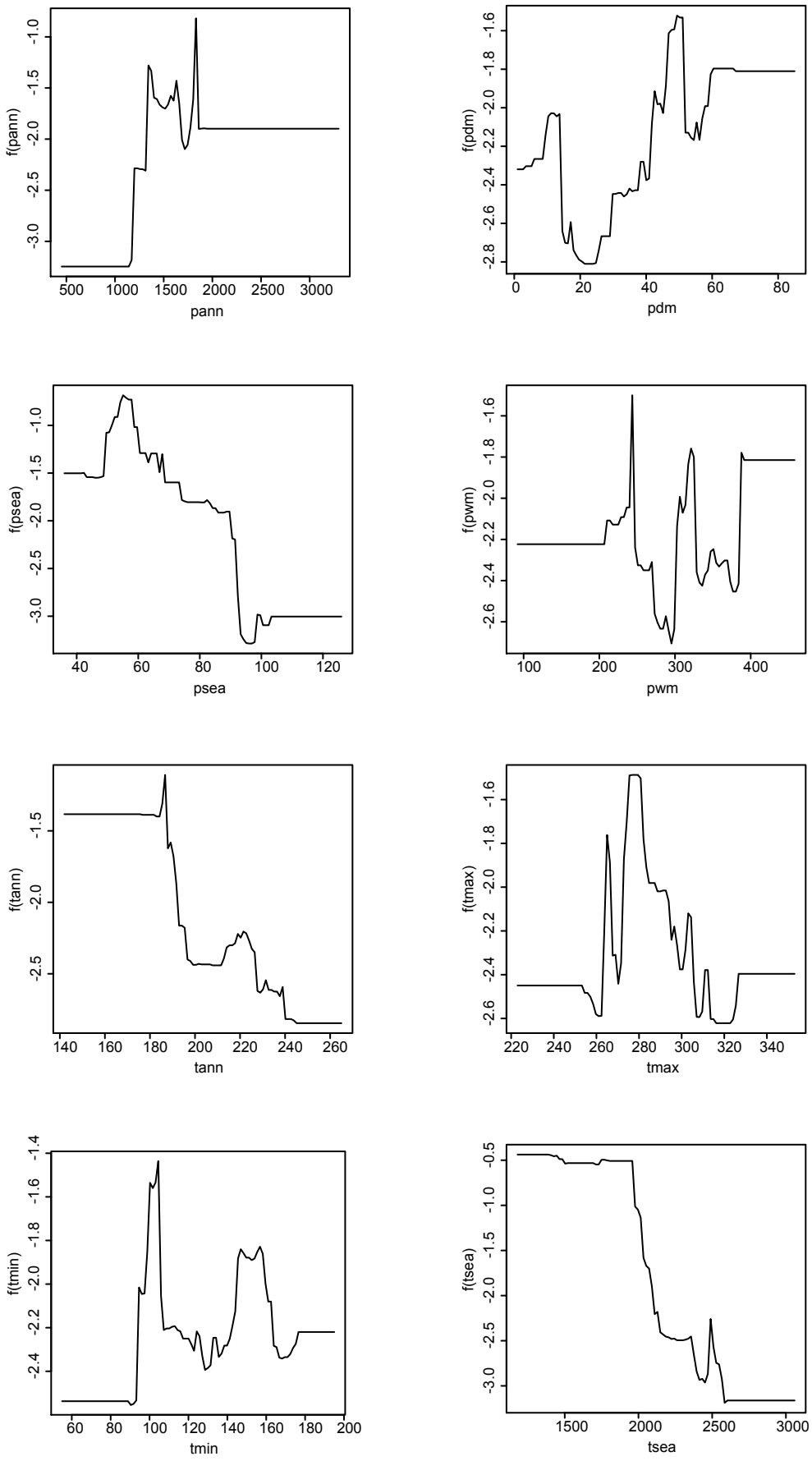


Figure S4.16: Response curves of the *Madaglymbus* clade to the individual climate variables.

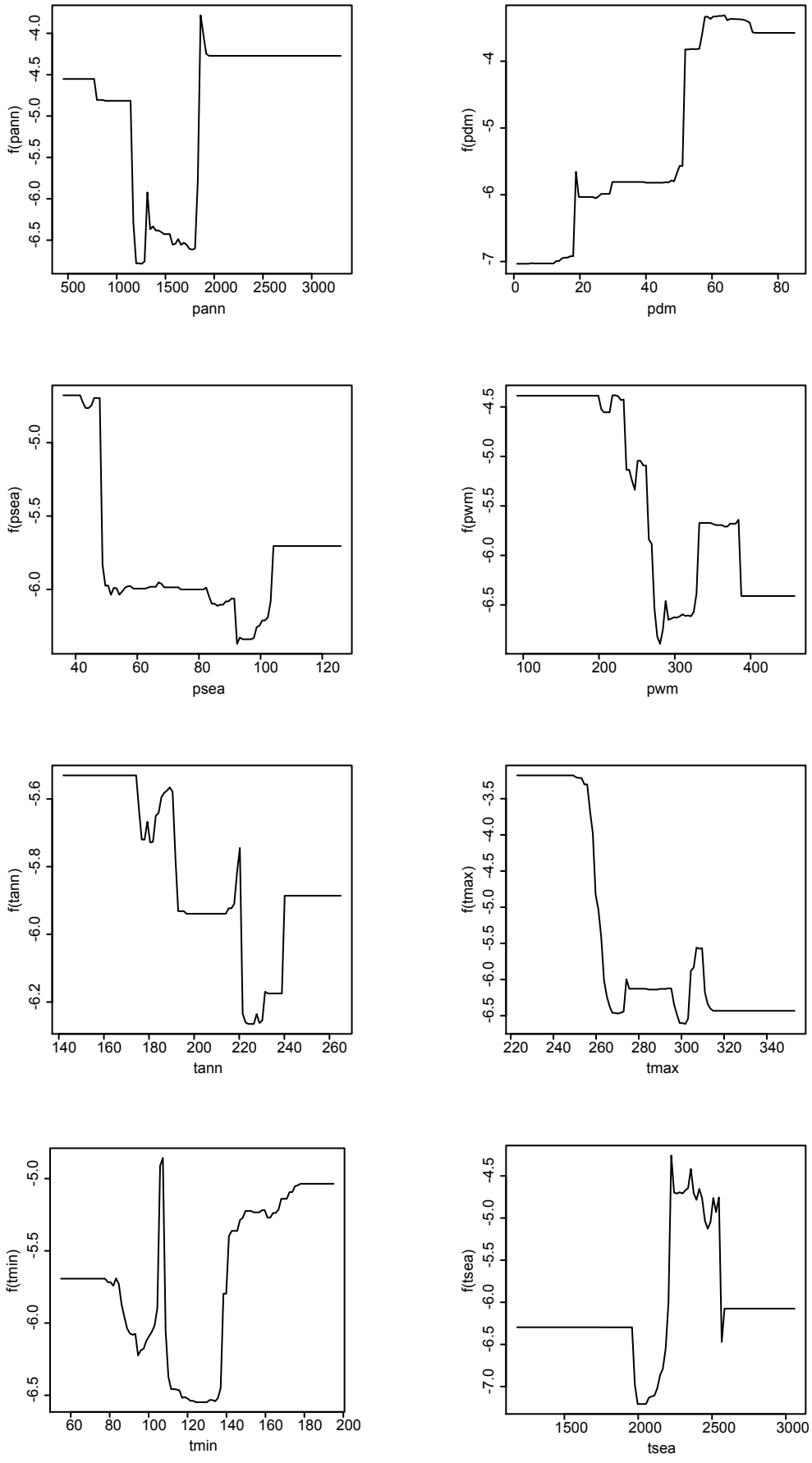


Figure S4.17: Response curves of the *Prodaticus* clade to the individual climate variables.

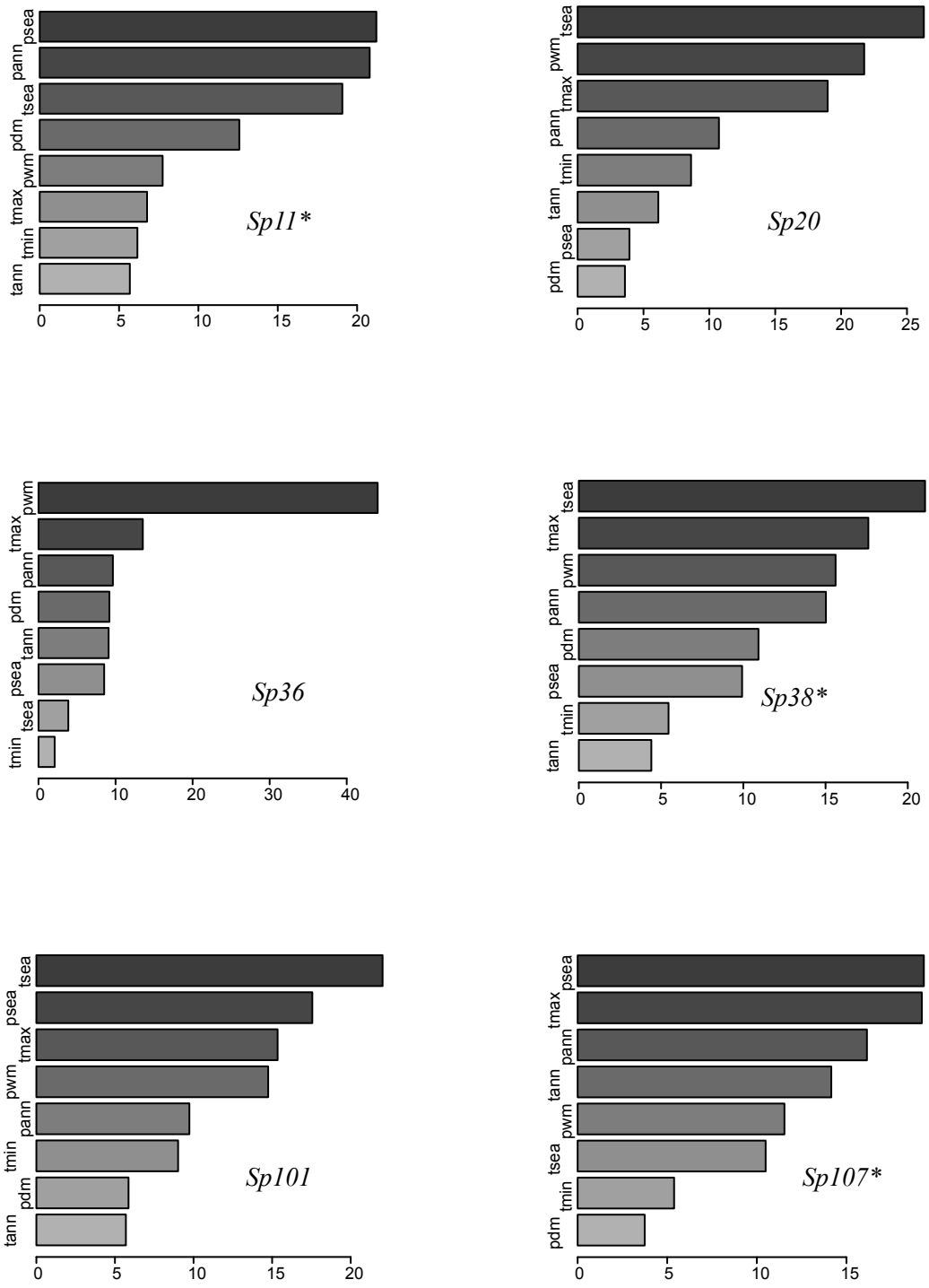


Figure S4.18: Individual contribution of the climatic variables in the GBM models from BIOMOD (part 1). The x axis represents the relative influence in %.

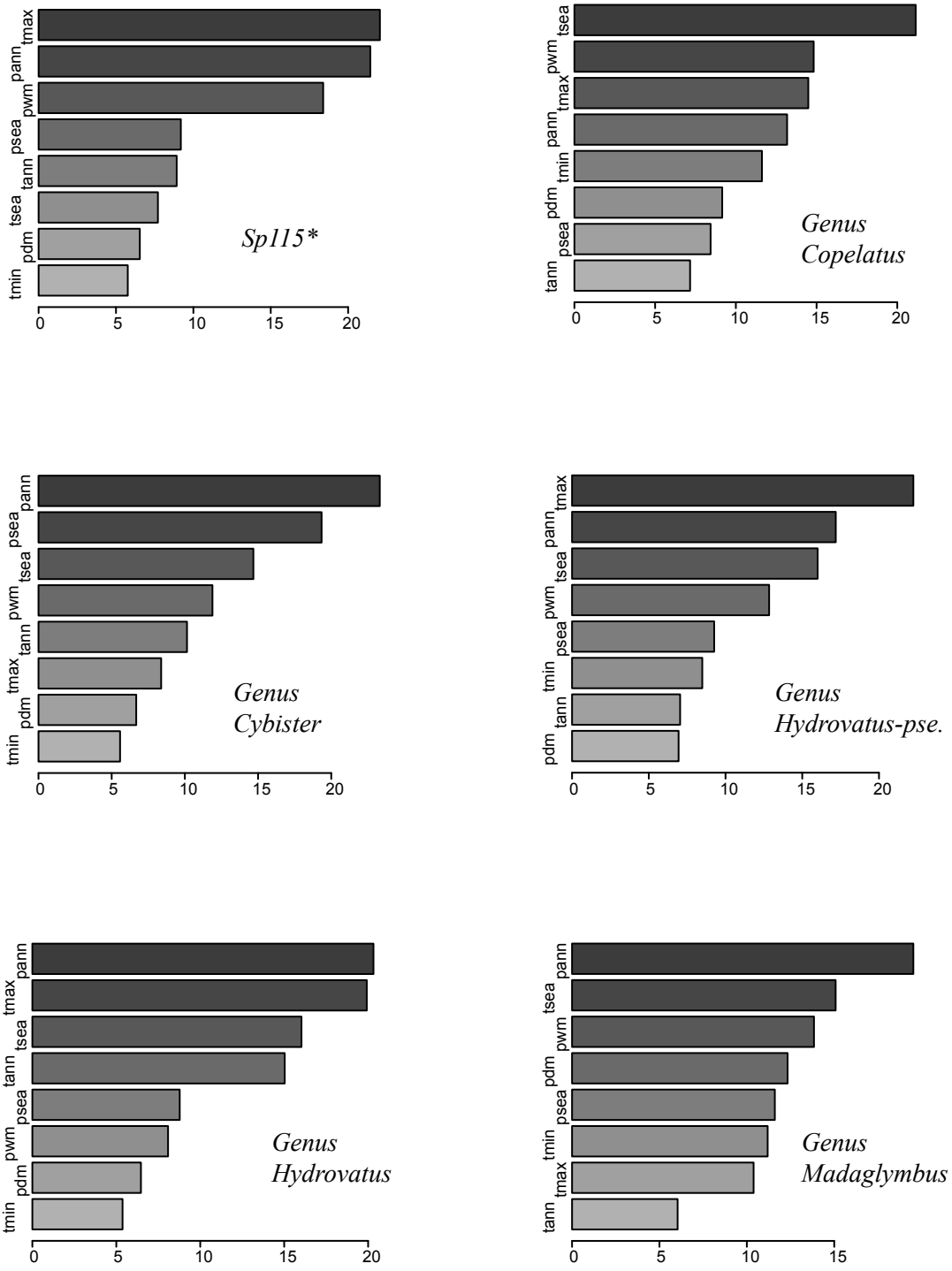


Figure S4.19: Individual contribution of the climatic variables in the GBM models from BIOMOD (part 2). The x axis represents the relative influence in %.

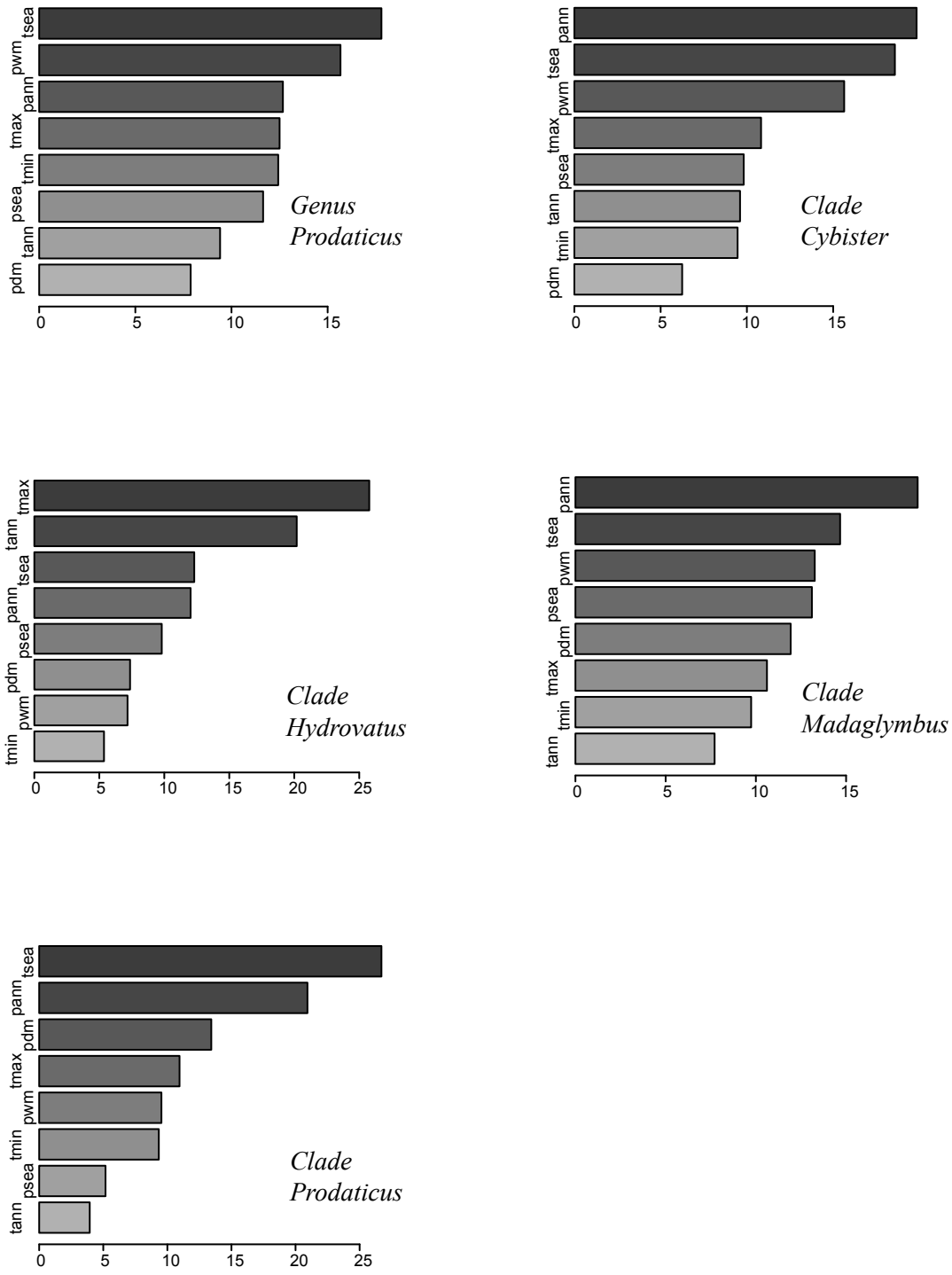


Figure S4.20: Individual contribution of the climatic variables in the GBM models from BIOMOD (part 3). The x axis represents the relative influence in %.

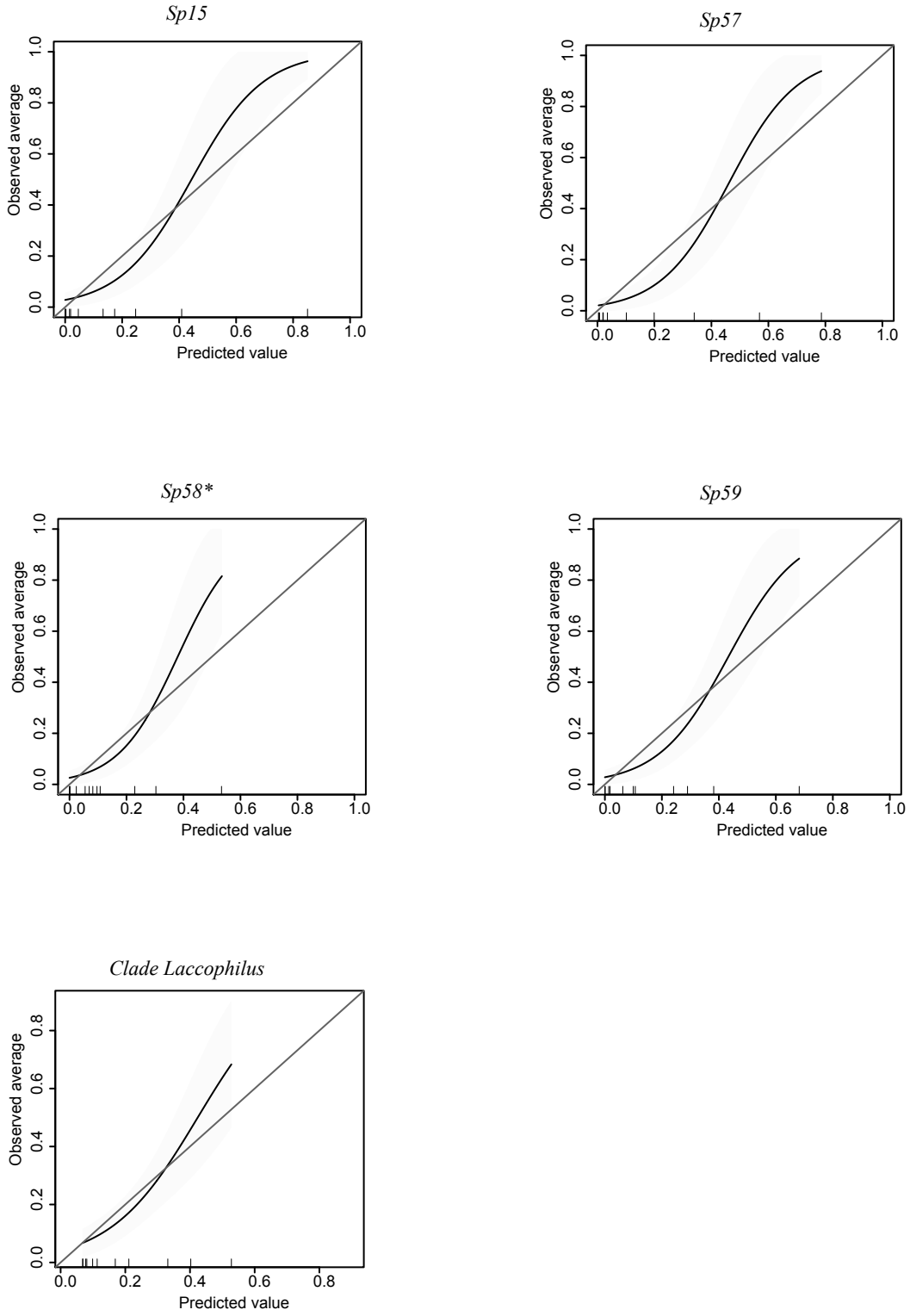


Figure S4.21: Response curves of the different Generalised Additive Models based on the predictions and the observed presences and absences.

	Tann	Tsea	Tmax	Tmin	Pann	Pwm	Pdm	Psea
sp11	0	4.4	15.7	1.2	0	10.1	68.5	0
sp15	3.8	22.1	2.3	2.2	1.1	15.9	50.9	1.6
sp20	0	12.9	30	12.5	0.1	18.1	22.9	3.5
sp43	0.5	79.5	0	15.2	0	0	3.8	0.8
sp58	0.2	52.8	9.7	8.8	0	6.9	21.7	0
sp65	1.1	25	0.4	0.2	0	11.7	61.6	0
sp76	0	23	33.9	8.4	1	18.1	14.6	1
sp32	0	22.5	41.9	6.5	1	13.8	13	1.4
sp57	1.3	26.6	1.7	0.8	1.2	10.2	54.7	3.5
sp63	0.7	20.2	13.4	1.7	0	20.6	38.9	4.5
sp101	0	56.1	17	16.5	1.2	4.1	5.1	0
sp124	0	66.9	0.2	14.5	0	4	14.4	0

Table S4.1: Individual contribution (in %) of the climatic variables in the Maxent models for the twelve GMYC species.

Genus Laccophilus

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-0.2408	0.2205	-1.092	0.2749
poly(pwm, 3)1	-6.0183	3.8692	-1.555	0.1198
poly(pwm, 3)2	7.442	3.456	2.153	0.0313 *
poly(pwm, 3)3	13.7595	3.3804	4.07	4.69e-05 ***
poly(tsea, 2)1	-3.3406	3.3163	-1.007	0.3138
poly(tsea, 2)2	8.1178	3.7094	2.188	0.0286 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Null deviance:	194.57	df: 141
Residual deviance:	141.05	df: 136
AIC:	153.05	

Clade copelatus

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	1.24E+01	6.17E+00	2.004	0.0451 *
I(tmax^3)	-6.50E-07	2.82E-07	-2.307	0.0211 *
poly(tmin, 3)1	1.92E+01	1.20E+01	1.604	0.1087
poly(tmin, 3)2	9.31E+00	5.62E+00	1.656	0.0977 .
poly(tmin, 3)3	1.45E+01	5.77E+00	2.506	0.0122 *
poly(pdm, 3)1	-2.88E+00	5.66E+00	-0.509	0.611
poly(pdm, 3)2	1.40E+01	8.18E+00	1.707	0.0879 .
poly(pdm, 3)3	-2.32E+01	1.06E+01	-2.2	0.0278 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Null deviance:	95.79	df: 141
Residual deviance:	76.328	df: 134
AIC:	92.328	

Table S4.2: Summary statistics for the Generalised Linear Models. Both the null and the residual deviances are given, together with the detailed dependent variables.