UNIVERSITY OF LJUBLJANA
BIOTECHNICAL FACULTY

Eva TURK

BIOGEOGRAPHY, MACROEVOLUTIONARY PATTERNS AND POPULATION GENETICS IN GOLDEN ORBWEAVER SPIDERS (NEPHILIDAE)

DOCTORAL DISSERTATION

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BIOGEOGRAFIJA, MAKROEVOLUCIJSKI VZORCI IN POPULACIJSKA GENETIKA PRI PAJKIH ZLATIH MREŽARJIH (NEPHILIDAE)

DOKTORSKA DISERTACIJA

Ljubljana, 2022

Based on the Statute of the University of Ljubljana and the decision of the Biotechnical Faculty senate, as well as the decision of the Commission for Doctoral Studies of the University of Ljubljana adopted on December 8, 2020, it has been confirmed that the candidate meets the requirements for pursuing a PhD in the interdisciplinary doctoral programme in Biosciences, Scientific Field Biology. Assist. Prof. Dr. Simona Kralj-Fišer is appointed as supervisor and Assoc. Prof. Dr. Matjaž Kuntner as co-supervisor.

Doctoral dissertation was conducted at the Jovan Hadži Institute of Biology, Scientific Research Centre of the Slovenian Academy of Sciences and Arts and at the Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung, Taiwan.

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Date of defense:

Eva Turk

KEY WORDS DOCUMENTATION

DN Dd
DC UDC 595.44(043.3)
CX Biogeography, speciation, extinction, population genetics, Nephilidae
AU TURK, Eva, MSc in Human Evolution and Behaviour
AA KRALJ-FIŠER, Simona (supervisor), KUNTNER, Matjaž (co-supervisor)
PP SI-1000 Ljubljana, Jamnikarjeva 101
PB University of Ljubljana, Biotechnical Faculty, Interdisciplinary Doctoral Programme in Biosciences, Scientific Field Biology
PY 2022
TI BIOGEOGRAPHY, MACROEVOLUTIONARY PATTERNS AND POPULATION GENETICS IN GOLDEN ORBWEAVER SPIDERS (NEPHILIDAE)
DT Doctoral dissertation
NO IX, 110 p., 8 fig., 6 ann., 121 ref.
LA en
AL en/sl
AB Golden orbweavers (Nephilidae) are a conspicuous family of spiders, recognized for their extreme sexual size dimorphism, web gigantism and other curious features. In four chapters, we explore their historical biogeography, diversification dynamics and genetic and geographical structuring at the population level using a variety of statistical and molecular approaches. In the first chapter, we combine an original species phylogeny of golden orbweavers with data on extant species distribution and estimates of dispersal probabilities among geographical areas to reconstruct the geographical origin and subsequent dispersal routes of the clade. We develop and employ a novel method of dispersal probability attribution, adding precision to the analysis and increasing reconstruction credibility. We find support for an Indomalayan and/or Australasian geographical origin of nephilids. In the second chapter, we infer the dynamics of two main macroevolutionary processes, speciation and extinction, in the nephilid phylogeny and detect heterogeneity in both. We test two environmental and two organismal factors for correlation with diversification, but find none. In the third chapter, we further develop biogeographical reconstruction methodology on coin spiders (genus *Herennia*), whose dispersal biology is largely unknown. We test two models assuming different main dispersal methods, and find the ballooning dispersal model more parsimonious than the slow stochastic "walking" dispersal model. The analyses also reveal the wide distribution of *H. multipuncta* is not human-driven in origin, but natural. The fourth chapter compares population genetic and geographic structuring in two golden orbweaver species with markedly different life histories. Populations of both species show genetic structure, but no geographical structure, implying strong gene flow among them.

KLJUČNA DOKUMENTACIJSKA INFORMACIJA

ŠD Dd
DK UDK 595.44(043.3)
KG Biogeografija, speciacija, izumiranje, populacijska genetika, Nephilidae
AV TURK, Eva, MSc in Human Evolution and Behaviour
SA KRALJ-FIŠER, Simona (mentor), KUNTNER, Matjaž (somentor)
KZ SI-1000 Ljubljana, Jamnikarjeva 101
ZA Univerza v Ljubljani, Biotehniška fakulteta, Interdisciplinarni doktorski študij Bioznanosti, znanstveno področje Biologija
LI 2022
IN BIOGEOGRAFIJA, MAKROEVOLUCIJSKI VZORCI IN POPULACIJSKA GENETIKA PRI PAJKIH ZLATIH MREŽARJIH (NEPHILIDAE)
TD Doktorska disertacija
OP IX, 110 str., 8 sl., 6 pril., 121 vir.
IJ en
JI en/sl

AI Zlati mrežarji (Nephilidae) so družina pajkov, znani po ekstremnem spolnem dimorfizmu, gigantizmu mrež in drugih nenavadnih lastnostih. V štirih poglavjih z naborom statističnih in molekularnih metod raziščemo njihovo biogeografsko zgodovino, dinamiko diverzifikacije ter genetsko in geografsko strukturiranost na nivoju populacij. V prvem poglavju združimo originalno vrstno filogenijo zlatih mrežarjev s podatki o današnji distribuciji vrst in ocenami verjetnosti disperzije med geografskimi regijami, in rekonstruiramo njihov geografski izvor in poti razširjanja. Pri tem razvijemo in uporabimo novo metodo ocenjevanja verjetnosti disperzije, ki izboljša natančnost analize in poveča verodostojnost rekonstrukcije. Naši rezultati podprejo Indomalajo in/ali Avstralazijo kot najverjetnejšo izvorno geografsko regijo zlatih mrežarjev. V drugem poglavju analiziramo dinamiko dveh glavnih makroevolucijskih procesov, speciacije in izumiranja, na filogeniji zlatih mrežarjev in zaznamo heterogenost v obeh. Dva okoljska in dva organizemska dejavnika testiramo za korelacijo z diverzifikacijo, a je ne najdemo pri nobenem dejavniku. V tretjem poglavju nadalje razvijamo metodologijo biogeografskih rekonstrukcij na primeru rodu *Herennia*, čigar disperzijska biologija je večinoma nepoznana. Testiramo dva modela, ki predpostavljata različna glavna načina disperzije. Model, ki predpostavlja razširjanje z zračnimi tokovi kot glavni način disperzije se izkaže za bolj parsimoničnega od modela, ki predpostavlja počasno stohastično disperzijo z lazenjem. Analize pokažejo, da široka današnja razširjenost vrste *H. multipuncta* ni posledica človekove aktivnosti, temveč je naravnega izvora. Četrto poglavje primerja populacijsko genetsko in geografsko strukturiranost dveh vrst zlatih mrežarjev z različno t. i. življenjsko zgodovino (ang. *life history*). Populacije obeh vrst kažeta genetsko, a ne geografske strukturiranosti, kar kaže na močan genski pretok med njimi.

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Turk E., Kralj-Fišer S., Kuntner M. 2021. Exploring diversification drivers in golden orbweavers. Scientific Reports, 11: 9248, doi: 10.1038/s41598-021-88555-3: 11 p.

Turk E., Bond J. E., Cheng R.-C., Čandek K., Hamilton C. A., Gregorič M., Kralj-Fišer S., Kuntner M. 2021. A natural colonisation of Asia: Phylogenomic and biogeographic history of coin spiders (Araneae: Nephilidae: *Herennia*). Diversity, 13, 11: 515, doi: https://doi.org/10.3390/d13110515: 14 p.

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ABBREVIATIONS AND SYMBOLS

mya	million years ago
eSSD	extreme sexual size dimorphism
COI	cytochrome c oxidase subunit I
RADseq	restriction site-associated DNA sequencing
MSG	multiplexed shotgun genotyping

1 INTRODUCTION

1.1 BIOLOGY OF GOLDEN ORBWEAVERS

Golden orbweavers (Araneae: Nephilidae) are a predominantly tropical family of spiders, known for exhibiting several intriguing phenotypes. The most conspicuous among them is the female-biased extreme sexual size dimorphism (eSSD) (Coddington et al., 1997; Kuntner and Coddington, 2020). In the giant orb weaver, *Nephila pilipes*, females can be up to 500 times heavier than males (Kuntner et al., 2012), which represents the most extreme case of sexual size dimorphism among all terrestrial animals. Another example of their peculiar traits is web gigantism (Figure 1). Females, but not males, build asymmetric orb webs of sometimes exaggerated proportions (Kuntner, 2017; Kuntner et al., 2019).

Figure 1: Gigantic orb web of a giant orb weaver (*Nephila pilipes*) female (photo: Kuntner M., 2010).

The unravelling of phylogenetic relationships among the 37 currently valid species has long proven problematic, mainly due to controversial familial classification (Coddington, 1990; Hong-Chun et al., 2004; Kuntner, 2006; Kuntner et al., 2008, 2013, 2019; Blackledge et al., 2009; Dimitrov and Hormiga, 2009; Dimitrov et al., 2017). The first study to construct a global, time-calibrated nephilid phylogeny using five mitochondrial genes, three nuclear genes and morphological data estimated the origin of nephilids to 40 million years ago (mya) (Kuntner et al., 2013). Recently, however, a study using phylogenomic data produced a robust and well-sampled species-level phylogeny, time-calibrated by two fossils (Kuntner et al., 2019). It pushed the estimated time of nephilid origin back significantly, to the Early Cretaceous (133 mya) (Kuntner et al., 2019).

Due to their conspicuousness, nephilids are a popular model in evolutionary research. But while their sexual biology (Schneider and Elgar, 2005; Kasumovic et al., 2007; Kuntner et al., 2009; Kralj-Fišer et al., 2011; D. Li et al., 2012), eSSD (Kuntner et al., 2012; Kuntner and Elgar, 2014; Schneider et al., 2015) and phylogenetics (Scharff and Coddington, 1997; Hong-Chun et al., 2004; Kuntner et al., 2008, 2013, 2019) have been studied extensively, some aspects of their biology remain poorly understood. Relevant for this dissertation, this is also true for large-scale evolutionary patterns, such as their historical biogeography and the dynamics of diversification, as well as genetic and geographic structuring at the population level.

1.2 EXTANT AND HISTORICAL DISTRIBUTIONS

Biogeography is the study of a taxon’s distribution through space and presents an essential piece of information for comprehensive understanding of organismal biology. While ecological biogeography focuses on extant distributions, historical biogeography inspects the changing of geographical distribution through geological time (Posadas et al., 2006). In order to credibly infer past distributions from present ones, resolved phylogenetic relationships among the studied taxa are among the main prerequisites. As dispersal among biogeographical regions is dependent on factors such as connectivity and distance among them, quantification of dispersal probabilities greatly enhances the precision and credibility of historical biogeographic reconstruction models. Ideally, these dispersal probability estimates would reflect the changing geographic and geological conditions in the studied area (e.g. plate tectonic movements), organism-specific dispersal biology and fossil data.

Golden orbweavers are an interesting subject for biogeographical research due to a wide extant distribution, presumed variable dispersal propensities and old phylogenetic age.

Extant species of golden orbweavers are distributed predominantly tropically and subtropically, with some species ranging into temperate regions (Su et al., 2011). Most species inhabit the African and Indomalayan tropics, with genera commonly occurring sympatrically (Kuntner et al., 2019). Some species, such as *Nephila pilipes* and *Herennia multipuncta*, have wide ranges, spanning thousands of kilometres, while others, such as *Indoetra thisbe* and most species of *Herennia*, are island endemics (Kuntner, 2005, 2006; Su et al., 2007). The only truly temperately distributed species is *Trichonephila clavata*, occurring between the Himalayas and Japan (Su et al., 2011), and, due to recent human introduction, in North America (Hoebeke et al., 2015).

From the large variability of extant range sizes, it can be assumed that dispersal propensity varies between the seven nephilid genera. The aforementioned *Nephila pilipes* has been shown to be an excellent aerial disperser, capable of active long-distance travel via ballooning (Lee et al., 2015). In ballooning, a spiderling climbs to a wind-exposed surface and releases silken threads into a wind current, which then blows it away (Bell et al., 2005; Kuntner and Agnarsson, 2011a). This type of dispersal allows for succesful gene flow maintenance across large distances, for example between the Caribbean islands and North American mainland (Čandek et al., 2020a). In certain genera, e.g. *Clitaetra*, *Nephilingis* and *Herennia*, however, this type of dispersal has not been observed. They presumably lack active long-range dispersal, resulting in narrow distributions (Kuntner and Agnarsson, 2011b, 2011a).

Past distribution of golden orbweavers is largely unknown, as fossils are scarce (Kuntner et al., 2013). Some fossilised spiders from the Dominican (Wunderlich, 1986) and Baltic amber (Wunderlich, 2004), aged 40 to 16 million years, have been recognised as nephilids, and served for phylogenetic calibration in past studies of nephilid phylogenetics (Kuntner et al., 2013). Most recently, Poinar and Buckley (2012) described an inclusion from Burmese amber containing one adult male and one juvenile spider that resemble modern nephilids. This species, named *Geratonephila burmanica*, has recently been interpreted as a possible stem nephilid (Kuntner et al., 2019). Burmese amber originates from a region of Myanmar, sitting on a small Gondwanan tectonic block with uncertain geological history, called the West Burma block (Cruickshank and Ko, 2003).

The amber itself is thought to be Cretaceous in origin (Cruickshank and Ko, 2003; Shi et al., 2012).

There is of yet no biogeographical study that would apply modern methodological approaches to a robust, well-sampled nephilid phylogeny to infer their geographical origin and colonization routes. The most comprehensive study so far included twelve species of *Nephila*, most of which have since been reclassified to *Trichonephila* (Su et al., 2011). It constructed a phylogeny on the basis of one nuclear (18S) and two mitochondrial markers (COI and 16S) and uncovered a phylogenetic divide between (sub)tropical/temperate Australasian species and African/American species. It reconstructed the ancestral range of the Australasian clade to tropical Asia and the ancestral range of the African/American clade to Africa. Asia or Africa was identified as the ancestral area of all included species (Su et al., 2011). The authors placed diversification events within the studied group to mid to late Neogene, when the Earth was cyclically warming and cooling, suggesting these climatic changes drove speciation (Su et al., 2011).

The aim of the first chapter of this dissertation was to reconstruct the family's biogeographic history from its hypothesised origin until the present on an updated phylogeny using refined statistical modelling. We posed two alternative hypotheses regarding the origin of the clade, assuming that the recent clade age estimation at 133 mya (Kuntner et al., 2019) is reasonably accurate. The first hypothesis, "Out of Africa", stemmed from the fact that Africa, specifically the Afrotropics, exhibit the largest extant taxonomic diversity of golden orbweavers. Africa may thus prove as their geographical origin, diversification arena, and colonization gateway. The alternative hypothesis leaned on the assumption that *Geratonephila* is, indeed, a representative of stem nephilids, placing the origin of nephilids on (and around) the West Burma Block. It remains unresolved, however, whether this fragment broke away from Australia and then rafted towards SE Asia alone or with the Indian plate (Poinar, 2019). This broadly defined "Out of West Burma" hypothesis therefore expected Australasia or Indomalaya (or both) to optimize as the most likely geographical origin of golden orbweavers.

1.3 MACROEVOLUTIONARY RATE DYNAMICS

Variation in species richness among comparable clades of organisms is striking, ranging from hyper species-rich lineages at one extreme to single representatives, sitting at the ends of long branches, at the other (e.g. the orchids vs the ginkgo). This heterogeneity is the product of two fundamental macroevolutionary processes, speciation and extinction (Raup, 1985). When speciation (cladogenesis) is more frequent than extinction (lineage termination), the lineage diversifies, while in the opposite case, it eventually goes extinct (Raup, 1985). Unlike these mechanisms of diversity heterogeneity, however, its drivers remain unclear (reviewed in Wiens, 2017).

Speciation and extinction (and thus their net difference, diversification) are themselves influenced by an array of factors and the complex interactions among them. Some macroevolutionary literature divides diversification drivers into intrinsic and extrinsic factors (Slowinski and Guyer, 1993; Rabosky, 2006; Bouchenak-Khelladi et al., 2015). Intrinsic factors are specific phenotypic traits (or their states), that correlate with, and presumably influence, taxonomic diversity (Slowinski and Guyer, 1993). The result is the so-called ‘trait-driven’ diversification. A commonly studied trait in this context is body size, negatively correlated with species richness in animals (Hutchinson and MacArthur, 1959; Čandek et al., 2020b). Proposed explanations for this trend include larger population sizes in smaller animals with access to more ecological niches (Hutchinson and MacArthur, 1959), higher levels of mobility and shorter generation times (Marzluff and Dial, 1991), and lower energetic requirements (Stanley, 1973; Brown and Nicoletto, 1991) of smaller animals. If the evolutionary acquirement of a trait is followed by rapid speciation, the trait is referred to as a ‘key innovation’ (Slowinski and Guyer, 1993). In spiders, traits related to web architecture and silk use are often interpreted as key innovations (Bond and Opell, 1998; Blackledge et al., 2009; but see Fernández et al., 2018).

In contrast, major tectonic events, formation of geographic barriers, alterations in wind and sea currents, changes in local and global climatic conditions and the subsequent availability and variability of ecological niches are some of the numerous, closely intertwined extrinsic factors, influencing ‘habitat-driven’ speciation and extinction (MacArthur et al., 1966; Brown and Nicoletto, 1991; Marzluff and Dial, 1991; Easterling et al., 2000; Moen and Morlon, 2014; Leprieur et al., 2016; Simões et al., 2016). Extrinsic factors studied in spiders include global climatic history (Fernández et al., 2018; Shao and Li, 2018; Luo et al., 2020), (micro)habitat availability (Dimitrov et al., 2012; Eberle et al., 2018) and diversification of other (prey) organisms (Liu et al., 2016).

The phylogeny of golden orbweavers exhibits a large variation in species richness among genera, currently ranging from one in *Indoetra* to 14 in *Herennia* (Kuntner et al., 2019; Kuntner et al. in preparation). Genera exhibit diverse ecologies and life histories, which could prove responsible for the discrepancies in species richness. No prior studies on diversification drivers in nephilids exist. Thus, in the second study of this dissertation, we aimed to explore the macroevolutionary dynamics in golden orbweavers and investigate whether diversification in this clade is related to the selected organismal and/or environmental factors. These included geographical distribution (climate and landmass type), phenotypic extremeness and dispersal propensity.

Elevated species richness in lower latitudes is a well-known phenomenon, also observed in spiders (Whitehouse et al., 2009; Piel, 2018). As almost all nephilid species are confined to geographic areas between the Tropics of Cancer and Capricorn, we broadly tested the effect of tropic vs. subtropic/temperate distribution on diversification. The other tested binary distributional factor was island vs. continental distribution. Some genera, like *Herennia*, contain a number of island endemic species. We expected heightened speciation rates in such clades, due to genetic isolation.

For both continuous traits, dispersal propensity and phenotypic extremeness, we hypothesised a bell-shaped relationship between the degree of trait expression and speciation rate. Put differently, we expected heightened speciation in genera with intermediate phenotypes and intermediate dispersal propensity. Extreme phenotypes must have ensured certain fitness advantages, otherwise they would not have persisted through the spiders’ evolutionary history, but they must also carry costs. At the other hand, the least extreme phenotypes avoid these costs, but also fail to provide the advantages of, for example, exaggerated female body size. We were particularly interested in whether extreme phenotypes represent an 'evolutionary dead end', i.e. a trait that initially provided fitness advantages, but later in evolution lost its benefit and became costly, potentially even causing lineage extinction (Bromham et al., 2016). This would be corroborated if increased extinction rates were detected in the most phenotypically extreme genera, such as *Nephila*.

Similarly, we hypothesised that the inherent levels of dispersal propensity and behaviours related to dispersal are important intrinsic factors influencing diversification. They are necessarily coupled with extrinsic factors, allowing and limiting ballooning, e.g. changes in global wind patterns and emergence of geographical barriers restricting air currents. Following the intermediate dispersal model of biogeography (Claramunt et al., 2012; Agnarsson et al., 2014; Čandek et al., 2020a), poor dispersers maintain a narrow distribution and thus disperse (and consequently speciate) only rarely. In contrast, excellent dispersers inhabit vast areas, but also successfully maintain gene flow across them, which in turn prevents speciation via genetic isolation. Intermediate dispersal propensity was thus hypothesised as optimal for maximising diversification potential in golden orbweavers.

1.4 SMALL-SCALE BIOGEOGRAPHY IN *HERENNIA*

As previously pointed out, modern biogeographic analyses should take into account both environmental (geographical and geological) conditions and organism-specific biology, reflected in varying dispersal probabilities. One of the outcomes of the first chapter of this thesis was the development of a novel approach to the quantification of dispersal probabilities, basing them on physical distances between biogeographic regions through time. This method is especially suitable for analyses on larger phylogenetic and geographic scales, where geological reconstructions are reasonably accurate, and when organismal dispersal biology is well documented. When this is not the case, however, the method should be modified. In the third part of the thesis, we aimed to develop, test and discuss an adjusted approach to biogeographical inference within a golden orbweaver genus.

Coin spiders (genus *Herennia*) are the most speciose genus among golden orbweavers, with 11 described and three yet undescribed species (Kuntner et al., 2019; Kuntner et al. in preparation). They exhibit several intriguing features, unique among golden orbweavers. As opposed to other genera, their so-called arboricolous webs are not fully suspended in the air column, but attached to tree trunks. Except for the spotted coin spider (*H. multipuncta*), all species have a relatively narrow distribution, in many cases confined to single islands. While other coin spider species inhabit pristine forests, *H. multipuncta* is synanthropic, regularly found in managed habitats (Kuntner, 2005). Biogeography of the genus has previously been addressed in a revision paper (Kuntner, 2005), where Australasia was suggested as both the geographical origin of the genus and the arena of their speciation.

The first goal of this chapter was to infer evolutionary relationships in the largest set of coin spider species to date. Secondly, we aimed to expand this species-level phylogeny by including several samples of each species, collected at various localities throughout species ranges. To gain insight into its unusually large range, we included an especially large sample of *H. multipuncta* specimens.

In biogeographic reconstruction, we aimed to follow the developed rationale of Chapter 1, but modified it to cater to the specifics of the studied clade. Directly transforming measured physical distances between pairs of biogeographical regions into dispersal probabilities would not be suitable in this case. The area inhabited by coin spiders has a complex geological past which is difficult, if not impossible, to reconstruct with sufficient precision. Therefore, we planned to develop an adaptation of the method, where this kind of uncertainty could be accommodated. Another issue was that very little is known about the dispersal biology of coin spiders. Ballooning behaviour has so far not been documented or reported by existing literature. Therefore, we aimed to develop and test in parallel two biogeographical models, one assuming ballooning as the predominant means of dispersal, and the other simple passive “walking” during search for vacant habitat as their primary mode of dispersal. Considering the high occurrence of island endemism in the genus, frequent ballooning does not seem plausible, so we expected passive dispersal to produce a more parsimonious biogeographic reconstruction.

Finally, we aimed to test the hypothesis that the unusually large range of *H. multipuncta* is anthropogenic. The assumption was that this species, today frequently inhabiting managed habitats, largely dispersed around its range via boats or other means of human transport. An indication of support for this hypothesis would be a co-occurrence of the phylogenetic splits between *H. multipuncta* populations and human presence in this region. Moreover, if human-induced dispersal was the main driver of range expansion in this species, specimens from the same populations can be expected to group together in the phylogenetic tree.

1.5 GENETIC AND GEOGRAPHICAL STRUCTURE IN NEPHILID POPULATIONS

The last chapter of this dissertation focused on within-species genetic structure. Much like we hypothesised the diverse ecologies, life histories and environments of nephilid species to be reflected in biogeographical and diversification trends, these divergences could also be reflected in genetic structure of different species. Factors such as geography (including range size, range fragmentation, environment seasonality, etc.), population size, solitary vs. aggregation lifestyle, and dispersal behaviour are complex, intertwined factors, that can be assumed to have an effect on genetic population structuring and its relationship with geography.

The goal of Chapter 4 was to examine and compare patterns in population genetic structure of two species of nephilids, exhibiting different ecologies and life histories: the giant orb weaver, *Nephila pilipes*, and the Joro spider, *Trichonephila clavata*. The first is a common species of low-elevation rainforests, found throughout Asia and Australia (Su et al., 2007). It is solitary and extremely sexually size dimorphic (Kuntner et al., 2019). In contrast, *T. clavata* is the only temperately distributed nephilid, maintaining a relatively narrow range in high latitude and/or high elevation habitats from the Himalayas to Japan (Su et al., 2011). It is a smaller, less sexually size dimorphic species, and, unlike the giant orb weaver, often found in loose aggregations (own observations).

Prior studies recovered genetic structure without geographical patterns in these two (Lee et al., 2004; Jung et al., 2006; Su et al., 2007) and other species of nephilids (Kuntner and

Agnarsson, 2011a; Čandek et al., 2020a). Both existing studies on *N. pilipes* used COI as the only genetic marker, a now-outdated method, largely replaced by modern approaches involving whole-genome surveying, increasing analytical resolution. In contrast, the only previous study on *T. clavata* did employ a whole-genome surveying method called amplified fragment length polymorphism fingerprinting (AFLP). In recent years, AFLP has been rivalled by a similar, more expensive but high-throughput approach called restriction site-associated DNA sequencing (RADseq). First proposed by Baird et al. (2008), the aim of RADseq is to discover and genotype single nucleotide polymorphisms (SNPs), adjacent to cut sites of a selected restriction enzyme throughout the genome. The differences between individuals' hundreds or thousands of informative homologous loci can then be used for phylogenetic inference or other bioinformatics analyses, much like the differences in COI sequences. Because of its whole-genome coverage, RADseq is mainly used to infer genetic variance at the population level. Additionally, because a reference genome is not necessary for sequence assembly, RADseq is an ideal tool for studying non-model organisms, such as golden orbweavers (Andrews et al., 2016).

Several variants of the original protocol exist, but the main pipeline is as follows (Andrews et al., 2016). Extracted DNA is digested by one or more restriction enzymes. Sequencing adaptors, required by next-generation sequencing platforms, are added. They may include unique barcodes, used to distinguish between individual samples later on. DNA from all individuals is pooled (multiplexed) in a single library. Optionally, the samples are size-selected. Details relating to the order and type of enzymes, adaptors and barcodes used differ between versions of RADseq. Fragments are amplified via PCR and sequenced on a next generation sequencing platform. Downstream bioinformatics follow the same basic procedure regardless of the RADseq variant, but can be modified depending on the type of obtained RAD data. Sequences are first de-multiplexed and barcodes trimmed. The reads are filtered to ensure sufficient quality. If no reference genome exists, sequences are assembled *de novo*, using purpose-build software, such as Stacks (Rochette et al., 2019) or pyRAD (Eaton, 2014).

The aim of Chapter 4 was to retest the previously observed genetic and geographical patterns, or the lack of them, using a version of RADseq called multiplexed shotgun genotyping (MSG) (Andolfatto et al., 2011). This method retains the key benefits of RADseq, but uses enzymes with more cut sites (‘frequent cutters’), so adaptors are attached to a large number of smaller DNA fragments, randomly oriented in respect to sequencing direction, which increases their chance to reveal informative sequence differences. Another benefit of MSG is that it requires smaller amounts of DNA, only about 10 ng per individual, than other methods.

Because genetic and geographical population structure is influenced by numerous intertwining factors, this chapter was explorative, with no *a priori* predictions. However, as both species occupy large ranges, the absence of structuring on all levels seemed unlikely. We expected the spiders’ inability to maintain gene flow across ranges of such sizes would lead to genetic segregation of nearby populations, in turn leading to geographical structure in haplotype composition. Furthermore, we expected that any detected differences in genetic and geographic structure between the species could be interpreted in light of an interplay between geography (climate), life history traits and species ecology.

1.6 AIMS

In summation, the broad aims of this dissertation were to i) determine the geographical origin and subsequent pattern of dispersal of golden orbweavers to their extant range, ii) explore the trends in macroevolutionary processes throughout the clade’s evolutionary history, identify the differences in the trends among genera and test whether these differences can be attributed to selected organismal and environmental factors, iii) infer the biogeographic history of coin spiders (genus *Herennia*) and search for evidence for a human-induced spread of *Herennia multipuncta*, and iv) infer genetic population structure in two distinct nephilid species, *Nephila pilipes* and *Trichonephila clavata*, and relate the resulting differences to geography and species biology.

2 SCIENTIFIC WORKS

2.1 PUBLISHED SCIENTIFIC WORKS

2.1.1 Biogeographical history of golden orbweavers: Chronology of a global conquest

Turk E., Čandek K., Kralj-Fišer S., Kuntner M. 2020. Biogeographical history of golden orbweavers: Chronology of a global conquest. Journal of Biogeography, 47, 6: 1333-1344

Aim: A wholistic biogeographic reconstruction should combine a phylogeny with specifics of organismal biology, plate tectonics, and consequent probabilities of historic dispersal events. Here, we demonstrate this approach by reconstructing the geographical origin and sequence of intercontinental colonization of the golden orbweaving spiders, a global clade. We test two alternative hypotheses about their ancestral range. Due to the highest contemporary species diversity in Africa, the “Out of Africa” hypothesis predicts the Afrotropics as their most likely ancestral area. The alternative, “Out of West Burma” hypothesis aims to explain a Burmese amber fossil as stem nephilid. Because the West Burma block probably detached from Australia, then rafted towards Laurasia, either on its own or with India, this hypothesis predicts either Australasia or Indomalaya (or both) as the ancestral area.

Location: Worldwide.

Taxon: Golden orbweaving spiders, family Nephilidae.

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Results: The best supported model reconstructs both Australasia and Indomalaya as ancestral area. In several parts of the phylogeny, these areas persist for the estimated 130-

million-year evolutionary history. However, numerous intercontinental shifts in nephilid biogeographic history are also inferred. Since nephilid origins are clearly Gondwanan, our study supports the interpretations that Burmese amber contains Gondwanan biota.

Main conclusions: These results are consistent with the Out of West Burma hypothesis but reject the Out of Africa hypothesis. That certain clades persist in their ancestral ranges while others may shift continents aligns well with the known nephilid biology. Our methodological approach that assesses organismal specific dispersal probabilities through concrete distances measured though time slices of the Earth's history can be applied to biogeographic reconstruction of any lineage.

Received: 2 October 2019 | Revised: 4 February 2020 | Accepted: 13 February 2020

DOI: 10.1111/jbi.13838

RESEARCH PAPER

Journal of Biogeography WILEY

Biogeographical history of golden orbweavers: Chronology of a global conquest

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Funding information
Javna Agencija za Raziskovalno Dejavnost RS, Grant/Award Number: 1000-17-0618, J1-9163, P1-0255 and P1-0236

Handling Editor: Evan Economo

Abstract

Aim: A wholistic biogeographical reconstruction should combine a phylogeny with specifics of organismal biology, plate tectonics and consequent probabilities of historic dispersal events. Here, we demonstrate this approach by reconstructing the geographical origin and sequence of intercontinental colonization of the golden orbweaving spiders, a global clade. We test two alternative hypotheses about their ancestral range. Due to the highest contemporary species diversity in Africa, the 'Out of Africa' hypothesis predicts the Afrotropics as their most likely ancestral area. The alternative, 'Out of West Burma' hypothesis aims to explain a Burmese amber fossil as stem nephilid. Because the West Burma block probably detached from Australia, then rafted towards Laurasia, either on its own or with India, this hypothesis predicts either Australasia or Indomalaya (or both) as the ancestral area.

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Results: The best supported model reconstructs both Australasia and Indomalaya as ancestral area. In several parts of the phylogeny, these areas persist for the estimated 130-million-year evolutionary history. However, numerous intercontinental shifts in nephilid biogeographical history are also inferred. Since nephilid origins are clearly Gondwanan, our study supports the interpretations that Burmese amber contains Gondwanan biota.

Main conclusions: These results are consistent with the Out of West Burma hypothesis but reject the Out of Africa hypothesis. That certain clades persist in their ancestral ranges while others may shift continents aligns well with the known nephilid biology. Our methodological approach that assesses organismal specific dispersal probabilities through concrete distances measured though time slices of the Earth's history can be applied to biogeographical reconstruction of any lineage.

KEYWORDS
ancestral areas, Burmese amber, Gondwana, jump dispersal, *Nephila*, Nephilidae, out of West Burma hypothesis, plate tectonics, spiders

1 | INTRODUCTION

A wholistic biogeographical reconstruction of a lineage should combine a robust, species-level, time-calibrated phylogeny with probabilities of historic dispersal events. Ideally, these probabilities would vary by time slices, being derived from details of biology of the studied organisms combined with accurate reconstructions of plate tectonics history, including measured distances among geographical areas. Numerous elegant biogeographical studies have combined some of these elements (e.g. Condamine, Sperling, & Kergoat, 2013; Dornburg, Moore, Beaulieu, Eytan, & Near, 2015; Dupin et al., 2017; Herrera et al., 2015; Nauheimer, Metzler, & Renner, 2012). However, to the best of our knowledge, wholistic approaches that would base the differences in dispersal probabilities through time slices on concrete measurements have not become mainstream in biogeography. Here, we outline such approach through a global biogeographical study of golden orbweaving spiders (family Nephilidae), using a well sampled and robust phylogeny (Kuntner et al., 2019), as well as estimated probabilities of their historic dispersal events.

Nephilids are renowned for their extreme phenotypes, most notably their pronounced, female-biased sexual size dimorphism (Coddington, Hormiga, & Scharff, 1997). In fact, they represent the most extreme case of sexual size dimorphism among all terrestrial animals, with females up to 500 times heavier than males (Kuntner, Zhang, Gregorič, & Li, 2012). Females, but not males, build asymmetric orb webs of sometimes exaggerated proportions (Kuntner, 2017; Kuntner et al., 2019). Nephilids are interesting also biogeographically because of their hypothesized ancient origin in the Mesozoic (Kuntner et al., 2019), their present wide distribution across most continents, and their varying dispersal propensities. Namely, some species are excellent aerial dispersers capable of long-distance, often overwater, travel, while others are not known to disperse over large distances and are distributed narrowly, such as some species of *Clitaetra*, *Nephilingis* and *Herennia* (Kuntner & Agnarsson, 2011a, 2011b). Ballooning (becoming airborne by releasing silken threads into a wind current) is a likely means of aerial dispersal in nephilids, as demonstrated in *Nephila pilipes* (Lee, Kuntner, & Li, 2015). Thus, they are also capable of sustaining gene flow across relatively large distances, for example, between islands of the Caribbean and North American mainland (Čandek, Binford, Agnarsson, & Kuntner, 2020).

Nephilid species distributions are predominantly tropical and subtropical with some species ranging into temperate regions (Figure 1; Su et al., 2011). Genera are often sympatric, with the majority of the 37 valid species (Kuntner et al., 2019, Table 1) found in the Afrotropics and Indomalaya. Specifically, *Herennia* and *Nephilengys* are co-distributed and range from India to Australia (Kuntner, 2005, 2007). *Clitaetra* inhabits tropical and subtropical Africa and some of the islands of the western Indian Ocean (Kuntner, 2006), and *Indoetra* is endemic to Sri Lanka (Kuntner, 2006; Kuntner et al., 2019). *Nephilingis* has Afrotropical species, but one extends disjunctly into South America (Kuntner, 2007; Kuntner et al., 2019). A newly reclassified *Nephila* (Kuntner et al., 2019) only contains two species: *Nephila constricta* is found in equatorial Africa (Su et al., 2011), while *N. pilipes* is widely distributed from South Asia through South-East Asia, and into Australia and the Pacific (Su, Chang, Lee, & Tso, 2007). The most species-rich nephilid genus, *Trichonephila*, has a wide distribution in tropical, subtropical and temperate regions of most continents (not Europe and Antarctica). As an exception among nephilid genera, some of the 12 extant *Trichonephila* species show temperate distributions. *Trichonephila clavata* is found from the Himalayas to Japan (Su et al., 2011), with a recent introduction to North America (Hoebeke, Huffmaster, & Freeman, 2015). *Trichonephila clavipes* is distributed from southern USA to Argentina (Kuntner, 2017), and is, along with the neotropical *Trichonephila sexpunctata*, one of the two extant nephilid species endemic to the Americas. *Trichonephila edulis* is widespread in Australia and seasonally occurs in New Zealand (Paquin, Vink, & Dupérré, 2010). The remaining *Trichonephila* species are tropical and subtropical.

Very few nephilid fossils are known (see Kuntner, Arnedo, Trontelj, Lokovšek, & Agnarsson, 2013), mostly from the Dominican (Wunderlich, 1986) and Baltic amber (Wunderlich, 2004). At least some of these fossils aged 40–16 million years have been confirmed to be nephilids, and were therefore used as phylogenetic calibration points (Kuntner et al., 2013). Most recently, Burmese amber has been hypothesized to be relevant for nephilid origins (Poinar & Buckley, 2012). Mined in the region of Myanmar that sits on the West Burma tectonic block (Cruickshank & Ko, 2003), it represents a rich source of fossilized plant and animal material. Both palaeontological—fossils aged 97–110 million years (Cruickshank & Ko, 2003)—and geological evidence (Shi et al., 2012) suggest Burmese amber to be Cretaceous. At least its insect inclusions suggest that the West Burma Block was Gondwanan in origin. How West Burma arrived at its present location, however, is unresolved. A recent hypothesis is that it may have broken off from Australia, then either rafted to its present location on its own or attached to the Indian plate (Poinar, 2018). Based on the inclusions in Burmese amber, Poinar (2018) suggests the split between the West Burma Block and Gondwana could not have occurred before Early Cretaceous. Poinar

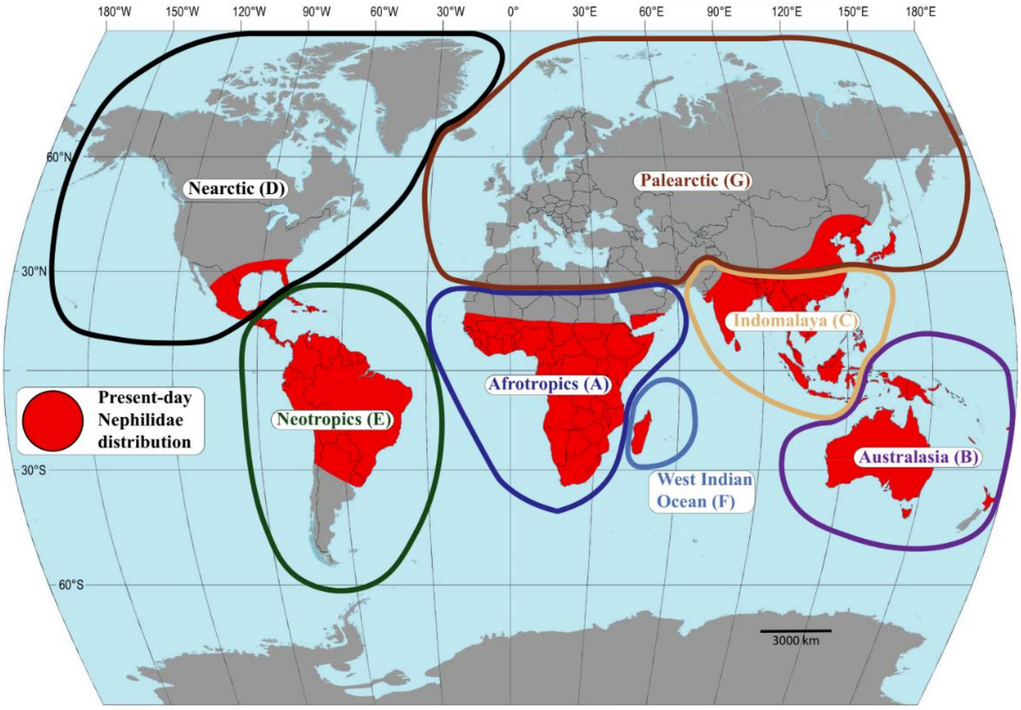

FIGURE 1 Present-day distribution of the spider family Nephilidae on a Miller global projection. Encircled are the seven biogeographical areas (A–G) used in this study [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Evaluation scores of six types of biogeographical models tested on a nephilid phylogeny using RASP. (LnL) log-likelihood; (AIC_c) Akaike information criterion corrected for small sample sizes; (AIC_c_wt) weighted AIC_c values. The asterisk indicates a statistically significant difference between the adequacy of fit of the basic and +*j* variant of each model. The model with the best overall fit is shown in bold

Model name	Ln L	AIC_c	AIC_c_wt	Δ −*j*/+*j*
DEC	−82.59	169.6	4.7e-05	*
DEC + j	−72.68	152.2	0.29	
DIVALIKE	−79.44	163.3	0.0011	*
DIVALIKE + j	**−71.81**	**150.4**	**0.68**	
BAYAREALIKE	−97.99	200.4	9.7e-12	*
BAYAREALIKE + j	−74.8	156.4	0.034	

and Buckley (2012) describe an inclusion from this amber containing one adult male and one juvenile spider, resembling modern nephilids. They name the species *Geratonephila burmanica* and suggest the presence of an adult male and a juvenile on the same web implies sociality (Poinar & Buckley, 2012). While the latter hypothesis has been rejected, *G. burmanica* has recently been interpreted as a possible stem nephilid (Kuntner et al., 2019).

The aims of the present study are to reconstruct the biogeographical history of the clade, and more precisely to test the hypothesized Gondwanan origin of Nephilidae (Kuntner, 2006). Assuming that the most recent clade age estimation at 133 Ma with a range of 97–146 Ma (Kuntner et al., 2019) is reasonably accurate, we test two alternative hypotheses about the nephilid biogeographical origin. The rationale for the first, 'Out of Africa' hypothesis, is that the continent that has retained the most taxonomic diversity from the Cretaceous to the present should correspond with the nephilid ancestral area (Lomolino, Riddle, & Whittaker, 2017). Since the Afrotropics have the most nephilid species today, the prediction of our first hypothesis is that present-day Africa should optimize as the most likely nephilid ancestral area. Assuming that *Geratonephila* from Burmese amber is indeed a stem nephilid, the second hypothesis posits that the geographical origin of nephilids was the Gondwanan fragment West Burma Block. This fragment probably got detached from Australia, then travelled to collide with SE Asia either as a distinct entity or attached to the Indian plate (Poinar, 2018). The age intervals of the fossil and the West Burma break-off partially overlap, thus we lack the resolution to precisely predict the nephilid ancestral range to either Australasia or Indomalaya. The broadly defined 'Out of West Burma' hypothesis would thus be supported if Australasia or Indomalaya (or both) optimized as the most likely nephilid ancestral area.

2 | MATERIALS AND METHODS

2.1 | Taxon selection

In order to reconstruct the biogeographical history of this lineage of spiders, a resolved species-level phylogeny is needed. Until recently, this has proven as problematic, not the least because precise familial taxonomic history has been controversial (Blackledge et al., 2009; Coddington, 1990; Dimitrov et al., 2012, 2017; Dimitrov & Hormiga, 2009; Hong-Chun, Kai-Ya, Da-Xiang, & Yang, 2004; Kallal, Dimitrov, Arnedo, Giribet, & Hormiga, 2019; Kuntner, 2006; Kuntner et al., 2013, 2019; Kuntner, Coddington, & Hormiga, 2008). In this paper, we treat nephilids at the family rank (Kuntner et al., 2019), although its rank continues to be debated (Kallal et al., 2019). The first comprehensive, global nephilid species-level phylogeny containing branch length information was constructed using three nuclear (18S rRNA, 28S rRNA, histone 3 subunit A) and five mitochondrial (COI, 12S rRNA, 16S rRNA and tRNA-leu and *nad1*) genes, additionally complemented with morphological data (Kuntner et al., 2013). The study dated nephilid origins to 40 Ma and *Nephila* origins to 16 Ma.

A recent, large-scale phylogenomic study, however, reassessed the nephilid evolutionary relationships and disputed the above hypothesized ages (Kuntner et al., 2019). This study applied Anchored Hybrid Enrichment phylogenomics to arrive at a robust, well-supported, and species-level phylogeny. The analysis targeted 585 single-copy orthologous loci, proven to reliably retrieve shallow and deep phylogenetic nodes in spiders (Hamilton, Lemmon, Lemmon, & Bond, 2016). The resulting chronogram, including 22 of 37 extant nephilid species, plus two fossils for time calibration (including *Geratonephila* from Burmese amber, see above), pushed nephilid origins back to the Early Cretaceous (133 Ma). The study also produced a phylogeny with added nephilid species without available phylogenomic data (Kuntner et al., 2019, Figure 3). Here, we further expanded this most recent phylogeny by adding undescribed *Herennia* species *H. nsp1-eva*, *H. nsp2-maj* and *H.* nsp3-tsoi to the original matrix of 31 nephilid species. This addition increased the total taxon sampling to 34 of the total 40 known (if undescribed) nephilid species (85%). To the 14 outgroup taxa from Kuntner et al. (2019) we added *Uloborus diversus*. A full matrix of aligned DNA sequences is available in Appendix S1.

2.2 | Time calibrated phylogeny

We constructed an original phylogeny based on CO1 sequences and constrained it according to the recently published phylogenomic nephilid tree (Kuntner et al., 2019). This COI matrix, building on that of Kuntner et al. (2019) with the addition of COI sequences of three previously undescribed species of *Herennia* (GenBank accession codes *H.* nsp2-maj MN855577, *H.* nsp1-eva MN855578, *H.* nsp3-tsoi MN855579), was aligned with MUSCLE (Edgar, 2004) implemented in MEGA7 (Kumar, Stecher, & Tamura, 2016). We then ran BEAST 2 (Bouckaert et al., 2014) via CIPRES (Miller, Pfeiffer, & Schwartz, 2010) on all CO1 sequences from 49 taxa. We used bModelTest (Bouckaert & Drummond, 2017) as the nucleotide substitution model and employed a relaxed log normal clock. Following Bidegaray-Batista and Arnedo (2011), the ucld.mean prior was set to have a normal distribution with a mean of 0.0199 and standard deviation of 0.001, while the ucldStdev prior was set to have an exponential distribution with a mean of 0.666. We constrained the ages of all nephilid nodes dated by Kuntner et al. (2019, Figure 2), with age estimates rounded to whole numbers and a standard deviation of 0.1. Four MCMC chains were set to run for 50 million generations.

2.3 | Ancestral area estimation

2.3.1 | Biogeographical areas

We quantified each species' geographical distribution by indicating its presence or absence in seven biogeographical areas, six of which correspond with biogeographical realms (Figure 1: A = Afrotropics, B = Australasia, C = Indomalaya, D = Nearctic, E = Neotropics and G = Palearctic). We defined an additional biogeographical area as islands of the west Indian Ocean (Figure 1: *F* = Madagascar, Réunion, Mauritius, Seychelles, Comoros) due to high level of nephilid endemism. Nephilid species ranges derived from the IUCN Red List (https://www.iucnredlist.org/).

2.3.2 | Biogeographical models

To reconstruct nephilid historical biogeography we used the software package RASP (Reconstruct Ancestral State in Phylogenies; Yu, Harris, Blair, & He, 2015) version 4.0 beta, which implements the R package BIOGEOBEARS (Matzke, 2013). We fit the data to all six BIOGEOBEARS models: DEC, DEC + j, DIVALIKE, DIVALIKE + j, BAYAREALIKE and BAYAREALIKE + j. These models differ in the types of cladogenetic events they allow. DEC models allow for narrow sympatry, subset sympatry and narrow vicariance, DIVALIKE models allow for narrow sympatry, narrow vicariance and widespread vicariance, and BAYAREALIKE models allow for narrow and widespread sympatry (Matzke, 2013). The '*+j*' variants of models account for jump dispersal, also known as founder event speciation, a common method of colonization of isolated landmasses and intercontinental dispersal. This type of model, specifically DEC + j, has recently been criticized for not modelling cladogenetic events (sympatric speciation, vicariance) as probabilistic with respect to time, unlike anagenetic events of local extinction and dispersal (Ree & Sanmartín, 2018). Consequently, the role of cladogenetic events is overestimated relative to time-dependent anagenetic range evolution. This effect is emphasized in the + *j* variant, because (a) it adds another possible cladogenetic event and (b) the probability of jump dispersal is expressed with a free

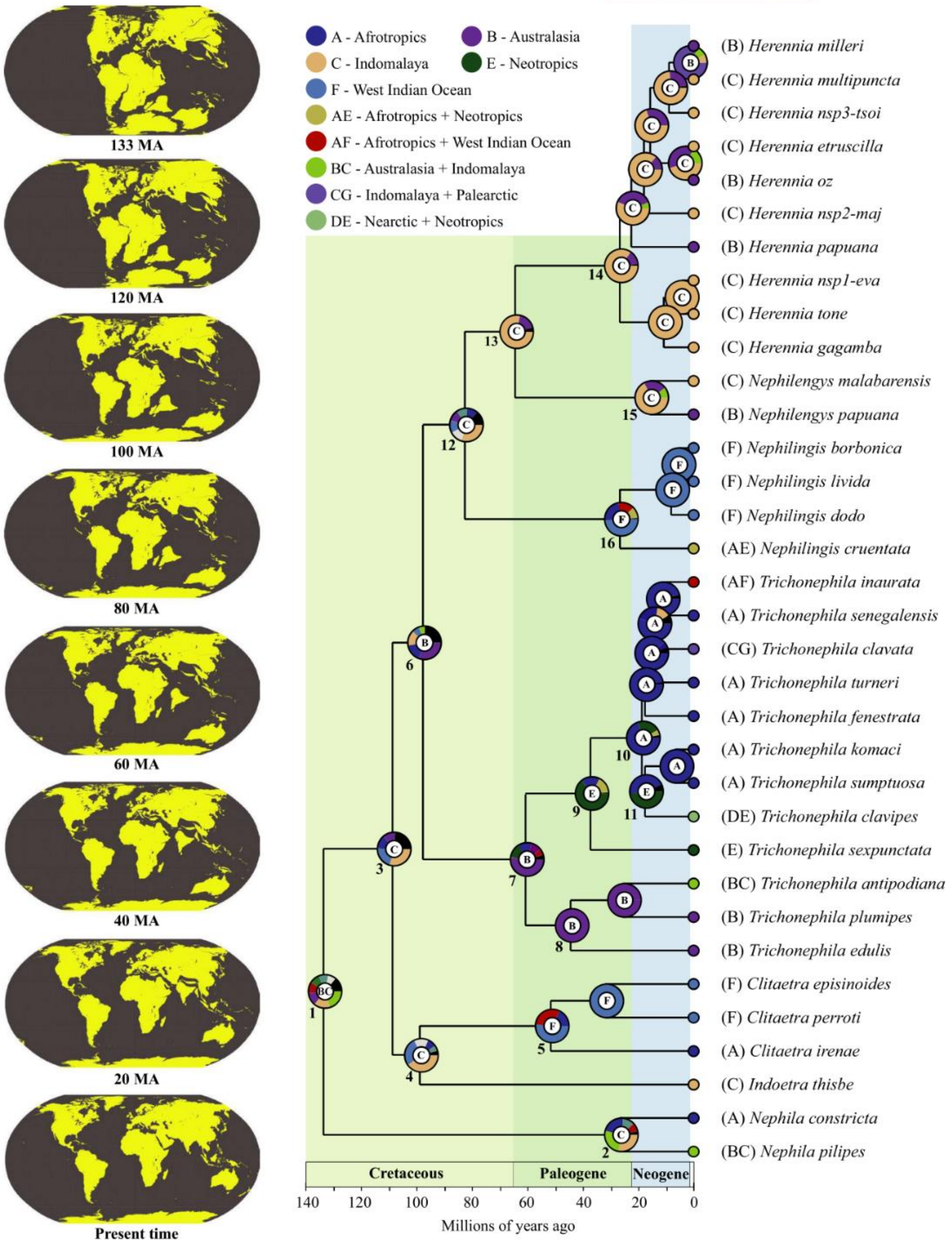

FIGURE 2 RASP global ancestral area reconstruction on a nephilid phylogeny, following the best supported model DIVALIKE + j. The letter at each node signifies the biogeographical area that received the highest support for that node. The colours of each circle reflect the relative proportion of support each biogeographical area received for that node. Letters next to extant taxa indicate their current distribution. Note that a combination of two letters indicates a distribution in both areas. The left panel shows the positions of continents from the time of nephilid origin until the present day, following Muller et al. (2019). Clade numbers match those in Table 2 and in the text [Colour figure can be viewed at wileyonlinelibrary.com]

parameter *j*, that, if maximized, makes non-jump dispersal events at deep nodes impossible (Ree & Sanmartín, 2018). However, both due to the scale investigated here and to the biology of the study organisms (orbweaving spiders are known to cross large distances via ballooning; e.g. Bell, Bohan, Shaw, & Weyman, 2005), we considered all six models.

2.3.3 | Dispersal probabilities

To fine-tune the analysis, we used time stratification to reflect the differences in probability of dispersal between geographical areas at different points in time. We used the physical distance between the closest points of the main landmasses of each pair of geographical areas as a proxy for dispersal probabilities. We measured the distances every 10 million years from 130 to 0 Ma using GPlates plate tectonics visualization software (Müller et al., 2018) on a recent tectonic reconstruction model (Müller et al., 2019), resulting in 294 measurements rounded to 10 km (see Appendices S2 and S3). We then transformed the measured distances to dispersal probabilities with low values reflecting large physical distances and vice versa. For a zero kilometre distance between areas (i.e. if areas are in physical contact), we set the highest dispersal probability to 95%, because dispersal is very likely, but not necessary. Moreover, we set the distance of 4,000 km as low (5%) dispersal probability, corresponding to the maximum distance spiders are thought to balloon. Namely, it has been inferred that Hawaii, one of the most isolated islands inhabited by spiders, have staged several colonization events from North America in the genus *Tetragnatha* alone (Gillespie, Croom, & Palumbi, 1994). The distance between Hawaii and the North American mainland is just under 4,000 km, as is the distance between another remote, spider-populated South Pacific island, Heard Island (Pugh, 2003, 2004) and Australia. We assumed a decreasing linear relationship of the remaining points between the two extremes and calculated the probabilities for all intermediate distances accordingly. All distances greater than 4,000 km were assigned an equal, 5% probability.

2.3.4 | Model evaluation

Prior to biogeographical analysis, we dropped all outgroups from the constructed phylogenetic tree, retaining only nephilid taxa. We then ran RASP, with a maximum number of areas occupied set to two. To compare the resulting model fit, RASP provides Akaike information criterion (AIC) values and Akaike weights (AIC_w). While AIC provides a common means of comparing the adequacy of the fit of alternative models to the data, small differences between AIC values are often misinterpreted and thus lead to overly confident conclusions (Wagenmakers & Farrell, 2004). To avoid this problem, AIC values can be transformed into AICw values, which, in turn, provide a straightforward measure of each model's relative probability (Wagenmakers & Farrell, 2004). Here, we report AIC_c values (AIC

TABLE 2 Three likeliest ancestral areas in 16 selected nephilid clades (labeled in Figure 2), as reconstructed by the DIVALIKE + j model in RASP. Letters correspond to the following geographical areas: A = Afrotropics, B = Australasia, C = Indomalaya, D = Nearctic, E = Neotropics, F = West Indian Ocean, G = Palearctic. A combination of letters signifies an ancestral distribution in both areas. All probabilities are rounded to a full number, MRCA = most recent common ancestor

Clade no.	Clade description	First (%)	Second (%)	Third (%)
1	Common nephilid ancestor	BC: 20	C: 16	B: 12
2	*Nephila* MRCA	C: 28	BC: 26	A: 23
3	All but *Nephila* MRCA	C: 30	F: 22	A: 12
4	*Clitaetra* + *Indoetra* MRCA	C: 40	F: 28	CF: 14
5	*Clitaetra* MRCA	F: 52	AF: 31	A: 18
6	*Trichonephila* + *Nephilingis* + *Nephilengys* + *Herennia* MRCA	B: 30	A: 17	C: 15
7	*Trichonephila* MRCA	B: 52	E: 15	A: 13
8	*Trichonephila antipodiana* + *Trichonephila plumipes* + *Trichonephila edulis* MRCA	B: 100	–	–
9	MRCA of all remaining *Trichonephila*	E: 64	A: 18	AE: 16
10	Clade 9 minus *T. sexpunctata*	A: 70	E: 22	AE: 5
11	*Trichonephila komaci* + *Trichonephila sumptuosa* + *Trichonephila clavipes* MRCA	E: 48	A: 45	DE: 4
12	*Nephilingis* + *Nephilengys* + *Herennia* MRCA	C: 30	CF: 13	F: 12
13	*Nephilengys* + *Herennia* MRCA	C: 78	B: 17	BC: 4
14	*Herennia* MRCA	C: 84	B: 14	BC: 2
15	*Nephilengys* MRCA	C: 68	B: 22	BC: 10
16	*Nephilingis* MRCA	F: 49	A: 23	AF: 16

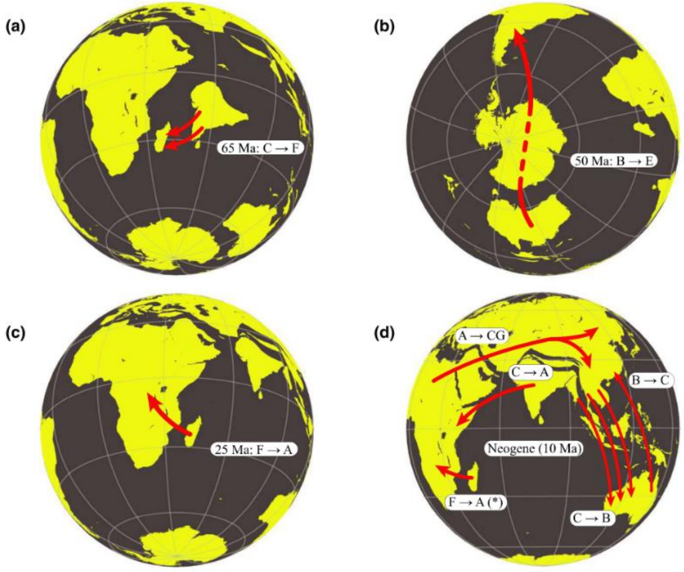

FIGURE 3 Examples of reconstructed intercontinental dispersal events in a nephilid phylogeny. The midpoint of the phylogenetic branch along which the dispersal event occurred was taken as the age of the event. Each arrow presents one independent dispersal event. (a) Dispersal from Indomalaya to West Indian Ocean islands occurred twice, by the ancestor of clade 5 and ancestor of clade 16. (b) Dispersal from Australasia to the Neotropics, presumably via Antarctica, in the ancestor of clade 9. (c) Dispersal from the West Indian Ocean islands to the Afrotropics in the ancestor of *Clitaetra irenae*; (d) Dispersal events in the Neogene in the genera *Nephila* (C→A), *Trichonephila* (A→CG), *Nephilingis* (F→A), *Nephilengys* (C→B) and *Herennia* (B→C, 2x C→B). *The clade's (*Nephilingis cruentata*) subsequent dispersal from the Afrotropics to the Neotropics is not shown. Geographical areas: A = Afrotropics, B = Australasia, C = Indomalaya, D = Nearctic, E = Neotropics, F = West Indian Ocean, G = Palearctic [Colour figure can be viewed at wileyonlinelibrary.com]

scores corrected for small sample sizes) and their corresponding weighted values (AIC_c_wt).

3 | RESULTS

The biogeographical reconstructions are based on the chronogram from BEAST (Appendix S4) that precisely places the three undescribed *Herennia* species: *H. nsp1-eva* from Sulawesi is sister to *H. tone* from the Philippines; *H. nsp2-maj* from Vietnam is sister to a clade that contains both Indomalayan and Australasian species; *H. nsp3-tsoi* from Taiwan is sister to a clade containing *H. multipuncta* (Indomalayan) and *H. milleri* (Australasian). All other nephilid nodes are topologically constrained and thus identical to Kuntner et al. (2019).

In all three RASP model pairs, +*j* variants provide significantly different results than their basic alternatives (Table 1). DIVALIKE + j shows the lowest AIC_c and highest AICc_wt and log-likelihood (LnL) values, all indicating it as the best supported model for our data.

Table 2 summarizes the three most likely ancestral areas based on DIVALIKE + j model for 16 selected clades (for all nodes, see Figure 2). The nephilid ancestor (Clade 1; see Figure 2 and Table 2) was most likely distributed over Indomalaya and Australasia (20%). The plausible alternatives have marginally lower probabilities: Indomalaya (16%), Australasia (12%). All these results are consistent with the Out of West Burma hypothesis. An Afrotropical most recent common ancestor (MRCA) of all nephilids is much less plausible (3%), rejecting the Out of Africa hypothesis.

Several dispersal scenarios are implied (Figure 3a–d). The ancestral area of the two *Nephila* species (Clade 2) is ambiguous, with a few percent difference among the probabilities (Table 2): Indomalaya (28%), Indomalaya + Australasia (26%) and Afrotropics (23%). Within this clade, *N. constricta* likely colonized the Afrotropics (Figure 3d). The MRCA of all remaining nephilids (Clade 3) shows a likely Indomalayan distribution (30%). The ancestral area for *Clitaetra* + *Indoetra* (Clade 4) is 40% Indomalayan. The ancestor of *Clitaetra* (Clade 5) likely colonized the West Indian Ocean islands (52%; Figure 3a), with a subsequent colonization of Africa (Figure 3c); note that not all African *Clitaetra* species are included in this phylogeny. The remaining nephilids (Clade 6) had a likely Australasian ancestor (30%) whose distribution was maintained in the *Trichonephila* MRCA (Clade 7; 52%). While one of the *Trichonephila* clades (Clade 8; *T. antipodiana*, *T. plumipes* and *T. edulis*) retains this ancestral distribution (with a range expansion to Indomalaya in *T. antipodiana*), the MRCA of remaining *Trichonephila* species (Clade 9) most likely (64%) colonized the Neotropics (Figure 3b). In this clade, *T. sexpunctata* retained its Neotropical distribution, but its sister clade is reconstructed to have shifted to the Afrotropics (Clade 10; 70%). This species-rich clade shows predominantly African species distributions, with a range expansion to West Indian Ocean in *Trichonephila inaurata*, a shift to Indomalaya and Palearctics in *T. clavata* (Figure 3d), and, interestingly, another colonization of Neotropics (Clade 11). Whose ancestor achieved the latter recolonization is ambiguous (see Section 4).

The ancestor of the clade sister to *Trichonephila* (Clade 12) shows a range shift from Australasia to Indomalaya (30%). This Indomalayan range is retained in most distal splits. Thus, the origins of both *Herennia* (Clade 14; 84%) and *Nephilengys* (Clade 15; 68%), as well as their common ancestor (Clade 13; 78%) are all strongly supported as Indomalayan. However, some of *Herennia* and *Nephilengys* species (*H. milleri*, *H. oz*, *H. papuana* and *N. papuana*) shift back to Australasia in Neogene (Figure 3d). In contrast, *Nephilingis* MRCA (Clade 16) likely shifted from Indomalaya to West Indian Ocean islands (49%; Figure 3a), where most of the genus diversity remains, albeit with *N. cruentata* having shifted to the Afrotropics and Neotropics (Figure 3d).

4 | DISCUSSION

We present a historic biogeographical reconstruction of most nephilid species. We use this reconstruction to test alternative hypotheses on the precise geographical origin of this clade. The first hypothesis, Out of Africa, based on the observation that most extant species diversity lies in the Afrotropics, receives no support. Moreover, the Out of West Burma hypothesis is consistent with our results that reconstruct Indomalaya and Australasia as nephilid ancestral range. Our results suggest numerous range shifts during the roughly 130 Ma long evolution of this clade, and we discuss some of these likely events below.

4.1 | Setting dispersal probabilities using geological data

While biogeographical studies often include time stratification of dispersal probabilities between areas, the estimates are usually rough-scaled and arbitrary (e.g. Chamberland et al., 2018; Eberle, Fabrizi, Lago, & Ahrens, 2017; Weaver, Cruz, Johnson, Dupin, & Weaver, 2016). Our study adds precision to a global analysis by applying measured distances among geographical entities at geological time intervals. This level of precision was possible due to the availability of a detailed plate tectonics reconstruction model (Müller et al., 2019). We combine these data with spider dispersal biology, resulting in a credible estimation of dispersal probabilities in this lineage.

4.2 | Nephilid historic biogeography

Our results support the hypothesis that Burmese amber contains Gondwanan biota (Poinar, 2018). Regardless of the precise origin of nephilids, they are clearly Gondwanan, which means that the West Burma Block, together with its amber, is not Laurasian in origin, but indeed broke off from Gondwana in the Cretaceous and then rafted to its present location.

None of the possible ancestral areas for the nephilid MRCA receives an overwhelming support, which is unsurprising given the clade's age and present wide distribution. However, the three best supported candidates, Indomalaya, Australasia and Indomalaya + Australasia, received a combined support of 48% (Table 2). Our Out of West Burma hypothesis was defined broadly, lacking precise resolution as to the origin of nephilids. Thus, all three most plausible MRCA reconstructions would be consistent with this hypothesis. Although Kuntner et al. (2019) hypothesize that the sister clade to nephilids is a clade that contains a huge diversity of araneid genera, other authors argue that a single enigmatic Australian genus, *Paraplectanoides*, might in fact be the more appropriate outgroup to nephilids (Kallal & Hormiga, 2018; Scharff et al., 2019). If the former is true, one cannot unequivocally reconstruct the ancestral area of nephilids plus araneids. If the latter, then one would expect support for an Australasian origin of that deeper node (*Paraplectanoides* plus nephilids), perhaps adding additional resolution to the origin of nephilids that may then be more decisively Australasian. Regardless, this would corroborate Out of West Burma, and refute Out of Africa.

The frequency of dispersal events as implied by the phylogeny suggests that a few lineages, such as *Herennia*, largely persisted in their ancestral ranges for tens of millions of years, while others, such as *Trichonephila*, frequently shifted continents. This disparity aligns well with the known nephilid species biology—the species differ greatly in their dispersal propensity (Kuntner & Agnarsson, 2011b; Lee, Jiang, Su, & Tso, 2004). Interestingly, simple range expansion of a single species occurred much less frequently in the phylogeny than intercontinental jump dispersal with subsequent speciation. The reconstruction only implies four range expansion events: *N. pilipes* from Indomalaya to Indomalaya + Australasia, *T. inaurata* from the Afrotropics to the Afrotropics + West Indian Ocean, *T. antipodiana* from Australasia to Australasia + Indomalaya and, perhaps, *T. clavipes* from Neotropics to Neotropics + Nearctic (but, see below).

Moreover, the reconstruction suggests some fairly dramatic jump dispersals, especially in the *Trichonephila* clade. A surprising one is the shift of the Australasian *Trichonephila* MRCA (Clade 7) to the Neotropics (Clade 9) in mid Paleogene (Figure 3b). The distance between Australasia and Neotropics 50 Ma was approximately 6,500 km, well beyond our 4,000 km threshold for 5% dispersal probability. However, this was around the time of the Paleocene-Eocene Thermal Maximum, when the poles were ice-free (Zachos, Pagani, Sloan, Thomas, & Billups, 2001). Additionally, the Drake

Passage between South America and Antarctica had not yet opened (Livermore, Nankivell, Eagles, & Morris, 2005), providing a dispersal route between Australasia and Neotropics. Trans-Antarctic dispersal in both directions was indeed frequent in animals (Sanmartín & Ronquist, 2004), seen for example in mammals (Woodburne & Case, 1996), frogs (Duellman, Marion, & Hedges, 2016) and bees (Almeida, Pie, Brady, & Danforth, 2012).

Of the descendent species in Clade 9, only one, *T. sexpunctata*, retained a Neotropical distribution and remains the only nephilid species endemic to South America. The MRCA of all other species (Clade 10) receives high support (69%) for a transition to the Afrotropics. One of its subclades then diversified in the Afrotropics and this diversification formed the basis for our 'Out of Africa' hypothesis. The other subclade, Clade 11, is reconstructed as 48% Neotropical versus 45% Afrotropical. While the basal species in this clade, *T. clavipes*, is presently American, the remaining two species shifted back to the Afrotropics. Thus, a reconstruction of an Afrotropical Clade 11 would be more parsimonious, implying a single, not two, intercontinental dispersals. Regardless, we might assume from the diversity of fossil nephilids found in Dominican amber (Wunderlich, 1986, 1988) that they underwent a radiation once colonizing the Americas, but were subsequently affected by extinction event(s). This hypothesis could be tested by reconstructing a morphological phylogeny of fossil and extant *Trichonephila* as it is currently not known how closely the Dominican amber fossils are related to *T. clavipes*.

While Out of Africa predicted the Afrotropics as the origin of nephilids, our results instead uncover Africa among the geographical areas colonized only recently. The first MRCA reconstructed as African (Clade 10) is approximately 17 million years old (Kuntner et al., 2019). This estimated age, combined with the phylogenetic topology, would suggest that nephilids underwent a fast diversification, perhaps as a response to newly available ecological niches encountered in Africa. Concurrently, the genus *Nephilingis* (Clade 16) colonized the West Indian Ocean islands from Indomalaya (Figure 3a) and diversified there, albeit to a smaller extent. One species, *Nephilingis cruentata*, further colonized Africa and South America (Figure 3d). Judging from the areas inhabited by *N. cruentata*, we hypothesize that its colonization to Africa was natural, while that to South America, where it is confined to a few coastal areas (Kuntner, 2007), may have been recent, and anthropogenic.

Su et al. (2011) argue that diversification patterns of Asian/Australian *Nephila* (now *Trichonephila*) spiders were driven by major global climatic changes in the Neogene. Our results are consistent with this hypothesis. The dramatic decrease in global temperature, ice accumulation and lowered sea levels in the Neogene (e.g. Jansen, Sjøholm, Bleil, & Erichsen, 2012; Woodruff, 2003; Zachos et al., 2001), might have had a profound effect on ecological niche diversity, wind directions and habitat distribution. With the collision of the Turkish and Arabian plates around 20 Ma, early Neogene also saw the formation of the first land bridge between Africa and Eurasia (Rögl & Steininger, 1983). This was followed by the so-called Afro-Eurasian faunal exchange (Steininger, Rabeder, & Rögl, 1985), which slightly predates the dispersal of the ancestor of *T. clavata* out of the Afrotropics into the Palearctic region of East Asia and Indomalaya (Figure 3d). It is the only extant *Trichonephila* species to inhabit higher elevations, as well as temperate regions.

Interestingly, the basalmost nephilid genus, *Nephila*, is reconstructed to have retained the nephilid ancestral distribution, with a dispersal to the Afrotropics in the Neogene (Figure 3d), where *N. constricta* persists to date. Despite their large adult female body sizes, *Nephila* are active dispersers as tiny juveniles (Lee et al., 2015), so it is surprising that *Nephila* is not even wider spread over other continents. Knowing that *Nephila* is positioned on one of the longest phylogenetic branches in this part of the phylogeny, we hypothesize that its current snapshot of species diversity is not representative of its entire evolutionary history. Rather it may be an evolutionary leftover of a history that may have involved numerous extinctions.

5 | CONCLUSIONS

Our methodological approach adds rigor to biogeographical analysis as it includes not only a robust phylogenetic basis, but also assesses organismal specific dispersal probabilities through concrete distances measured though time slices of the Earth's history. We believe that by modifying this pipeline to fit lineage specific biologies, this methodology can be applied to biogeographical reconstruction of any organism.

ACKNOWLEDGEMENTS

We thank R.-C. Cheng for the help obtaining sequence data, S. Zahirovic for providing crucial geological models and for helpful comments, and J. Bond for a thorough review. Support for this research came from the Slovenian Research Agency, grants P1-0236, P1-0255, J1-9163, and 1000-17-0618.

DATA AVAILABILITY STATEMENT

The new sequences are available on GenBank (MN855577-9). The alignment and the geological data are available as supplements to this paper.

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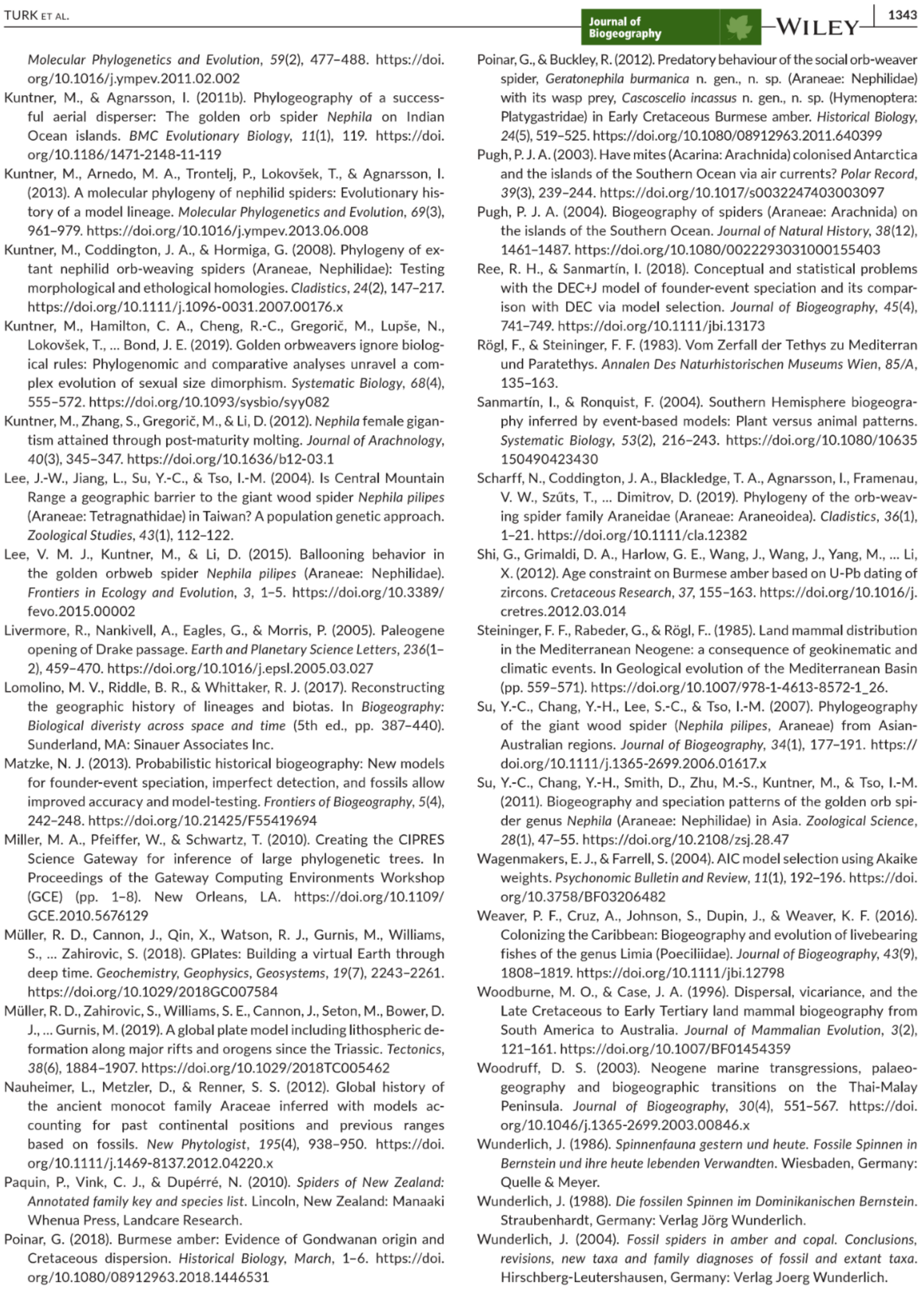

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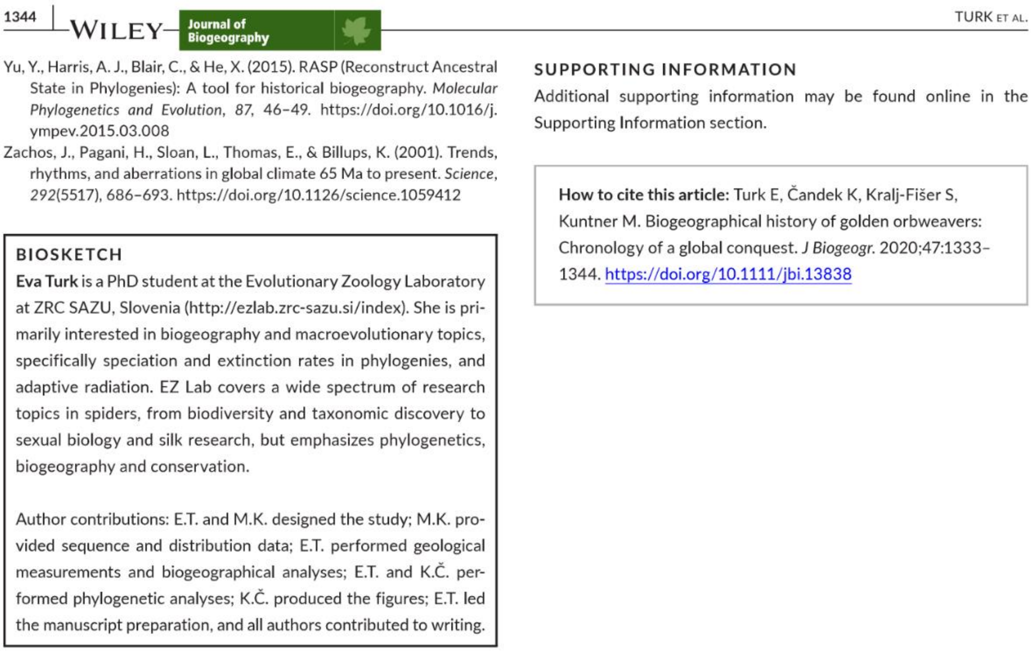

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BIOSKETCH

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Author contributions: E.T. and M.K. designed the study; M.K. provided sequence and distribution data; E.T. performed geological measurements and biogeographical analyses; E.T. and K.Č. performed phylogenetic analyses; K.Č. produced the figures; E.T. led the manuscript preparation, and all authors contributed to writing.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Turk E, Čandek K, Kralj-Fišer S, Kuntner M. Biogeographical history of golden orbweavers: Chronology of a global conquest. *J Biogeogr*. 2020;47:1333–1344. https://doi.org/10.1111/jbi.13838

2.1.2 Exploring diversification drivers in golden orbweavers

Turk E., Kralj-Fišer S., Kuntner M. 2021. Exploring diversification drivers in golden orbweavers. Scientific Reports, 11: 9248, doi: 10.1038/s41598-021-88555-3: 11 p.

Heterogeneity in species diversity is driven by the dynamics of speciation and extinction, potentially influenced by organismal and environmental factors. Here, we explore macroevolutionary trends on a phylogeny of golden orbweavers (spider family Nephilidae). Our initial inference detects heterogeneity in speciation and extinction, with accelerated extinction rates in the extremely sexually size dimorphic *Nephila* and accelerated speciation in *Herennia*, a lineage defined by highly derived, arboricolous webs, and pronounced island endemism. We evaluate potential drivers of this heterogeneity that relate to organisms and their environment. Primarily, we test two continuous organismal factors for correlation with diversification in nephilids: phenotypic extremeness (female and male body length, and sexual size dimorphism as their ratio) and dispersal propensity (through range sizes as a proxy). We predict a bell-shaped relationship between factor values and speciation, with intermediate phenotypes exhibiting highest diversification rates. Analyses using SSE-class models fail to support our two predictions, suggesting that phenotypic extremeness and dispersal propensity cannot explain patterns of nephilid diversification. Furthermore, two environmental factors (tropical versus subtropical and island versus continental species distribution) indicate only marginal support for higher speciation in the tropics. Although our results may be affected by methodological limitations imposed by a relatively small phylogeny, it seems that the tested organismal and environmental factors play little to no role in nephilid diversification. In the phylogeny of golden orbweavers, the recent hypothesis of universal diversification dynamics may be the simplest explanation of macroevolutionary patterns.

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Exploring diversification drivers in golden orbweavers

Eva Turk[1,2]✉, Simona Kralj-Fišer[1] & Matjaž Kuntner[1,3,4,5,6]

Heterogeneity in species diversity is driven by the dynamics of speciation and extinction, potentially influenced by organismal and environmental factors. Here, we explore macroevolutionary trends on a phylogeny of golden orbweavers (spider family Nephilidae). Our initial inference detects heterogeneity in speciation and extinction, with accelerated extinction rates in the extremely sexually size dimorphic *Nephila* and accelerated speciation in *Herennia*, a lineage defined by highly derived, arboricolous webs, and pronounced island endemism. We evaluate potential drivers of this heterogeneity that relate to organisms and their environment. Primarily, we test two continuous organismal factors for correlation with diversification in nephilids: phenotypic extremeness (female and male body length, and sexual size dimorphism as their ratio) and dispersal propensity (through range sizes as a proxy). We predict a bell-shaped relationship between factor values and speciation, with intermediate phenotypes exhibiting highest diversification rates. Analyses using SSE-class models fail to support our two predictions, suggesting that phenotypic extremeness and dispersal propensity cannot explain patterns of nephilid diversification. Furthermore, two environmental factors (tropical versus subtropical and island versus continental species distribution) indicate only marginal support for higher speciation in the tropics. Although our results may be affected by methodological limitations imposed by a relatively small phylogeny, it seems that the tested organismal and environmental factors play little to no role in nephilid diversification. In the phylogeny of golden orbweavers, the recent hypothesis of universal diversification dynamics may be the simplest explanation of macroevolutionary patterns.

Extant biological lineages exhibit a wide variation in species richness, ranging from hyper species rich lineages undergoing explosive radiation at one extreme to single representatives, sitting at the ends of long branches at the other. Heterogeneity in species richness is the product of the interplay between two fundamental macroevolutionary processes, speciation and extinction[1]. If speciation and cladogenesis are more frequent than extinction and lineage termination, the lineage diversifies and potentially radiates. If the opposite is true, the lineage eventually goes extinct[1]. While these mechanisms of diversity heterogeneity are understood, its drivers remain unclear.

Macroevolutionary literature generally assumes the differences in clade sizes result from accelerated or inhibited diversification (i.e. speciation minus extinction), which is in turn the consequence of a complex interplay between extrinsic factors (specific environmental conditions) on one hand, and organismal, intrinsic factors (e.g. states of particular traits) on the other[2–4]. Major tectonic events, emergence of new geographic barriers, changing patterns of wind and sea currents, shifts in local and global climatic conditions and the subsequent availability and variability of ecological niches are examples of closely intertwined extrinsic factors, influencing habitat-driven speciation and extinction[5–11]. In contrast, intrinsic factors are organismal traits (or their states) that correlate with, and presumably influence, taxonomic diversity. Body size has been repeatedly tested as a correlate of species richness due to ease of measurement and comparability of data among taxa. It has been found to correlate negatively with species richness in animals[12,13], presumably because smaller animals have larger population sizes with access to more ecological niches[12], lower energetic requirements[9,14], shorter generation times[8] and higher levels of mobility[8].

Among animals, spiders provide an excellent platform for macroevolutionary studies, as they comprise a vast number of species with extremely diverse ecologies, life histories and evolutionary histories. Within spiders,

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Scientific Reports | (2021) 11:9248 | https://doi.org/10.1038/s41598-021-88555-3 nature portfolio 1

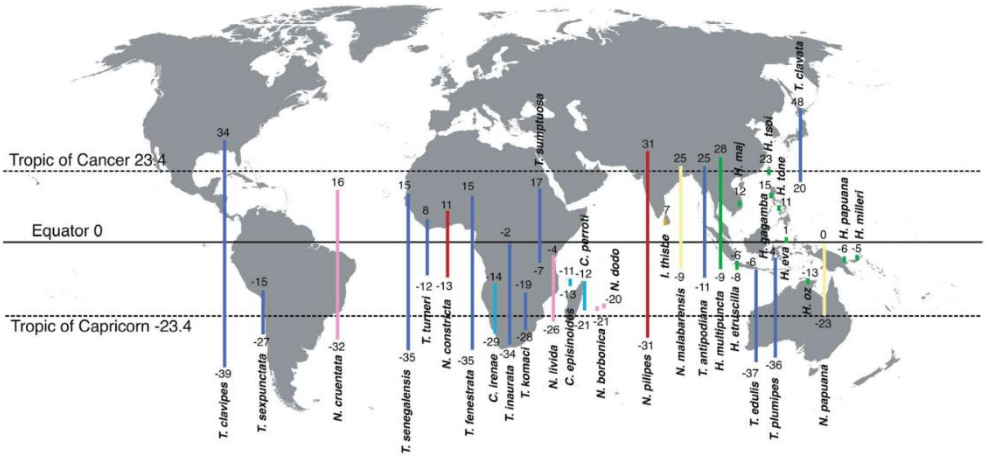


Figure 1. Approximate latitudinal ranges of 34 species of nephilid spiders included in the phylogeny. Latitudinal bars are colour-coded by genus: light blue – *Clitaetra*, dark blue—*Trichonephila*, red—*Nephila*, yellow—*Nephilengys*, orange—*Indoetra*, pink—*Nephilingis*, green—*Herennia*. Numbers denote latitudinal degrees of each species' approximate range limits. The figure combines own distributional data with IUCN Red List data (https://www.iucnredlist.org/).

golden orbweavers (Nephilidae) make an interesting subject for macroevolutionary analyses due to their well understood phylogeny and considerable age (estimated at 133 million years[15]), as well as their variation in phenotypes and species richness among the seven genera (1 sp. in *Indoetra* to 12 spp. in *Trichonephila*). Among their extreme phenotypes are extreme sexual size dimorphism (SSD), where females are up to 10 times larger than males, and web gigantism[15–18]. Here, we explore the macroevolutionary dynamics in nephilid spiders and search for evidence that would suggest diversification in this clade is driven by organismal and environmental factors. We infer the overall trends in speciation and extinction in the phylogeny and test several candidate factors, potentially responsible for the observed heterogeneity in diversity: geographical distribution (climate and landmass type), phenotypic extremeness and dispersal propensity.

A latitudinal species richness gradient, with higher diversity in lower latitudes, is a well-known pattern found across animal taxa, including spiders[19,20]. This is also true for nephilids, where species are predominantly tropically distributed and seldom extend beyond the Tropics of Cancer and Capricorn (Fig. 1). We broadly test the latitudinal species richness pattern by evaluating the association between diversification and a binary factor of tropical versus subtropical distribution. We test island versus continental distribution as another binary environmental factor, under the premise that diversification is accelerated in clades with many cases of island endemism.

For the two continuous traits, body length and dispersal propensity, we hypothesise a bell-shaped relationship between each trait and diversification—in other words, we expect to find the highest rates of diversification in genera with intermediate phenotypes and intermediate dispersal propensity (Fig. 2). For gigantism to evolve, it must provide certain fitness advantages, namely increased fecundity of large females[21] and increased foraging success of large webs[22]. However, judging by the rarity of phenotypical extremeness and low species diversity of these lineages, extreme traits must also imply costs. Kuntner and Coddington[18] suggest multiple possible disadvantages of giant female body size (increased predation risk, large nutrient demands), giant female web size (functionality constraints, larger numbers of kleptoparasites) and extreme SSD (genital mismatch, heterospecific mating, permanent sperm depletion). Traits that initially provide fitness benefits, but later in evolution lose their advantage and become costly (and potentially even cause lineage extinction) are termed 'evolutionary dead end' traits[23], and extreme phenotypes in nephilids could be among them. We thus expect phenotypically extreme genera will exhibit lower rates of diversification, presumably due to elevated rates of extinction. On the other hand, genera with milder phenotypes do not enjoy the benefits of exaggerated phenotypes, like increased fecundity of giant females, which could likewise slow their diversification. Intermediately expressed phenotypes might thus prove to be the optimal state for maximising diversification potential.

Secondly, some nephilid genera, such as *Nephila* and *Trichonephila*, have wide distributions and disperse over large distances via ballooning, whereas others, such as *Nephilingis* and *Herennia*, exhibit low levels of dispersal propensity and maintain a narrow distribution[24,25]. Dispersal into a previously unoccupied area provides a spectrum of vacant ecological niches, a state previously shown to promote speciation in spiders[26]. Thus, inherent levels of dispersal propensity and behaviours related to dispersal might be influential intrinsic diversification factors, necessarily coupled with environmental conditions (i.e. extrinsic factors) allowing and limiting ballooning, such as changes in global wind patterns and emergence of geographical barriers restricting air currents. According to the intermediate dispersal model[27–29], poor dispersers maintain a narrow distribution and only occupy new space (and consequently speciate) rarely. At the other extreme, excellent dispersers occupy large ranges, but

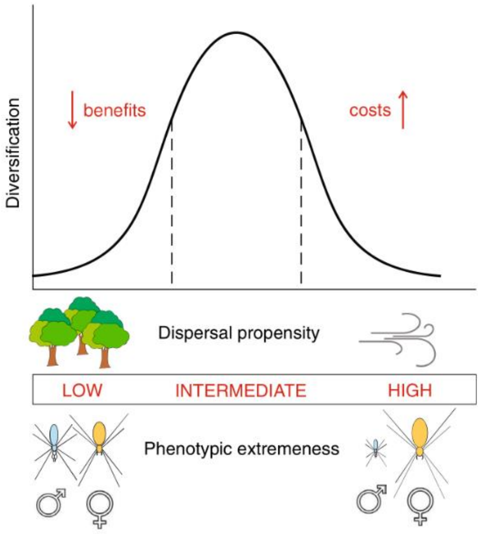


Figure 2. The hypothesised bell-shaped relationship between diversification and two continuous factors, dispersal propensity and body length sexual dimorphism. We test the prediction that intermediately expressed phenotypes will exhibit the highest rates of diversification.

also successfully maintain gene flow across them, inhibiting population fragmentation and, with it, speciation. Highest levels of diversification should thus be found in intermediate dispersers (Fig. 2) that are able to overcome dispersal barriers occasionally, but not often enough to maintain gene flow with neighbouring populations.

Additionally, we explore whether web type, another manifestation of extreme phenotypes in nephilids and an example of their extended phenotype, influences diversification. Types of webs differ between nephilid genera, but not within them. If web type has an effect on the rates of speciation and extinction, the underlying reasons may be related to differences in captured prey type, prey quantity and protection from predators each web type provides. The methods, results and discussion relating to this analysis are available in Supplementary Information online.

Results

Accumulation of lineages through time. The semi-log lineage-through-time plot on a nephilid phylogeny shows a relatively linear overall trend, without an obvious upturn towards the present (Fig. 3). The gamma statistic score ($\gamma = 0.908$, $p = 0.36$) and MCCR test ($p = 0.26$) do not imply an early burst in diversification, followed by its decrease. Instead, the accumulation of new lineages is denser towards the present (Fig. 3).

Macroevolutionary rate inference. BAMM analysis detects no distinct shifts in nephilid speciation or extinction rate dynamics. Similarly, the rate-through-time plots show no cumulative trend in either process across the clade's evolutionary history (see Supplementary Figs. S1 and S2 online). However, the mean phylorate plots for both rates do display differences in rate dynamics across the phylogeny (Fig. 4). The highest rate of speciation is recovered in *Herennia*, but in other parts of the phylogeny, speciation rate is relatively uniform, showing an overall increase from the root to the present. On the other hand, a strongly accelerated extinction rate is recovered in the species poor genus *Nephila*, and to a lesser extent in *Indoetra* and *Herennia* (Fig. 4). The remaining genera exhibit much lower, uniform extinction rates. RevBayes corroborates heterogeneity in macroevolutionary rates across nephilids, with an inferred increase in diversification in *Herennia* and *Trichonephila* (see Supplementary Fig. S3 online). Lastly, MEDUSA indicates one shift in diversification rate, placed at the base of *Herennia*, at approximately 65 Ma (Fig. 4). In this clade, it detects significantly greater diversification ($r = 0.08$) relative to the background rate ($r = 0.02$).

Correlating candidate factors to macroevolutionary rates. We used SSE-class models to test whether nephilid diversification correlates with the states of selected organismal and environmental factors. For both selected binary environmental factors, continental vs. island distribution and tropical vs. subtropical distribution, there is no significant difference between full models and those with constrained speciation, extinction and transition rates from one state to the other (Table 1). This result implies no difference in the three macroevolutionary processes between species from different environmental conditions. The only marginally significant result ($p = 0.06$) suggests a difference in speciation rate between tropically and subtropically distrib-

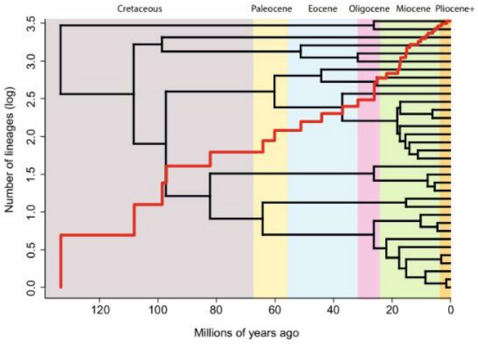


Figure 3. A lineage-through-time plot, showing species number accumulation (in red) on a logarithmic scale over the nephilid phylogenetic topology.

uted lineages, with higher rates in the tropics. Similarly, there were no improvements in model fit compared to basic BiSSE (null model) when rates were allowed to change at 26 million years ago (mya) for island versus continental ($p = 0.79$) and at 45 mya for tropical versus subtropical distribution ($p = 0.88$). When each rate was individually allowed to change at the time switch point, there were likewise no significant improvements of model fit (Table 1).

Lastly, we tested which function, if any, describes the relationship between diversification (with extinction held constant) and continuous traits of female body length, male body length and body length SSD as examples of extreme phenotypes, and dispersal propensity. None of the models are even marginally statistically supported for any trait (Table 1), suggesting the evolution of the selected continuous traits is unrelated to diversification in nephilids.

Discussion

Nephilid evolutionary history has been a subject of much scientific inquiry, particularly due to the striking variety of phenotypes and species richness, relative to the family's size. Ours is the first examination of the dynamics of two main macroevolutionary processes, speciation and extinction, on a robust nephilid phylogeny. Using a suite of different approaches to inferring diversification dynamics, we test potential organismal and environmental drivers of the rates' heterogeneity and find no clear support for the hypothesized influence of extreme phenotypes and dispersal propensity on diversification of golden orb weavers.

Accumulation of lineages through time. Summarizing the phylogeny, the semi-log LTT plot shows a roughly linear trend, with only a hinted upturn in the last 30 million years, but with an accelerated accumulation of new lineages towards the present (Fig. 3). A linear trend is in accordance with a pure-birth model of evolution (further supported by the gamma and MCCR test statistics), suggesting negligible extinction throughout nephilid evolutionary history. An upturn in the LTT plot, on the other hand, could be interpreted as the 'pull of the present'—an apparent increase in diversification towards the present, that is in fact the consequence of a non-zero extinction rate. Because younger clades have not yet had time to go extinct, this could inflate recent diversification rates[30–32]. If so, the pattern detected here could indicate that our studied lineage may have recently broken its diversification stasis, entering a phase with livelier speciation and extinction. The presence or absence of an upturn in the plot towards the present is ambiguous, however, and the lack of a clear pattern might be a consequence of the small absolute number of taxa in the phylogeny.

Macroevolutionary rate inference. In BAMM rate-through-time plots, the very slight increase in speciation and a completely flat trend in extinction are a surprising result, further corroborating the pure-birth model of evolution. The failure of BAMM to recover any rate shifts is also unexpected, although Kodandaramaiah and Murali[33] show that it underestimates rate shift numbers in small phylogenies, such as this one, and recommend a careful interpretation of a zero rate shift result in such cases. Interestingly, despite the lack of rate shifts, BAMM recovers large differences in rates along different branches (Fig. 4). *Herennia* is the only clade that stands out by speciation rates, and MEDUSA even recovers a rate shift at its base. This may be related to the high occurrence of island endemism in the genus, its persistence within a single biogeographic region, and thus speciation by vicariance[34,35].

On the other hand, BAMM infers the largest extinction rates in the genus *Nephila*, potentially pointing towards the 'dead-end' nature of *Nephila*'s life history[18]. Moreover, both *Nephila* and *Indoetra*—a monotypic genus also exhibiting heightened levels of extinction, are genera with only one or two extant species, sitting at the tips of very long branches. One can speculate these branches may have contained other species in the past that went extinct during the course of their long evolutionary histories—be it due to extreme phenotypes or other reasons. Accelerated extinction is also recovered in *Herennia*—a relatively young clade, where extinction cannot

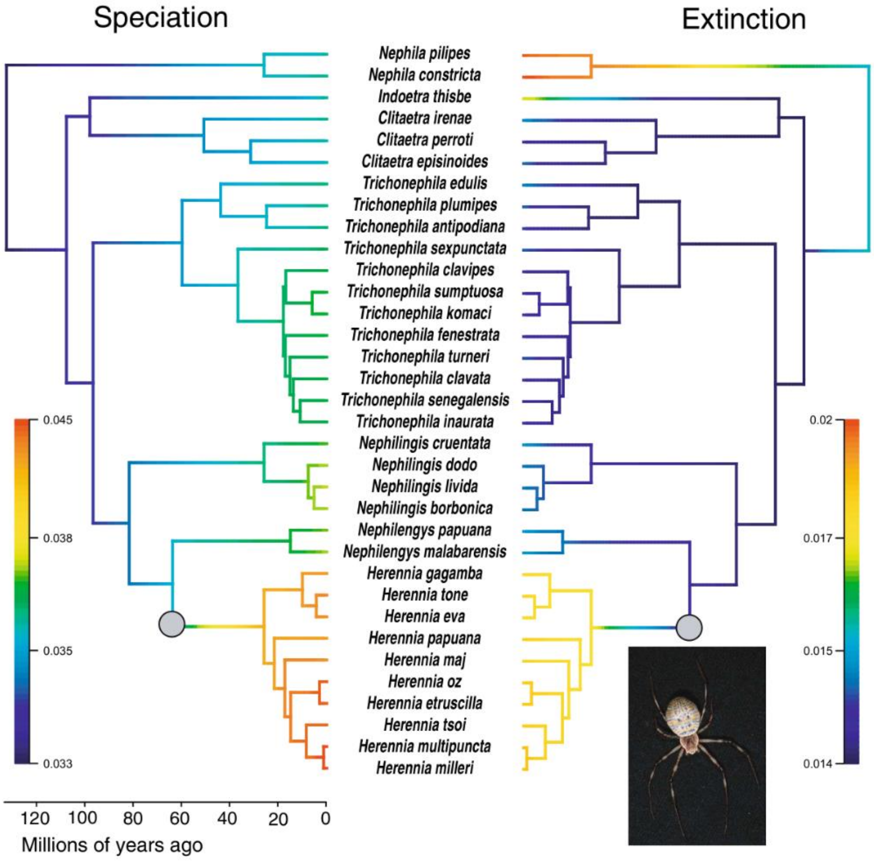
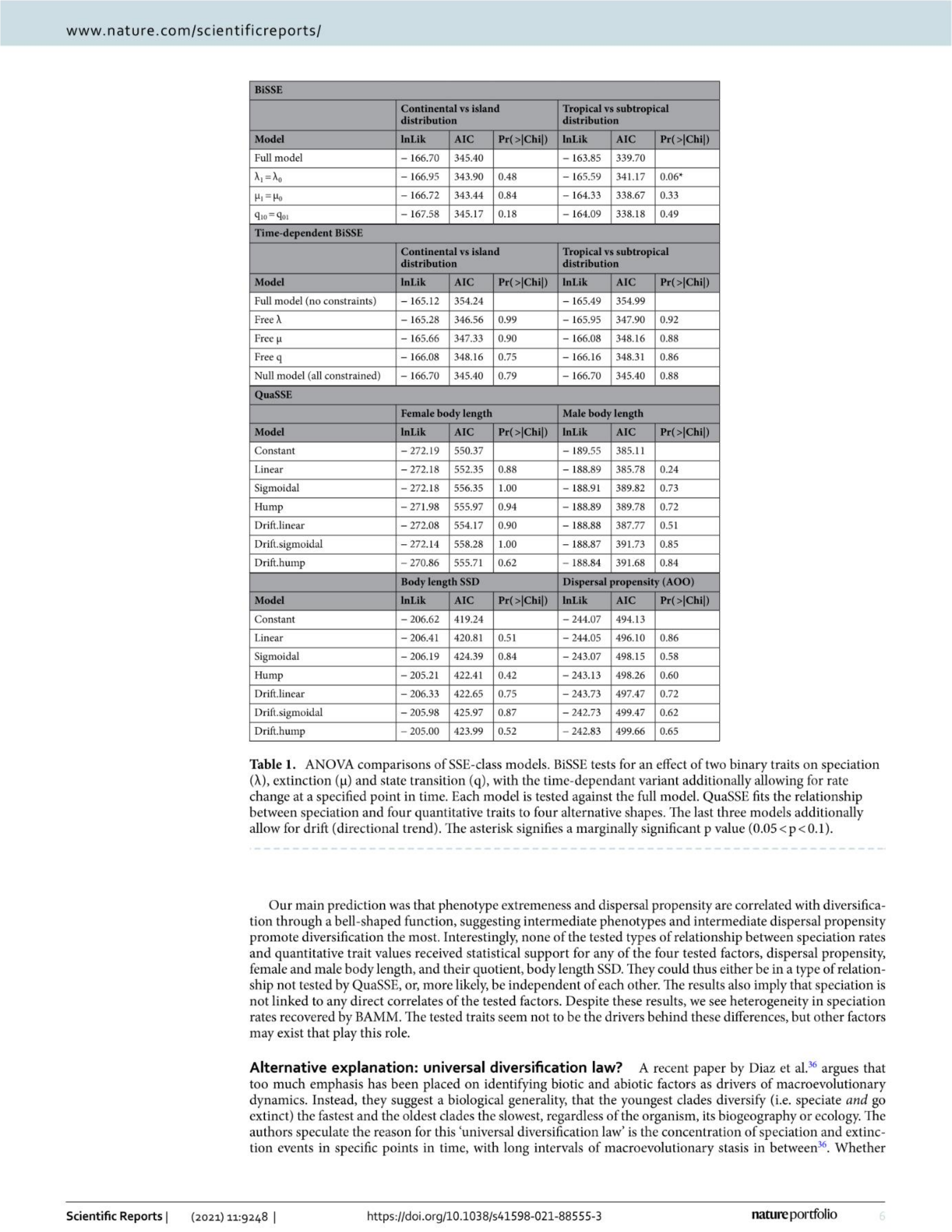


Figure 4. Macroevolutionary rate inference using two methods. BAMM phylorate plots show speciation and extinction rate dynamics in the nephilid phylogeny. Colours denote relative intensity of speciation and extinction along branches. Grey dots signify the location of a shift in diversification rate, inferred by MEDUSA. It detected increased diversification in the genus *Herennia* (visually presented by *H. eva*; own photograph) relative to the rest of the phylogeny.

be attributed to the same causes. Here, we hypothesise it relates to the species' limited ranges, often confined to single islands, and consequently smaller population sizes, making them more susceptible to extinction.

Branch-specific diversification analysis with RevBayes corroborates livelier diversification in *Herennia*, but also identifies *Trichonephila* as a rapidly diversifying clade. BAMM, on the other hand, recovers no exceptional rates for either speciation or extinction in *Trichonephila*, despite the clade's comparatively large extant diversity. This discrepancy in the results between the two closely related methods may be a manifestation of the differences in rate calculation, referred to in the Methods, and implies the importance of method selection in such analyses.

Phenotype extremeness and dispersal propensity do not affect macroevolutionary rates. Neither of the two binary traits tested with BiSSE and its time-dependent variant reveal a strong correlation with diversification, although tropical versus subtropical distribution does show a marginally significant relationship with speciation. This result is not surprising, as the tropics do in fact host a much larger taxonomic diversity of nephilids than the subtropics (Fig. 1). On the other hand, a lack of correlation between speciation and island distribution is unexpected, as some genera, especially *Herennia* and *Nephilingis*, contain island endemic species. This is closely related to the idea of dispersal propensity as a major intrinsic factor of diversification, in that these species are such poor dispersers they do not maintain gene flow even with adjacent islands and should exhibit speciation by vicariance.



BiSSE						
	Continental vs island distribution			**Tropical vs subtropical distribution**		
Model	**lnLik**	**AIC**	**Pr(>|Chi|)**	**lnLik**	**AIC**	**Pr(>|Chi|)**
Full model	− 166.70	345.40		− 163.85	339.70	
$\lambda_1 = \lambda_0$	− 166.95	343.90	0.48	− 165.59	341.17	0.06*
$\mu_1 = \mu_0$	− 166.72	343.44	0.84	− 164.33	338.67	0.33
$q_{10} = q_{01}$	− 167.58	345.17	0.18	− 164.09	338.18	0.49
Time-dependent BiSSE						
	Continental vs island distribution			**Tropical vs subtropical distribution**		
Model	**lnLik**	**AIC**	**Pr(>|Chi|)**	**lnLik**	**AIC**	**Pr(>|Chi|)**
Full model (no constraints)	− 165.12	354.24		− 165.49	354.99	
Free λ	− 165.28	346.56	0.99	− 165.95	347.90	0.92
Free μ	− 165.66	347.33	0.90	− 166.08	348.16	0.88
Free q	− 166.08	348.16	0.75	− 166.16	348.31	0.86
Null model (all constrained)	− 166.70	345.40	0.79	− 166.70	345.40	0.88
QuaSSE						
	Female body length			**Male body length**		
Model	**lnLik**	**AIC**	**Pr(>|Chi|)**	**lnLik**	**AIC**	**Pr(>|Chi|)**
Constant	− 272.19	550.37		− 189.55	385.11	
Linear	− 272.18	552.35	0.88	− 188.89	385.78	0.24
Sigmoidal	− 272.18	556.35	1.00	− 188.91	389.82	0.73
Hump	− 271.98	555.97	0.94	− 188.89	389.78	0.72
Drift.linear	− 272.08	554.17	0.90	− 188.88	387.77	0.51
Drift.sigmoidal	− 272.14	558.28	1.00	− 188.87	391.73	0.85
Drift.hump	− 270.86	555.71	0.62	− 188.84	391.68	0.84
	Body length SSD			**Dispersal propensity (AOO)**		
Model	**lnLik**	**AIC**	**Pr(>|Chi|)**	**lnLik**	**AIC**	**Pr(>|Chi|)**
Constant	− 206.62	419.24		− 244.07	494.13	
Linear	− 206.41	420.81	0.51	− 244.05	496.10	0.86
Sigmoidal	− 206.19	424.39	0.84	− 243.07	498.15	0.58
Hump	− 205.21	422.41	0.42	− 243.13	498.26	0.60
Drift.linear	− 206.33	422.65	0.75	− 243.73	497.47	0.72
Drift.sigmoidal	− 205.98	425.97	0.87	− 242.73	499.47	0.62
Drift.hump	− 205.00	423.99	0.52	− 242.83	499.66	0.65

Table 1. ANOVA comparisons of SSE-class models. BiSSE tests for an effect of two binary traits on speciation (λ), extinction (μ) and state transition (q), with the time-dependant variant additionally allowing for rate change at a specified point in time. Each model is tested against the full model. QuaSSE fits the relationship between speciation and four quantitative traits to four alternative shapes. The last three models additionally allow for drift (directional trend). The asterisk signifies a marginally significant p value ($0.05 < p < 0.1$).

Our main prediction was that phenotype extremeness and dispersal propensity are correlated with diversification through a bell-shaped function, suggesting intermediate phenotypes and intermediate dispersal propensity promote diversification the most. Interestingly, none of the tested types of relationship between speciation rates and quantitative trait values received statistical support for any of the four tested factors, dispersal propensity, female and male body length, and their quotient, body length SSD. They could thus either be in a type of relationship not tested by QuaSSE, or, more likely, be independent of each other. The results also imply that speciation is not linked to any direct correlates of the tested factors. Despite these results, we see heterogeneity in speciation rates recovered by BAMM. The tested traits seem not to be the drivers behind these differences, but other factors may exist that play this role.

Alternative explanation: universal diversification law? A recent paper by Diaz et al.[36] argues that too much emphasis has been placed on identifying biotic and abiotic factors as drivers of macroevolutionary dynamics. Instead, they suggest a biological generality, that the youngest clades diversify (i.e. speciate *and* go extinct) the fastest and the oldest clades the slowest, regardless of the organism, its biogeography or ecology. The authors speculate the reason for this 'universal diversification law' is the concentration of speciation and extinction events in specific points in time, with long intervals of macroevolutionary stasis in between[36]. Whether these concentrations happen due to regular fluctuations in global climate, complex ecosystem dynamics or some other factor remains unanswered.

While the concentrations of speciation and extinction events, proposed by Diaz et al.[36], might not be as obvious at the scale of our small, comparatively young phylogeny, it is interesting to note that lively branch splitting occurs in the last 20 million years (i.e. the Neogene) in all nephilid genera except *Clitaetra* and *Indoetra*. Before that, branch splitting is sparse and evenly distributed in time, resembling a period of diversification stasis. In contrast to this pattern of relatively recent lively cladogenetic events, it seems that earlier nephilid evolutionary history had more shifts among biogeographic realms. Namely, Turk et al.[34] reconstructed much more frequent migration across biogeographic regions in the Paleogene and Cretaceous compared with the Neogene, when each genus diversified almost exclusively within a single biogeographic region. This is especially notable in a subclade of *Trichonephila*, which colonized the Afrotropics in the beginning of the Neogene and continued rapid diversification predominantly within Africa. In this case, empty ecological niches could have acted as a major extrinsic driver of adaptive radiation, although this remains to be tested.

Limitations in methodology. Heterogeneity in diversification across space and time has long been a subject of interest in evolutionary biology, but research has long proven difficult due to poorly reconstructed phylogenies lacking time calibration, scarce fossil records and a lack of specialized statistical tools. Advancements in molecular phylogenetics enabled the production of robust phylogenies and with them the development of superior macroevolutionary rate estimation methods (reviewed in e.g.[32,37,38]). However, there is an extensive ongoing debate in macroevolutionary literature on how and when macroevolutionary rates should be estimated (e.g.[39]). This is especially true for extinction rate estimation in clades with no fossil records and with non-uniform diversification rates, like ours (see "Methods"). The field is far from reaching a consensus, with some work disputing the validity of rate estimation altogether[40].

It is important to acknowledge a crucial limiting factor in our analyses, the relatively small size of the phylogeny. Several studies have shown empirically that the number of terminals in a lineage can profoundly influence the performance of an array of macroevolutionary analyses, where small phylogenies show limits in statistical power and accuracy[33,41,42]. Some taxonomic groups, like nephilids studied here, have relatively low species numbers, but their macroevolutionary rates nonetheless remain an interesting object for research. One might speculate that the lack of support for our main hypotheses is at least in part due to the small absolute number of taxa, making the relationship with diversification difficult to detect. We hope to see advancements in this field in the future, providing adequate macroevolutionary methodology for smaller taxonomic units.

Conclusion

While there might be biotic and abiotic factors that further stimulate or inhibit diversification, species emergence, persistence and demise are complex processes, requiring the right conditions at the right time to unfold. A universal pattern of diversification might indeed explain why we fail to find correlation between any of the tested traits and diversification—it is simply not as dependent on organismal and environmental traits as predicted. Considering the same patterns of diversification are found across the tree of life, results of studies like ours might be best explained in the simplest way possible.

Methods

All diversification analyses were performed in *R* v.3.5.0[43], on a time-calibrated, ultrametric phylogeny after Turk et al.[34] (see Supplementary Data online). This phylogeny includes 34 out of 40 currently described nephilid species, meaning 85% taxon sampling.

Accumulation of lineages through time. Using the R package *phytools* v.0.7-70[44], we produced a lineage-through-time (LTT) plot to visualise species accumulation through time, calculated the gamma (γ) statistic and ran the Monte Carlo constant rates test (MCCR) (both after[45]). Both statistics test whether observed diversification in a phylogeny deviates from diversification rates, expected in a pure-birth model, with the MCCR test additionally accounting for incomplete taxon sampling. A significant value of both statistics is generally interpreted as recent deceleration in diversification rate, implying an early burst in diversification[46]. Although a popular method of detecting potential early bursts, it has been criticized for being overly sensitive to recent changes in diversification rates compared to those in the early history of the tree, regardless of the tree's size and completeness of sampling[46].

Macroevolutionary rate inference. We inferred overall macroevolutionary dynamics in the phylogeny with BAMM v.2.5.0 (Bayesian Analysis of Macroevolutionary Mixtures)[47]. It uses reversible jump Markov Chain Monte Carlo to survey and assess a large number of potential diversification models for a given phylogenetic tree, each with a unique configuration and intensity of macroevolutionary processes[47]. The main aim of BAMM is to identify points of rapid change in rate intensity, known as rate shifts. An important benefit of this method is that it does not assume the rate of the processes to be constant through time, but to behave dynamically[47].

We used BAMM to infer rates of speciation and extinction. Speciation (λ) and extinction (μ) priors were set using the 'setBAMMpriors' function in the R package *BAMMtools* v.2.1.7[48]. Due to the relatively small phylogeny, the expected number of rate shifts was set to 1. Additionally, incomplete taxon sampling was accounted for in the control file by specifying which genera lack complete species representation and to what extent. BAMM was set to run for 10 million generations on four MCMC chains, sampled every 1000 generations. We guaranteed chain convergence by confirming that effective sample size (ESS) values were > 200. Results were analysed using

BAMMtools and visualised in the form of rate-through-time plots and mean phylorate plots, which display varying rates by colour-coding branch segments.

Despite its popularity, BAMM has seen criticism regarding its reliability ([49], but see[50]). Extinction rate estimates in particular should be interpreted cautiously, as they are potentially biased and often differ dramatically from true extinction rates in simulated phylogenies[47]. One of the reasons for this is BAMM's assumption of no rate shifts on unobserved, extinct branches. Thus, we also estimated branch-specific diversification rates with RevBayes[51], a similar approach which solves extinction-related caveats of BAMM by drawing diversification rates from a discrete, not continuous, distribution, allowing correct probability calculation across all possible rates. RevBayes output was visualised using the associated R package *RevGadgets* v.1.0.0 (https://github.com/revbayes/RevGadgets).

Finally, as an alternative to Bayesian methods, we ran a similar, maximum-likelihood analysis with MEDUSA[52], implemented in the R package *geiger* v.2.0.6.4[53]. MEDUSA calculates the likelihood of obtaining a given tree with its particular phylogenetic (shape, branch lengths) and taxonomic (node age, extant species richness) properties, given specific values of birth rate (i.e. speciation) and death rate (i.e. extinction)[52]. Diversification rates are allowed to vary among clades, but are held constant through time, as opposed to BAMM. It identifies the likeliest birth and death rate values and calculates the model's AIC score. It then proceeds to fit alternative, increasingly complex models of diversification to the phylogeny, calculating AIC scores of each. The process is stopped when a more complex model is no longer a significant improvement over the previous one[52]. Incomplete sampling was accounted for in the 'richness' file.

Correlating candidate factors to macroevolutionary rates. We proceeded to test potential factors influencing nephilid speciation and extinction using state-dependent (SSE) models of diversification, all included in the R package *diversitree* v.0.9-13[54] (see Supplementary Data online). We used BiSSE (Binary-state Speciation and Extinction Model)[55] to estimate how speciation and extinction rates are affected by the state of a binary (two-state) character. In essence, BiSSE simultaneously models character change and the effect of this change on diversification. It acquires the probability that extant species would evolve the way they did, given a specific model of the character's effect on evolution. Provided a phylogenetic tree and a binary character, BiSSE calculates this probability according to six parameters: speciation and extinction rates for state 0 and state 1, and rates of the two character state transitions. BiSSE applies maximum likelihood to estimate the parameters and with them tests the hypothesis that speciation and extinction rates do or do not depend on the state of the chosen character[55]. Because regular BiSSE assumes constant rates over time, we also applied the function *bisse.td*, which allows rates to change at a specified point in time.

We tested two binary traits for effects on nephilid diversification: island versus continental distribution and tropical versus subtropical distribution (the latter included two species with predominantly temperate distribution). We first estimated all six parameters in the 'full model', and then repeated the analyses with three types of constraints: equal speciation rates ($\lambda_1 = \lambda_0$), equal extinction rates ($\mu_1 = \mu_0$), and equal transition rates between states ($q_{10} = q_{01}$). The full model was tested against each constrained model with ANOVA. Significant differences between them would suggest that macroevolutionary process do in fact depend on the state of the trait.

For time-dependent BiSSE testing island versus continental distribution, the age of the genus *Herennia*, which contains most cases of island endemism, was set as the point in time when rates were allowed to change (i.e. 26 mya). For tropical versus subtropical distribution, the approximate age of the oldest species, currently inhabiting subtropical or temperate climates, was set as the time switch point (i.e. 45 mya). For each trait, we created a *full* model, where speciation, extinction and state transition rates before and after the switch point were unconstrained. We then separately allowed each rate to vary across the two time periods, while keeping the other two rates constrained. The resulting three models and the *null* model (with all rates constrained across both time periods—in other words, the regular BiSSE model) were individually compared against the full model for improvement of fit.

BiSSE has been used in numerous studies and proved especially powerful for large trees, e.g.[56]. However, Rabosky and Goldberg[57] point out that it is concerningly easy to obtain a statistically significant association between speciation rate and a quickly evolving neutral trait. They demonstrate this Type I error empirically and question the validity of conclusions made from BiSSE analyses in existing literature[57].

To test the main proposed correlates of diversification, phenotype extremeness and dispersal propensity, we used QuaSSE (Quantitative-state Speciation and Extinction)[58], the most complex of the SSE-class analyses, where speciation and extinction rates are modelled as functions of a continuous trait. It models and compares four alternative types of functions: constant, linear, sigmoidal and hump (modal) functions. We expect the latter to optimise as the best supported type of relationship between trait values and diversification.

We used body length, a standard in SSD research, as the tested extreme phenotype trait. We tested female and male body length separately, as well as body length SSD, calculated as the mean female body length divided by mean male body length for each species[16]. The phylogenetic tree used in the analysis for male body length and body length SSD omitted four species of *Herennia* (*H. gagamba*, *H. maj*, *H. milleri* and *H. oz*) for which the males are unknown.

To test the intermediate dispersal hypothesis, we used each species' estimated area of occupancy (AOO) as a proxy for dispersal propensity, under the rationale that species with greater dispersal propensity will occupy and maintain presence on a larger geographical area. AOO is an established metric that records the area of suitable habitat, presently occupied by a selected species (after IUCN Standards and Petition Committee). AOO values were recovered from the IUCN Red List (https://www.iucnredlist.org/) and from unpublished data for *Herennia milleri*, *Herennia tone* and *Trichonephila edulis* (M. Kuntner and P. Cardoso, unpublished data). AOO values for species with single known specimens (*Herennia eva*, *H. maj* and *H. tsoi*) were arbitrarily assigned the minimal

recorded nephilid AOO, that of *Nephilingis dodo*. Due to the extremely large variation in AOO values among species, we log-transformed the data prior to QuaSSE calculations.

Macroevolutionary literature has seen much debate on whether rates of extinction can be estimated from phylogenies lacking fossil data[30,48,55,59,60]. Cautious interpretation of extinction rate estimates is advised repeatedly and estimation is even discouraged when diversification is not uniform across the phylogenetic tree[59]. This is clearly the case in the nephilid phylogeny with a wide variation in species richness across genera, while the tree itself is small, reducing statistical power. Considering these limiting factors on top of QuaSSE's calculation complexity, we kept the background extinction rate constant throughout the analysis and fitted the four candidate functions to speciation only. Additionally, we tested alternative versions of all four functions where drift (directional trend) is not constrained to zero. Positive values of the drift parameter signify an increase in the modelled trait with time, and negative values signify a decrease. All resulting QuaSSE models were compared with ANOVA and the difference in fit assessed via Akaike Information Criterion (AIC) values.

Data availability

All data generated and analysed during this study are included in this published article and its Supplementary Information and Supplementary Data files.

Received: 29 December 2020; Accepted: 14 April 2021

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Acknowledgements

We thank Wayne Maddison and Stephen de Lisle for methodological discussions, Pedro Cardoso for data sharing, and Fabien L. Condamine for a thorough review and further methodological advice. Support for this research came from the Slovenian Research Agency, Grants P1-0236, P1-0255, J1-9163, and 1000-17-0618.

Author contributions

E.T. and M.K. designed the study; M.K. provided phenotypic and distribution data; E.T. performed macroevolutionary analyses; E.T. and M.K. produced the figures; E.T. led the manuscript preparation, and all authors contributed to interpretation and writing.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-88555-3.

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2.1.3 A natural colonisation of Asia: Phylogenomic and biogeographic history of coin spiders (Araneae: Nephilidae: *Herennia*)

Turk E., Bond J. E., Cheng R.-C., Čandek K., Hamilton C. A., Gregorič M., Kralj-Fišer S., Kuntner M. 2021. A natural colonisation of Asia: Phylogenomic and biogeographic history of coin spiders (Araneae: Nephilidae: *Herennia*). Diversity, 13, 11: 515, doi: 10.3390/d13110515: 14 p.

Reconstructing biogeographic history is challenging when dispersal biology of studied species is poorly understood, and they have undergone a complex geological past. Here, we reconstruct the origin and subsequent dispersal of coin spiders (Nephilidae: *Herennia* Thorell), a clade of 14 species inhabiting tropical Asia and Australasia. Specifically, we test whether the all-Asian range of *Herennia multipuncta* is natural vs. anthropogenic. We combine Anchored Hybrid Enrichment phylogenomic and classical marker phylogenetic data to infer species and population phylogenies. Our biogeographical analyses follow two alternative dispersal models: ballooning vs. walking. Following these assumptions and considering measured distances between geographical areas through temporal intervals, these models infer ancestral areas based on varying dispersal probabilities through geological time. We recover a wide ancestral range of *Herennia* including Australia, mainland SE Asia and the Philippines. Both models agree that *H. multipuncta* internal splits are generally too old to be influenced by humans, thereby implying its natural colonisation of Asia, but suggest quite different colonisation routes of *H. multipuncta* populations. The results of the ballooning model are more parsimonious as they invoke fewer chance dispersals over large distances. We speculate that coin spiders' ancestor may have lost the ability to balloon, but that *H. multipuncta* regained it, thereby colonising and maintaining larger areas.

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A Natural Colonisation of Asia: Phylogenomic and Biogeographic History of Coin Spiders (Araneae: Nephilidae: *Herennia*)

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Abstract: Reconstructing biogeographic history is challenging when dispersal biology of studied species is poorly understood, and they have undergone a complex geological past. Here, we reconstruct the origin and subsequent dispersal of coin spiders (Nephilidae: *Herennia* Thorell), a clade of 14 species inhabiting tropical Asia and Australasia. Specifically, we test whether the all-Asian range of *Herennia multipuncta* is natural vs. anthropogenic. We combine Anchored Hybrid Enrichment phylogenomic and classical marker phylogenetic data to infer species and population phylogenies. Our biogeographical analyses follow two alternative dispersal models: ballooning vs. walking. Following these assumptions and considering measured distances between geographical areas through temporal intervals, these models infer ancestral areas based on varying dispersal probabilities through geological time. We recover a wide ancestral range of *Herennia* including Australia, mainland SE Asia and the Philippines. Both models agree that *H. multipuncta* internal splits are generally too old to be influenced by humans, thereby implying its natural colonisation of Asia, but suggest quite different colonisation routes of *H. multipuncta* populations. The results of the ballooning model are more parsimonious as they invoke fewer chance dispersals over large distances. We speculate that coin spiders' ancestor may have lost the ability to balloon, but that *H. multipuncta* regained it, thereby colonising and maintaining larger areas.

Keywords: coin spider; Nephilidae; phylogenomics; biogeography; dispersal probability

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Citation: Turk, E.; Bond, J.E.; Cheng, R.-C.; Čandek, K.; Hamilton, C.A.; Gregorič, M.; Kralj-Fišer, S.; Kuntner, M. A Natural Colonisation of Asia: Phylogenomic and Biogeographic History of Coin Spiders (Araneae: Nephilidae: *Herennia*). *Diversity* **2021**, *13*, 515. https://doi.org/10.3390/d13110515

Academic Editors: Mark Harvey and Michael Wink

Received: 10 September 2021
Accepted: 19 October 2021
Published: 22 October 2021

1. Introduction

Biogeography is a scientific field that integrates evolutionary hypotheses, contemporary and fossil taxonomic distributions and time calibrated phylogenies. Nevertheless, modern biogeography has struggled to become an exact science for several reasons. First, time calibrated phylogenies often yield unreliable topologies and/or divergence times [1] or produce very wide margins of error [2]. Consequently, time estimates of divergence and speciation events remain vague, and hypothesis testing imprecise (but, see

Magalhaes et al. [3]). Second, organism-specific biology is usually not accounted for within historical biogeographic reconstructions. When it is, the organism-specific resolution rarely goes beyond basic trait-state binning [4], e.g., winged versus pedestrian versus aquatic animals, etc. Third, although the Earth's tectonic and climatic histories represent essential variables for the distribution of organisms, their precise reconstructions through insets of time have not been integrated into biogeographic algorithms.

We have recently discussed this gap in methodology in a biogeographic study of a globally distributed spider family [5]. We suggested and demonstrated a novel method of fine-tuning biogeographical analyses by combining a robust phylogeny and specific organismal biology with dispersal probability estimates, based on concrete measurements between geographical regions in the geological past. This approach proved suitable for analyses across large geographical areas, where geological reconstructions are sufficiently accurate and for organisms whose dispersal biology is well understood. When the geological past of the area of interest is not as clear and species biology is unknown, however, this methodology has to be modified. Here, we produce a comprehensive phylogeny of coin spiders (Nephilidae: *Herennia*) using phylogenomic data and test and discuss an alternative approach to biogeographical inference to the one proposed previously.

Among the golden orbweaver spiders of the family Nephilidae (catalogued as Nephilinae-Araneidae; we here follow the family classification proposed by [6]), coin spiders (genus *Herennia* Thorell, 1877 [7]) are the most species-rich genus with over a dozen species distributed in tropical Asia and Australasia. As all nephilids, they exhibit extreme sexual size dimorphism with males but a fraction of female size [6]. Unlike the remainder of genera though, all *Herennia* build arboricolous ("tree-hugging") ladder webs on tree trunks [6,8]. A newly updated *Herennia* taxonomy (in preparation) recognises 14 species of coin spiders (three of which have not yet been formally described and thus feature manuscript names in quotation marks). With the exception of *H. multipuncta* Doleschall 1859 [9], they are distributed narrowly, mostly as island endemics. *Herennia gagamba* Kuntner 2005 [8] and *H. tone* Kuntner 2005 are found in the Philippines, *H.* "tsoi" Kuntner et al., (in preparation) in Taiwan, *H.* "maj" Kuntner (in preparation) in Vietnam, *H. etruscilla* Kuntner 2005 in Java, *H.* "eva" Kuntner (in preparation) in Sulawesi, *H. deelemanae* Kuntner 2005 in Borneo, *H. jernej* Kuntner 2005 in Sumatra, *H. sonja* Kuntner 2005 in Borneo and Sulawesi, *H. papuana* Thorell 1881 [10] in New Guinea and Australia, *H. agnarssoni* Kuntner 2005 in the Solomon Islands, *H. milleri* Kuntner 2005 in New Guinea and New Britain and *H. oz* Kuntner 2005 in northern Australia (Figure 1). In contrast, *H. multipuncta* is distributed throughout southern India, Indochina and the Philippine and Indonesian archipelagos (Figure 1). Unlike other species, which are obligatory arboricoles in pristine forests, *H. multipuncta* is synanthropic, frequently found in managed habitats, and lives in sympatry with other, narrower endemic species of the genus [8]. This fact has sparked speculation on the invasive origin of *H. multipuncta* super-range [8], but this hypothesis has remained untested.

Although coin spiders exhibit several intriguing biological features, many aspects of their biology remain unexplored. A prior revision of the genus discussed the taxonomy, biology and biogeography of the 11 then-known species [8]. It suggested purely Australasian speciation of coin spiders and proposed the "*Herennia* line", west of Wallace's and Huxley's lines, which was only crossed by one then-known species, *H. multipuncta*. Since then, several new species have been recognised, some of them inhabiting areas west of the proposed line (Kuntner et al., in preparation). A recent nephilid biogeographic study [5] inferred historical biogeography of 10 species with available genetic data; however, that study was global and, thus, its biogeographical resolution was necessarily insufficient to resolve the *Herennia* biogeographic history. Nonetheless, Turk et al. [5] suggested an Indomalayan origin of the genus with recent colonisation of Australasia by the ancestor of *H. milleri* and *H. multipuncta*.

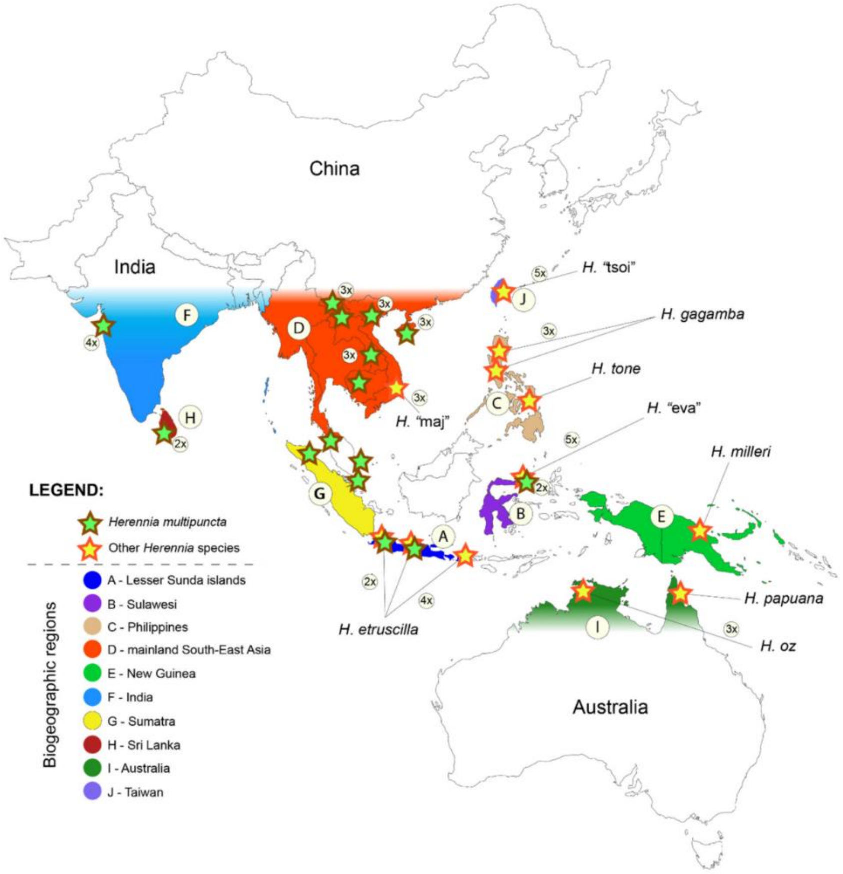

Figure 1. Sampling locations of *Herennia* individuals used in this study. Encircled numbers denote the number of individuals from the same sampling location. Geographic areas, used for historical biogeography inference (**A**–**J**), are colour-coded.

Here, we aimed to answer the following three main questions: (i) what is the sequence and chronology of coin spider dispersal from their origin to the present distribution, (ii) how would alternative dispersal biologies influence this pattern and (iii) is the unusually large range of *H. multipuncta* a result of human activity, and is the species invasive? We used phylogenomic data to construct a species-level phylogenetic scaffold, and then used classical phylogenetic markers to infer the most comprehensive population-level phylogeny and chronogram of coin spiders to date. We used this reference phylogeny to infer coin spiders' historical biogeography by adapting the methodology proposed by Turk et al. [5]. We tested two alternative models, each assuming a different type of dispersal, while accounting for the complex geological past of Australasia (Figure 2). The first model (A) assumes active dispersal via ballooning [11]. Ballooning behaviour has been observed in other nephilid species [12], but not yet in coin spiders. This type of dispersal promotes island colonisation, but also facilitates gene flow maintenance across large distances, thus inhibiting island endemism. The second model (B) assumes short distance random walking dispersal during the search for vacant habitat as the main method of dispersal. It allows for passive dispersal over long distances of connected lands given enough time. Neither model completely excludes rare chance occurrences of long-distance dispersal with wind currents.

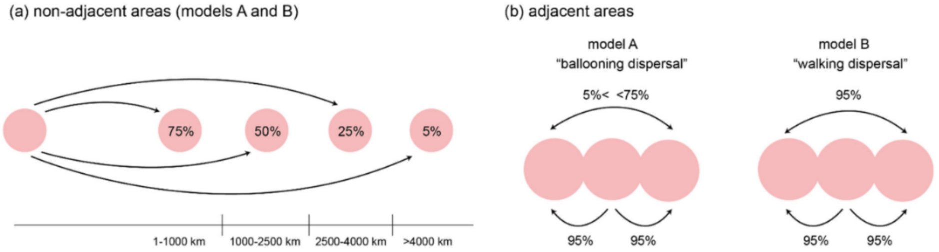

Figure 2. Methods of dispersal probability attribution. Each pink circle represents a geographical area. (**a**) For non-adjacent pairs of areas, both models attributed dispersal probabilities based on physical distances between the areas. Probabilities were inversely proportionate to the distance and binned into four categories. (**b**) When areas were in physical contact, model A attributed the maximal, 95% dispersal probability only between directly neighbouring areas, while indirectly connected areas were scored as non-adjacent pairs of areas. In contrast, model B treated all physically connected areas as a single area, attributing a 95% dispersal probability to all pairs of areas.

To address our main questions highlighted above, we inferred the most comprehensive species- and population-level phylogenies of coin spiders to date and calculated the most plausible biogeographic histories and dispersal trajectories using the two alternative models. If our data revealed *H. multipuncta* intraspecific splits are too recent to be resolved via phylogenetic time calibration, this would speak for a human-induced dispersal of *H. multipuncta* into other *Herennia* species ranges. In contrast, if intraspecific splits were resolved, and reconstructed as more ancient than human presence in this area (over 50,000 years [13]), then a natural colonisation would be implied. Generally, the estimated ages of nodes within *H. multipuncta* did not support the human-driven dispersal hypothesis. We speculate that ballooning ability and/or propensity was lost in coin spiders (except for *H. multipuncta*), resulting in a high occurrence of island endemism in the genus.

2. Materials and Methods

2.1. Species-Level Phylogeny

Currently, tissue material and genetic data are available for ten of the 14 known *Herennia* species, while the remaining four (*H. agnarssoni*, *H. deelemanae*, *H. jernej* and *H. sonja*) are only known from holotypes and could not be included here. Prior to our study, the best supported nephilid phylogeny used Anchored Hybrid Enrichment (AHE) data to resolve phylogenetic relationships among 22 species including five species of *Herennia* [6]. We here expand this taxon coverage to include eight *Herennia* species, of which *H. multipuncta* was represented by a specimen from Sri Lanka and one from Laos in order to test this widespread species monophyly (Supplementary Spreadsheet S1).

We employed the AHE targeted-sequencing approach for spiders (outlined in Hamilton et al. [14]) to target 585 single copy orthologous loci from across the genome. These loci have been shown to possess sufficient variation for resolving both shallow and deep-scale evolutionary relationships throughout the Araneae, e.g., [14,15]. These data have also been used to recover inter- and intrageneric relationships, as well as inter- and intraspecies relationships within a range of spider families [6,16–21]. Library preparation, enrichment, sequencing, assembly, alignment and phylogeny construction from AHE data followed the procedures described in Kuntner et al. [6]. The full AHE matrix is available as Supplementary File S1.

For two species, *H. papuana* and *H. tone*, we were unable to recover AHE data. To identify their phylogenetic placement, we ran maximum likelihood (RAxML) and Bayesian (MrBayes) phylogenetic inference analyses only on COI sequences, obtained for the ten species. The primers and PCR amplification protocols to obtain partial COI sequences followed Kuntner et al. [22] (matrix available as Supplementary File S2; COI GenBank accession codes OK017092 to OK017142). The placements of *Herennia* "maj" and *H. milleri*, the latter likely due to low COI sequence quality, were poorly supported and inconsistent with the AHE topology. To eliminate the influence of these "rogue taxa" on *H. papuana* (topological placement of *H. tone* was consistent regardless of their presence), these two terminals were removed from the COI only dataset and the analyses were repeated. Results produced by MrBayes were inspected with Tracer to ensure effective sample sizes were >200.

Divergence dating calibration was performed in BEAST2 [23], again using only COI sequences. We used a relaxed log normal clock and set the bModelTest [24] as the nucleotide substitution model and a birth-death tree prior. The ucldMean prior was set as normally distributed with a mean of 0.0199 and a standard deviation of 0.001 following Bidegaray-Batista and Arnedo [25]. The topology was constrained as described above, whereas clade ages and their confidence intervals were constrained in three nodes that correspond directly to those acquired by Kuntner et al. [6]. BEAST ran on four MCMC chains for 10 million generations. Results were checked with Tracer and summarised with TreeAnnotator with a 10% burnin.

2.2. Population-Level Phylogeny

In the population-level phylogeny, species were represented by a varying number of samples, ranging from one in *H. tone, H. milleri* and *H. papuana* to 26 in *H. multipuncta*. Again, we inferred the phylogeny in BEAST2, using COI and 28S sequences. For 28S sequences, primers and PCR amplification protocols again followed Kuntner et al. [22] (concatenated matrix available as Supplementary File S3; 28S GenBank accession codes OK017174 to OK017212). Where an individual lacked data for both genes, only one was used. We employed a relaxed log normal clock and set the bModelTest. The Coalescent Bayesian Skyline prior, allowing for stochastic changes in population sizes through time, was chosen as the tree prior following Ritchie et al. [26]. COI priors were set as before, while the ucldMean prior for 28S was set as normally distributed with a mean of 0.0011 and standard deviation of 0.0003 after Bidegaray-Batista and Arnedo [25]. The ages of all species-level splits were constrained to those recovered in the previous phylogeny. BEAST ran on four MCMC chains for 70 million generations to ensure large enough effective sample sizes. Again, a consensus tree was obtained with TreeAnnotator with a 10% burnin.

2.3. Inference of Biogeographic History

For biogeographic analyses, we pruned the reference population-level phylogeny so that each population (locality) was represented by a single specimen (hereafter referred to as "pruned phylogeny"). The individual with the most complete sequence was chosen as the representative of each population. This narrowed the population tree to 29 tips, with the number of representatives per species ranging from one in *H. oz*, *H. milleri, H. papuana* and *H. tone* to 13 in *H. multipuncta*. We treated the known areas of species occupancies within 10 biogeographic regions. These consisted of three continental landmasses—mainland South-East Asia, Australia and India—and seven islands or archipelagos—Sulawesi, Sumatra, New Guinea, Sri Lanka, Taiwan, the Philippines and the island pair Java and Lombok, for simplicity referred to as Lesser Sunda islands. As the tips in the phylogenetic tree are individual samples, necessarily only inhabiting a single biogeographic region, each tip was attributed one region.

Following the rationale developed in Turk et al. [5], dispersal probabilities were fine-tuned to reflect the varying geographical configuration of biogeographic regions during the area's lively geological past. Physical distance among landmasses was used as a proxy for dispersal probabilities, scored separately in six time slices, each spanning 5 million years. As the geological history of the area, especially Indonesian islands, is extremely complex and thus difficult to reconstruct with precision, we binned dispersal probabilities into five categories. Following the argumentation in Turk et al. [5], we attributed a 95% dispersal probability to pairs of geographic regions in physical contact, where dispersal is likely but not necessary, and a 5% dispersal probability where distances exceed 4000 kilometres. For distances between regions of 1000 kilometres or less, a 75% dispersal probability was assigned, for distances between 1000 and 2500 kilometres, a 50% dispersal probability, and for distances between 2500 and 4000 kilometres, a 25% dispersal probability (Figure 2a). If a region had not yet emerged or was sunk during a time slice, it was disallowed in the "areas allowed" option in RASP (see below).

In contrast to the previous paper, however, we attributed these probabilities in two ways, differing in the definition of physical contact among regions (Figure 2b). In time slices where, for example, three regions were consecutively connected by land, model A attributed a 95% dispersal probability only between the middle and marginal areas, but not between the marginal areas themselves, thus accounting for the larger physical distance between them, despite physical connection via the middle area. This model, which we term "ballooning dispersal", puts emphasis on long-distance dispersal via ballooning as the main method of dispersal in coin spiders. Model B, in contrast, attributed a 95% dispersal probability for all three pairs of regions in the previous example. Biologically, model B assumes coin spiders largely disperse over land with small-step, gradual expansion; we term this "walking dispersal". Given enough time, this model assumes that spiders can reach all physically connected areas equally likely.

Geological data (Supplementary Spreadsheet S1) were compiled from a tectonic reconstruction model [27] via GPlates plate tectonics visualisation software [28] and geological literature [29–33]. We reconstructed the genus' historical biogeography with RASP 4.0 beta [34], comparing all six included biogeographical models. The maximum number of ancestral areas occupied was set to three. We evaluated model fit through weighted $\mathrm{AIC_c}$ values ($\mathrm{AIC_c}$_wt), expressing the model's relative probability, corrected for small sample sizes.

3. Results

3.1. Phylogenies

The AHE phylogeny placed eight *Herennia* species unequivocally and with overwhelming support in all nodes (Figure 3a). The oldest split from the MRCA of all coin spiders was recovered in the Vietnamese *H.* "maj". *Herennia* "eva" and *H. gagamba* then branched off as sister species, followed by *H.* "tsoi" and *H. multipuncta*, *H. milleri* and finally *H. etruscilla* and *H. oz* as sister species. As for the COI sequences, both RAxML and MrBayes consistently placed *H. tone* as sister to *H.* "eva" with a 99% support in MrBayes (Figure 3b). After the elimination of "rogue" taxa that interfered with topological stability, *H. papuana* was placed as sister to ((*H.* "tsoi", *H. multipuncta*), (*H. milleri*, *H. etruscilla*, *H. oz*)) consistently using both methods, again with a high node support of 92% in MrBayes (Figure 3b).

In the population-level phylogeny (Supplementary Figure S1), samples of the same species always grouped together; however, samples from the same locality often did not (e.g., *H. etruscilla* populations from Java, and *H. multipuncta* populations from Laos, Vietnam, Malaysia, Yunnan and Hainan). In the pruned phylogeny containing one tip per population, used in biogeographical reconstruction (see Figure 4), the divergence dating revealed frequent within-species cladogenesis during the last few million years.

3.2. Biogeographical Reconstruction: Model A

RASP identified DIVALIKE+j as the best model for the data (Table 1, Figure 4). The node uniting all *Herennia* taxa received strong support for a wide ancestral distribution in Australia, mainland SE Asia and the Philippines (61%). Although *H.* "maj" persisted only in mainland SE Asia, the most recent common ancestor (MRCA) of all other species persisted in the Philippines and Australia (65%). The clade containing *H. gagamba*, *H. tone* and *H.* "eva" remained in the Philippines, with the latter species colonising Sulawesi sometime during the last four million years.

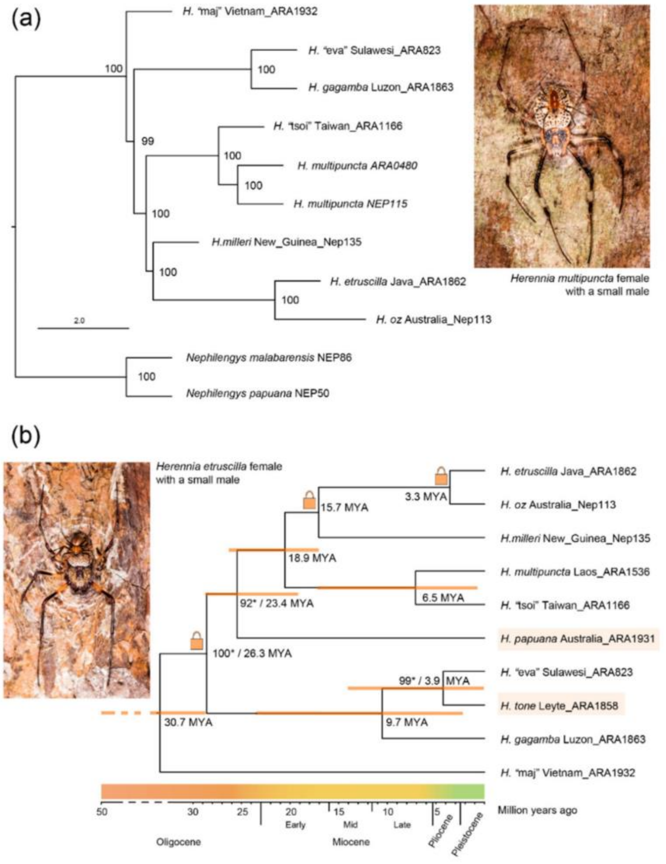

Figure 3. Species-level phylogenies of coin spiders. (**a**) AHE-only phylogeny produced with ASTRAL [35], resolving the relationships between eight species of *Herennia* with *Nephilengys* as the outgroup as per Kuntner et al. [6]; (**b**) Species-level phylogeny of the available ten species of *Herennia*, calculated from COI data. Highlighted are two species lacking AHE data, *H. papuana* and *H. tone*. The lock symbols denote age-constrained nodes. Supports for nodes not present in the AHE-only phylogeny (marked with an asterisk) were recovered by MrBayes.

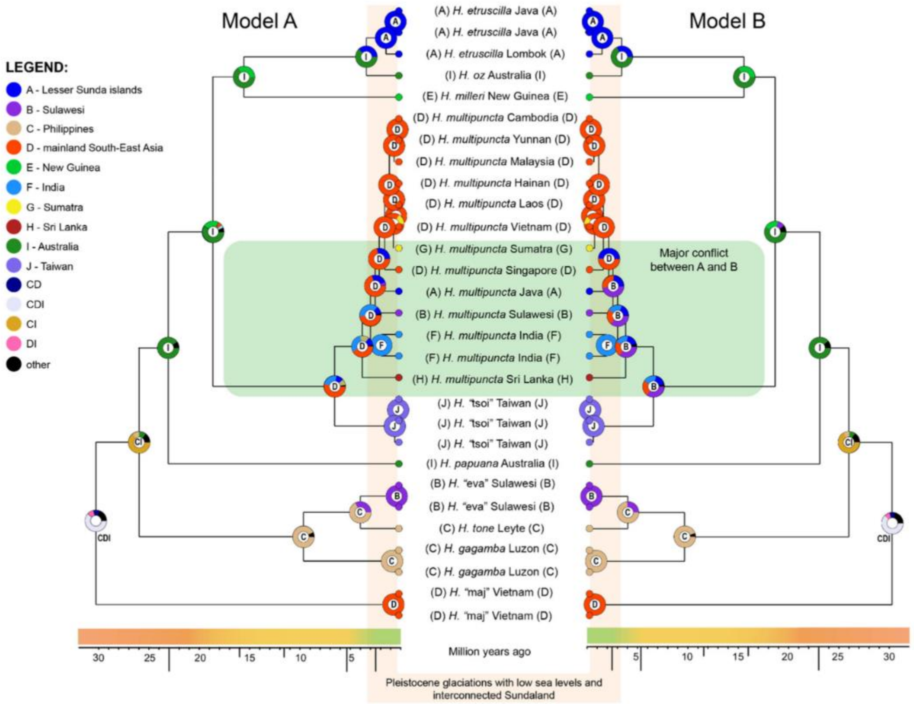

Figure 4. RASP ancestral area reconstruction of two alternative biogeographic models on a COI + 28S population-level phylogeny of *Herennia*. Model A assumes long-distance dispersal via ballooning as the main method of dispersal in coin spiders, while model B predicts that coin spiders mainly disperse over land with small-step, gradual expansion. Encircled letters signify the likeliest ancestral area in that node, with a combination of several letters indicating an inferred distribution in all those areas. Green area marks the major conflicts in ancestral area reconstruction between the two models.

Table 1. RASP model scores for models A and B. Best supported RASP model is shown in bold. An asterisk (*) in the last column indicates that the model variant allowing jump dispersal (+j) was a significantly better fit for the data than the variant without it.

Model A	LnL	AICc	AICc_wt	Δ −j/+j
DEC	−62.28	129	2.5×10^{-7}	*
DEC+j	−47.08	101.1	0.29	
DIVALIKE	−55.86	116.2	0.0002	*
DIVALIKE+j	**−46.19**	**99.33**	**0.7**	
BAYAREALIKE	−72.01	148.5	1.5×10^{-11}	*
BAYAREALIKE+j	−50.27	107.5	0.012	
Model B				
DEC	−62.86	130.2	3.1×10^{-7}	*
DEC+j	−48	103	0.25	
DIVALIKE	−56.68	117.8	0.0001	*
DIVALIKE+j	**−46.92**	**100.8**	**0.74**	
BAYAREALIKE	−73.4	151.3	8.1×10^{-12}	*
BAYAREALIKE+j	−50.89	108.7	0.014	

The MRCA of all other species remained in Australia only, with *H. papuana* persisting in the same area until the present. The MRCA of *H.* "tsoi" and *H. multipuncta* shifted from Australia to mainland SE Asia, from where *H.* "tsoi" colonised Taiwan, while *H. multipuncta* remained in SE Asia with consecutive colonisations of Sri Lanka, India, Sulawesi, Java and Sumatra during the last four million years. From the remaining Australian MRCA, *H. milleri* colonised New Guinea, while its sister Australian clade diversified into the Australian *H. oz* and *H. etruscilla*, which shifted to the Lesser Sunda islands in the last few million years.

3.3. Biogeographical Reconstruction: Model B

Again, DIVALIKE+j received the highest statistical support in RASP (Table 1, Figure 4). All nodes except the MRCA of *H.* "tsoi" and *H. multipuncta* and their intra-species nodes received nearly identical support as in model A. Here, the reconstruction for the MRCA of *H.* "tsoi" and *H. multipuncta* was ambiguous, with similar support for Sulawesi (35%), mainland SE Asia (23%) and India (21%). The basal *H. multipuncta* was attributed the same three regions in almost identical shares (36, 23 and 20%, respectively). The species was reconstructed to have colonised Sri Lanka and India from Sulawesi. Next, it colonised (and remained in) the Lesser Sunda islands (49%) or mainland SE Asia (51%), and then dispersed across the SE Asian mainland and to Sumatra.

Comparing the fit of DIVALIKE+j between the two models through the calculation of the Bayes factor from the log of the likelihood scores ($B = 0.98$; after Kass and Raftery [36]) revealed a non-significant difference.

3.4. Human-Induced Dispersal of H. multipuncta?

Neither of the two models supported the hypothesis of a recent, human-induced dispersal of *H. multipuncta*. Node ages, recovered for phylogenetic splits among the included populations in the pruned phylogeny, ranged from 3.72 million years ago (mya) in the MRCA of all included populations to 0.13 mya (132,200 years) in the split between the Yunnanese and Cambodian populations. In the latter, the confidence interval ranged from 516,800 to 800 years ago, a time frame potentially compatible with human-induced dispersal, albeit between two areas in relatively close proximity. The only other node whose estimated age fit within the timeframe of human presence in the area was the split between the Vietnamese and Lao populations. It was dated to 277,300 years ago, with a confidence interval ranging from 757,500 to 26,900 years ago. The two areas are adjacent with no obvious barriers between them, implying that natural dispersal is very likely.

4. Discussion

In this study, we provide a test case of what next generation biogeographic inference should optimally encompass: a robust phylogenetic/phylogenomic framework for a comprehensive population-level ancestral area reconstruction that at the same time accounts for geological dynamics and species biology. To this end, we used a modified version of our previously proposed methodology [5], comparing two methods of dispersal probability quantification. Both models suggested a wide ancestral range and relatively old splits (from 3.72 to 0.13 mya) between terminals of *H. multipuncta*, strongly implicating a natural, not anthropogenic colonisation of the areas that constitute an extremely wide contemporary range of this species.

4.1. Phylogenetic Placements

The species-level phylogeny recovered unexpected relationships among species with overwhelming support (Figure 3a,b). Within the available taxon sample, *Herennia* "maj" was the first species to split from the coin spider MRCA, a placement that was not recovered in a prior COI-based phylogeny in Turk et al. [5]. As surprising as this may be, we believe the relationship is not artefactual, given our understanding of the robustness of AHE phylogenomic topologies in nephilids (*H.* "maj" was not included in Kuntner et al. [6]).

In the population-level phylogeny, species were represented by a varying number of specimens. In *H. multipuncta*, the largest sample, specimens from the same population failed to group together (Supplementary Figure S1). Perhaps genetic differences between them are not (yet) large enough, as they maintain modest gene flow among populations. This matches our emerging hypothesis of heightened dispersal propensity in this species, relative to others, as proposed below.

4.2. Biogeographic Inference with Two Models

Unlike our previous study [5], we only have limited knowledge about the dispersal biology of coin spiders. As in all motile animals, they can be assumed to, at least, undergo gradual, slow dispersal through suitable habitat during stochastic search for vacant space. On the other hand, we might expect coin spiders to practice active ballooning, as observed in the related *Nephila pilipes* [12], but no field or experimental evidence that would support this assertion currently exists. Even in the absence of ballooning, however, chance dispersal across geographic barriers, such as the sea, have to occur, otherwise they would not be able to simultaneously inhabit landmasses that have not been connected during the relevant timeframe, such as mainland SE Asia and Australia. The two proposed types of dispersal have different consequences: ballooning would make it easier to colonise new areas and maintain gene flow across the entire range, but the evolution of island endemism is less likely. Conversely, relying on "walking" dispersal with occasional, chance long-distance dispersal would facilitate genetic isolation and thus speciation and island endemism, but would restrict gene flow maintenance across large ranges. Regardless, we do not assume that all coin spider species necessarily exhibit identical dispersal behaviours and propensities.

Thus, in the absence of a better understood, and experimentally tested, organism-specific dispersal biology, we resorted to engaging in two biogeographical approaches (Figure 2). Model A assumed less likely dispersal between areas further apart, even if connected by land, because ballooning was taken as the default method of dispersal. In contrast, model B assigned equal dispersal probabilities to all physically connected areas, as it assumed spiders spread "on foot", gradually, over millions of years. Although such passive, "walking" dispersal is much slower, range expansion of, for example, only 10 m per year (which we deem as a distance easily overcome by orbweaving spiders) would theoretically allow for a spread across 10,000 kilometres over the course of a single million years, which is approximately the extent of the entire extant genus range. While the comparison of the two best-fitting models using the Bayes factor did not show significant differences between them, they nonetheless provided somewhat discrepant results.

Deep nodes near the root of the phylogeny were reconstructed with nearly identical probability proportions for ancestral areas by both models (Figure 4). A wide ancestral distribution over mainland SE Asia, Australia and the Philippines fragmented, with the ancestral mainland population leading to the Vietnamese *H.* "maj", and the Australian and Philippine populations giving rise to all other species. Interestingly, mainland SE Asia was not re-colonised until 24–29 million years later, depending on the model. In both models, *H. tone* and *H. gagamba* maintain the ancestral range in the Philippines, while *H.* "eva" disperses to Sulawesi sometime during the last four million years. This inferred colonisation is plausible assuming either model of dispersal, requiring an active or chance dispersal across approximately 600 km of sea [32]. Either type of dispersal on this route may have been further facilitated by a possible island chain connecting Sulawesi to the Philippines in the Neogene [33].

The most likely ancestral areas of the (*H.* "tsoi" + *H. multipuncta* + *H. milleri* + *H. oz* + *H. etruscilla*) and (*H. milleri* + *H. oz* + *H. etruscilla*) MRCAs are reconstructed to Australia by both models (Figure 4). The latter clade features another recent over-water colonisation of *H. etruscilla* from Australia to Lesser Sunda islands. A salient difference between the two models is the inferred dispersal of *H. multipuncta* (Figure 4). In model A, the MRCA of *H. multipuncta* and *H.* "tsoi" is already distributed in mainland SE Asia, from where *H.* "tsoi" colonises Taiwan during the last 6 million years. This does not exceed the understood age of the island (approximately 9 million years [29]), which has remained close to, and even connected with, the Asian mainland by a land bridge during Pleistocene glaciations [30]. In *H. multipuncta*, model A suggests gradual expansion from mainland SE Asia to Sri Lanka, India, across Indonesian islands and finally within mainland SE Asia itself. On the other hand, model B inconclusively places the MRCA of *H. multipuncta* and *H.* "tsoi" to Sulawesi, a challenging proposition. At 6 mya, the distance between Sulawesi and Taiwan, which *H.* "tsoi" supposedly crossed, was not considerably shorter than today (approximately 2300 kilometres), having been separated by the Philippine archipelago.

Deeper nodes within *H. multipuncta* are also ambiguous in model B, with similar probabilities for several ancestral areas, but it generally suggests gradual dispersal of the species from Sulawesi to Sri Lanka and India, colonisation of Java directly or via mainland SE Asia, and finally a spread within SE Asia and colonisation of Sumatra. Dispersal from Sulawesi to Sri Lanka during this time would require crossing a >4000-kilometre distance either by chance dispersal, which is rarely observed on such a scale in spiders in general (as discussed in Turk et al. [5]), or over land. As opposed to islands such as Sri Lanka, New Guinea and Taiwan, however, it is unclear whether Sulawesi formed land connections with the mainland or mainland-connected islands, such as Borneo, during the time of Plio-Pleistocene sea level changes [33]. Furthermore, a subsequent colonisation of India, requiring another >4000-kilometre dispersal event shortly after the first, seems highly unlikely. On the other hand, all sampled *H. multipuncta* populations except those from Sulawesi, India and Sri Lanka were connected by land during the Pleistocene on the landmass Sundaland [32]. Dispersing around this landmass could indeed be performed by "walking" dispersal, but, as seen above, even model B infers several oversea dispersals, either by chance or actively.

Curiously, species such as *H.* "maj" seem to maintain a narrow distribution despite living on the Asian mainland, where they could, across millions of years, passively disperse over a much wider area (assuming our model B with no other limiting factors). We speculate such species are either restricted to specific habitats that are not continuous, decreasing the chance of successful active or passive dispersal, or are confined to their range through ecological competition with other species and genera in adjacent areas.

4.3. A Natural "Coinquest" of H. multipuncta

The pruned population-level phylogeny showed relatively recent splitting between the sampled populations of *H. multipuncta* (median node ages ranging from 3.72 to 0.13 mya), however, not recent enough to infer human-related dispersal. If that were the case, nucleotide sequence divergencies would have been predictively low, having only accumulated changes over the past few thousand years (contra to what we see in our phylogeny). In the two cases where confidence intervals of node ages do overlap with human presence in the species' range, the pairs of geographical areas are either adjacent or in close proximity, making the exclusion of natural colonisation difficult.

Why, then, is a super-wide present-day range of *H. multipuncta* unique among all *Herennia*? We hypothesise that although nephilid ancestors perform active ballooning, retained in *Nephila* and *Trichonephila* [9,34,35], coin spiders secondarily lost the ability to balloon. Such a loss of dispersal ability is a common phenomenon in island spider biology, severely limiting gene flow maintenance and often leading to single-island endemism [37,38]. *Herennia multipuncta* might have regained this ability, allowing it to disperse across suitable habitat and inhabit most of the genus range, sometimes overlapping with more ancient *Herennia* spp ranges. This would also allow it to sustain some degree of gene flow among populations, which would, in turn, explain the recovered phylogenetic picture of non-monophyly of sympatric specimens of certain populations (from Vietnam, Laos, Malaysia, Hainan and Yunnan). In many parts of its range, *H. multipuncta* is sympatric with other species (*H. etruscilla* in Java, *H.* "eva" in Sulawesi, *H.* "maj" in Vietnam, *H. jernej* in Sumatra, etc.) and, according to our dated population phylogeny, this has been the case for millions of years. This pattern suggests that *H. multipuncta* does not outcompete other sympatric species, perhaps due to subtle differences in ecological niches. In fact, adaptiveness to different habitats might be another trait, specific to *H. multipuncta*, that enabled its easier and more successful dispersal. These interpretations could be tested in an ecological framework in the near future, as could the ability of *H. multipuncta*, but not other *Herennia*, to disperse via ballooning.

4.4. Limitations in Methodology and Future Work

One source of statistical bias in the present study is the incomplete representation of coin spider species in the phylogeny and with it the lack of representation of the missing species' geographical distributions. The precise phylogenetic placement of the Bornean *H. deelemanae*, Sumatran *H. jernej*, Bornean and Sulawesian *H. sonja,* as well as *H. agnarssoni* from the Solomon Islands has never been tested outside of a morphological, cladistic framework [8]; therefore, the influence of their distributions on biogeographical reconstruction remains unclear. Furthermore, at the population level, species are not equally represented in terms of specimen number and range cover, also potentially biasing evolutionary relationships and divergence times between populations. That said, specimen collections of coin spiders are scarce. The present study was performed with all genetic material currently available to us.

One of the topics addressed in the study was the dispersal behaviour of coin spiders. Ideally, the presence or absence of active ballooning ought to be tested experimentally. Considering that ballooning is difficult to observe in nature, future research could include subjecting juvenile coin spiders to wind tunnel experiments [12] in a laboratory environment. If performed on multiple species, such an experiment might serve as a test of our hypothesised regained ballooning behaviour in *H. multipuncta*, but not in other species.

5. Conclusions

We have demonstrated the importance of the understanding of organismal biology in biogeographic reconstructions. In organisms where dispersal is not well understood, testing alternative modes of dispersal through parallel statistical models might prove helpful in uncovering the most likely dispersal biology without direct field observation. By modifying our previously proposed pipeline to account not only for the specifics of the geological history of the area, but also dispersal specifics of the studied organisms, we are further contributing to the development of biogeographic methodology.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d13110515/s1, File S1: AHE matrix (Fasta) with partition file, File S2: COI species-level matrix (Nexus), File S3: concatenated COI and 28S population-level matrix (Nexus), Figure S1: full population-level phylogeny, Spreadsheet S1: geology and geography data.

Author Contributions: Conceptualisation, E.T. and M.K.; methodology, E.T., K.Č., C.A.H. and M.K.; formal analysis, E.T., K.Č., C.A.H., J.E.B., R.-C.C. and M.K.; investigation, all authors; writing—original draft preparation, E.T. and M.K.; writing—review and editing, all authors; visualisation, K.Č.; funding acquisition, M.K. and S.K.-F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Slovenian Research Agency, grants P1-0236, P1-0255, J1-9163 and 1000-17-0618.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The new genetic sequences are available on GenBank (accession codes: OK017092 to OK017142 (COI), OK017174 to OK017212(28S)) and also as a supplement to this paper. AHE and geological data are available as a supplement to this paper.

Acknowledgments: We thank A. V. Abramov, I. Agnarsson, G. J. Anderson, M. Bedjanič, D. Court, A. Harmer, M. S. Harvey, S. Huber, P. Jaeger, D. Li, W. Maddison, W. Schawaller and I.-M. Tso for their kind assistance in field work and/or specimen acquisition. We thank I. Magalhaes and two anonymous referees for constructive criticism.

Conflicts of Interest: The authors declare no conflict of interest.

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2.2 ADDITIONAL SCIENTIFIC WORK

2.2.1 Genetic structure in nephilid populations

2.2.1.1 Introduction

In the last chapter, we explored the patterns of genetic variability within and between two species of golden orbweavers. Broadly speaking, population genetic structure refers to any detectable pattern in genetic composition (i.e. allele frequencies) in a population (i.e. a group of organisms, potentially capable of reproduction), due to non-random mating (Chakraborty, 1993; Bohonak, 1999). Natural populations, for example species, are divided into smaller subgroups as a consequence of various factors, the most prominent among them being geography. Populations of a particular species can be separated by mountains, rivers, deserts or other barriers to gene flow. Even when no such barriers exist, gene flow is not uniform throughout the range, because individuals tend to stay and reproduce close to their geographical origin. If a population becomes genetically isolated from the rest, any new mutations remain confined to it until gene flow with other subpopulations is re-established. If it remains isolated, speciation can ultimately follow. Conversely, populations that maintain a degree of gene flow are resilient to genetic differentiation (Chakraborty, 1993). Other factors influencing population structure include bottleneck (i.e. sharp reduction in population size) and expansion events, founder effect (i.e. the establishment of a new population by a small number of individuals) and chance (Bohonak, 1999).

For highly dispersive taxa, such as orbweaver spiders, gene flow is efficiently maintained among populations, even when separated by large distances. This, in turn, lowers genetic structure on the population level (Bohonak, 1999). Among golden orbweavers, dispersal behaviour is best documented in the giant orb weaver (*Nephila pilipes* Fabricius 1793), readily exhibiting ballooning behaviour in the juvenile stage (Lee et al., 2015). Following the above theory, population structure of the species should be relatively homogenous. This has been recovered for the central part of species range in a previous study, described below, albeit using today outdated molecular tools. The aim of this chapter was to retest previous findings using modern molecular and statistical approaches with greater analytical power. For comparison, we performed a parallel analysis on a related golden orbweaver, the Joro spider (*Trichonephila clavata* Koch 1878) with contrasting ecological and life history characteristics. Besides exploring within-species population structures, we were also interested in how structures would compare between species.

Figure 2: A giant orb weaver female (*Nephila pilipes*; left) and a Joro spider female (*Trichonephila clavata*; right) (photos: Turk E., 2019; Kuntner M., 2019).

The giant golden orbweaver (Figure 2) is a large spider, abundant in low-elevation rainforests throughout South and South-East Asia, Australia and the Pacific (Su et al., 2007). Like their habitat, *N. pilipes* populations are aseasonal (Higgins, 2002), allowing for a continuous generation turnover. The species is solitary and exhibits one of the highest degrees of eSSD among all spiders (Kuntner et al., 2019). The previously mentioned existing study investigated phylogeographical patterns in *N. pilipes* throughout its range, using COI as the sole genetic marker (Su et al., 2007). It recovered 67 haplotypes, grouped into five main lineages. The largest included samples from the central part of species range, while the remaining four were distributed around the geographical margins of the sampled area. The authors attributed this differentiation at the range periphery to the isolation of subpopulations in pockets of suitable habitat during Quaternary glacial periods (see discussion; Su et al., 2007). In another study, Lee et al. (2004) examined the effect of the Taiwanese Central Mountain Range on the population genetic structure of the species, also using a COI fragment as the only genetic marker. They recovered 11 haplotypes from three major lineages, but no geographical structure in haplotype composition of Taiwanese populations. This indicated strong gene flow, minimizing the barrier effect of the Central Mountain Range (Lee et al., 2004).

The Joro spider (Figure 2), on the other hand, is a smaller, less sexually size dimorphic species, with narrower distribution in subtropical to temperate habitats from the Himalayas to Japan (Su et al., 2011), with a recent introduction to North America (Hoebeke et al., 2015). Subtropical and especially temperate habitats exhibit large fluctuations in environmental conditions such as temperature and food availability (Miyashita, 1986, 1992). The range of *T. clavata* extends into high-latitude lowland forests and scrublands, as well as high-elevation mountainsides, where the period of favourable environmental conditions is limited (Kim et al., 1999), and the species consequently exhibits distinct phenological peaks (Miyashita, 1986). In Japan, for example, spiders hatch in May, mature in September and lay eggs in October and November (Miyashita, 1986). Adults then die and the eggs overwinter (Miyashita, 1986). Unlike *N. pilipes, T. clavata* individuals frequently aggregate into loose colonies (own observations; Figure 3). Active dispersal behaviour in this species is not well documented, although assumed (Miyashita, 1992; Jung et al., 2006; Hoebeke et al., 2015), and neither is the effect of high-elevation relief in mountainous regions of its areal on dispersal.

A study on Korean and Japanese populations of *T. clavata* used amplified fragment length polymorphism fingerprinting (AFLP) to evaluate population genetic diversity and found it concentrated within populations, rather than among them (Jung et al., 2006). The authors suggested this is characteristic of spiders with high dispersal propensities that easily maintain gene flow among populations, presumably through ballooning. The study failed to recover an association between genetics and geography, which was again attributed to strong dispersal, and speculated that any genetic divergences found in a highly dispersive species might be attributed to historical processes, such as geographical isolation (Jung et al., 2006). Similar observations of genetically homogenous populations have been made in other *Trichonephila* species, for example *T. inaurata*, which maintains gene flow between Africa and West Indian Ocean islands (Kuntner and Agnarsson, 2011a), and *T. clavipes*, which maintains gene flow between the North American mainland and the Caribbean (Čandek et al., 2020).

Figure 3: Aggregation of Joro spider (*Trichonephila clavata*) individuals into a loose colony (photo: Kuntner M., 2017).

The methodology employed by most existing studies has seen much advancement since the early 2000s. Here, we retested the observed patterns in both species with restriction site-associated DNA sequencing (RADseq) (Baird et al., 2008). This method uses single nucleotide polymorphisms (SNPs) as genetic markers, allowing for analyses of greater resolution. Due to the complexity of factors, potentially influencing genetics at this level, concrete hypotheses would only be arbitrary; nevertheless, we expected this methodology would uncover previously unknown genetic and geographic structure in both species. In broad, we expected within-species structure could be related to palaeoclimatic and palaeogeological factors, such as shifts in global climate and island formation, and extant range size, demanding adaptation to local environment.

If inferred, we speculated that between-species differences in genetic structure patterns could be explained in the context of discrepant environmental and organismal factors. One example is the aforementioned difference in environmental (a)seasonality, allowing for a constant gene flow among *N. pilipes* populations, but limiting it to favourable seasons in *T. clavata*. Temperate climates might also demand more local adaptations, increasing geographical structuring. On the other hand, *N. pilipes* exhibits a larger range, spread over two continents and numerous islands. Despite the mobility of *N. pilipes* via ballooning, gene flow may be harder to sustain across such a large range; range size might thus prove to be a factor lowering the species' genetic homogeneity. Among organismal factors, aggregation of individuals in *T. clavata* might facilitate genetic divergence of populations, promoting within-population mating.

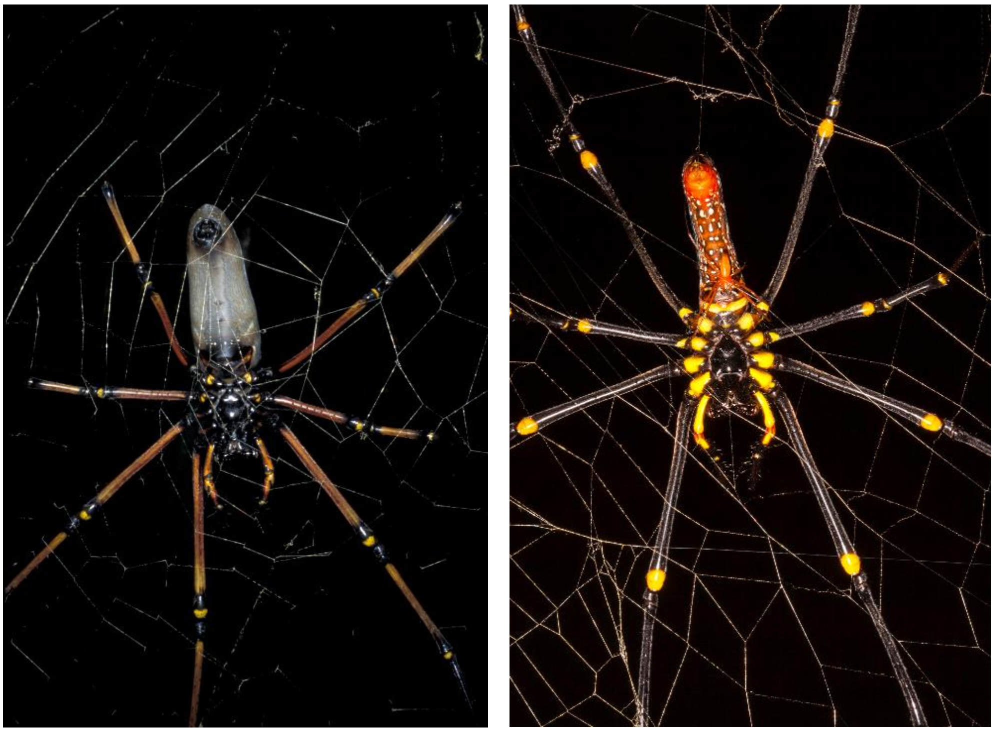


Figure 4: Melanic (left) and common form (right) of giant golden orbweaver (*Nephila pilipes*) females (photos: Kuntner M., 2002; 2010).

Additionally, we aimed to use the assembled dataset to test whether melanic individuals of *N. pilipes*, sometimes referred to as a separate species, "*Nephila kuhli*" or "*Nephila kuhlii*" (Chetia and Kalita, 2012), segregate phylogenetically from the remaining samples.

Melanic females have completely to predominantly black abdomens and reddish legs (Figure 4), but their males are morphologically identical to those collected with the common spotted form. Although relatively rare, melanic females can be found throughout the species range, particularly in India (own observations). It is not clear whether dark colouration is an adaptation or not. Tso et al. (2002) found melanic females to reflect less ultraviolet light and consequently capture significantly less prey than common females. They speculated the melanic form persist in the population because it is less visible to predators (Tso et al., 2002). Regardless of the colouration's effect on fitness, a scattered placement of melanic individuals throughout the phylogenetic tree would contradict the hypothesis of "*N. kuhli*" as a separate species, but instead confirm it as a colour variant of *N. pilipes*.

2.2.1.2 Methods

Taxon selection

We obtained 94 specimens of *Nephila pilipes* from 39 populations and 40 specimens of *Trichonephila clavata* from 16 populations. Each population was represented by 1 to 3 individuals, depending on material availability. Specimens were treated as a single population if their collection coordinates were identical or in close vicinity. While we aimed to maximize the geographic range coverage of sampling, population distribution depended entirely on material availability. Specimens were acquired from existing collections in Slovenia and Taiwan and through own fieldwork (for original collection sites, see Annex A). We field-collected *N. pilipes* in Hong Kong and *T. clavata* in Hunan, China, both in September 2019.

Sampling localities were divided into rough geographic regions. In *T. clavata*, these were Japan (2 populations, 6 specimens), Ryukyu islands (2 pop., 3 spec.), Taiwan (2 pop., 6 spec.), Fujian (3 pop., 8 spec.), Hunan (4 pop., 10 spec.) and Yunnan (3 pop., 7 spec.). In *N. pilipes*, the regions were India (3 pop., 7 spec.), sub-Himalaya (2 pop., 6 spec.), mainland SE Asia (7 pop., 16 spec.), Malay peninsula (5 pop., 8 spec.), the Philippines (11 pop., 29 spec.), Taiwan (3 pop., 8 spec.), Ryukyu islands (4 pop., 11 spec.), Bali (1 pop., 3 spec.), Sulawesi (2 pop., 3 spec.) and Australia (1 pop., 3 spec.). This sample contained nine melanic *N. pilipes* females, collected in India (3), Taiwan (2), the Philippines (2), Japan (1) and Laos (1).

Multiplexed shotgun genotyping

All laboratory (and subsequent bioinformatic) analyses were performed during a research visit at Yong-Chao Su’s laboratory at Kaohsiung Medical University, Kaohsiung, Taiwan. Muscle tissue from the specimens’ legs was homogenized in a lysis buffer and incubated at 56°C for 12 hours. DNA extraction from each tissue sample was performed using Maxwell RSC Blood DNA Kit on the automated extraction system Maxwell RSC Instrument (both by Promega Corporation, USA), following the manufacturer’s instructions. Mean DNA concentration for all 134 samples was 56.5 ± 45.5 ng/µl. Library preparation followed a customised version of the multiplexed shotgun genotyping (MSG) protocol (Andolfatto et al., 2011). The samples were divided into 3 plates (48 + 48 + 38), each subjected to the following protocol separately. The same 48 barcode adaptors were used in each plate.

We diluted the DNA samples to a standard 5 ng/µl concentration (50 ng of DNA per each 10 µl sample), added the restriction enzyme *MseI* and incubated them for 3 hours at 37°C in a TurboCycler Thermal Cycler (Blue-Ray Biotech Corporation, Taiwan). To inactivate the enzyme, we incubated the samples at 65°C for 20 minutes. Next, we ligated unique six-base pair barcode adaptors to the samples in the TurboCycler at 16°C for 3 hours and deactivated the ligation reaction by incubating at 65°C for 10 minutes. We pooled DNA fragments from all samples in the plate together and bound them to magnetic purification beads. We captured the beads with a magnet and washed away the rest of the sample. After several washes, we detached the DNA fragments from the beads and resuspended them in a buffer. Size selection of DNA fragments between 300 and 450 base pairs was performed automatically using Pippin Prep (Sage Science, USA). They were amplified with Phusion High Fidelity PCR Kit (New England Biolabs, USA) and again purified with magnetic beads, following the Agencourt AMPure (Beckman Coulter, USA) purification protocol. Resulting multiplexed samples were sent for sequencing on an Illumina platform (at Genomics Co., Taiwan).

Bioinformatic analyses

Note that all bioinformatic analyses presented here were preliminary. Repeated analyses and further data exploration with alternative statistical approaches are in progress and will be included in upcoming publications of this work.

We assembled the resulting sequences using Stacks 2 v. 2.54 (Rochette et al., 2019). For both species, the reads were mapped against a reference genome of the related species *Trichonephila clavipes* (GenBank accession code: GCA_019973935.1), and single nucleotide polymorphisms (SNPs) were called following the *ref_map.pl* pipeline. Different filtering parameters specifying the number of SNPs per locus and the completeness of representation of loci across individuals were tested to identify the optimal balance between SNP number and locus representation. A 35% minimum locus representation across individuals was chosen as optimal in *N. pilipes*, resulting in 3049 informative SNPs when using single SNPs per catalogue locus and 29,512 when using multiple SNPs per catalogue locus. In *T. clavata*, a 50% minimum locus representation was chosen, resulting in 5753 informative SNPs when using single SNPs per catalogue locus and 33,928 when using multiple SNPs per catalogue locus.

Although RADseq datasets with very high degrees of missing data have been shown to reliably resolve phylogenetic relationships (Tripp et al., 2017), we excluded samples with >90% of missing data from subsequent bioinformatic analyses following their poor performance in initial runs. This included three samples of *T. clavata* and 14 samples of *N. pilipes*, among them one melanic sample (EEG760). Two *N. pilipes* populations were thusly excluded, from West Bengal, India (India_4; 3 samples) and Gunung Senyum, Malaysia (Malaysia_2; 1 sample).

To produce phylogenetic trees, we ran BEAST2 (Bouckaert et al., 2014) on the multiple SNP per locus matrix of each species to include as much genetic information as was available. We used the general time reversible (GTR) substitution model and employed an uncorrelated relaxed clock with a log normal distribution. A Yule (pure birth) speciation model was used as a tree prior. The analyses ran for 10^9 generations on four MCMC chains.

Genetic population structure was inferred from SNPs using STRUCTURE v. 2.3.4 (Pritchard et al., 2000). STRUCTURE clusters multilocus genotype data, such as SNPs, to infer genetic structure and assign each individual to a population using Bayesian statistics. A 'population' in this sense is characterised by a specific set of allele frequencies at specific loci, similar to a haplotype. The number of such populations in a dataset is typically unknown, so several are tested and compared for goodness of fit. Each individual is assigned the probability it originates in one or several populations, if its genotype suggests admixture. When samples are collected at various geographical locations, the analysis can take this information into account as location priors. It assumes a higher likelihood that the sample originates in the area of collection, and a lower likelihood that it is an immigrant from elsewhere (Pritchard et al., 2000).

For each species, STRUCTURE first ran for 100,000 generations with a 10% burnin on the single SNP per catalogue locus matrix of each species. Admixture was allowed and population IDs were used as sampling location priors (LOCPRIOR) to increase clustering accuracy. Correlated allele frequencies were assumed. The initial run tested the probability of 1 to 10 populations (K), with 10 iterations for each K. The results were summarised with STRUCTURE HARVESTER (Earl and VonHoldt, 2012), a web programme that assesses and visualises likelihoods for each tested K and identifies the best K for the data using the Evanno method (Evanno et al., 2005). STRUCTURE determines the best K by comparing posterior probability estimates for each K, however, Evanno et al. (2005) argued that this method does not always correctly identify the true K. Instead, they suggested the use of ΔK, a statistic reflecting the difference in log probability of two successive K values, found to show a clear peak at the true value of K (Evanno et al., 2005). To correct for stochastic effects, related to replicated analyses of the same dataset, membership coefficient (Q) matrices of all iterations of the same K were summarized with CLUMPP v. 1.1.2 (Jakobsson and Rosenberg, 2007).

2.2.1.3 Results

Ultrametric tree for *T. clavata* inferred resolved phylogenetic relationships among specimens, with high support in almost all phylogenetic nodes (see Annex B1).

Unexpectedly, specimens from the same population exhibited low levels of relatedness. Only one sampled population formed a monophyletic group, that from Xishuangbanna, China (Yunnan_2), while the remaining populations were polyphyletic (Figure 5). All but one Taiwanese sample grouped together, with a sister specimen from Amami, a Japanese island at the north of the Ryukyu island chain. Chinese samples from Hunan, Fujian and Yunnan were mostly intermixed, forming no obvious phylogenetic patterns.

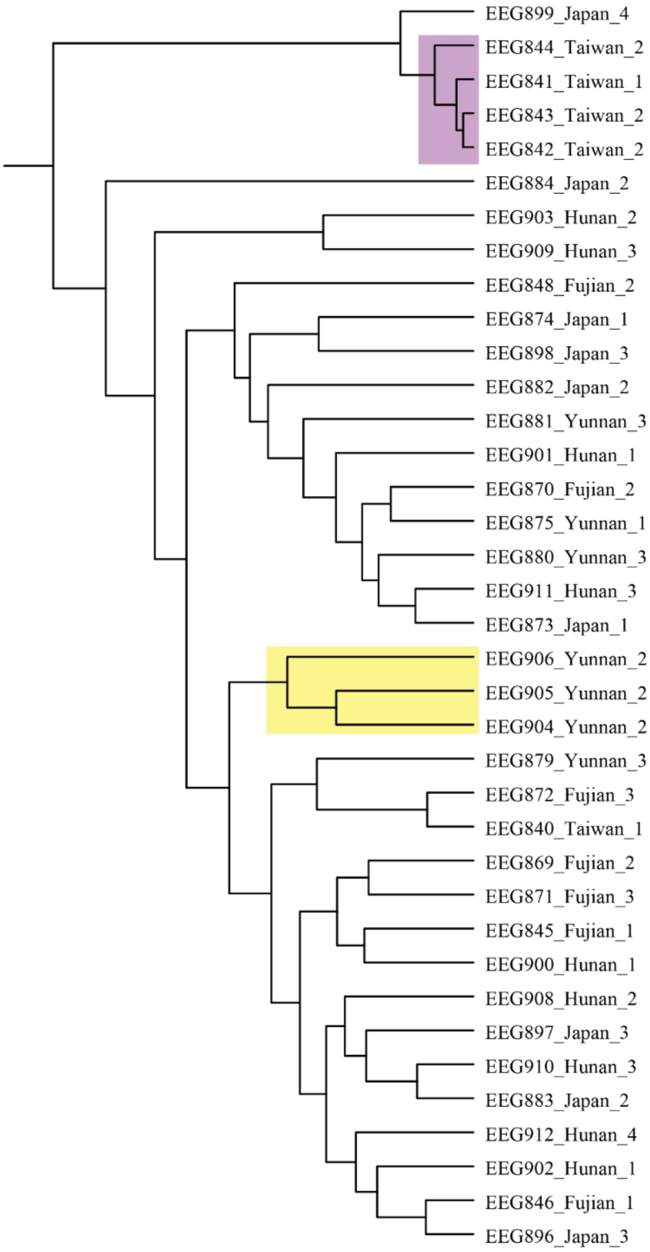


Figure 5: **Ultrametric phylogeny of the included *Trichonephila clavata* samples.** Coloured areas denote cases where all samples from the same population or more than two samples from the same geographical region, but not necessarily the same population, formed a clade.

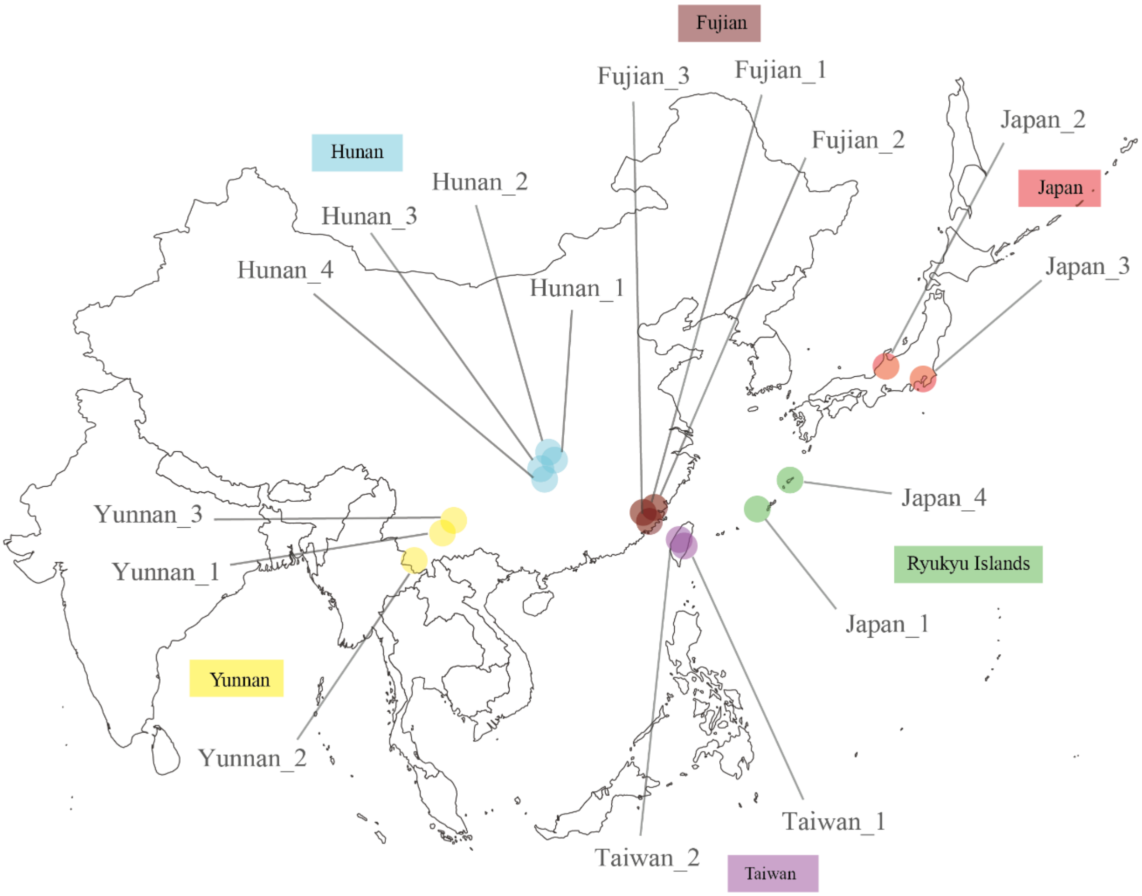


Figure 6: **Collection sites of the included *Trichonephila clavata* populations.** Population names correspond to those in Figure 5. Genetic structure of each population is not shown as it proved homogenous across all samples.

STRUCTURE HARVESTER identified K=2 (ΔK=120,6) as the optimal number of genetically homogenous clusters (subpopulations) for the included specimens of *T. clavata* (see Annex C for ΔK plot). However, cluster I was represented in all included specimens in at least 90,4%, showing strong predominance over cluster II. Due to the very low representation of cluster II and the fact that the Evanno method cannot evaluate ΔK for K=1 (Evanno et al., 2005), we treated *T. clavata* as a single cluster species. No association between geography and genetic structure could therefore be inferred either (Figure 6 thus only features population collection sites).

As opposed to *T. clavata*, most nodes in the *N. pilipes* population phylogeny received relatively low support (see Annex B2). Here, too, populations were mostly intermixed, with some localities, notably the Philippines, exhibiting loose phylogenetic grouping (Figure 7). The basalmost split in the tree (Clade 1) divided four samples from the Ryukyu islands from all remaining samples. The second split (Clade 2) branched off one sample from Sulawesi, Indonesia; note that the second sample from Sulawesi was positioned in the more distal part of the tree, exhibiting similar genetic structure. At the next proximal node, all remaining samples were divided into two larger clades (Clades 3 and 4). In just one case all samples from the same population formed a clade, those from Taroko National Park in Taiwan (Taiwan_2). Philippine samples formed a larger clade, with samples originating from various populations throughout the archipelago, and a smaller clade with samples originating from two central islands and Palawan. Four of the seven Indian samples grouped together, relatively far from the only sub-Himalayan sample from Assam. All other geographic areas showed even clearer polyphyletic patterns, with very little or no phylogenetic aggregation. Melanic females were likewise spread throughout the phylogeny (Figure 7), refuting "*N. kuhli*" as a separate species.

For *N. pilipes*, K=4 (ΔK=120,4) was identified as the optimal number of clusters (see Annex C for ΔK plot). Clusters I (blue; Figures 7 and 8) and II (orange) were widespread, however, when comparing cluster components to the phylogenetic tree, cluster I was prevalent in Clade 4, while cluster II was prevalent in Clade 3. Clusters III (grey) and IV (yellow) were less abundant, but were predominant in the basal-most Clade 1 from the Ryukyu islands. Cluster IV was also relatively common in the large Philippine clade, while cluster III was scattered around the phylogeny, mostly in Philippine, Indian and Ryukyu island samples. Melanic females showed no similarities in cluster membership (Figure 7). Dividing samples by geographical region and plotting average cluster memberships per population again failed to reveal a clear pattern of geographical aggregation of the four detected clusters (Figure 8). Broadly speaking, clusters I and II seemed more prevalent among mainland populations, while clusters III and IV were more frequent in island populations. This observation had some exceptions, for example the sub-Himalayan population exhibited an almost exclusive representation of cluster III and Australian population had both clusters III and IV in limited proportions.

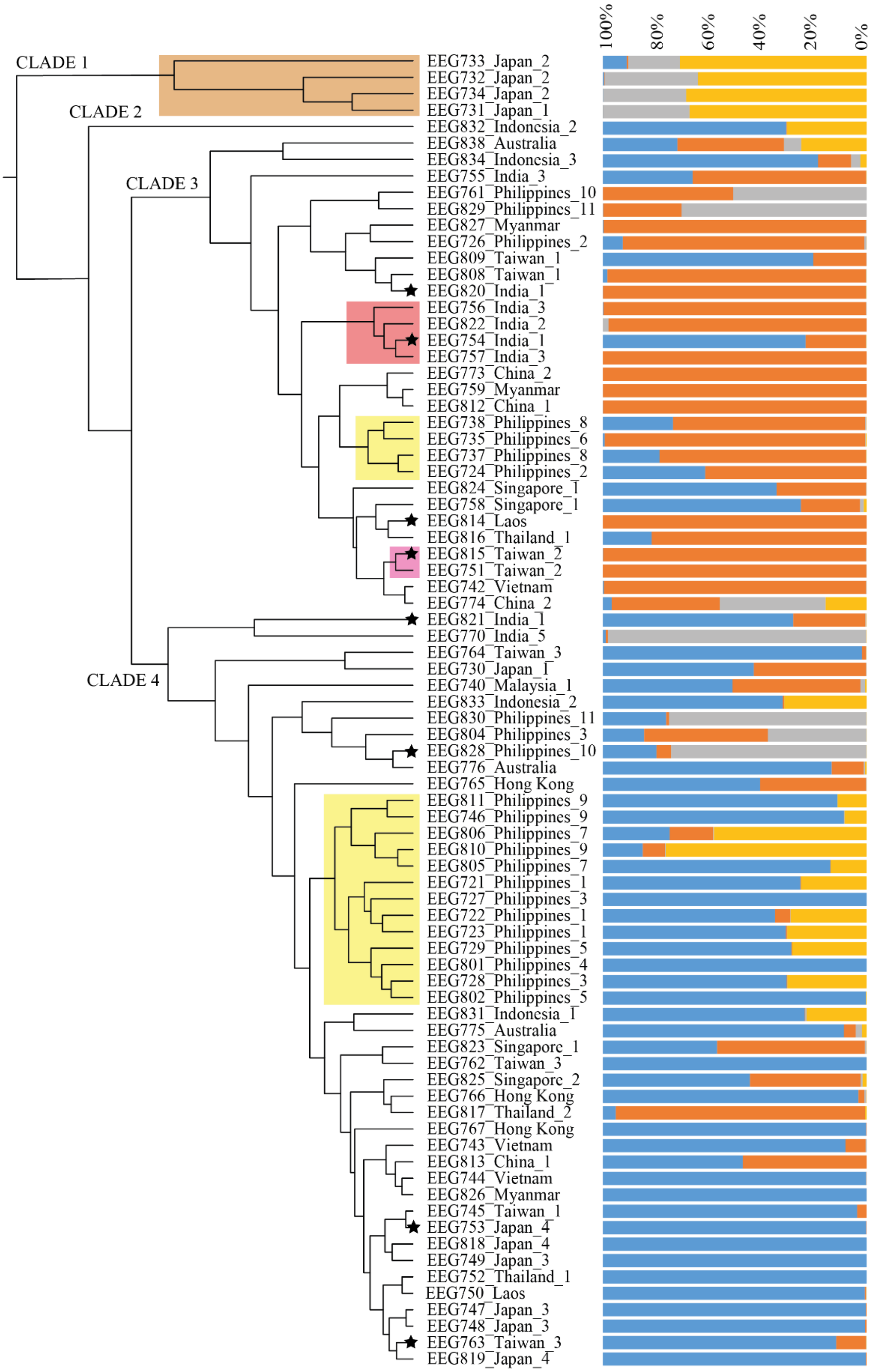
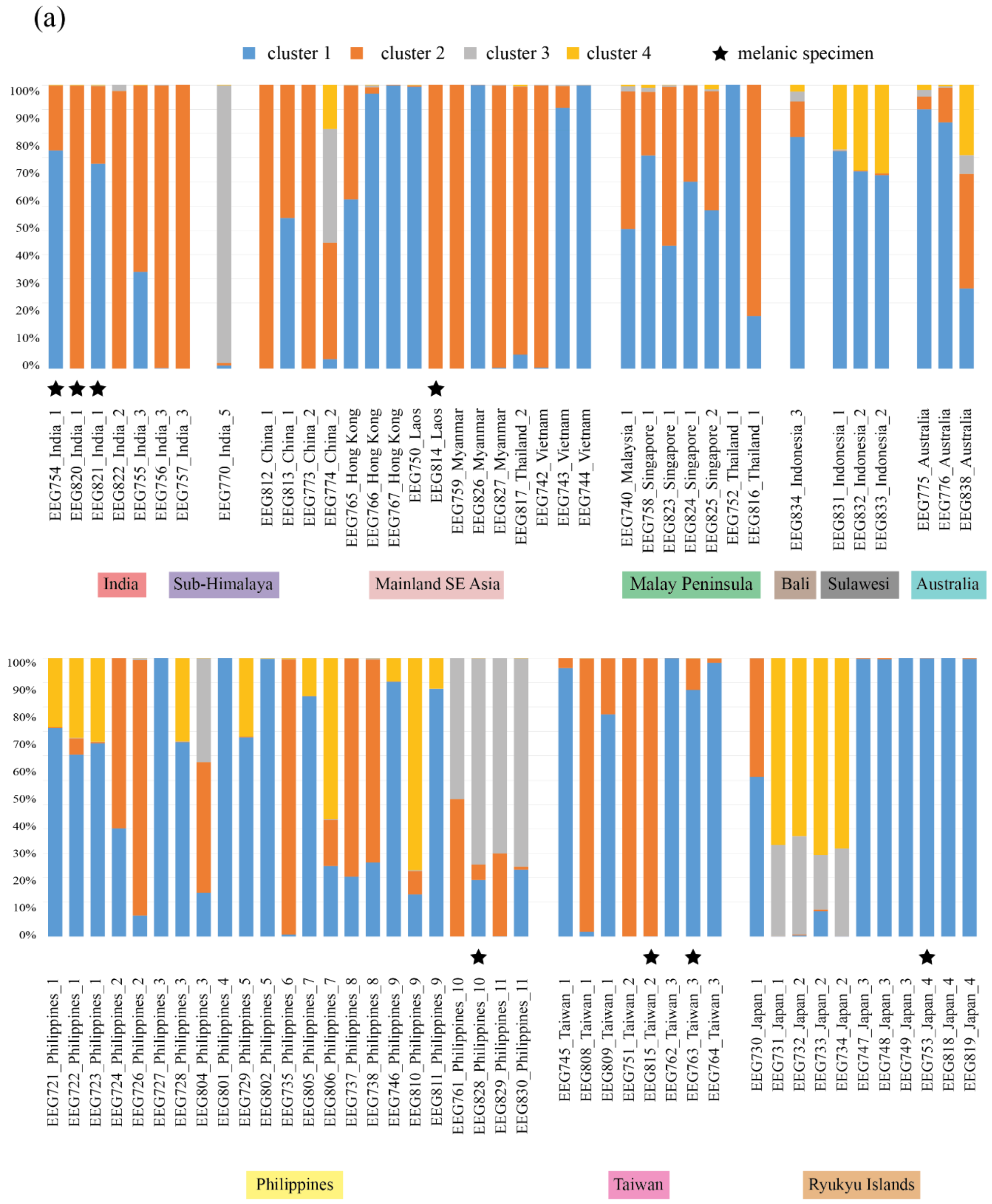


Figure 7: **Ultrametric phylogeny and STRUCTURE results in *Nephila pilipes*.** Coloured areas in the phylogeny denote cases where all samples from the same population or more than three samples from the same region, but not necessarily the same population, formed a clade. Coloured bars reflect cluster membership of each individual and correspond to those in Figure 8. Black stars denote melanic samples.



Continuation

Continuation of Figure 8

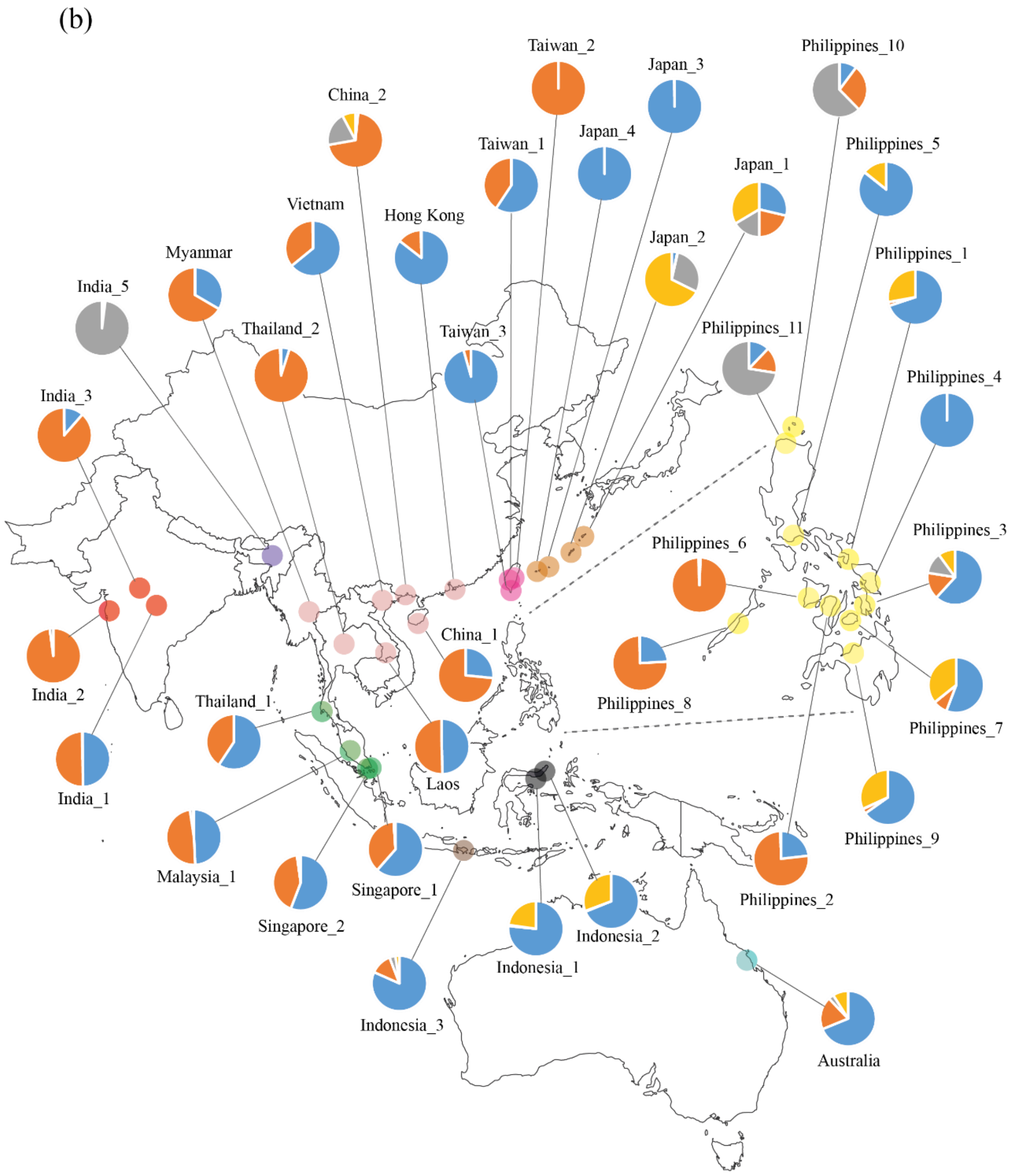


Figure 8: **Best-fit STRUCTURE result (K=4) in *Nephila pilipes*.** (a) Colour-coded bars show cluster membership of each individual. (b) Pie charts reflect the average cluster membership of all individuals in a population. Cluster colour-codes and locality colours match those in panel (a). The Philippine archipelago is enlarged for clarity.

2.2.1.4 Discussion

In this chapter, we inferred population genetic structure of two species of golden orbweavers in order to unravel potential causes for within-species genetics structure and between-species differences, if detected. We hypothesised factors such as past climate and geology would be most influential on haplotype diversity and distribution within species, while we expected to attribute between-species differences to discrepancies in range size and climate. Additionally, we aimed to examine whether genetic structure has a geographic component – in other words, whether some parts of the species range exhibit different haplotype compositions than others.

Surprisingly, both species exhibited a large degree of unrelatedness among specimens within a population. This was apparent both in the phylogenetic trees and in the STRUCTURE output. Only one population per species formed a clade (albeit several nodes in *N. pilipes* were poorly supported), suggesting lively gene flow occurs among populations in both species. Considering ballooning behaviour is regularly practiced by *N. pilipes* juveniles (Lee et al., 2015), this result is not entirely unexpected, however, it provides an indication of surprisingly effective gene flow maintenance across the included range, spanning almost 10,000 km. On the other hand, dispersal behaviour is not well documented in *T. clavata*. Our results thus provide support for the assumption that, at least among the included populations, ballooning is practiced just as regularly in the Joro spider as in the giant orbweaver. Within the included range, geographic barriers, such as mountain ranges and bodies of water, do not seem to present a barrier for gene flow in either species, as previously indicated by Lee et al. (2004). *Trichonephila clavata* does, however, also inhabit mountain valleys in the Himalayas. The magnitude of mountain barriers there may have a more pronounced impact on genetic structure by limiting contact between populations, but as our sample did not include Himalayan individuals, this remains to be tested.

Unexpectedly, STRUCTURE revealed almost complete genetic homogeneity in *T. clavata*. As one of the two inferred clusters exhibited overwhelming dominance over the other (in addition to methodological limitations as explained in the Results), the rarer cluster was dismissed altogether and, at least for our sample, *T. clavata* treated as a genetically homogenous (single-cluster) species. The implied high level of mobility and gene flow maintenance across large distances was somewhat surprising, as we expected environment seasonality and complex geography to induce some degree of genetic structure in this species. These results were, however, in line with previous findings on the absence of genetic structure in *T. clavata* by Jung et al. (2006).

In *N. pilipes*, four distinct clusters were inferred. Clusters I and II were most prevalent and showed rough phylogenetic clustering in clades 4 and 3, respectively. Within each of these clades, samples were scattered throughout species range, showing limited geographic association. Due to their phylogenetic ubiquity, we hypothesised these two haplotypes are old in origin, and have admixed relatively recently in the species' evolutionary history (but, see below). They were nearly absent, however, from the basal-most clade in the phylogeny, constituting of four samples from the Japanese Ryukyu islands of Amami (Japan_1) and Okinawa (Japan_2). These samples were inferred as admixed between clusters III and IV, comparatively rare in the rest of the phylogeny. The remaining Amami sample and all six samples from the Ryukyu islands of Irabu (Japan_3) and Iriomote (Japan_4) belonged predominantly to cluster I.

Ryukyu islands presumably drifted away from the East Asian mainland about 1.5 mya (Osozawa et al., 2012) and have since then acted as the margin of the species range. Su et al. (2007) proposed that the retreat of rainforests during Quaternary glacial periods had a profound effect on population dynamics in this species, isolating populations at range margins within rainforest refugia and thus generating genetic structure. During interglacial periods, some of these populations expanded their range, giving rise to admixed populations, while others remained narrowly distributed due to specific climatic and geological conditions. On the SE Asian mainland, in contrast, rainforests are thought to have been continuously present at least along rivers, allowing for constant admixture of populations and thus an unstructured genetic pattern (Su et al., 2007). This rainforest refugia hypothesis was compatible with our results to some degree. Populations from continental Asia belonged mostly to clusters I and II. If, as hypothesised previously, these haplotypes are old in origin, they might have evolved in two distinct mainland SE Asian populations with limited or no gene flow during glacial periods. Later, perhaps during interglacial periods, each of them dispersed, resulting in extant wide distributions of both haplotypes, and simultaneously admixed. If the haplotypes are indeed old, however, it is curious admixture has not been stronger, considering the overlap of the haplotypes' extant ranges.

On the other hand, clusters III and IV were distributed relatively marginally, mainly in the northern (cluster III) and eastern (cluster IV) parts of the included range. Cluster III was mainly inferred in populations from the Philippines, northern Ryukyu islands, Guangxi and sub-Himalayan India, while cluster IV was mainly inferred in Philippine, Sulawesi and northern Ryukyu populations. Elsewhere, these clusters were only recovered in trace proportions. Many of these marginal areas are islands, of which some have not been land-connected to the continent throughout the species' evolutionary history. We can hypothesise these haplotypes emerged in isolated populations at range periphery and were later introduced into other populations via long-distance dispersal. Alternatively, following the basal position of clade 1, clusters III and IV could be ancestral. The reduction of forests and with it population sizes during glacial periods might have caused a bottleneck effect, resulting in the prevalence of these haplotypes in marginal, newly isolated areas. A bottleneck effect of glaciation events has, indeed, been previously inferred in spiders (e.g. Magalhaes et al., 2014; Bidegaray-Batista et al., 2016; Postiglioni et al., 2019). Interestingly, Taiwanese samples lacked clusters III and IV, despite Taiwan's position between Ryukyu and Philippine islands, characterized by high proportions of said clusters. A larger Taiwanese sample might reveal their presence in Taiwan as well.

Melanic specimens of *N. pilipes* did not exhibit phylogenetic relatedness, clearly refuting the monophyly of these females and, with it, the hypothesis that they represent a separate species. This conclusion is corroborated by the invariable morphology of males, found with both female morphs (own observations). Additionally, melanic females did not show uniformity in genetic structure. Dark colouration was thus not linked to any of the inferred haplotypes, but may rather develop in response to certain environmental factors, perhaps predator presence as proposed by Tso et al. (2002).

As expected, we recovered between-species differences in genetic structure, but found *T. clavata* to be, by far, the less genetically structured species. Temperate climates do not seem to influence genetic composition, neither through local adaptations nor limitations related to short favourable seasons. A lack of effect of environment seasonality on population structure was also found in other arthropods, e.g. eusocial paper wasps (Lengronne et al., 2012). Aggregation into loose colonies does not seem to affect genetic structure either. Seeing as individuals collected at the same locality almost never grouped as sister taxa, Joro spiders do not seem to mate predominantly within their colonies.

We also proposed range size could play a role in the discrepancy in genetic structure between the species. *Nephila pilipes* did indeed show finer structure than *T. clavata,* but range size itself did not seem to be as important as past range fragmentation. Range size, but not life-history traits, has also been shown as a predictor of genetic diversity in other organisms (Duminil et al., 2007). Isolation by distance, i.e. an increase in genetic divergence with geographic distance due to decreasing frequency of dispersal, is another frequently studied factor influencing genetic structure (Frantz et al., 2009); however, as already hypothesised in previous studies (Jung et al., 2006; Su et al., 2007), it does not seem to occur in the two studied species.

All this considered, we propose species age to be the simplest underlying factor determining the degree of genetic structure. The ages of *N. pilipes* and *T. clavata* were estimated to approximately 26 and 14 million years, respectively (Turk et al., 2020). Here presented species phylogenies lacked time calibration, impeding speculation on whether the proposed climate-induced genetic isolation events occurred during the Quaternary climatic oscillations or during earlier climatic processes. Regardless, we propose greater haplotype divergence in *N. pilipes* than in *T. clavata* can ultimately be attributed to its older age, allowing more time for haplotype accumulation by subjection to more structure-inducing factors.

The inferred gene flow maintenance also implied hindered speciation in these lineages. If the African sister species to *N. pilipes*, *N. constricta*, shares similarly high levels of mobility, this would shed some light onto the small extant diversity of the genus (along with potentially elevated past extinction rates, inferred in Chapter 2). On the other hand, *Trichonephila* is a relatively species-rich genus, making gene flow maintenance across large distances surprising also from this aspect.

As previously stated, the analyses included in this chapter will be revisited and supplemented by alternative methodological approaches (e.g. ADMIXTURE, sPCA) in future work. Additionally, an important factor to note is that although we accumulated the largest dataset of genetic markers in these two species to date, and effort was made to maximise sample size and whole-range representation, our sample is still modest when considering the vast natural ranges and population sizes of both species. Sampling strategy and sample size have been shown to strongly influence statistical inference with STRUCTURE (Lawson et al., 2018). In order for the natural haplotype composition to be accurately reflected in the sample, much more comprehensive sampling would be required. Moreover, collection locations were not random, but biased towards populated, accessible locations. Samples from certain parts of species ranges, for example Himalayan Joro spiders, are scarce or even absent from known collections, so field sampling should also be part of potential future sampling efforts.

2.2.1.5 References

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3 DISCUSSION AND CONCLUSIONS

3.1 DISCUSSION

We argue that for a holistic examination of an organism, the understanding of large-scale evolutionary processes is important. This, in turn, cannot be achieved without combining evolutionary biology with other fields of natural science, notably geography, geology, climatology, palaeontology, ecology, genetics and statistics. In this thesis, we aimed to combine currently available information from these fields to shed new light on the evolution of golden orbweaver spiders. Their conspicuousness makes them an interesting organism not only for arachnologists, but also for the lay public. As a phylogenomic study has recently produced a well-sampled, robust phylogeny of the family (Kuntner et al., 2019), we built on it to investigate biogeographic history of specific genera and the family as a whole, tested potential drivers of their diversification and explored genetic patterns at the species level.

Historical biogeography at different taxonomic levels was the topic of the first and third chapters of the thesis. Chapter 1 reconstructed a dynamic history of range shifts within the family and, in the largest scale, corroborated the hypothesis that West Burma block amber contains Gondwanan biota (Poinar, 2019), and that these inclusions must have been transported to its present-day location by tectonic processes. Topically related was the third chapter, reconstructing biogeography in a single nephilid genus, coin spiders (*Herennia*). It uncovered a wide ancestral distribution of the genus and a natural colonization of the widespread *H. multipuncta* throughout Asia. The reconstruction model assuming ballooning dispersal in the genus provided more parsimonious results than the "walking" model, however, many extant species of *Herennia* are single-island endemics. We proposed that coin spiders have lost the ability to balloon, but that *H. multipuncta* must have regained it. Perhaps more in line with our results, however, is the explanation that dispersal was selected against only *after* island colonization. Upon reaching a remote island, further dispersal tends to be unsuccessful. Selection therefore starts favouring individuals with lowered dispersal propensity, which remain in the island habitat (Gillespie et al., 2012). Organisms with non-directional dispersal, such as ballooning spiders, are especially prone to this phenomenon, as they are unable to intentionally return to their original population (Gillespie et al., 2012).

Although our results refute human-induced dispersal of *H. multipuncta*, other potential cases of such dispersal exist within the family. For example, *Trichonephila clavata* has recently been introduced from Asia to North America (Hoebeke et al., 2015), presumably with transport from China. Trans-oceanic dispersal by ships could be assumed in yet another golden orbweaver, *Nephilingis cruentata*. It inhabits sub-Saharan Africa and southern Brazil and often lives synanthropically, on buildings or other man-made objects, including ships (Kuntner, 2007). Since the route from West Africa to Brazil was once heavily used by slave ships, it seems very plausible that *N. cruentata* crossed the Atlantic in this manner and successfully colonized the Brazilian coast. This hypothesis is yet to be tested with molecular tools.

One of the main achievements of the two biogeographical studies was our original improvement of the established methodological pipeline in biogeographical reconstructions. While time stratification of dispersal probabilities is now relatively commonplace, it is often arbitrary (e.g. Weaver et al., 2016; Eberle et al., 2017; Chamberland et al., 2018). The manner in which time stratification is performed should be optimised to include factors, important for the evolution of the specific studied organism. Some spiders, for example the primitively segmented members of the family Liphistiidae, are sedentary, confined to burrows throughout their life. As they are poor dispersers, formation of a new river can present a strong limiting factor to dispersal, sometimes cutting gene flow off completely (Xu et al., 2018). In this case, time-stratification of a biogeographic analysis would be based on river formation time estimates, while long-distance dispersal would not be assumed as these spiders do not balloon.

Compared to members of Liphistiidae, orbweavers can be regarded as excellent dispersers. But ballooning in itself is passive, with the spider unable to influence the direction or distance travelled. Consequently, the changing configuration of tectonic plates (and with it wind currents) through millions of years must have strongly influenced dispersal success. At a large scale, such as in Chapter 1, a direct conversion of physical distances separating biogeographical areas into dispersal probability estimates is suitable. At smaller scales, such as in Chapter 3, however, geological reconstructions are sometimes not accurate enough to allow direct transformation of measured distances into dispersal probabilities. This is another example of how biogeographic reconstructions can be modified to account for as much available information as possible.

Integration of palaeotectonics and palaeoclimate should also be considered during result interpretation. Some dispersal scenarios, suggested by the family-level reconstruction in Chapter 1, seem extremely improbable when applying them to the present-day landmass configuration. For example, in the genus *Trichonephila*, dispersal across 6,500 kilometres between Australia and South America was implied at 50 mya. However, taking into account this was the time of the Paleocene-Eocene Thermal Maximum, when the Earth's poles were not covered in ice, this shift seems plausible or even probable, given the suspected frequency of dispersal of various animal clades through this Antarctic corridor (e.g. Woodburne and Case, 1996; Sanmartín and Ronquist, 2004; Almeida et al., 2012; Duellman et al., 2016).

For all our biogeographical analyses, we used RASP (Reconstruct Ancestral State in Phylogenies) software (Yu et al., 2015), implementing the widely used R package BioGeoBEARS (Matzke, 2013). Historical biogeography can be inferred using a variety of statistical models, differing in the array of allowed cladogenetic processes (i.e. types of sympatry and vicariance). Reconstruction at shallow nodes is usually relatively uniform regardless of the model used, but at deeper nodes, results can be widely discrepant among models. Biogeographical literature brought forth much debate regarding model suitability for different biogeographical questions, with an emerging consensus to subject the data to a variety of models and then select the best through a model fit score (Yu et al., 2015). RASP implements this approach by fitting the data to tree biogeographical models, each in two variants – with or without allowing for "jump dispersal", in this context signifying founder-event speciation (Magalhaes et al., 2021). It identifies the model that explains the data best based on calculated weighed supports for each model. Therefore, we deemed this methodology as suitable for our studies.

Methodological challenges have also characterized the advancement of macroevolutionary research. Studies of large-scale evolutionary processes have long been impeded by poorly reconstructed phylogenies lacking branch lengths and scarce fossil records, however, the advance of molecular phylogenetics facilitated new approaches (Ricklefs, 2007; Morlon, 2014). Among the most popular methods for macroevolutionary rate modelling are BAMM (Bayesian Analysis of Macroevolutionary Mixtures) (Rabosky, 2014), MEDUSA (Modelling Evolutionary Diversification Using Stepwise Akaike Information Criterion) (Alfaro et al., 2009), and SSE-class models, such as BiSSE (Binary-State Speciation and Extinction) (Maddison et al., 2007). These methods differ in the specific aim of the analysis and the type of implemented statistical approach. BAMM and SSE-class models have been used in macroevolutionary studies in spiders at various taxonomic levels (Liu et al., 2016; Soto et al., 2017; Eberle et al., 2018; F. Li and Li, 2018; Fernández et al., 2018; Shao and Li, 2018), but never on nephilids specifically.

Chapter 2 of this thesis filled this knowledge gap by examining the dynamics of speciation and extinction and tested whether they relate to any of the selected organismal and environmental traits. Surprisingly, none of the tested traits exhibited an association with diversification, which was especially unexpected for dispersal propensity. The intermediate dispersal model, which predicts peak diversity in intermediate dispersers, has been found at large scales, e.g. across terrestrial and freshwater organisms of the west Indian Ocean (Agnarsson et al., 2014), but we failed to recover it in nephilids.

Recently, Diaz et al. (2019) suggested that speciation and extinction are in fact not dependent on any specific trait, thus far tested in clades across the tree of life, but rather follow a universal pattern of interchanging periods of concentrated speciation and extinction events, and evolutionary stasis. Thus, they suggested clade age is the sole determinant of diversification, with elevated rates in younger clades. The authors showed that this time scaling is not a statistical artefact, but an authentic property of the tree of life (Diaz et al., 2019). This provided some context for future explorations of macroevolutionary processes, suggesting that regardless of factors driving diversification in the short-run (e.g. intermediate dispersal propensity), rate trends ultimately cannot deviate from a universal dynamic of diversification.

Lastly, Chapter 4 explored genetic variability in two golden orbweaver species, *Nephila pilipes* and *Trichonephila clavata*. A lack of clear association between genetic structure and geography, inferred in both species, is typically found in highly dispersive taxa, such as birds (e.g. Morinha et al., 2017). Especially in *N. pilipes*, we speculated severe restrictions to gene flow must have existed in the past for genetic divergence to occur in such effective dispersers. In accordance with Su et al. (2007), we proposed that Quaternary climatic oscillations, inducing interchanging retreats and expansions of tropical habitat, catalysed genetic divergence and admixture of populations. While this species was previously recognized as an efficient ballooner (Lee et al., 2015), successful cross-range gene flow maintenance was unexpected in *T. clavata*. Considering the low dispersal propensities of coin spiders, discussed in Chapter 3, such large discrepancies in dispersal tendencies within a spider family as small as golden orbweavers is intriguing and has not been formerly recognized.

Neither analysis in this chapter found support for the existence of a separate, melanic giant orbweaver species, "*N. kuhli*". Tso et al. (2002) found that melanic females reflect significantly less ultraviolet light than common-form females, attracting less prey. They suggested this decreased foraging success exists in a trade-off with decreased visibility to predators (Tso et al., 2002). We expand this hypothesis by proposing that it may also be more energetically consuming to develop and maintain the common form than the melanic form. Two alternative rationales could then be given. One, well-fed juvenile females develop the common colouration, while starved juveniles cannot afford it energetically and thus grow dark in colour. Two, starvation causes juvenile females to develop the common colouration to increase foraging success in adulthood, while for well-fed juveniles, common colouration is not worth the energetic input. Any of these hypotheses would also be compatible with the omnipresence of melanic females throughout the species range and their inferred unrelatedness. Future work could easily test these hypotheses in a laboratory setting.

A common feature of all studies, presented in this dissertation, is the complexity of factors that influence evolutionary and biogeographic processes. Natural systems are characterized by incredibly intertwined and constantly changing biotic and abiotic factors that are difficult or impossible to capture in formal statistical analyses. As previously emphasized, interdisciplinary approaches are indispensable in all aspects of evolutionary biology. In future studies, effort should be made to maximise the included amount of biologically relevant information.

3.2 CONCLUSIONS

Each part of this thesis examined a different aspect of the same broad topic, the evolutionary history of golden orbweavers. Morphology, behaviour, distribution, migration, speciation and extinction are intertwined, co-dependent factors in organismal evolution – so much so, that it is often impossible to study them in isolation. Each aspect provided a piece of information that contributed to and refined our interpretation of the results that followed. The main conclusions of the thesis are as follows.

1. Golden orbweavers likely originated on a Gondwanan landmass constituting modern Australasia and/or Indomalaya. Subsequently, some genera persisted in their ancestral areas for millions of years, while others colonized tropical and subtropical habitats around the world.
2. Heterogeneity in species richness among nephilid genera cannot be attributed to any of the tested organismal and environmental factors, including phenotype extremeness and dispersal propensity. The pattern might be best explained by a recently proposed ‘universal diversification law’, where the main predictor of diversification rate is clade age.
3. Within the genus *Herennia*, assuming ballooning as the main method of dispersal produced a more parsimonious biogeographic reconstruction than assuming “walking” dispersal, requiring fewer chance dispersals across large distances. The hypothesis that the wide range of the spotted coin spider, *Herennia multipuncta*, is anthropogenic in origin, was rejected.
4. Sampled populations of the giant golden orbweaver, *Nephila pilipes*, show a degree of genetic structure, while populations of the Joro spider, *Trichonephila clavata*, do not. Neither species exhibited a clear association between genetic structure and geography. Unexpectedly, both species seem to maintain strong gene flow across their respective ranges, inhibiting genetic divergence.

4 SUMMARY

4.1 SUMMARY

In the first chapter, we reconstructed the geographical origin and sequence of intercontinental colonization of golden orbweavers, combining a robust phylogeny with geological (plate tectonic) data, specifics of organismal biology (dispersal behaviour) and probabilities of historic dispersal events. We posed two alternative hypotheses about their ancestral range. The basis of the first, termed ‘Out of Africa’, was the fact that Africa hosts the highest contemporary species diversity. This hypothesis predicted that the biogeographical realm Afrotropics would optimise as the most likely ancestral area of the spider family. The alternative, ‘Out of West Burma’ relies on the assumption that a Burmese amber fossil, *Geratonephila*, is indeed as stem nephilid. Because the West Burma block likely detached from the Australian plate and then travelled towards its present location either on its own or with India, this hypothesis predicted Australasia and/or Indomalaya as the ancestral area.

We constructed an original expanded phylogeny of nephilid spiders and inferred their global biogeographical history. To fine-tune the analysis, we used a novel method of evaluating varying dispersal probabilities among geographical areas through time. We measured physical distances between pairs of areas on a geological reconstruction model and used them as a proxy for dispersal probabilities, thus accounting for plate tectonics. The best supported biogeographical model revealed both Australasia and Indomalaya as the likeliest ancestral area of nephilids. Some species were revealed to persist in the same geographical region for the entire estimated 130-million-year evolutionary history. In others, however, many, sometimes dramatic, intercontinental dispersals were inferred, which aligned with the known nephilid dispersal biology. Regardless of the precise area of origin, nephilids are clearly Gondwanan. Our study thus corroborated the interpretations that Burmese amber contains Gondwanan biota.

In the second chapter, we explored macroevolutionary trends in the golden orbweaver phylogeny. Variation in species richness among comparable clades of organisms is the consequence of the interplay between two main macroevolutionary processes, speciation and extinction. These can in turn be influenced by intrinsic (organismal; phenotypic traits and their states) and extrinsic (environmental) factors. We evaluated potential drivers of diversity heterogeneity among nephilid genera, related to the organisms themselves and to their environment. We tested two binary extrinsic factors, tropical versus subtropical and island versus continental species distribution, and two continuous organismal factors for correlation with diversification. One was phenotypic extremeness, tested through female and male body length separately, as well as their ratio, the sexual size dimorphism index. The second continuous tested trait was dispersal propensity, quantified using the species' respective range sizes as a proxy. Following the theory of the intermediate dispersal model, we predicted a bell-shaped relationship between continuous trait values and speciation, with intermediate phenotypes exhibiting highest diversification rates.

We detected heterogeneity in speciation and extinction among nephilid genera using all employed methods. Accelerated speciation was detected in the genus *Herennia*, defined by pronounced island endemism, while extinction was heightened in the extremely sexually size dimorphic *Nephila*. None of the trait-diversification correlation analyses showed statistically significant results, the only exception being a marginal support for higher speciation rates in tropical habitats. We thus failed to find support for our prediction that intermediate phenotypes relate to heightened speciation rates, further suggesting that phenotypic extremeness and dispersal propensity do not explain diversification dynamics in golden orbweavers. While the small size of the used phylogeny probably posed certain methodological limitations, we concluded that the examined factors play little to no role in speciation and extinction in this spider family. We speculated that a recently presented hypothesis of universal diversification dynamics may be the most parsimonious explanation of the inferred macroevolutionary patterns.

In the third chapter, we returned to biogeography, this time inferring the geographical origin and subsequent dispersal in coin spiders (Asian and Australasian nephilid genus *Herennia*). Their dispersal biology is not well documented, while the studied geographical area is characterized by a complex geological past, further complicating biogeographic reconstruction. We inferred a species-level phylogeny using phylogenomic data and used it as scaffold for a population-level phylogeny, calculated using classical phylogenetic markers. As coin spider dispersal is not well understood, biogeographical inference employed a modified version of our novel methodology, introduced in Chapter 1. We designed two alternative biogeographical models, differing in the main assumed method of dispersal, all the while accounting for the complex geological past of the relevant geographical region. Model A assumed coin spiders readily balloon, promoting island colonization on one hand, and facilitating gene flow maintenance among populations at the other. Model B assumed short-distance stochastic "walking" during search for vacant habitat as the main method of dispersal, allowing for a passive spread across connected lands given enough time. Neither model excluded rare chance long-distance dispersal with wind currents.

Both models reconstructed a wide ancestral range of *Herennia*, encompassing Australia, mainland SE Asia, and the Philippines. Subsequent dispersal routes were fairly similar in both biogeographical models, with discrepancies within *H. multipuncta*. In model A, the ancestor of *H. multipuncta* inhabited mainland SE Asia, from where it consecutively colonized other regions, e.g. Sri Lanka, India and islands of the Indonesian archipelago. On the other hand, model B suggested Sulawesi as the ancestral region from where the species colonized most remaining regions. This scenario would call for two dispersal events over distances larger than 4000 km in just a few million years, making model A more parsimonious. As an additional conclusion of this study, the internal splits of *H. multipuncta* proved too old for its dispersal to be human-driven, thus implying a natural spread across Asia. We hypothesised that coin spiders' ancestor may have lost the ability to balloon, but that *H. multipuncta* regained it, thereby colonizing and maintaining gene flow across a large areal.

In the fourth chapter, we explored genetic structure in two golden orbweaver species, the giant golden orbweaver, *Nephila pilipes,* and the Joro spider, *Trichonephila clavata*. Taxa with high dispersal propensities have little difficulty maintaining gene flow among populations, even when they are far apart. Consequently, genetic differentiation of populations is inhibited and genetic structure homogenous. *Nephila pilipes* juveniles have been shown to regularly exhibiting ballooning behaviour, whereas ballooning frequency of *T. clavata* juveniles is not well documented. Previous studies have recovered genetic structure in both species, however, the employed molecular tools are now mostly outdated. We retested their findings with restriction site-associated DNA sequencing, targeting single nucleotide polymorphisms adjacent to restriction enzyme cut sites.

The study was largely explorative, however, we expected to uncover yet unknown patterns in genetic structure in both species and find them associated to spatial distribution within the included range. If differences in genetic structure between the species emerged, we hypothesised to explain them in the context of discrepant life history traits and environmental factors. Additionally, we tested the hypothesis that melanic *N. pilipes* females are not just a colour variant of the common form, but in fact belong to a separate species. While rare, melanic females, characterized by black abdomens and reddish legs, can be encountered throughout the species range. The adaptive value of this colouration remains unclear.

Same-population individuals in both species showed a surprisingly low degree of relatedness in phylogenetic analyses. Individuals collected at the same geographical location only seldom exhibited monophyly, suggesting gene flow among populations is not obstructed by mountain or water barriers in either species. Analyses with STRUCTURE recovered genetic homogeneity of the included *T. clavata* specimens, which was unexpected but provided some indication of frequent aerial dispersal and successful gene flow maintenance in this species. In *N. pilipes*, we inferred four genetically distinct clusters. Two of them were widespread throughout the phylogeny, each showing prevalence in one of the two major phylogenetic clades. Remaining two clusters were predominant in the basal-most clade from Ryukyu islands, but relatively rare in other parts of the phylogeny. We did not find a clear association between genetic structure and geography in either species.

We discussed these results in the light of palaeoclimatic and palaeogeologic changes during climatic oscillations in the last few million years. Our findings were in line with a previous hypothesis, suggesting that the changes in rainforest distribution during the Quaternary glacial and interglacial periods played a major role in shaping genetic diversity within *N. pilipes*. During glacial periods, rainforests retreated in size, forming habitat refugia at their margins. The newly isolated spider populations, hosted by these refugia, did not sustain gene flow with other populations, thus enabling genetic divergence. In turn, the expansion of rainforests during interglacial periods re-enabled genetic contact among populations and with it admixture.

Both our phylogenetic and genetic structure analyses failed to find support for the hypothesis that melanic specimens of *N. pilipes* constitute a separate species, "*N. kuhli*". Comparing the genetic structuring of both examined species, we concluded discrepancies in environment seasonality, range size and aggregation into colonies does not leave a significant mark on their respective genetic compositions. In turn, we proposed species age might be the simplest ultimate factor, predicting population genetic structure.

4.2 POVZETEK

Zlati mrežarji (družina Nephilidae) so tropski in subtropski pajki, znani po nenavadnih fenotipih. Med najzanimivejšimi je ekstremni spolni dimorfizem (Coddington in sod., 1997; Kuntner in Coddington, 2020). Pri eni od vrst, orjaškem mrežarju (*Nephila pilipes*), so lahko samice do petstokrat težje od samcev, kar je en najekstremnejših primerov velikostnega spolnega dimorfizma med vsemi kopenskimi živalmi (Kuntner in sod., 2012). Izrednih velikosti so lahko tudi asimetrične mreže, ki jih pletejo samice zlatih mrežarjev, kar imenujemo gigantizem mrež (Kuntner, 2017; Kuntner in sod., 2019).

Evolucijski odnosi med 37 trenutno priznanimi vrstami so dolgo veljali za problematične, predvsem zaradi kontroverzne klasifikacije na taksonomskem nivoju družine (Coddington, 1990; Hong-Chun in sod., 2004; Kuntner, 2006; Kuntner in sod., 2008, 2013, 2019; Blackledge in sod., 2009; Dimitrov in Hormiga, 2009; Dimitrov in sod., 2017). Prva študija, ki je izračunala globalno, časovno kalibrirano filogenijo na podlagi petih mitohondrijskih genov, treh jedrnih genov in morfoloških podatkov, je starost zlatih mrežarjev ocenila na 40 milijonov let (Kuntner in sod., 2013). Nedavno pa je druga študija na filogenomskih podatkih izračunala novo, bolje podprto filogenijo, kalibrirano z dvema fosiloma (Kuntner in sod., 2019). Izvor družine je pomaknila dlje v preteklost, 133 milijonov let nazaj v zgodnjo kredo (Kuntner in sod., 2019).

Zaradi svojih nenavadnih lastnosti so zlati mrežarji priljubljen model evolucijskih študij. A čeprav so njihova spolna biologija (Schneider in Elgar, 2005; Kasumovic in sod., 2007; Kuntner in sod., 2009; Kralj-Fišer in sod., 2011; D. Li in sod., 2012), spolni dimorfizem (Kuntner in sod., 2012; Kuntner in Elgar, 2014; Schneider in sod., 2015) in filogenetika (Scharff in Coddington, 1997; Hong-Chun in sod., 2004; Kuntner in sod., 2008, 2013, 2019) dobro raziskani, ostajajo drugi vidiki njihove biologije slabo poznani. Ta disertacija je podrobneje raziskala tri: biogeografsko zgodovino, vzroke za velike razlike v vrstni pestrosti med rodovi in genetsko strukturo na nivoju populacij.

V prvem poglavju smo raziskovali biogeografsko zgodovino zlatih mrežarjev. Zgodovinska biogeografija (ang. *historical biogeography*) je veda o spreminjanju razširjenosti določenega taksona skozi prostor in čas, in je nujna za celostno razumevanje njegove biologije. Zlati mrežarji so biogeografsko zanimivi predvsem zaradi starega domnevanega izvora v mezozoiku, današnje široke razširjenosti na večini kontinentov in domnevnih razlik v disperzijskem potencialu med rodovi. Danes so zlati mrežarji prisotni predvsem v tropskih in subtropskih regijah po vsem svetu, redkeje tudi v zmernih podnebjih (Su in sod., 2011). Vrstna pestrost je največja v tropski Afriki in Indomalaji, kjer so rodovi pogosto simpatrični. Nekatere vrste se aktivno razširjajo z zračnimi tokovi in na ta način premagujejo dolge razdalje, pogosto prečkajo tudi vodna telesa. Pri tem načinu disperzije, t. i. balonanju (ang. *ballooning*), mladi pajki na vetru izpostavljenih mestih v zrak spuščajo tanke svilene niti, ki jih ujamejo zračni tokovi in pajka odpihnejo (Bell in sod., 2005). Na ta način lahko vrste ohranjajo genski pretok na velike razdalje, na primer med karibskimi otoki in severnoameriško celino (Čandek in sod., 2020a). Nekatere vrste, na primer iz rodov *Herennia*, *Clitaetra* in *Nephilingis*, so razširjene ozko, morda prav zaradi odsotnosti balonanja.

Cilj prvega poglavja disertacije je bila rekonstrukcija biogeografske zgodovine in s tem najverjetnejšega geografskega izvora zlatih mrežarjev. O slednjem smo postavili dve hipotezi. Afriška hipoteza je predpostavljala, da se bo za najverjetnejši izvor družine pokazala tropska Afrika, saj je danes vrstna pestrost tam največja. Druga, burmanska hipoteza pa je slonela na predpostavki, da je fosilna vrsta *Geratonephila*, najdena v burmanskem jantarju, predstavnik izvornih zlatih mrežarjev. Burmanski jantar se nahaja na zahodnoburmanskem tektonskem fragmentu (ang. *West Burma Block*), ki se je odlomila od avstralske, nato pa v zgodnji kredi sama ali pritrjena na indijsko ploščo odpotovala do današnje lege v jugovzhodni Aziji (Poinar, 2019). Sledeč tej hipotezi bi se morala za najverjetnejši geografski izvor zlatih mrežarjev pokazati Avstralazija ali Indomalaja (ali obe).

Predpogoj za biogeografsko študijo je dobro podprta filogenija s čim večjo zastopanostjo taksonov. Najbolje podprto obstoječo filogenijo (Kuntner in sod., 2019) smo dodatno razširili s tremi neopisanimi vrstami rodu *Herennia*, kar je število vključenih vrst povečalo na 34 od 40 znanih vrst, t. j. 85%. Geografsko distribucijo vsake vrste smo kvantificirali glede na njeno prisotnost ali odsotnost v šestih biogeografskih regijah, ki so ustrezale uveljavljenim biogeografskim kraljestvom: Afrotropi (subsaharska Afrika in Arabski polotok), Avstralazija (Avstralija, Nova Zelandija in Nova Gvineja), Indomalaja (Indija in JV Azija), Nearktika (Severna Amerika), Neotropi (Srednja in Južna Amerika) in Palearktika (preostala Evrazija in severna Afrika). Kot sedmo biogeografsko regijo smo določili otoke zahodnega Indijskega oceana (Madagaskar, Reunion, Mavricij, Sejšeli in Komori) zaradi visoke stopnje endemizma zlatih mrežarjev na tem območju.

Biogeografsko zgodovino zlatih mrežarjev smo rekonstruirali s programom RASP (Reconstruct Ancestral States in Phylogenies; Yu in sod., 2015), ki implementira programski paket BioGeoBEARS (Matzke, 2013) v okolju »*R*«. Program dano filogenijo in podatke o današnji distribuciji analizira po šestih biogeografskih modelih, ki se razlikujejo po naboru dovoljenih kladogenetskih dogodkov. Analizo je mogoče z dodatnimi nastavitvami časovno razslojiti in za vsak sloj določiti poljubno verjetnost disperzije med vsakim parom biogeografskih regij. Za razliko od ostalih študij, kjer je način kvantifikacije teh verjetnosti pogosto arbitraren in grob, smo jih v naši študiji izračunali na podlagi dejanskih fizičnih razdalj med regijami tekom različnih geoloških obdobij. Na računalniškem rekonstrukcijskem modelu premikanja tektonskih plošč (Müller in sod., 2019) smo izmerili razdaljo med najbližjima točkama vsakega para regij vsakih 10 milijonov let, med 130 in 0 milijoni let nazaj. Končnih 294 meritev smo nato pretvorili v verjetnost disperzije (od 0 do 1) tako, da smo kratkim razdaljam pripisali visoko verjetnost disperzije in obratno. Če sta bili regiji v določenem časovnem sloju v fizičnem stiku, smo določili najvišjo, 95% verjetnost disperzije, saj je ta zelo verjetna, a ne nujna. Najnižjo, 5% verjetnost smo določili za razdalje nad 4000 km. To je približna razdalja med najodročnejšimi kraji, naseljenimi s pajki, in njim najbližjo celino (npr. med Havaji in Severno Ameriko). Med tema ekstremoma smo predpostavili padajočo linearno zvezo med razdaljo in verjetnostjo disperzije.

Model z najvišjo podporo je izvor zlatih mrežarjev umestil v Indomalajo in Avstralazijo, kar je podprlo našo burmansko hipotezo. Med rodovi so se pokazali različni poteki disperzije. Nekateri rodovi, na primer *Herennia*, vztrajajo na istih geografskih območjih že milijone let, medtem ko so drugi, na primer *Trichonephila*, območja pogosto zamenjali. Analiza je pokazala nekaj zanimivejših primerov disperzije prek velikih razdalj (ang. *long-distance dispersal*). Na primer, v rodu *Trichonephila* je bila v srednjem paleogenu rekonstruirana disperzija iz Avstralazije v Neotrope, torej v Južno Ameriko. V tem času je bila razdalja med tema kontinentoma približno 6500 km, a je bil to hkrati čas paleocensko-eocenskega termalnega maksimuma, ko so bili Zemljini poli kopni (Zachos in sod., 2001). Poleg tega se kanal med Južno Ameriko in Antarktiko še ni odprl (Livermore in sod., 2005), kar je dodatno olajšalo migracijo med Avstralazijo in Neotropi, rekonstruirano tudi pri drugih skupinah organizmov (Sanmartín in Ronquist, 2004).

Čeprav je ena od naših hipotez kot izvorno območje zlatih mrežarjev predpostavljala Afriko, se je ta pokazala kot ena zadnjih koloniziranih geografskih regij. Po najbolje podprtem modelu so jo zlati mrežarji osvojili šele pred približno 17 milijoni leti, čemur je sledila hitra diverzifikacija, morda zaradi novo razpoložljivih ekoloških niš.

Naši rezultati so podprli domnevo ene prejšnjih študij, da je bila diverzifikacija zlatih mrežarjev močno podvržena globalnim podnebnim spremembam v neogenu (Su in sod., 2011). Močno znižanje globalnih temperatur, akumulacija ledu na polih in znižanje morske gladine so nedvomno vplivali na diverziteto ekoloških niš, vetrne tokove in razporejenost habitatov, posledično pa na razporejenost organizmov. Naša študija je v biogeografsko analizo kot prva vključila verjetnosti disperzije, ki temeljijo na konkretnih meritvah fizičnih razdalj med geografskimi regijami skozi čas. S tem je poleg reševanja vprašanj o biogeografski zgodovini zlatih mrežarjev prispevala tudi k razvoju biogeografske metodologije.

Drugo poglavje disertacije je raziskovalo razloge za velike razlike v vrstni pestrosti med rodovi zlatih mrežarjev. Vrstna pestrost je rezultat prepletanja dveh osnovnih makroevolucijskih procesov, speciacije in izumiranja, ki pa sta sama posledica prepletanja zunanjih (ekstrinzičnih, okoljskih) in notranjih (intrinzičnih, organizemskih) dejavnikov (npr. Bouchenak-Khelladi in sod., 2015). Tektonski premiki, nastanek novih geografskih ovir, spremembe v zračnih in morskih tokovih, spremembe v lokalnih in globalnih podnebnih pogojih in posledično razpoložljivosti in raznolikosti ekoloških niš so primeri tesno prepletenih zunanjih dejavnikov, ki vplivajo na t. i. habitatno-gnano (ang. *habitat-driven*) speciacijo in izumiranje. Na drugi strani so notranji dejavniki določene lastnosti (ali njihova stanja) organizmov samih, ki korelirajo s taksonomsko diverziteto in nanjo domnevno vplivajo. Primer pogosto proučevanega notranjega dejavnika je telesna velikost. Pri različnih živalskih skupinah obstaja negativna korelacija med telesno velikostjo in vrstno pestrostjo (Hutchinson in MacArthur, 1959). Med predlaganimi vzroki za ta vzorec so večje velikosti populacij z dostopom do več ekoloških niš, nižje energetske potrebe, hitrejši generacijski obrati in višja stopnja mobilnosti pri manjših živalih (Hutchinson in MacArthur, 1959; Stanley, 1973; Brown in Nicoletto, 1991; Marzluff in Dial, 1991).

Rodovi zlatih mrežarjev se močno razlikujejo po vrstni pestrosti. Število vrst se giblje med 1 pri rodu *Indoetra* in 14 pri rodu *Herennia*. V drugem poglavju disertacije smo analizirali splošno dinamiko intenzivnosti speciacije in izumiranja na filogeniji in nato testirali več dejavnikov, potencialno odgovornih za heterogenost v vrstni pestrosti med rodovi. Prva med njimi sta dva binarna okoljska dejavnika: tip klime in tip kopnega. Pri mnogih živalskih skupinah, tudi pajkih, je znan gradient vrstne pestrosti, ki se manjša od ekvatorja proti poloma (Whitehouse in sod., 2009; Piel, 2018). Ta vzorec smo splošno testirali pri zlatih mrežarjih s preverjanjem korelacije med diverzifikacijo in tipom podnebja (tropski proti subtropsko-zmernem podnebnem pasu). Drugi testirani binarni dejavnik je bila celinska proti otoški razširjenosti, kjer smo pričakovali višji nivo speciacije v rodovih z več primeri otoškega endemizma.

Kontinuirana dejavnika, testirana za korelacijo z diverzifikacijo, sta bila nivo spolnega dimorfizma v telesni velikosti ter disperzijski potencial. Pričakovali smo zvonasti korelacijski krivulji, ki bi nakazovali, da kažejo rodovi s srednje izraženim spolnim velikostnim dimorfizmom in srednje izraženim disperzijskim potencialom najaktivnejšo diverzifikacijo. Ekstremen spolni dimorfizem z gigantizmom samic mora osebkom zagotavljati določene evolucijske prednosti, kot na primer povišano plodnost večjih samic, sicer se ne bi razvil. A redkost ekstremnega dimorfizma in maloštevilnost ekstremno dimorfnih vrst nakazuje, da je tovrsten fenotip tudi evolucijsko potraten, na primer zaradi večje vidnosti plenilcem ali večjih energetskih potreb večjih samic. Po drugi strani vrste z blago izraženim spolnim dimorfizmom niso deležne evolucijskih ugodnosti ekstremnejših fenotipov, kar bi prav tako lahko upočasnjevalo diverzifikacijo. Lastnosti, ki so za evolucijski doprinos (fitnes) sprva koristne, kasneje v evoluciji pa svojo korist izgubijo, postanejo za organizem potratne in celo vodijo v izumrtje vrste, imenujemo 'evolucijske slepe ulice'. Ekstremni fenotipi bi se lahko pokazali kot ena od takih lastnosti pri zlatih mrežarjih.

Rodovi se razlikujejo tudi po velikosti arealov in, domnevno, disperzijskem potencialu. Disperzija na novo območje lahko spodbudi diverzifikacijo zaradi nezasedenih ekoloških niš, ki organizmu postanejo na voljo (Eberle in sod., 2018). Disperzijski potencial bi zato lahko predstavljal pomemben notranji dejavnik diverzifikacije, neločljivo povezan s številnimi zunanjimi dejavniki, ki vplivajo na disperzijo (npr. vetrovnimi tokovi). Po modelu srednje disperzije (ang. *intermediate dispersal model*) organizmi z nizkim disperzijskim potencialom le redko kolonizirajo nova območja, zato ohranjajo ozko razširjenost. Na drugi strani pa organizmi z visokim disperzijskim potencialom nova območja pogosto osvojijo, a z njimi tudi uspešno ohranjajo genski pretok, kar zavira genetsko izolacijo in s tem speciacijo. Diverzifikacija naj bi bila tako najintenzivnejša pri organizmih s srednjim disperzijskim potencialom, ki disperzijske ovire premagajo občasno, a ne dovolj pogosto, da bi preko njih vzdrževali genski pretok (Claramunt in sod., 2012; Agnarsson in sod., 2014; Čandek in sod., 2020a).

Splošno dinamiko makroevolucijskih procesov na filogeniji smo analizirali z dvema programoma, ki se razlikujeta predvsem po tipu implementirane statistike: BAMM (Bayesian Analysis of Macroevolutionary Mixtures; Rabosky, 2014) in MEDUSA (Modeling Evolutionary Diversification Using Stepwise Akaike Information Criterion; Alfaro in sod., 2009). Prednost prve metode je predvsem v tem, da procesov ne modelira kot konstantne skozi čas, temveč so lahko dinamični. Obe metodi med drugim poskušata identificirati mesta naglih sprememb v intenzivnosti makroevolucijskih procesov na filogeniji. Korelacijo med intrinzičnimi in ekstrinzičnimi dejavniki ter diverzifikacijo smo testirali s t. i. SSE (ang. *state-dependent speciation and extinction*) modeli. Za binarna dejavnika smo uporabili metodo BiSSE (Binary-state Speciation and Extinction; Maddison in sod., 2007), za kvantitativna dejavnika pa sorodno QuaSSE (Quantitative-state Speciation and Extinction; FitzJohn, 2010). Fenotipsko ekstremnost smo kvantificirali z indeksom spolnega dimorfizma, t. j. količnikom povprečne telesne dolžine samic in telesne dolžine samcev pri vsaki vrsti. Disperzijski potencial smo kvantificirali z velikostjo areala vsake vrste, sledeč predpostavki, da vrste z večjim disperzijskim potencialom uspešno kolonizirajo in vzdržujejo večji areal.

BAMM na filogeniji ni zaznal točk hitrih sprememb v intenzivnosti speciacije ali izumiranja, vseeno pa je pokazal razlike v intenzivnosti procesov med različnimi deli filogenije. Povišano speciacijo je zaznal v rodu *Herennia*, povišano izumiranje pa v rodovih *Nephila, Indoetra* in *Herennia*. MEDUSA je za mesto intenzivnejše diverzifikacije označila rod *Herennia*. Nobena od korelacij med izbranimi intrinzičnimi in ekstrinzičnimi dejavniki ter diverzifikacijo se, presenetljivo, ni izkazala za statistično značilno. Edina izjema je bila mejno značilna korelacija med speciacijo in tipom klime, z intenzivnejšo speciacijo v tropskih klimatih.

Domnevali smo, da je povišana stopnja speciacije v rodu *Herennia* povezana s pogostostjo otoškega endemizma v tem rodu. Na drugi strani je bila povišana stopnja izumiranja ugotovljena pri najbolj spolno dimorfnem rodu med zlatimi mrežarji, *Nephila*. Ta rezultat bi lahko govoril v prid naši hipotezi o ekstremnem spolnem dimorfizmu kot evolucijski slepi ulici. Glede na dolgo filogenetsko vejo, na koncu katere sta edina predstavnika rodu *Nephila*, smo domnevali, da je bil rod v preteklosti vrstno pester, a je večina vrst tekom njegove dolge evolucijske zgodovine izumrla. Tudi naše pričakovanje, da bo odnos med dvema kvantitativnima lastnostma in diverzifikacijo zvonast, ni bilo podprto. Zaključili smo, da je diverzifikacija neodvisna od vseh testiranih lastnosti in njihovih korelatov.

Nedavno je bila v literaturi predlagana ideja, da makroevolucijski procesi sploh niso odvisni od biotskih in abiotskih dejavnikov, temveč mlajši kladi vedno diverzificirajo hitreje, starejši pa počasneje, ne glede na organizem, njegovo biogeografijo ali ekologijo (Diaz in sod., 2019). Speciacija in izumiranje sta po tej teoriji koncentrirana v času, vmes pa so obdobja evolucijske stagnacije. Čeprav je tak vzorec na relativno mladi filogeniji zlatih mrežarjev težko zaznaven, smo zaključili, da bi bil tovrsten 'univerzalen zakon diverzifikacije' lahko najboljša razlaga dobljenih rezultatov.

Tretje poglavje se je vrnilo k biogeografskim temam, tokrat na nivoju rodu. Rod *Herennia* je vrstno najbolj pester rod zlatih mrežarjev, razširjen v tropski Aziji in Avstralaziji. Od ostalih rodov se razlikuje predvsem po obliki mrež, ki so lestvičaste in se prilegajo drevesnim deblom. Med 14 poznanimi vrstami (od tega so tri neopisane) je široko razširjena le ena, *H. multipuncta*. Ostale vrste imajo majhne areale, pogosto omejene na en sam otok ali skupino otokov, npr. *H. etruscilla* z Jave, *H. gagamba* in *H. tone* s Filipinov, *H. jernej* s Sumatre in *H. milleri* z Nove Gvineje in Nove Britanije (Kuntner, 2005). Po drugi strani je *H. multipuncta* razširjena v južni Indiji, Indokini ter na filipinskem in indonezijskem arhipelagu. Za razliko od ostalih vrst, ki živijo v neokrnjenih gozdovih, je *H. multipuncta* sinantropna in pogosto naseljuje upravljane habitate. Pogosto se pojavlja v simpatriji z drugimi, ožje razširjenimi vrstami iz rodu. Razlog za dramatično razliko v velikosti arealov te in ostalih vrst ni poznan, poleg tega ostaja nedokumentiran glavni način njihove disperzije.

Cilj tretjega poglavja je bila rekonstrukcija biogeografske zgodovine rodu, testirati, kako različni domnevani načini disperzije vplivajo na to rekonstrukcijo, in preveriti, ali je nenavadno velik areal vrste *H. multipuncta* naraven ali rezultat človekove dejavnosti. Vrstno filogenijo smo izračunali na podlagi filogenomskih podatkov, pridobljenih z metodo *Anchored Hybrid Enrichment* (Lemmon in sod., 2012). Drevo smo nato s klasičnima genetskima markerjema, genoma za COI in 28S rRNA, razširili na populacijski nivo, pri čemer je število predstavnikov posamezne vrste obsegalo od enega do 26 osebkov (največ pri *H. multipuncta*). Populacijsko filogenijo smo uporabili za rekonstrukcijo biogeografske zgodovine rodu podobno, kot v prvem poglavju, a smo zaradi nepoznavanja prevladujoče metode disperzije pri rodu *Herennia* metodologijo prilagodili. Verjetnosti disperzije smo ponovno izračunali na podlagi fizičnih razdalj med biogeografskimi regijami skozi čas, ker pa je geološka zgodovina proučevanega območja, predvsem indonezijskega otočja, zelo kompleksna in težavna za rekonstrukcijo, smo verjetnosti disperzije združili v pet kategorij. Zgornji (95%) in spodnji (5%) limit sta ostala definirana enako, kot v prvem poglavju, fizični stik med regijami pa je bil definiran na dva različna načina. Model A je 95% verjetnost disperzije pripisal le parom regij v neposrednem stiku, model B pa je takšno verjetnost pripisal vsem regijam, ki so med seboj fizično povezane, torej tudi posredno povezanim parom regij. V biološkem smislu je model A predpostavljal balonanje kot glavni način disperzije, model B pa počasno, stohastično disperzijo z lazenjem pri iskanju prostega habitata.

Vrstna filogenija je pokazala nekaj nepričakovanih evolucijskih odnosov med vrstami, na primer položaj *H. maj* kot sestrske vrste vsem ostalim. V populacijski filogeniji so se vzorci iste vrste konsistentno združevali v klade, vzorci istih populacij pa pogosto ne. Rekonstruirana zgodovinska razširjenost se v večjem delu filogenije med biogeografskima modeloma A in B ni razlikovala. Analizi sta ugotovili široko izvorno distribucijo rodu v Avstraliji, celinski JV Aziji in na Filipinih, nato pa postopno kolonizacijo ostalih regij iz ali preko izvornih treh. Razlike med modeloma so nastale le pri rekonstrukciji zgodnje biogeografske zgodovine vrste *H. multipuncta*. Po modelu A je bila vrsta skozi vso svojo zgodovino prisotna na celinski JV Aziji, od koder je postopoma kolonizirala ostale geografske regije, Šri Lanko, Indijo, Sulavezi, Javo in Sumatro. Po modelu B pa je bila njena prvotna distribucija omejena na Sulavezi, od koder je kolonizirala Šri Lanko, Indijo in Javo, in šele nato zavzela in se razširila po celinski JV Aziji. Model B je tako v ozkem časovnem razmaku predpostavljal dve uspešni disperziji prek razdalj, večjih od 4000 km. Zaradi te in drugih malo verjetnih komponent rekonstrukcije modela B, smo model A določili za bolj parsimoničnega.

Noben od modelov ni podprl hipoteze o nedavni, antropogeni razširitvi vrste *H. multipuncta*. Filogenetske divergence med populacijami so bile namreč prestare, da bi časovno sovpadale s človekovo prisotnostjo na relevantnem območju. Zakaj, torej, je današnji areal te vrste toliko večji od arealov ostalih vrst? Postavili smo hipotezo, da so vrste rodu *Herennia* tekom evolucije izgubile spodobnost balonanja, domnevno prisotne pri predniških zlatih mrežarjih. To je pogost pojav pri pajkih po uspešni kolonizaciji izoliranih otokov. Ker je vsakršna nadaljnja disperzija zelo verjetno neuspešna, naravna selekcija odbira posameznike z nizkim disperzijskim potencialom (Gillespie in sod., 2012). To omeji vzdrževanje genskega pretoka med populacijami in s tem pospeši speciacijo. Predpostavili smo, da pri vrsti *H. multipuncta* do te izgube ni prišlo oziroma je sposobnost balonanja pridobila nazaj, in se uspešno razširila prek današnjega areala. Glede na pogosto in dolgotrajno simpatrijo z drugimi vrstami iz rodu smo zaključili še, da *H. multipuncta* drugih vrst ne izpodriva, morda zaradi razlik v ekoloških nišah.

V četrtem poglavju disertacije smo dve vrsti zlatih mrežarjev proučili še na nivoju populacijske genetske strukture. V splošnem populacijska genetika išče vzorce v genetski sestavi (t. j. alelnih frekvencah) populacije (t. j. skupine organizmov, potencialno sposobne razmnoževanja), ki so posledica nenaključnega parjenja osebkov (Chakraborty, 1993; Bohonak, 1999). Naravne populacije so zaradi različnih ekoloških, geografskih in drugih dejavnikov običajno razdeljene na več subpopulacij, ki lahko med seboj vzdržujejo genski pretok, ali pa so izolirane. Mutacije, ki se pojavijo v izoliranih subpopulacijah, ostanejo nanjo omejene, dokler ne pride do (ponovne) vzpostavitve genetskega stika z drugo populacijo. Subpopulacije, ki ohranjajo genski pretok z drugimi subpopulacijami, so odpornejše na genetsko diferenciacijo (Chakraborty, 1993).

Močno disperzivni organizmi lažje ohranjajo genski pretok med populacijami tudi, ko so razdalje med njimi velike. Tak primer je tudi vrsta zlatega mrežarja *Nephila pilipes*, ki se v juvenilni fazi pogosto razširja z balonanjem (Lee in sod., 2015). Obstoječe študije so našle relativno homogeno genetsko strukturo vrste v nekaterih delih njenega areala (Lee in sod., 2004; Su in sod., 2007). V tem poglavju smo to ugotovitev preverili z modernejšimi molekularnimi orodji in ugotovljeno genetsko strukturo primerjali s strukturo še enega zlatega mrežarja, vrste *Trichonephila clavata*. Vrsti se med seboj razlikujeta po več organizemskih in okoljskih lastnostih. *Nephila pilipes* naseljuje tropske gozdove po celotni južni in jugovzhodni Aziji, Avstraliji in Pacifiku, in sodi med najbolj spolno dimorfne vrste pajkov nasploh (Su in sod., 2007; Kuntner in sod., 2019). Po drugi strani je *T. clavata* manj spolno dimorfna vrsta, ki naseljuje subtropske in zmerne habitate med Himalajo in Japonsko (Su in sod., 2011). Za razliko od tropskih gozdov so zmerna okolja sezonska in ponujajo omejen časovni okvir ugodnih okoljskih razmer (Kim in sod., 1999). Medtem, ko je *N. pilipes* samotarska vrsta, se osebki vrste *T. clavata* pogosto združujejo v ohlapne kolonije (lastna opažanja).

Zaradi števila in prepletenosti dejavnikov, ki vplivajo na genetsko strukturo na populacijskem nivoju, je bila ta študija v prvi vrsti eksplorativna, brez konkretnih hipotez. Vseeno smo pričakovali, da bomo z natančnejšo metodo odkrili prej nepoznano genetsko strukturo pri obeh vrstah in v njej prepoznali geografske vzorce. V grobem smo domnevali, da bo znotrajvrstna struktura povezana s paleoklimatskimi in paleogeološkimi dejavniki, na primer s spremembami v globalnem podnebju, in z velikostjo vrstnega areala, saj bi večji areali lahko zahtevali več prilagoditev na lokalne okoljske razmere. Če bi se pokazale razlike v genetski strukturi med vrstama, smo pričakovali, da bi jih lahko razložili v okviru že omenjenih okoljskih in organizemskih razlik med vrstama.

V študiji smo preverili tudi hipotezo, da so melanistični osebki vrste *N. pilipes* predstavniki druge vrste, imenovane »*Nephila kuhli*« (Chetia in Kalita, 2012). Melanistične samice imajo popolnoma ali delno črno obarvan zadek in rdečkaste noge, samci, najdeni ob njih, pa so morfološko enaki samcem ob običajnem tipu samic (lastna opažanja). Čeprav so redke, melanistične samice najdemo po vsem arealu vrste. Če res predstavljajo svojo vrsto, bi morali melanistični osebki na filogenetskem drevesu tvoriti monofiletsko skupino.

Iz obstoječih zbirk in z lastnim terenskim delom smo pridobili 94 primerkov vrste *N. pilipes* in 40 primerkov vrste *T. clavata*. Iz tkiv smo izolirali DNA in jo uporabili za sodobno metodo sekvenciranja, imenovano *restriction site-associated sequencing* oziroma *RADseq* (Baird in sod., 2008). Ta metoda za genetske markerje uporablja polimorfizme posameznih nukleotidov (ang. *single nucleotide polymorphism* ali *SNP*), kar omogoča večjo ločljivost sledečih analiz v primerjavi s klasičnimi genetskimi markerji, kot je gen za COI. Z RADseq pridobimo nabor tovrstnih polimorfizmov na fragmentih DNA, tik ob mestih vezave izbranega restrikcijskega encima. Končno podatkovno bazo, ki je vključevala tisoče homolognih SNP za vsak osebek, smo nato uporabili za izračun filogenij in analizo genetske strukture populacij s programom STRUCTURE (Pritchard in sod., 2000).

Filogenetski drevesi sta pri obeh vrstah razkrili presenetljivo stopnjo nesorodnosti med osebki istih populacij. Pri obeh vrstah so monofiletsko skupino tvorili le osebki ene od populacij, vse ostale pa so bile polifiletske. Filipinski vzorci *N. pilipes* so se sicer združevali v večje monofiletske klade, a ne nujno osebki iz istih populacij. Sledeč tem rezultatom smo sklepali, da obe vrsti vzdržujeta močan genski pretok med vključenimi populacijami. Za *N. pilipes* to sicer ni nepričakovano, so pa ti rezultati zanimiva indikacija, da vrsta genski pretok vzdržuje na skoraj 10.000 kilometrov širokem območju.

Pri *T. clavata* disperzijsko vedenje ni dobro dokumentirano, zato naši rezultati predstavljajo pomemben posreden dokaz pogostega balonanja tudi pri tej vrsti. Geografske ovire, kot so gorovja in vodna telesa, nobeni od vrst očitno ne predstavljajo ovire za disperzijo. *Trichonephila clavata* sicer naseljuje tudi območje Himalaje, kjer so gorske ovire morda dovolj izrazite, da omejujejo disperzijo na posamezne doline, kar bi pustilo sled v genetski strukturi. Ker pa so himalajski primerki redki ali celo odsotni iz obstoječih zbirk, v to študijo niso bili vključeni, in ta hipoteza ostaja nepreverjena.

STRUCTURE je v vzorcu vrste *T. clavata* identificiral dva genetska tipa, a smo zaradi izredno nizke zastopanosti enega od tipov vrsto obravnavali kot genetsko homogeno. Tak rezultat je bil nepričakovan, a je nakazal, da je disperzija z balonanjem pogosta tudi pri tej vrsti. Pri *N. pilipes* je STRUCTURE identificiral štiri genetske tipe. Tipa I in II sta bila pogosta in široko razširjena na filogeniji, tipa III in IV pa sta bila prisotna le v nekaterih, večinoma robnih populacijah. Zaznali nismo nobene jasne povezave med geografijo in genetsko strukturo populacij *N. pilipes*.

Dobljene rezultate smo razložili predvsem v luči paleoklimatskih in paleogeoloških sprememb med podnebnimi nihanji v zadnjih nekaj milijonih let. Naši rezultati so bili skladni s hipotezo, da so imele spremembe v porazdelitvi deževnih gozdov med kvartarnimi ledenimi in medledenimi dobami pomembno vlogo pri oblikovanju genetske diverzitete *N. pilipes* (Su in sod., 2007). Med ledenimi dobami so se deževni gozdovi skrčili, na njihovih robovih pa so se oblikovali habitatni žepi. Tako izolirane pajčje populacije niso bile sposobne vzdrževanja genskega pretoka z drugimi populacijami, kar je vodilo v genetsko divergenco. V medledenih dobah pa je ponovna širitev deževnih gozdov omogočila stik med populacijami in s tem genetsko mešanje.

Tako filogenetske kot strukturne analize niso našle nobene podpore za hipotezo, da so melanistične samice vrste *N. pilipes* predstavnice druge vrste. Ob primerjavi obeh proučevanih vrst smo zaključili, da razlike v sezonskosti okolja, velikosti areala in združevanju v kolonije ne vplivajo na genetsko sestavo populacij. Zaključili smo, da bi lahko filogenetska starost vrste predstavljala osrednji dejavnik, ki napoveduje njeno genetsko strukturiranost.

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ACKNOWLEDGEMENTS

I would first like to thank the members of my doctoral commission, dr. Rok Kostanjšek, dr. Tomaž Skrbinšek and dr. Jason E. Bond, for your willingness to participate in the making of this thesis. Your encouragement and constructive criticism were greatly appreciated.

I thank my colleagues at Jovan Hadži Institute of Biology ZRC SAZU for making day-to-day work over the past five years thoroughly enjoyable. I will miss our morning debates over coffee very much. Special appreciation goes to the members of the EZ lab for introducing me to the extraordinary life of spiders. I am especially thankful to Klemen Čandek, who has been an indispensable source of help and laughter since day 1. I am grateful to have made a lifelong friend during my doctoral study.

I wish to thank dr. Yong-Chao Su and members of his laboratory for enabling me to conduct a substantial part of my research at their facilities at the Kaohsiung Medical University. Thank you for patiently teaching me new molecular and statistical tools and for your continued help following my stay in Taiwan. Special thanks go to dr. Weiyu Chen, who generously offered me accommodation in Kaohsiung and presented to me the wonderful world of Taiwanese cuisine.

I also thank my family and friends outside of work who supported me and helped me maintain great work-life balance. I thank my parents, Inga and Boris, for supporting me and my (career) decisions, even when they seem unwise. I thank my university friends, in Slovenia and abroad, for being wonderful the entire past 10+ years.

My final and deepest thanks go to my advisor and co-advisor. Simona and Matjaž, I am quite sure that doctoral study is not meant to be as enjoyable as you made it for me. Your guidance has led me to acquire and refine a number of scientific skills that have without doubt made me a better biologist. I am especially grateful for all the experiences you made possible for me or encouraged me to pursue, including field work, research visits and congresses literally half way across the world. I am very much aware that such a great personal and professional relationship between a PhD candidate and their advisors is rare and I am genuinely grateful to have worked with you.

Funding

This work was supported by the “Young Researcher” graduate student funding and other grants from the Slovenian Research Agency - ARRS (J1-9163, P1-0236 and P1-0255).

ZAHVALA

Najprej bi se rada zahvalila članom doktorske komisije, dr. Roku Kostanjšku, dr. Tomažu Skrbinšku in dr. Jasonu E. Bondu, za vašo pripravljenost za sodelovanje pri pripravi te disertacije. Zahvaljujem se vam za vaše spodbudne besede in konstruktivno kritiko.

Zahvaljujem se svojim sodelavcem z Biološkega inštituta Jovana Hadžija ZRC SAZU, da so vsakodnevno delo tekom zadnjih petih let naredili nadvse prijetno. Močno bom pogrešala naše debate ob jutranji inštitutski kavi. Posebna zahvala gre članom skupine EZ lab, da so mi predstavili izjemno življenje pajkov. Zlasti bi se zahvalila Klemnu Čandku, ki je bil od prvega dne zame nepogrešljiv vir pomoči in smeha. Vesela sem, da sem med doktorskim študijem spoznala prijatelja za vse življenje.

Hvala tudi dr. Yong-Chao Suju in članom njegove skupine, da so mi omogočili izvedbo velikega dela mojih raziskav v svojih laboratorijih na Kaohsuing Medical University. Hvala, ker ste me potrpežljivo učili novih molekularnih in statističnih orodij, tudi po mojem odhodu s Tajvana. Posebej bi se rada zahvalila dr. Weiyu Chen, ki mi je velikodušno ponudila namestitev v Kaohsuingu in mi predstavila čudoviti svet tajvanske kuhinje.

Zahvaljujem se tudi svoji družini in prijateljem izven službe, ki so me ves čas podpirali in pomagali ohranjati odlično ravnovesje med delom in prostim časom. Zahvaljujem se svojima staršema, Ingi in Borisu, ker me podpirata pri (kariernih) odločitvah tudi, ko se zdijo nespametne. Hvala kolegom s fakultete, v Sloveniji in tujini, da ste vseh zadnjih 10+ let izvrstni.

Nazadnje in najgloblje se želim zahvaliti svoji mentorici in svojemu somentorju. Simona in Matjaž, verjetno ni mišljeno, da se imajo študentje med doktorskim študijem tako lepo, kot sem se, predvsem zaradi vaju, imela jaz. Zaradi vajinega vodstva sem pridobila in izpopolnila številne znanstvene veščine, ki so me brez dvoma naredile boljšega biologa. Posebej sem vama hvaležna za vse izkušnje, ki sta mi jih omogočila ali me za njih spodbujala, vključno s terenskim delom, raziskovalnimi obiski in kongresi dobesedno na drugi strani sveta. Zavedam se, da tako odličen oseben in profesionalen odnos med doktorskim kandidatom in njegovimi mentorji ni pogost ali samoumeven, zato sem iskreno hvaležna, da sem lahko delala z vama.

Financiranje

Izdelavo doktorske disertacije je s financiranjem programa "Mladi raziskovalec" in drugih znanstvenih programov omogočila Javna agencija za raziskovalno dejavnost Republike Slovenije – ARRS (J1-9163, P1-0236 in P1-0255).

ANNEX A1

Collection details for *Trichonephila clavata* specimens used in Chapter 4

Code	**Population name**	**Collecting site**	**Sex**		
			F	M	Juv
EEG 845	Fujian_1	Qing Yun Mt., Yongtai Town, Fujian, China	1		
EEG 846	Fujian_1	Qing Yun Mt., Yongtai Town, Fujian, China	1		
EEG 847*	Fujian_1	Qing Yun Mt., Yongtai Town, Fujian, China	1		
EEG 848	Fujian_2	Tian Men Mt., Fujian, China	1		
EEG 869	Fujian_2	Tian Men Mt., Fujian, China	1		
EEG 870	Fujian_2	Tian Men Mt., Fujian, China	1		
EEG 871	Fujian_3	Jiu Long Yan, Fujian, China	1		
EEG 872	Fujian_3	Jiu Long Yan, Fujian, China	1		
EEG 900	Hunan_1	Hu Ping Mt Slope, Hunan, China			1
EEG 901	Hunan_1	Hu Ping Mt Slope, Hunan, China	1		
EEG 902	Hunan_1	Hu Ping Mt Slope, Hunan, China			1
EEG 903	Hunan_2	Hu Ping Mt Peak, Hunan, China	1		
EEG 907*	Hunan_2	Hu Ping Mt Peak, Hunan, China	1		
EEG 908	Hunan_2	Hu Ping Mt Peak, Hunan, China	1		
EEG 909	Hunan_3	Tianzi Mt, Zhangjiajie National Forest Park, Hunan, China	1		
EEG 910	Hunan_3	Tianzi Mt, Zhangjiajie National Forest Park, Hunan, China	1		
EEG 911	Hunan_3	Tianzi Mt, Zhangjiajie National Forest Park, Hunan, China	1		
EEG 912	Hunan_4	Tianmen Mountain, Hunan, China			1
EEG 873	Japan_1	Daruma Yama park, Mt. Daruma, Kumejima, Okinawa, Japan		1	
EEG 874	Japan_1	Kumejima, Okinawa, Japan	1		
EEG 882	Japan_2	Kanazawa, Ishikawa, Japan			1
EEG 883	Japan_2	Kanazawa, Ishikawa, Japan			1
EEG 884	Japan_2	Kanazawa, Ishikawa, Japan			1
EEG 896	Japan_3	Koganei, Tokyo, Japan			1
EEG 897	Japan_3	Koganei, Tokyo, Japan			1
EEG 898	Japan_3	Koganei, Tokyo, Japan			1
EEG 899	Japan_4	Uken, Kagoshima, Japan			1
EEG 839*	Taiwan_1	Low altitude experimental station, Heping district, Taichung, Taiwan			1
EEG 840	Taiwan_1	Low altitude experimental station, Heping district, Taichung, Taiwan			1
EEG 841	Taiwan_1	Low altitude experimental station, Heping district, Taichung, Taiwan			1
EEG 842	Taiwan_2	Po Kong Keng, Sanyi township, Miaoli county, Taiwan			1
EEG 843	Taiwan_2	Po Kong Keng, Sanyi township, Miaoli county, Taiwan			1
EEG 844	Taiwan_2	Po Kong Keng, Sanyi township, Miaoli county, Taiwan			1
EEG 875	Yunnan_1	Linjiang park, Eshan country, Yuxi City, Yunnan, China	1		

Continuation

Continuation of Annex A1

Code	**Population name**	**Collecting site**	**Sex**		
			F	M	Juv
EEG 904	Yunnan_2	Menghai, Xishuangbanna, Yunnan, China	1		
EEG 905	Yunnan_2	Menghai, Xishuangbanna, Yunnan, China	1		
EEG 906	Yunnan_2	Menghai, Xishuangbanna, Yunnan, China	1		
EEG 879	Yunnan_3	Shilin, Yunnan, China	1		
EEG 880	Yunnan_3	Shilin, Yunnan, China	1		
EEG 881	Yunnan_3	Shilin, Yunnan, China	1		

Note: an asterisk (*) denotes samples, excluded from bioinformatic analyses due to >90% of missing data.

ANNEX A2

Collection details for *Nephila pilipes* specimens used in Chapter 4

Code	**Population name**	**Collecting site**	**Sex**			**Melanic**
			F	M	Juv	
EEG 775	Australia	Mackay, Queensland, Australia			1	
EEG 776	Australia	Mackay, Queensland, Australia	1			
EEG 838	Australia	Mackay, Queensland, Australia	1			
EEG 812	China_1	Jinmujiao, Jiyang District, Sanya City, Hainan, China	1			
EEG 813	China_1	Jinmujiao, Jiyang District, Sanya City, Hainan, China	1			
EEG 773	China_2	Shiwan Mountain National Forest Park, Guangxi, China	1			
EEG 774	China_2	Shiwan Mountain National Forest Park, Guangxi, China	1			
EEG 765	Hong Kong	Aberdeen Park, Hong Kong Island, Hong Kong	1			
EEG 766	Hong Kong	Aberdeen Park, Hong Kong Island, Hong Kong	1			
EEG 767	Hong Kong	Aberdeen Park, Hong Kong Island, Hong Kong	1			
EEG 754	India_1	Tadoba tiger reserve, Maharasthra, India	1			●
EEG 820	India_1	Tadoba tiger reserve, Maharasthra, India	1			●
EEG 821	India_1	Tadoba tiger reserve, Maharasthra, India	1			●
EEG 822	India_2	Mumbai, Sanjay Ghandi NP, Maharasthra, India	1			
EEG 755	India_3	Melghat tiger reserve, Maharasthra, India	1			
EEG 756	India_3	Melghat tiger reserve, Maharasthra, India	1			
EEG 757	India_3	Melghat tiger reserve, Maharasthra, India	1			
EEG 835*	India_4	Sukna, West Bengal, India	1			
EEG 836*	India_4	Sukna, West Bengal, India	1			
EEG 837*	India_4	Sukna, West Bengal, India	1			
EEG 770	India_5	Assam, India	1			
EEG 771*	India_5	Assam, India	1			
EEG 772*	India_5	Assam, India	1			
EEG 831	Indonesia_1	Forest above Ratatotok, Sulawesi, Indonesia			1	
EEG 832	Indonesia_2	Tangkoko Reserve, Sulawesi, Indonesia			1	
EEG 833	Indonesia_2	Danowudu forest fragment, Sulawesi, Indonesia			1	
EEG 768*	Indonesia_3	Ubud, Bali, Indonesia	1			
EEG 769*	Indonesia_3	Ubud, Bali, Indonesia	1			
EEG 834	Indonesia_3	Ubud, Bali, Indonesia			1	
EEG 730	Japan_1	Uken, Amami, Kagoshima, Japan		1		
EEG 731	Japan_1	Uken, Amami, Kagoshima, Japan	1			
EEG 732	Japan_2	Nago, Okinawa, Japan			1	
EEG 733	Japan_2	Nago, Okinawa, Japan			1	
EEG 734	Japan_2	Nago, Okinawa, Japan	1			
EEG 747	Japan_3	Makiyama Observation Deck, Irabu Island, Japan	1			

Continuation

Continuation of Annex A2

Code	**Population name**	**Collecting site**	**Sex**			**Melanic**
			F	M	Juv	
EEG 748	Japan_3	Makiyama Observation Deck, Irabu Island, Japan	1			
EEG 749	Japan_3	Makiyama Observation Deck, Irabu Island, Japan	1			
EEG 753	Japan_4	Iriomote, Okinawa, Japan	1			•
EEG 818	Japan_4	Iriomote, Okinawa, Japan			1	
EEG 819	Japan_4	Iriomote, Okinawa, Japan		1		
EEG 750	Laos	Pakse, Laos	1			
EEG 814	Laos	Pakse, Laos	1			•
EEG 740	Malaysia_1	Gombak, Malaysia (scored at Selangor State Park)	1			
EEG 741*	Malaysia_2	HL Gunung Senyum, Malaysia			1	
EEG 759	Myanmar	Kalekho Atet, Kayin State, Myanmar	1			
EEG 826	Myanmar	Kalekho Atet, Kayin State, Myanmar	1			
EEG 827	Myanmar	Kalekho Atet, Kayin State, Myanmar	1			
EEG 721	Philippines_1	Irosin, Bicol, Philippines	1			
EEG 722	Philippines_1	Irosin, Bicol, Philippines	1			
EEG 723	Philippines_1	Irosin, Bicol, Philippines	1			
EEG 724	Philippines_2	Manugal resort, Negros, Visaya, Philippines	1			
EEG 725*	Philippines_2	Manugal resort, Negros, Visaya, Philippines	1			
EEG 726	Philippines_2	Manugal resort, Negros, Visaya, Philippines	1			
EEG 727	Philippines_3	Rd to Lake Mahognao, Leyte, Visaya, Philippines	1			
EEG 728	Philippines_3	Rd to Lake Mahognao, Leyte, Visaya, Philippines	1			
EEG 804	Philippines_3	Rd to Lake Mahognao, Leyte, Visaya, Philippines	1			
EEG 801	Philippines_4	Calbiga cave, Samar, Visaya, Philippines		1		
EEG 729	Philippines_5	Mt. Banahaw, Kinabuhayan, S. Luzon, Philippines	1			
EEG 802	Philippines_5	Mt. Banahaw, Kinabuhayan, S. Luzon, Philippines	1			
EEG 735	Philippines_6	Valderrama resort, Panay, Philippines	1			
EEG 736*	Philippines_6	Valderrama resort, Panay, Philippines	1			
EEG 803*	Philippines_6	Valderrama resort, Panay, Philippines	1			
EEG 805	Philippines_7	Loboc cave, Bohol, The Philippines	1			
EEG 806	Philippines_7	Chocolate Hills, Bohol, The Philippines	1			
EEG 807*	Philippines_7	Hanging Bridge, Bohol, The Philippines	1			
EEG 737	Philippines_8	Isaub, Palawan, The Philippines	1			
EEG 738	Philippines_8	Isaub, Palawan, The Philippines	1			
EEG 739*	Philippines_8	Isaub, Palawan, The Philippines	1			
EEG 746	Philippines_9	Taytay, Ditucalan, Iligan City, Philippines	1			
EEG 810	Philippines_9	Taytay, Ditucalan, Iligan City, Philippines	1			
EEG 811	Philippines_9	Taytay, Ditucalan, Iligan City, Philippines	1			
EEG 760*	Philippines_10	Calayan Island, Philippines	1			•
EEG 761	Philippines_10	Calayan Island, Philippines			1	
EEG 828	Philippines_10	Calayan Island, Philippines			1	•

Continuation

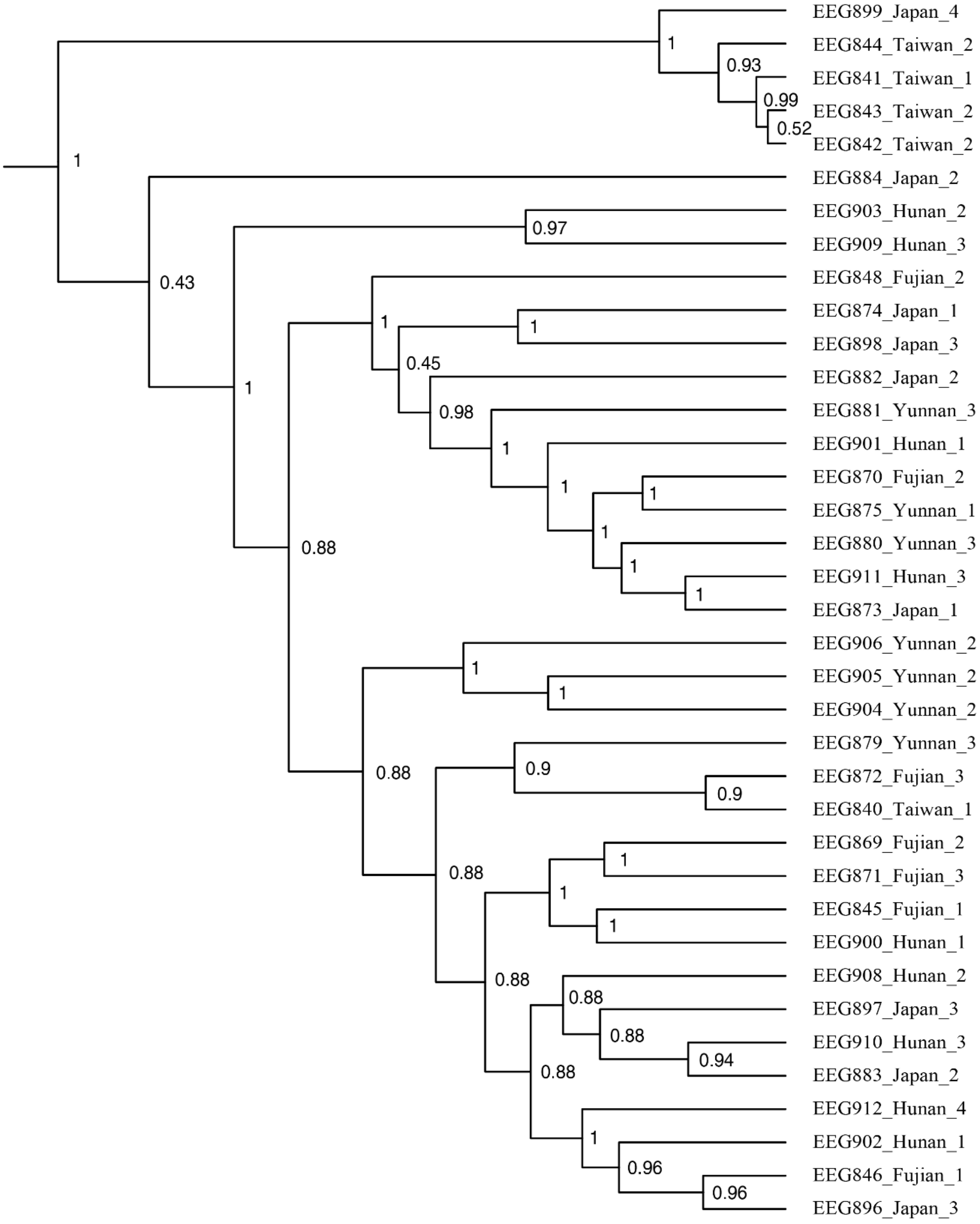
Continuation of Annex A2

Code	**Population name**	**Collecting site**	**Sex**			**Melanic**
			F	M	Juv	
EEG 829	Philippines_11	Kalbario-Patapat Natural Park, Ilocos Norte, Philippines			1	
EEG 830	Philippines_11	Kalbario-Patapat Natural Park, Ilocos Norte, Philippines			1	
EEG 758	Singapore_1	Pulau Ubin, Singapore	1			
EEG 823	Singapore_1	Pulau Ubin, Singapore	1			
EEG 824	Singapore_1	Pulau Ubin, Singapore		1		
EEG 825	Singapore_2	Sentosa Island, Singapore		1		
EEG 745	Taiwan_1	Husong, Namaxia, Kaohsiung, Taiwan	1			
EEG 808	Taiwan_1	Husong, Namaxia, Kaohsiung, Taiwan	1			
EEG 809	Taiwan_1	Husong, Namaxia, Kaohsiung, Taiwan			1	
EEG 751	Taiwan_2	Shakadang Trail, Taroko National Park, Taiwan	1			
EEG 815	Taiwan_2	Shakadang Trail, Taroko National Park, Taiwan			1	•
EEG 762	Taiwan_3	Dahan Shan forest road, Pingtung County, Taiwan	1			
EEG 763	Taiwan_3	Dahan Shan forest road, Pingtung County, Taiwan	1			•
EEG 764	Taiwan_3	Dahan Shan forest road, Pingtung County, Taiwan	1			
EEG 752	Thailand_1	Forest SW of Pa Klok, Phuket, Thailand	1			
EEG 816	Thailand_1	Forest SW of Pa Klok, Phuket, Thailand			1	
EEG 817	Thailand_2	Sridit waterfall, Petchabun, Thailand			1	
EEG 742	Vietnam	Melinh station, Vietnam	1			
EEG 743	Vietnam	Melinh station, Vietnam	1			
EEG 744	Vietnam	Melinh station, Vietnam	1			

Note: an asterisk (*) denotes samples, excluded from bioinformatic analyses due to >90% of missing data.

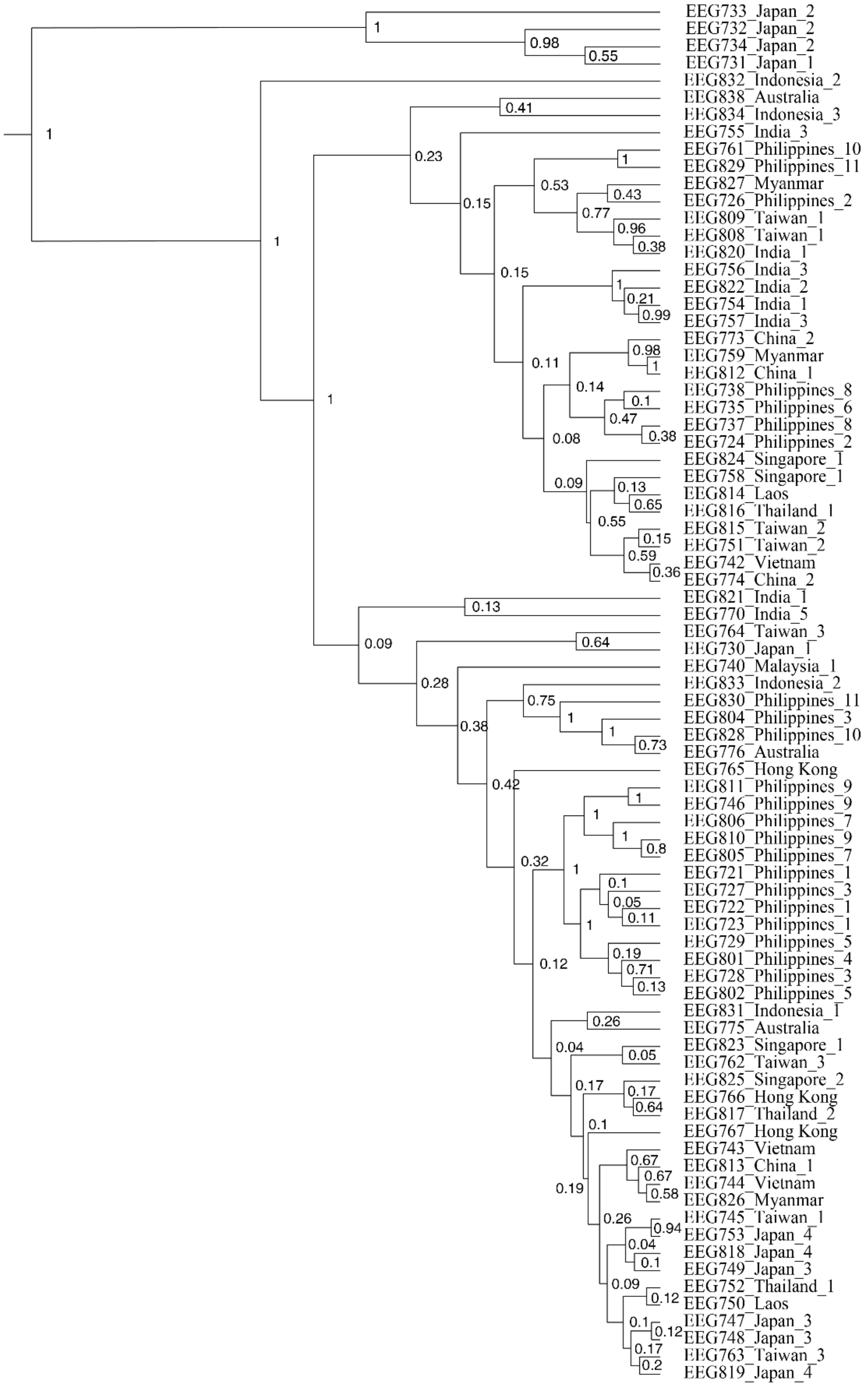
ANNEX B1

Node supports in the *Trichonephila clavata* population phylogenetic tree



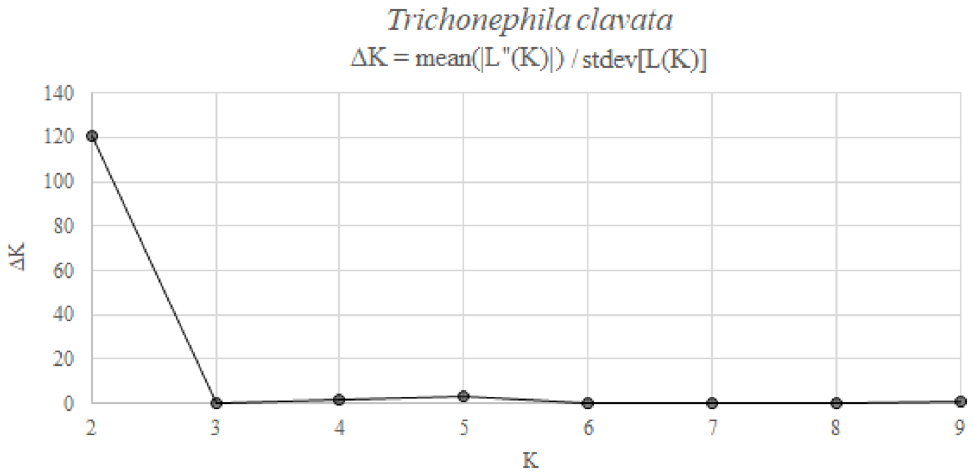
ANNEX B2

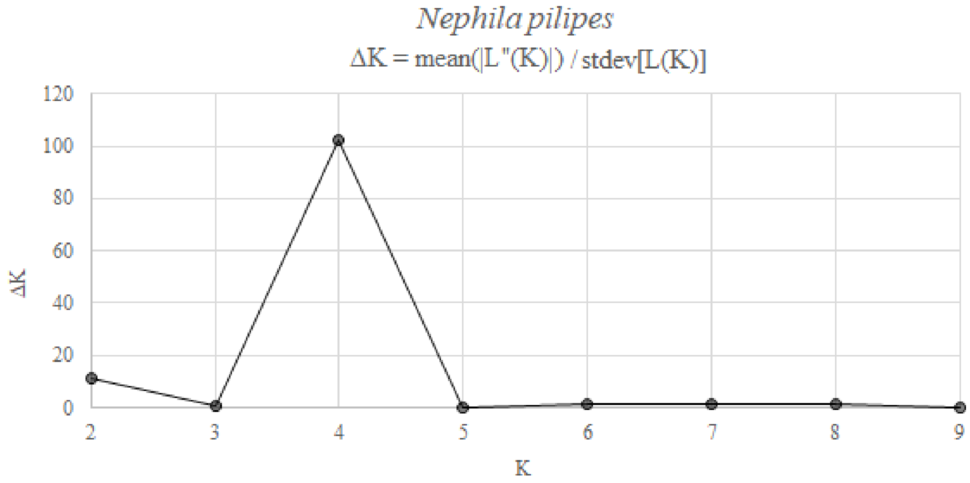
Node supports in the *Nephila pilipes* population phylogenetic tree



ANNEX C

Delta K (ΔK) plots for *Trichonephila clavata* and *Nephila pilipes* produced in STRUCTURE HARVESTER





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