SYSTEMATICS, TAXONOMY AND BIOGEOGRAPHY OF THE COSMOPOLITAN AND FOSSIL-RICH BUCKTHORN FAMILY (RHAMNACEAE JUSS.)

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DISSERTATION

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ABSTRACT

My thesis combines taxonomy, phylogenetics, divergence time estimations and ancestral area reconstructions, nomenclature, and other methods to shed light on the evolutionary processes that shaped the extant biodiversity of the buckthorn family (Rhamnaceae Juss.). In a first step, this thesis provides a major contribution to the taxonomic assessment and arrangement within the buckthorns. By describing the new genus *Pseudoziziphus* Hauenschild, and resurrecting four genera, this thesis highlights and compensates underestimated diversity. Simultaneously, it provides a phylogenetic framework based on the largest molecular data set in the family so far. In a second step, the taxonomic and phylogenetic framework was used to reconstruct solid divergence time estimates and ancestral area estimates for Rhamnaceae, with a focus on the ziziphoid lineage, and the genus complex *Alphitonia* Reissek ex Endl. *sensu lato*. By this, it was possible to unravel evolutionary processes within the history of Rhamnaceae and provide a biogeographic scenario for the ziziphoid lineages, including *Alphitonia* s.l.

Summary

The causes of Earth’s unevenly distributed biodiversity are diverse. In this thesis, I shed light on the processes that shaped the biodiversity within the phylogenetic lineage of buckthorns (Rhamnaceae). This rosid family overall comprises trees and shrubs, but also few herbs and lianas, distributed throughout the Earths terrestrial surface. Buckthorns can be found from Norway to Patagonia, from the dry Australian desert to the Amazonian rainforest, and from sea level to 3800 meters above the mean sea level in the Himalayas. Although the family is cosmopolitan, the lineages within the family are often restricted to certain parts of the world, such as Australia and Oceania, North America or the Mediterranean regions. To investigate how this pattern arose, I performed four studies in Rhamnaceae. First, I combined molecular phylogenetic analyses with classical taxonomy to detect how many genera of Rhamnaceae were actually valid. On the taxonomic side, with this approach I resurrected and described genera and identified synonyms. On the phylogenetic side, I highlighted which clades were supported and could be used as topological constraints for further analyses, and which clades were not supported. Second, I used the taxonomic knowledge to place fossil taxa to the previously detected supported clades as temporal constraints and performed molecular dating analyses on Rhamnaceae and close allies. By this approach, I reconstructed the temporal origin of the buckthorns to the Cretaceous. Furthermore, I could reconstruct age estimates for the internal nodes within the chronogram, such as the ziziphoid stem and crown age, allowing for detailed reconstructions of this lineage. Those reconstructions where then used to unravel the

biogeographic history of the ziziphoids as a whole, and the ziziphoid taxon *Alphitonia sensu lato* in detail.

With the exception of a few genera, the phylogenetic reconstructions of chapter one largely supported previously proposed taxonomic concepts within the buckthorn family (Rhamnaceae). In a nutshell, this means that the buckthorns were divided into three separate lineages: the large ziziphoids (about two thirds of all species), the large rhamnoids (about one third of all species), and the species poor ampelozizyphoids. Those three lineages were divided into three tribes in ampelozizyphoids and rhamnoids each, and five tribes plus a few genera *incertae sedis* in the ziziphoids, respectively. However, some necessary modifications within the classification system were identified. The species-rich *Ziziphus* Mill. (tribe Paliureae Reiss. ex Endl., ziziphoid lineage) was split into *Ziziphus* sensu stricto, *Sarcomphalus* P. Browne and *Pseudoziziphus* Hauenschild (tribe Rhamneae Hook. f., rhamnoid lineage). Remaining uncertainties requiring taxonomic treatment within the entire family: were identified among other aspects, at least several uncertainties in tribe Paliureae (including its monophyly) were detected. Furthermore, those doubts were likely to be resolved if this relatively poorly sampled tribe was investigated thoroughly with more genetic markers and species in further studies. Actually, this doubt in monophyly was confirmed in chapter three, where a larger molecular data set and additional taxa within the ziziphoid lineage was used. In this advanced phylogenetic reconstruction, the resurrected genus *Sarcomphalus* war identified as sister to *Colubrina* Rich. ex Brongn. (*inc. sed.*, ziziphoid lineage) and not within tribe Paliureae. In other tribes, such as in Gouanieae Reiss.

ex Endl (ziziphoid lineage), the phylogenetic relationships between several species-poor lineages remained unknown, for example, *Alvimiantha* Grey-Wilson, *Johnstonalia* Tortosa and *Pleuranthodes* Weberb. ex Engl. The latter genus was added in chapter three's reconstruction and confirmed within Gouanieae.

In chapter two, five monophyletic groups within former *Rhamnus* L. s.l. were identifi: *Frangula* Mill., *R.* sect. *Oreoherzogia* W. Vent, a new group comprising the North American *R.* sect. *Rhamnus*, *R.* sect. *Alaternus* Mill. and *R.* sect. *Rhamnus* s.s. The exclusion of *Frangula* from *Rhamnus* was proposed multiple times in history, last by Pool (2013), while also the re-inclusion as *R.* sect. *Frangula* was discussed frequently. This back and forth arose because *Frangula* and *R.* sect. *Rhamnus* differed in multiple traits, such as leaf venation, armed habits, and branching pattern. But the North American *Rhamnus* and *R.* sect. *Oreoherzogia* showed intermediate traits. Chapter two highlighted that the groups with intermediate traits formed a clade of their own, sister to *R.* sect. *Rhamnus* and *R.* sect. *Alaternus*, and apart from *Frangula*. Hence, all six North American species from *R.* sect. *Rhamnus* were excluded to keep *R.* sect. *Rhamnus* monophyletic. The six North American species were now first described as the new genus *Ventia* Hauenschild, *nom. superfl.*, but later changed in the corrigendum to *Endotropis* Raf., as one of the six species has already been lifted to generic rank, apart from *Rhamnus* by Rafinesque (1825). As Rafinesque's circumscription did not match the circumscription of the entire group, the genus descriptions and subordinate taxa were emended. Sister to *Endotropis*, *R.* sect. *Oreoherzogia* was resurrected to the genus *Atadinus* Raf. (also renamed in the corrigendum from *Oreoherzogia* W. Vent, *nom. superfl.*). This had already been proposed by Vent in 1960, including the same species, based on morphological characters. Furthermore, a few species were transferred from *Rhamnus* to *Atadinus* and *Frangula*, such as *Frangula borneensis* (Steenis) Hauenschild and *Atadinus sibthorpiana* (Roem. & Schult.) Hauenschild, as Rafinesque's concept of *Atadinus* only included *A. alpinus* (L.) Raf.

In chapter three, the phylogenetic and taxonomic inferences were used to perform temporal and biogeographic reconstructions, based upon the largest Rhamnaceae data set available to date. In this chapter, the focus was put on the ziziphoid lineage, as the key question for the ampelozizyphoids and rhamnoids (in prep.) differed from the questions framed for the ziziphoids. The early Upper Cretaceous was reconstructed as likely temporal origin of the most recent common ancestor (mrca) of all buckthorns, and Upper Cretaceous Africa and South America was reconstructed as most likely origin for the ziziphoid lineage. The key question raised in chapter three was if and how the break-up of the Gondwanan continent during the Mesozoic (including the Cretaceous) and the continental movements of the Gondwanan fragments during the Phanerozoic could have shaped the evolutionary processes in the ziziphoids. The biogeographic analyses illustrated vicariance events in the Upper Cretaceous and Paleocene, by taking Africa (and/or India) as raft to the northern hemisphere in the Paliureae s.s. (without *Sarcomphalus*) to Asia. Apart from these epochs, long-distance dispersal (LDD) was supported as the major driver towards the extant distribution pattern of biodiversity in the ziziphoids, such as the Cenozoic LDD events in tribes Colletieae Reiss. ex Endl. and Phyliceae Reiss. ex Endl. from Australia to South

America and Africa, respectively. Furthermore, the reconstructions indicated that the mrca of *Alphitonia* Reiss. ex Endl. and its close allies (*Alphitonia* s.l.) migrated with the northwards drifting Australia towards Southeast Asia.

In chapter four, the biogeographic history of the species-poor *Alphitonia* and related genera was analysed. In total, this group comprised two major lineages, one comprising *Alphitonia* and *Granitites* Rye, the other being *Emmenosperma* F. Muell. and *Jaffrea* H.C. Hopkins & Pillon. While *Granitites* and *Jaffrea* were southwestern Australian and New Caledonian endemics, respectively, *Alphitonia* and *Emmenosperma* were distributed in Australia, Southeast Asia and the Pacific Islands. This study supported the hypothesis that a common ancestor of the entire clade inhabited Australia in the Miocene, and gradual westward dispersal was detected in the Quaternary into Southeast Asia. Concerning the eastward dispersal into Polynesia, the reconstructed biogeographic scenarios were less clear, but nonetheless Australia was supported as the major source area. Finally, the analyses highlighted potential human-mediated dispersal of *Alphitonia ponderosa* Hillebr. from Western Polynesia to Hawaii.

Altogether, the four studies in Rhamnaceae presented here provide a major contribution to unravel the evolutionary processes in the history of the buckthorns that shaped its extant biogeography. My integrative approach combining molecular phylogenetic analyses, classical taxonomy, molecular dating analyses and biogeographic analyses provided a thorough workflow to solidly attack key questions related to biodiversity within lineages. And beyond Rhamnaceae, I highlighted that multiple biogeographic studies investigating the role of the Gondwanan break-up on evolutionary processes focused on in parts unfitting groups, for example closely related clades endemic to separate fragments of Gondwana which were too young to correlate divergence time estimates with intercontinental vicariance events, instead of widespread taxa that are old enough to have experienced the break-up.

ZUSAMMENFASSUNG

Die Ursachen dafür, dass die Biodiversität der Erde ungleich verteilt ist, sind vielfältig. In meiner Arbeit untersuchte ich die Prozesse, welche die Biodiversität der Kreuzdorngewächse (Rhamnaceae) beeinflussten. Diese Familie der Rosiden ist weltweit in den terrestrischen Lebensräumen beheimatet und besteht zum größten Teil aus Baum- und Straucharten, jedoch finden sich auch einige wenige Lianen und krautige Pflanzen. Kreuzdorngewächse findet man von Norwegen bis Patagonien, von den trockenen Wüsten Australiens bis hin zum Amazonas-Regenwald, und von Lebensräumen auf Meeresspiegelhöhe bis zu 3800 Meter über diesem im Himalaya. Obwohl die Familie als Ganzes kosmopolitisch verbreitet ist, sind einzelne Evolutionslinien innerhalb der Kreuzdorngewächse oft nur in einzelnen Teilen der Welt verbreitet, wie Australien und Neuseeland, Nordamerika oder Mittelmeerraum. Um zu untersuchen, wie es dazu kam, führte ich vier Studien durch. In den ersten Schritten kombinierte ich molekular-phylogenetische Analysen mit klassischer Taxonomie, um festzustellen, welche Gattungen der Kreuzdorngewächse tatsächlich als gültig angesehen werden konnten. Auf Seiten der Taxonomie konnte ich durch diese Herangehensweise Gattungen beschreiben, reaktivieren, sowie Synonyme und Neukombinationen benennen. Auf der phylogenetischen Seite konnte ich unterstützte Kladen hervorheben, welche in den folgenden Schritten als topologisches Grundgerüst genutzt werden konnten. In einem zweiten Schritt nutzte ich das taxonomische Vorwissen um ausgewählte fossile Arten der Kreuzdorngewächse auf dem Baum als zeitliche Vorabinformation zu den unterstützten Kladen zu platzieren, und führte eine molekulare Datierung eines Stammbaumes der Kreuzdorngewächse durch. Durch diese

Herangehensweise konnte ich den zeitlichen Ursprung der Familie auf die Kreidezeit bestimmen. Neben dem zeitlichen Ursprung konnte ich zudem Altersabschätzungen für einzelne Knoten innerhalb des Stammbaumes abschätzen, wie zum Beispiel den Ursprung der Ziziphoiden (Stamm und Krone). Diese Altersabschätzungen nutzte ich anschließend, um die biogeographische Geschichte der Ziziphoiden als Ganzes sowie des ziziphoiden Taxons *Alphitonia* und verwandte Arten zu erörtern.

Mit Ausnahme von wenigen Gattungen bestätigten meine phylogenetischen Hypothesen aus Kapitel eins die gültigen taxonomischen Konzepte innerhalb der Kreuzdorngewächse. In aller Kürze heißt dies, dass sich die Familie in drei getrennte Entwicklungslinien unterteilen lässt: die artenreichen Ziziphoiden (ungefähr zwei Drittel aller Arten), die artenreichen Rhamnoiden (ungefähr ein Drittel aller Arten), und die artenarmen Ampelozizyphoiden. Diese drei Entwicklungslinien unterteilen sich wiederum in drei Triben (bei Ampelozizyphoiden und Rhamnoiden), bzw. fünf Triben und einige Gattungen *incertae sedis* (in den Ziziphoiden). Es konnten jedoch einige Stellen im taxonomischen System aufzeigt werden, die Bearbeitung erforderten. So wurde die artenreiche Gattung *Ziziphus* Mill. (Tribus Paliureae Reiss. ex Endl., Ziziphoiden) in die Gattungen *Ziziphus sensu stricto, Sarcomphalus* P. Browne und *Pseudoziziphus* Hauenschild (Tribus Rhamneae Hook. f., Rhamnoiden) aufgeteilt. Des Weiteren wurden weitere Unsicherheiten im taxonomischen Konzept der Familie festgestellt, die eine Überarbeitung benötigten, wie zum Beispiel Zweifel an der Monophylie der Tribus Paliureae. Diese Unsicherheiten würden durch Analyse von weiteren genetischen Merkmalen und zusätzliche Arten innerhalb des Tribus ausgeräumt werden könnte. Tatsächlich war es später möglich mit genau diesen Mitteln in Kapitel drei diesen Zweifel an der Monophylie zu bestätigen. Durch eine erweiterten phylogenetische Rekonstruktion wurde die wiederaufgestellte Gattung *Sarcomphalus* außerhalb der Paliureae als Schwestergruppe zur Gattung *Colubrina* (*inc. sed*., Ziziphoiden) platziert. In anderen Triben, wie jene der Gouanieae Reiss. ex Endl. (Ziziphoiden), blieben die verwandtschaftlichen Beziehungen weiterhin unklar, zum Beispiel die Gattungen *Alvimiantha* Grey-Wilson, *Johnstonalia* Tortosa und *Pleuranthodes* Weberb. ex Engl. betreffend. Letztere Gattung konnte jedoch in Kapitel drei hinzugefügt und als den Gouanieae zugehörig bestätigt werden.

In Kapitel zwei wurden fünf monophyletische Gruppen in der ehemaligen Gattung *Rhamnus* L. s.l. (Rhamnoiden) identifiziert: *Frangula* Mill., *R.* sect. *Oreoherzogia* W. Vent, eine neue Klade mit den nordamerikanischen Arten der Sektion *R.* sect. *Rhamnus*, *R.* sect. *Alaternus* Mill. und *R.* sect. *Rhamnus* s.s. Das Abtrennen von *Frangula* aus *Rhamnus* wurde bereits mehrmals in der taxonomischen Geschichte der Gattung vollführt, zuletzt durch Pool (2013). Ebenso wurde die Gattung des Öfteren wieder in *Rhamnus* integriert. Dieses Hin und Her ist dadurch zu erklären, dass sich die Sektionen *R.* sect. *Frangula* und *R.* sect. *Rhamnus* zwar in vielen äußerlichen Merkmalen unterscheiden, wie der Blattaderung, Bedornung, und der Verzweigung. Die nordamerikanischen *Rhamnus*-Arten, sowie die Sektion *R.* sect. *Oreoherzogia* zeigen jedoch Merkmalsausprägungen, die oft zwischen beiden Gruppen liegen. In Kapitel zwei wurde aufgezeigt, dass eben jene dazwischenliegenden Gruppen tatsächlich in eigenen Kladen zu finden sind, die im

Gesamten eine Schwestergruppe zu *R.* sect. *Alaternus* und *R.* sect. *Rhamnus* bilden. Folglich wurden die nordamerikanischen Arten aus *Rhamnus* exkludiert, was die Gattung *Rhamnus* wieder monophyletisch machte. Diese exkludierten Arten wurden zunächst in der neuen Gattung *Ventia* Hauenschild, *nom. superfl.* Neu beschrieben und in einem Korrigendum schließlich in *Endotropis* Raf. umbenannt, da eine der Arten bereits in einer eigenen Gattung außerhalb von *Rhamnus* beschrieben worden war (Rafinesque 1825). Da Rafinesques Beschreibung der Gattung nur eine Art umfasste, wurde diese im Korrigendum auf die anderen nordamerikanischen Arten ausgeweitet. Als Schwester der neuen Gattung wurde die Sektion *R.* sect. *Oreoherzogia* als eigene Gattung (*Atadinus* Raf., ebenfalls umbenannt im Korrigendum) beschreiben, wie dies bereits 1960 durch Vent auf Basis morphologischer Merkmale vorgeschlagen wurde. Zuletzt wurden einige Arten in die Gattungen *Frangula* und *Atadinus,* wie z.B. *Frangula borneensis* (Steenis) Hauenschild und *Atadinus sibthorpiana* (Roem. Ex Schult.) Hauenschild transferiert und kombiniert, da dies nomenklatorisch nötig war.

In Kapitel drei wurden die phylogenetischen und taxonomischen Erkenntnisse aus den ersten beiden Kapiteln genutzt, um zeitliche und biogeographische Hypothesen zu rekonstruieren. Dazu wurde der bisher größte molekulare Datensatz verwendet. In diesem Kapitel wurde der Blick ausschließlich auf die Ziziphoiden gerichtet, vor allem deshalb, weil die Hauptfragestellung der anderen beiden Entwicklungslinien eine andere war. Die Analysen legten die frühe Obere Kreidezeit als Serie des jüngsten gemeinsamen Vorfahrens aller Kreuzdorngewächse nahe, und die afrikanischen und südamerikanischen Landmassen der Oberen Kreidezeit als Lebensraum der ersten Ziziphoiden. Die zentrale Frage der Studie war, ob und in welchem Umfang das Auseinanderbrechen des Superkontinents Gondwana im Mesozoikum und die Kontinentalplattenbewegungen des Phanerozoikums die evolutionären Prozesse der Ziziphoiden gestalteten. Die biogeographischen Analysen zeigten Vikarianz-Ereignisse der Oberen Kreidezeit und des frühen Paläogen auf und legten z.B. die Funktion Afrikas (und/oder Indiens) als Vektor der Paliureae s.s. (ohne *Sarcomphalus*) Richtung Asien nahe. Außerhalb dieser Zeitabschnitte konnten die disjunkten Areale der Colletieae Reiss. ex Endl. und anderen Kladen nur durch Fernausbreitung im Känozoikum erklärt werden. Zuletzt legten die Untersuchungen in Kapitel drei den Lebensraum des jüngsten gemeinsamen Vorfahren der Klade *Alphitonia* Reiss. ex Endl. s.l. in Australien nahe, welches sich im Känozoikum Richtung Asien bewegte.

Eben jene Klade um die Gattung *Alphitonia* und ihrer nahen Verwandten *Granitites* Rye, *Emmenosperma* F. Muell. und *Jaffrea* H.C. Hopkins & Pillon wurde in der biogeographischen und phylogenetischen Studie in Kapitel 4 untersucht. Während *Granitites* und *Jaffrea* auf Lebensräume in Südwestaustralien und Neukaledonien beschränkt waren, fand man *Alphitonia* und *Emmenosperma* Arten in Australien, Südostasien und dem polynesischen Raum vor. Diese Studie brachte Unterstützung für die Hypothese, dass Vorfahren der Gruppe im miozänen Australien lebten und sich erst in jüngster Vergangenheit (Quartär) graduell westwärts nach Asien und ostwärts in den Pazifischen Raum ausbreiteten, auch wenn der Weg nach Osten nicht vollständig rekonstruiert werden konnte. Zuletzt wurden die Möglichkeit der Einschleppung von *A. ponderosa* Hillebr. oder deren Vorfahren durch frühe polynesische Kulturen nach Hawaii aufgezeigt.

Zusammenfassend stellen die vier vorgelegten Arbeiten einen wichtigen Beitrag dar, die evolutionären Prozesse innerhalb der Kreuzdorngewächse und deren biogeographische Geschichte aufzuklären. Zudem stellt mein integrativer Ansatz, der u.a. molekularphylogenetische Methoden, klassische Taxonomie, molekulare Datierung und Biogeographie verbindet, auch einen funktionsfähigen Leitfaden für aussagekräftige Antworten auf viele biodiversitätsbezogene Fragen innerhalb genetischer Gruppen dar. Außerdem legen meine Studien dar, dass viele biogeographischen Studien, die sich mit der Frage, ob und in welcher Weise Vikarianz-Ereignisse durch das Auseinanderbrechen Gondwanas Einfluss auf die Geschichte von Organismen hatte, oft unpassende Taxe wählten: Taxa, welche zwar in disjunkten Arealen auf verschiedenen Fragmenten des einzigen Superkontinents verbreitet waren, deren jüngsten gemeinsame Vorfahren jedoch schlichtweg nicht alt genug waren, um das Auseinanderbrechen Gondwanas erlebt zu haben.

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Dissertation

213 pages, 512 references, 10 figures, 5 tables

My thesis combines taxonomy, phylogenetics, divergence time estimations and ancestral area reconstructions, nomenclature, and other methods to shed light on the evolutionary processes that shaped the extant biodiversity of the buckthorn family (Rhamnaceae Juss.). In a first step, this thesis provides a major contribution to the taxonomic assessment and arrangement within the buckthorns. By describing the new genus *Pseudoziziphus* Hauenschild, and resurrecting four genera, this thesis highlights and compensates underestimated diversity. Simultaneously, it provides a phylogenetic framework based on the largest molecular data set in the family so far. In a second step, the taxonomic and phylogenetic framework was used to reconstruct solid divergence time estimates and ancestral area estimates for Rhamnaceae, with a focus on the ziziphoid lineage, and the genus complex *Alphitonia* Reissek ex Endl. *sensu lato*. By this, it was possible to unravel evolutionary processes within the history of Rhamnaceae and provide a biogeographic scenario for the ziziphoid lineages, including *Alphitonia* s.l.

GENERAL INTRODUCTION

This dissertation focussed on one aspect of biodiversity. When we scientists assessed the world's biodiversity, we collected data on presence (rarely on absence) of taxa and in sum on the taxonomic composition of areas. The entirety of the hereby recognized presence of taxa and the taxonomic composition of regions resembled contemporary taxonomic diversity. When we focused on regions, we quickly realized that neither quantity, nor identity of taxa were distributed evenly (Myers et al., 2000; Whittaker, 1960). The given mixture and variability of species and species compositions was addressed as biodiversity. Also, biodiversity was unevenly distributed on earth, comprising areas of higher and lower biodiversity, was it taxonomic, genetic, or functional. Those peaks and troughs were not to be confused with biodiversity hot- and coldspots, which had to fulfil definite criteria besides high biodiversity. For example, the degree of species endemism in the target area and the degree of threat to extinction faced by the occurring species were criteria for biodiversity hotspots (Mittermeier et al., 2011; Myers et al., 2000). Aside of those diversity concepts stood the diversity within phylogenetic groups and the observed taxonomic, genetic, and ecological variability among those lineages. In my study, I focused on this aspect of diversity.

Biodiversity within lineages – Focusing on the diversity within phylogenetic groups provided advantages in addressing certain taxon-related questions, while other diversity concepts, such as the taxonomic composition of areas and biodiversity hotspots faced two limitations for the scope of my thesis. As a first advantage, focusing on biodiversity within a phylogenic group was not limited to a particular region. A biodiversity hotspot might correlate with high species numbers and taxonomic variety in one family, yet be dominantly inhabited by one single species of another family (Mittermeier et al., 2011). In contrast, when focusing on phylogenetic lineages, the regional centres containing high species numbers were given by the taxon itself. The second major advantage was given by the taxonomic level. Biodiversity hotspots and species composition were by definition linked to the taxonomic rank of species. A general biodiversity assessments could also be based on different levels of taxonomic hierarchy, but it required comparability among multiple, usually phylogenetically distinct taxa. However, there was neither an equal hierarchic taxonomic level among all biological taxa, nor among or within subgroups, like angiosperms, botanical orders, and families. The reason for this inequality was funded in two aspects: the lack of an entirely consistent and applicable species concept (Freudenstein et al., 2016), and the lack of consistent criteria for derived ranks (Konstantinidis and Stackebrandt, 2013; Zhao et al., 2016), although in both cases many were proposed. Hence, focusing on only one phylogenetic group, such as a single family, reduced the impact of hardly comparable taxonomic ranks. However, as a consequence, many of such phylogenetic case studies were needed to address questions on general biologic patterns, for example, how entire regions became populated by the organisms that can be observed nowadays (Favre et al., 2015). To put it strait, the chosen approach was a necessary trade-off, and within this introduction, I highlighted why I came to a decision in favour of the lineage-driven over the region driven approach. Yet, before focussing on this trade off, another limitation of both approaches had to be addressed: The species and their distributions observed nowadays were the results of a process throughout the history of living organisms (Cox et al., 2000; Lomolino et al., 2006). Likewise, the taxa of a clade, which were described based on living organisms found by scientists around the world, merely represented the tips of an ongoing process, dating back to the ancestor of this particular group, and beyond. Those extant species and their extant distributions were the complex result of dispersal, vicariance, migration, speciation, and extinction. Hence, the evolutionary success of a taxon largely depended on the dispersal and establishment ability and the ability to persist, i.e. to avoid extinction (Hoorn et al., 2013; Lomolino et al., 2006).

Biogeography – Parameters like speciation and extinction, as well as the capability of dispersal and migration were important to get insights into the distributional history of both, a taxon and the taxonomic composition of a region. Such studies that intended to unravel this history entered the field of biogeography. Historically, scientists documented the extant distribution of taxa and their closest relatives, and postulated hypotheses on how those taxa invaded the areas of current distribution (Lomolino et al., 2006). As biogeography was often linked to a large temporal scale, paleo data was of high value (Magallón, 2014). For example, fossil data provided information on extinct taxa, their morphology, and former areas of distribution. Paleoclimatic data gave insights in the environment of the ancestral taxa and a potential study region, or it might explain the absence of a taxon in a region nowadays occupied by this taxon (Burge and Manchester, 2008; Manchester, 1999; Palazzesi et al., 2014). Hence, paleo data did not only help to explain how a taxon invaded an area, it also helped to unravel why parts of its distributional range faced extinction events over time (Burge and Manchester, 2008). Biogeographic hypotheses were proposed straight-forward in taxa of steady distribution and regions formerly connected, or separated by fluctuating sea levels. But closely related taxa occurring on disjunct areas puzzled many scientists. The hypotheses explaining such disjunct patterns included long-distance dispersal (LDD) via vectors, dispersal via intercontinental land bridges, and plate tectonics which allowed vicariance as explanation (Cox et al., 2000; Lomolino et al., 2006). In the last years however, scientist illustrated that LDD might have played a huge role in many, especially botanic taxa, and that LDD often remained as most plausible explanation, after tectonically driven vicariance events were ruled out (Gillespie et al., 2012; Keppel et al., 2008; Martín-Bravo and Daniel, 2016; Renner et al., 2010; Schaefer and Renner, 2010; Shen et al., 2016).

Those more recent hypotheses were developed by the combination of spatial and temporal reconstructions within taxa. In its first step, this approach combined a phylogenetic hypotheses, based on molecular genetic data and a substitution model, with time-calibrated constraints, a tree model, and a molecular clock model to reconstruct dated phylogenies (dos Reis and Yang, 2011, 2013; Drummond and Rambaut, 2007; Drummond and Suchard, 2010; Heath et al., 2014; Sanderson, 1997, 2002; Sanderson et al., 2004; Warnock et al., 2012; Yang and Rannala, 2006). In a second step, those chronograms were used as topological and temporal priors for character mapping or model-based ancestral area reconstructions (Matzke, 2014; Ree et al., 2005; Ree and Smith, 2008; Ronquist, 1997; Yu et al., 2010). In the last decade, model based approaches proved to be highly valuable for developing biogeographic hypotheses, such as dispersal scenarios and/or reconstructions of areas of origin for extant taxa and their ancestors (Matzke, 2014; Ree and Smith, 2008; Ronquist, 1997). Yet, we had to keep in mind that none of the input sources is absolute data (e.g. a species or a phylogenetic hypothesis) and none of the deductions is logical (e.g. extrapolation or classification). Thus we had to work with models and uncertainties (e.g. mathematical distributions, constraints, and tree and clock models). Consequently, this approach incorporated *a priori* uncertainties and the challenge to minimize those

(Magallón, 2014; Warnock et al., 2015; Warnock et al., 2012). While some uncertainties were of statistical nature and may be mathematically addressed, others proposed more complex challenges. The most important of the latter group were both related to taxonomy: taxon sampling and its impact of the underlying tree topology and the distributional data on the one hand, and the attribution of fossil taxa to extant clades on the other hand (Magallón, 2014). Both limitations were crucial in the light of the initial trade off mentioned above. A correct assessment of taxonomic diversity required vast knowledge of morphological (and potentially anatomical and chemical) traits, which were differently relevant in different taxonomic groups. Furthermore, this capability to use morphologic and anatomic traits to differentiate between extent taxa was also relevant to attribute fossils. This knowledge could only be generated by experience over years. Hence I decided to focus on one family and visited multiple herbaria to achieve this expertise in this family. This allowed me identify and assess the relevant traits of this family, and consequently to minimize the two greatest sources of uncertainty: incomplete taxon sampling and false fossil attribution. In the following, I presented my workflow and highlight in which steps those two uncertainties intervene.

Workflow – This workflow allowed gaining insights into the biogeographic history of one or multiple taxa, while at the same time minimizing the above mentioned uncertainties. In short, I needed to acquire data, reconstruct solid phylogenetic hypotheses, reconstruct reliable node age estimates and ancestral areas, and then finally interpret the dispersal scenarios, based on the previous hypotheses. Data acquisition started by examining specimens and collections. I (re-)determined taxa and collected leave tissue from herbarium vouchers of multiple herbaria and botanical gardens, including Cairns (ATH), Canberra (CANB), Florence (FI), Halle (HAL), Jerusalem

(HUJ), Jena (JE), the Naturalis Herbarium Leiden (L), Leipzig (LZ), Leipzig Botanical Garden, Melbourne (MEL), Sydney (NSW), and Ulm (ULM). Furthermore, additional data was added to my data set via Gerardo A. Salazar from Mexico City (MEXU), Sabine Matuszak from Frankfurt (FR), Frankfurt Botanic Gardens, and Singapore (SING), Jana Ebersbach from the Munich herbarium (M), and data available on GenBank (Benson et al., 2012). Last, I added three specimens now located in Leipzig (LZ) collected by Sabine Matuszak during a fieldtrip in Yunnan, China. At this stage, my study already had by design two limitations: a) it potentially missed new or undescribed species, subspecies and varieties, and b) redetermination of GenBank data was limited by accessibility of the vouchers. I reduced the impact of those limitations by not only incorporating existing keys to Rhamnaceae (Bramwell and Bramwell, 1994; Chen and Schirarend, 2008; Grubov, 1949; Heywood, 1974; Johnston, 1971a, 1971b, 1974; Johnston and Johnston, 1978; Medan and Schirarend, 2004; Press and Short, 1997; Vent, 1960, 1962), but I also included the original descriptions and type material of buckthorn taxa for identification of plants, which were in large parts provided by The Biodiversity Heritage Library (Gwinn and Rinaldo, 2009), JSTOR (JSTOR, 1994), and the German National Library (Leipzig, Germany). Furthermore, wherever accessions based only on GenBank sequences with inaccessible vouchers created conflicts between the reconstructed phylogeny and the most recent taxonomic attribution, I added additional sequences of the same species from vouchers I examined.

In the next step, I needed to reconstruct robust phylogenetic hypotheses that provided a topology and reliable branch lengths. Although it was possible to reconstruct a phylogenetic hypothesis simultaneously to temporal reconstructions, an *a priori* phylogeny was needed to add constraints. At this point, the first advantage of the chosen study taxon, the buckthorn family (Rhamnaceae Juss.) became evident: recent preliminary studies, including at least in parts molecular approaches. Up to the beginning on my work on Rhamnaceae, three publications provided family-wide phylogenetic hypotheses of Rhamnaceae (Richardson et al., 2000a; Richardson et al., 2000b), and one of these studies provided a dated phylogeny of Rhamnaceae (Richardson et al., 2004). Other (younger) studies, however, highlighted problems concerning the monophyly of certain Rhamnaceae taxa (Bolmgren and Oxelman, 2004; Islam and Simmons, 2006) and rejected the temporal hypothesis of Richardson et al. (2004) (Correa et al., 2010). Consequently, I reconstructed my own phylogenetic hypothesis based on FastTree-Like analyses, Maximum Likelihood analyses and Bayesian estimates, as presented in chapters 1 and 2. Furthermore, I combined the phylogenetic hypothesis and the gathered taxonomic and nomenclatural data of Rhamnaceae to revise the most urgent taxonomic and topological issues addressed by previous studies (Islam and Simmons, 2006; Pool, 2013; Vent, 1962).

Subsequently, a set of paleo data needed to be acquired. This potentially included paleobotanical data such as fossil ages, as well as potential paleogeological data, such as the chronostratigraphic timing of island uplifts. An island cannot be inhabited by terrestrial taxa before its uplift; hence, emerging islands could be used as maximum age priors for extant taxa on this particular island (yet the taxa themselves might have been present on other landmasses). Contrary, a fossil attributed to a taxon illustrated its presence at a certain time and place, and thus could be used as a minimum age for a certain taxon (Lomolino et al., 2006). By summing up paleo data and linking it to multiple nodes of a topology, we got a picture of how old certain internal clades were. This fossil-constraining of nodes was one major reason for the necessity of a robust phylogenetic hypothesis, as varying topologies resulted in drastically different node age (and clade age) interpretations (Magallón, 2014; Renner and Schaefer, 2016). This effect was increased when such constraints were used as temporal constraints, i.e. for molecular dating. In this field, multiple studies highlighted the importance of correct setting of constraints, as they are one major source of error and bias in the approach of molecular dating (Clarke et al., 2011; Magallón, 2014; Warnock et al., 2015; Warnock et al., 2012). Consequently, this error needed to be minimized, starting with a substantial revision of the given fossil and paleogeological data. As most fossils were published and determined by paleobotanists, this may look redundant, yet paleo-science cannot be transferred directly into modern biogeography. A first reason was given by the nomenclature palaeontologists use, which, in the case of paleobotany, was treated independently in the International Code of Nomenclature for algae, fungi, and plants (McNeill et al., 2012). Hence, a fossil taxon did not necessarily resemble the corresponding rank in extent fossils, and maybe, even more important, the taxonomy of fossil taxa did not necessarily resemble the accepted taxonomy of extant species (Forey et al., 2004). For example, a fossil attributed to †*Rhamnus* Linnaeus, such as †*Rhamnus cleburni* Lesquereux, expressed leaves of the rhamnoid type, but was not attributed to the extant genus *Rhamnus* L. (Johnson, 2002; Peppe et al., 2007). If fossil-based calibration was performed only by matching nomenclature, the calibrated constraints were potentially simply wrong. Consequently, there was only one way to perform fossil-based molecular dating approaches: the evaluation of matching points between fossil and extant taxa. This need highlighted the strength of a phylogeny based approach within one family over a region-based approach including many families, as false attributions were likely promoted when the specific taxonomic expertise would decrease. Furthermore, the value the very fossil-rich Rhamnaceae was illustrated when fossil taxa had to be incorporated (Correa et al., 2010; Richardson et al., 2004).

Once the paleo data was selected, and the corresponding clades were identified, calibrated constraints needed to be set. A first crucial step was the placement of the fossil in the topology. In most cases, identifying the exact extant sister lineage to the fossil taxon was not possible, and thus I had to incorporate the possibility of the fossil being sister to all extant taxa of the corresponding clade. Hence, the fossil could be used to constrain the ancestral node of all taxa which potentially sister the fossil taxon, i.e. stem calibration (Magallón, 2014). Yet, there were multiple other means of setting calibrated constraints, including the insertion of fossil lineages as branches (Nauheimer et al., 2012b), and advanced prior functions exceeding simple uniform minimum (or maximum) calibrations (Drummond and Rambaut, 2007). Such advanced prior functions included normal distributions around a centre, allowing the model for example the incorporation of the potential timeframe in which a certain fossil taxon existed, or a lognormal prior, giving higher chances in a certain timeframe, yet not neglecting potential older ages. However, it was clear that such priors needed justification and evaluation (Warnock et al., 2015), as presented in chapter 3 and 4. Naturally, a fossil-based molecular dating approach was only possible in groups represented in the fossil records. In all other cases, scientist used outgroups and secondary calibration approaches, yet the latter were criticized a lot due to its inaccuracy of reconstructed node age estimates (Magallón, 2014; Muellner-Riehl et al., 2016; Schenk, 2016). Aside of setting fossil-calibrated constraints, a clock model, a tree prior, and a substitution model was selected. Depending on the underlying molecular data (e.g. if the sequence alignment contained gene coding regions, or rather spacer data), best fitting substitution models were identified. The selection of the tree prior was most dependent on the sampling, i.e. its completeness and the estimation of extinction-frequencies. Finally, strict clock behaviour needed to be tested, and the impact of priors in relation to the sequence data on the molecular clock needed to be evaluated (Magallón, 2014), before a clock model was selected. After the selection of suitable models, the ancestral ages of all nodes were reconstructed, and presented by a calibrated ultrametric tree (chronophylogram). This tree was then used as input for subsequent biogeographic analyses.

State-of-the-art biogeographic analyses comprised a plethora of analyses, including cladistic approaches, character mapping approaches, and model based approaches (Lomolino et al., 2006; Matzke, 2013; Matzke, 2014). In my thesis, I focussed on the latter approaches, and in detail on ancestral area reconstructions based on two major models: dispersal extinction cladogenesis (DEC) and dispersal vicariance (DIVA) analyses as implemented in BioGeoBEARS (Matzke, 2013; Ree and Smith, 2008; Ronquist, 1997). Both models were frequently in use by biogeographers in the last years (Grudinski et al., 2014; Matuszak et al., 2016; Nauheimer et al., 2012a; Yesson et al., 2009). Furthermore, the models have been discussed, and improved over the last years (Crisp et al., 2011; Kodandaramaiah, 2010; Matzke, 2013; Matzke, 2014; Nylander et al., 2008; Ronquist and Sanmartín, 2011; Yu et al., 2010; Yu et al., 2015). Using those models, biogeographers could unravel the geo-temporal evolutionary history of multiple taxa and regions, such as Boraginales, southern beeches, and gecko lizards (Cook and Crisp, 2005; Gamble et al., 2008; Luebert et al., 2017), and the related key questions what role vicariance and dispersal played in shaping the taxa’s extant distributions.

The role of the Gondwanan break-up towards the distribution of extend taxa - One of the most prominent events driving potential vicariance processes was the breakup of the Gondwanan supercontinent. Originally, Gondwana comprised the continental plates of Africa, Arabia, Antarctica, Australia, India, Madagascar, Zealandia, South America, and other, smaller fragments, such as Falkland. While the initial breakup was earlier, the gradual events since 130 million years are of special interest to botanists, as they fall in the age of angiosperms (Bell et al., 2005, 2010; Magallón and Castillo, 2009; Magallón et al., 2015; Soltis et al., 2008). In the Lower Cretaceous, the separation of the major fragments was initiated, yet only partially progressed (Seton et al., 2012). Within Upper Cretaceous, Africa (and Arabia), India and Madagascar separated further from the Antarctic centre of the Gondwanan remnants (Lomolino et al., 2006; McLoughlin, 2001; Metcalfe, 1996; Seton et al., 2012). The African-South American and the Indian-Madagascar separation was just initiated (Seton et al., 2012). Consequently, an organism could still migrate from the Australian fragment via Antarctica and South America into Africa, yet a potential route did not imply an actual migration. Up to the end of the Maastrichtian, Africa (and Arabia), India and Madagascar became surrounded by water bodies, and the separation of Australia, Zealandia, and South America from the Antarctic Gondwanan remnant was initiated. During the Paleogene, the opened gaps were enlarged, and finally, new contacts were achieved. Those contacts included the African contact with Eurasia, a migration of Africa and Madagascar towards each other, the collision of the Indian plate with Asia, the re-closure of the Isthmus of Panama, and, during the middle Cenozoic, the Australian contact with Southeast Asia. The Gondwanan break-up was finalized by the division of Arabia from

Africa, which started during the early Miocene (Bacon et al., 2015; Favre et al., 2015; Renner, 2016; Seton et al., 2012).

Fossil evidence supporting Gondwanan vicariance events was sparse, yet present in few taxa distributed throughout Gondwanan remnants, such as †*Glossopteris* Brongniart. fossils attributed to †*Glossopteris* were for example found in on Antarctica, Australia, Chile, and Norfolk Island (Cooke, 2016; Tewari et al., 2015), and the taxon was treated as index fossil of the Permian Gondwanan flora. Other support was illustrated by the use of ancestral area reconstructions, as shown for example in gecko lizards and Boraginales Juss. ex Bercht. & J. Presl (Gamble et al., 2008; Luebert et al., 2017). Yet, naturally vicariance patterns were blurred by younger dispersal and extinction events (Waters and Craw, 2006). Hence, current advanced methods incorporated multiple biogeographic mechanisms, including vicariance, dispersal, extinction, founder events, and potentially migration. Those methods included DEC, DIVA, and comparable models included e.g. in the BioGeoBEARS package (Matzke, 2012, 2013; Matzke, 2014). As mentioned above, a clear trend from vicariance to younger LDD events and/or alternative, younger routes alongside Laurasian routes, is also visible in studies of Gondwanan distributed taxa (Davis et al., 2002; Pirie et al., 2015; Renner et al., 2010). Often, this was due to the fact that younger ages than the epochs comprising the Gondwanan break-up exclude vicariance driven by this fragmentation, while ages within the time frame of do not exclude LDD. Consequently, a sufficiently old study taxon had to be selected, in order to test the impact of vicariance vs. LDD in the context of Gondwanan break-up events.

The study taxon - The buckthorn family (Rhamnaceae Juss.) was a suitable model organism to tackle biogeographic questions related to Paleogene vicariance, such as the

Gondwanan break-up. It comprised at least 900 species among three major lineages: the rhamnoids, the ampelozizyphoids and the ziziphoids (Medan and Schirarend, 2004; Richardson et al., 2000b). In total, Rhamnaceae comprised 11 tribes and 60+ genera. The buckthorns were cosmopolitans, distributed on all continents, from the Antarctic Islands throughout the tropics to as high latitudes as Canada, Scandinavia and Russia (Chen and Schirarend, 2008; Medan and Schirarend, 2004). The first major advantage was given by the age of Rhamnaceae. Although, when starting the work on the thesis, no solid temporal reconstruction was available for Rhamnaceae, indications of the group at least dating back to the Upper Cretaceous were given by the fossil record itself (Calvillo-Canadell and Cevallos-Ferriz, 2007; Richardson et al., 2004). Second, although Rhamnaceae as a whole were cosmopolitan, certain tribes showed high endemism to Southern Hemisphere continents, such as Phyliceae Reissek ex. Endl. in Africa (Richardson et al., 2001), the Colletieae Reissek ex. Endl. predominantly in South America (Aagesen, 1999), or Pomaderreae Reissek ex. Endl. in Australia (Ladiges et al., 2005). Consequently, prior to the start of my work, strong indications that Rhamnaceae were old enough to have experienced the Gondwanan break-up and the family comprised disjunct distributions among the Gondwanan fragments, were demonstrated.

A second advantage of Rhamnaceae for molecular dating was the tremendous amount of fossils, dating back to the Cretaceous (Behrensmeyer and Turner, 2013; Spicer et al., 2002). This included *Paliurus*-like fruits (Fig. 1A), which were unique in Rhamnaceae and the angiosperms (Burge and Manchester, 2008; Chen et al., 2017; Correa et al., 2010; Li et al., 2014; Schirarend and Olabi, 1994). Those fruits were attributed to two fossil genera, †*Archaeopaliurus* Correa, Manchester, Jaramillo & Gutierrez and

†*Paliurus* P. Miller and had been, in parts, used for molecular dating (Magallón et al., 2015; Onstein et al., 2015; Onstein and Linder, 2016). †*Paliurus* fossils were found in Northern Hemisphere sites throughout the Quaternary, the Neogene and major parts of the Paleogene, as well as Upper Cretaceous sites from India, the latter which was positioned in the Southern Hemisphere during those epochs. Other well-documented fossilized fruits and pyrenes included e.g. the genus (†)*Frangula* P. Miller. Those fossils, dating back to the Oligocene, were in parts attributed to extant species or species complexes, such as *Frangula alnus* s.l. Mill. to †*Frangula alnus* and *F. californica* s.l. (Eschsch.) A. Gray to †*Frangula californica*. Besides fruits attributed to numerous other Rhamnaceae lineages and genera (Czaja, 2006; Liu et al., 2015; Singh et al., 2010), the fossil record also comprised leaves (de Leon et al., 1998; Gregor, 1977; MacGinitie, 1953; Peppe et al., 2007; Wang, 2015), wood (Suzuki, 1982; Wheeler and Meyer, 2012), flowers (Chambers and Poinar, 2014; Correa et al., 2010; Millan and Crepet, 2014), and pollen (Menke, 1976; Moe, 1984; Schirarend, 1996). Consequently, while many studies only relied on one to few internal or even external fossils for molecular dating, Rhamnaceae comprised attributable fossils to multiple lineages (Fig 1B).

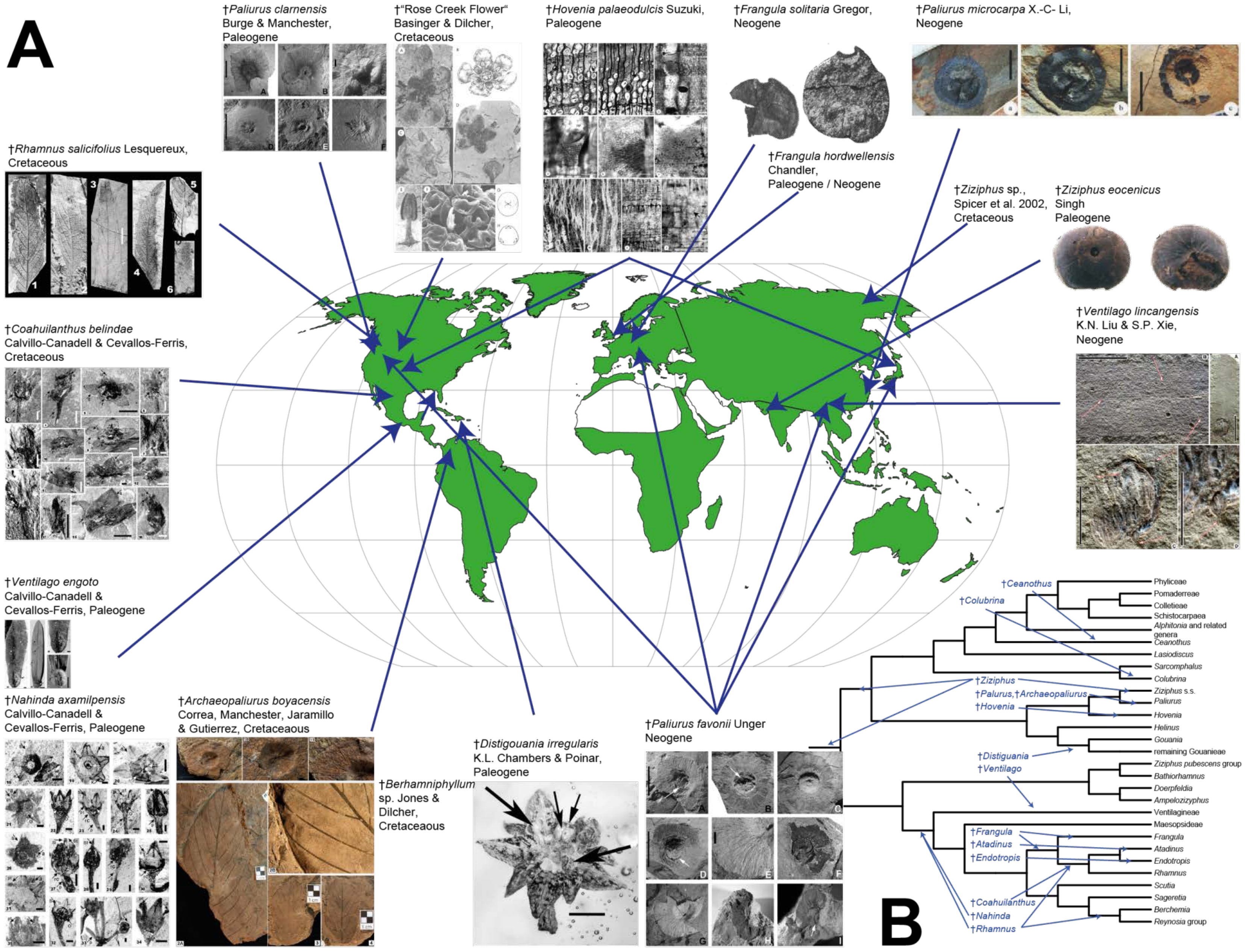
A
†Rhamnus salicifolius Lesquereux,
Cretaceous
1
3
4
5
6
†Paliurus clarnensis
Burge & Manchester,
Paleogene
A
B
C
D
E
F
†"Rose Creek Flower"
Basinger & Dilcher,
Cretaceous
†Hovenia palaeodulcis Suzuki,
Paleogene
†Frangula solitaria Gregor,
Neogene
†Frangula hordwellensis
Chandler,
Paleogene / Neogene
†Paliurus microcarpa X.-C- Li,
Neogene
a
b
c
†Ziziphus sp.,
Spicer et al. 2002,
Cretaceous
†Ziziphus eocenicus
Singh
Paleogene
†Ventilago lincangensis
K.N. Liu & S.P. Xie,
Neogene
†Coahuilanthus belindae
Calvillo-Canadell & Cevallos-Ferris,
Cretaceous
†Ventilago engoto
Calvillo-Canadell &
Cevallos-Ferris, Paleogene
†Nahinda axamilpensis
Calvillo-Canadell &
Cevallos-Ferris, Paleogene
†Archaeopaliurus boyacensis
Correa, Manchester, Jaramillo
& Gutierrez, Cretaceaous
1 cm
†Berhamniphyllum
sp. Jones &
Dilcher,
Cretaceaous
†Distigouania irregularis
K.L. Chambers & Poinar,
Paleogene
†Paliurus favonii Unger
Neogene
A
B
C
D
E
F
G
H
I
B
†Ceanothus
†Colubrina
†Ziziphus
†Paliurus,†Archaeopaliurus
†Hovenia
†Distiguania
†Ventilago
†Frangula
†Atadinus
†Endotropis
†Coahuilanthus
†Nahinda
†Rhamnus
Phyliceae
Pomaderreae
Colletieae
Schistocarpaea
Alphitonia and related genera
Ceanothus
Lasiodiscus
Sarcomphalus
Colubrina
Ziziphus s.s.
Paliurus
Hovenia
Helinus
Gouania
remaining Gouanieae
Ziziphus pubescens group
Bathiorhamnus
Doerpfeldia
Ampelozizyphus
Ventilagineae
Maesopsideae
Frangula
Atadinus
Endotropis
Rhamnus
Scutia
Sageretia
Berchemia
Reynosia group

Fig. 1: **A**, Approximate locality and chronostratigraphic system of selected fossil taxa related to the extant buckthorn family (Rhamnaceae Juss.). The map is modified from Hauenschild et al. (submitted). Fossil illustrations, locality data, and age of the fossils are taken and modified from original publications and revisions (Basinger and Dilcher, 1984; Burge and Manchester, 2008; Calvillo-Canadell and Cevallos-Ferriz, 2007; Chambers and Poinar, 2014; Correa et al., 2010; Gregor, 1977; Li et al., 2014; Liu et al., 2015; Peppe et al., 2007; Singh et al., 2010; Spicer et al., 2002; Suzuki, 1982; Wheeler and Meyer, 2012). **B**, Potential attribution of fossil genera or taxa attributed to fossil genera on a hypothetical cladogram of the buckthorn family after Hauenschild et al. (submitted).

A third criterion favouring Rhamnaceae as biogeographic model was related to taxonomy (Medan and Schirarend, 2004). At the starting point of my thesis, most genera were the target of recent phylogenetic studies. Hence, many Rhamnaceae taxa were sequenced for multiple markers, and a family-wide phylogeny was available (Richardson et al., 2000a; Richardson et al., 2000b). Subsequently, taxonomic and systematic revisions were performed, usually targeting subordinate Rhamnaceae taxa (e.g. tribes, genera, and species complexes), and often included molecular phylogenies. In detail, tribes Colletieae (Aagesen, 1999; Aagesen et al., 2005; Kellermann et al., 2005a; Tortosa et al., 1996), Gouanieae Reissek ex. Endl. (Buerki et al., 2011; Tortosa, 2005), Phyliceae (Richardson et al., 2001), Pomaderreae (Kellermann et al., 2007; Kellermann and Udovicic, 2008; Kellermann et al., 2005b), and the genera *Alphitonia* s.l. Reissek ex. Endl. (Bean, 2010; Fay et al., 2001; Hopkins et al., 2015), *Bathiorhamnus* Capuron (Callmander et al., 2008), *Ceanothus* L. (Burge et al., 2011;

Hardig et al., 2000), *Paliurus* Mill. (Burge and Manchester, 2008; Chen et al., 2017; Li et al., 2014), and *Ziziphus* Mill. (Islam and Simmons, 2006) were revised either prior to the beginning or during my work on Rhamnaceae. Hence, only a handful of groups had remained unrevised, the most prominent ones being tribe Rhamneae, of which only a small subset had been studied (Bolmgren and Oxelman, 2004; Pool, 2013), the species poor Ventilagineae Hook. f., and the genera *inc. sed. Colubrina* Rich. ex. Brongn..

A last advantage for biogeographic analyses was the robust phylogenetic relations of the buckthorn family within Rosales. Rhamnaceae were nested in a clade, sister to the species poor Elaeagnaceae Juss., the monotypic Dirachmaceae Hutch., and also monotypic Barbeyaceae Rendle (Zhang et al., 2011). This whole clade itself was sister to a clade comprising Ulmaceae Mirb., Cannabaceae Martinov, Urticaceae Juss., and Moraceae Gaudich, and all together the whole group was sistering Rosaceae Juss. (Magallón et al., 2015; Zhang et al., 2011). Robust phylogenetic relations beyond the target group allowed to expend the molecular data to incorporate close outgroups with only few species and, more important, the incorporation of fossil taxa attributed to Rhamnaceae, yet potentially sister to all extant buckthorns. Such fossils included a leaf from Russia referred to as †*Ziziphus* sp. and a rhamnaceous flower from the United States referred to as Rose Creek Flower, both dating back to the early Upper Cretaceous (Basinger and Dilcher, 1984; Spicer et al., 2002).

Now being equipped with solid preliminary work, a vast amount of data, and a workflow, I tackled the following hypotheses in my thesis: 1) I tested the robustness of the phylogenetic hypotheses within the buckthorns, by incorporating the underrepresented tribes Rhamneae and Ventilagineae. This revised hypothesis could then be used for molecular dating approaches. Furthermore, at this point, I could not completely rely on the taxonomy that was available in 2012, as Islam et al. (2006) pointed out major concerns in the monophyly of *Ziziphus* Mill. Consequently, the second step 2) was to provide a robust phylogenetic hypotheses and a taxonomic revision of the genus *Ziziphus*. Both points were addressed in chapter 1 of this thesis. As the inclusion of the species-rich tribe Rhamneae raised further taxonomic questions, a revision of *Rhamnus* s.l. L., and the reconstruction of a robust phylogenetic hypotheses of *Rhamnus* and its close allies was performed in chapter 2. After a robust phylogeny was reconstructed, that now covers all lineages in Rhamnaceae adequately, I placed the family and its subgroups in a temporal context. 3) The questions of how old were Rhamnaceae, its tribes, and major lineages was attacked in chapter 3. These questions, however, were in 2015 and 2016 also attacked by Renske Onstein, yet on a smaller data set and including the above mentioned taxonomic problems (Onstein et al., 2015; Onstein and Linder, 2016). Consequently, a comparison of my reconstructions with previous reconstructions was vital. 4) Finally, I was interested in the biogeographic questions: Where did certain lineages in Rhamnaceae come from? And were the ziziphoids and their internal lineages influences by the breakup of the Gondwanan supercontinent and thus vicariance. This was examined on the entire ziziphoid clade in chapter 3. Last but not least, the *Alphitonia* sensu lato group (within the ziziphoid clade) was looked upon in detail in chapter 4, applying the biogeographic on a Cenozoic timeframe. Appendix one comprises a study on *Allium* L., which highlighted the ability to transfer the knowledge and methods learned on Rhamnaceae to a different group of plants, and shifting the focus from continental fragments and islands to mountain ranges.

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Chapter 1: Phylogenetic relationships within the cosmopolitan buckthorn family (Rhamnaceae) support the resurrection of *Sarcomphalus* and the description of *Pseudoziziphus* gen. nov.

Phylogenetic relationships within the cosmopolitan buckthorn family (Rhamnaceae) support the resurrection of *Sarcomphalus* and the description of *Pseudoziziphus* gen. nov.

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Abstract Uncertainties persist in the delineation of tribes and genera within the cosmopolitan and species-rich Rhamnaceae. In this family, the identification of tribes and genera largely depends on combinations of traits rather than single synapomorphies, diagnostic morphological features being often shared among several clades. When taxonomic treatments based on morphological traits are in conflict with each other, phylogenetic reconstructions can help to guide taxonomic efforts. In this study, we present the largest molecular dataset to date for Rhamnaceae, by combining *trnL-trnF* and ITS sequence data for more than 400 taxa, either newly sequenced or retrieved from GenBank. Our sampling design includes all 11 Rhamnaceae tribes and 57 genera. We used fasttree-like searches, maximum likelihood estimates, and Bayesian analyses to generate a consensus phylogeny. Our reconstructions support most of the tribes as monophyletic, except Paliureae. Similarly, our analyses show the monophyly of most genera, with the exception of *Ziziphus* which appears to be paraphyletic. To preserve its monophyly, some species are excluded from *Ziziphus* and either attributed to the resurrected genus *Sarcomphalus* or to *Pseudoziziphus*, which is newly described here. Finally, our study highlights remaining uncertainties concerning phylogenetic relationships within Rhamnaceae, and identifies genera for which an increased sampling effort is encouraged.

Keywords molecular phylogenetics; *Pseudoziziphus*; Rhamnaceae; *Sarcomphalus*; taxonomy; *Ziziphus*

Supplementary Material Electronic Supplement (Figs. S1–S5) and DNA sequence alignment are available in the Supplementary Data section of the online version of this article at http://ingentaconnect.com/content/iapt/tax

■ INTRODUCTION

The buckthorns (Rhamnaceae Juss.) are a cosmopolitan family of shrubs, small trees and climbers. Recent taxonomic revisions divided Rhamnaceae into three major groups (Richardson & al., 2000a, b, 2004): the rhamnoid and the ziziphoid groups encompass by far the majority of extant species (more than 300 rhamnoid, and more than 600 ziziphoid species), whereas the ampelozizyphoid group is comparatively species-poor (10 spp.). In total, Rhamnaceae comprise about 60 genera distributed among 11 tribes, with some notable uncertainties: for example, the large genus *Ceanothus* L. (about 50 spp.) has not yet been attributed to any tribe (Burge & al., 2011). Rhamnoid and ziziphoid tribes are often cosmopolitan, distributed from the tropics to the subarctic and from sea level to treeline (Medan & Schirarend, 2004). In contrast, ampelozizyphoid taxa are restricted to tropical regions of northern South America, Cuba and Madagascar.

Within-family taxonomy of the monophyletic Rhamnaceae (Richardson & al., 2000a) is difficult because morphological diversity of floral traits is relatively low, and because identification often relies on a combination of traits that are shared between genera rather than on synapomorphies (Richardson & al., 2000b; Medan & Schirarend, 2004). In each of the three Rhamnaceae groups, a better understanding of phylogenetic relationships was achieved by studies considering both morphological and genetic data. In the ziziphoid group, for example, molecular and classical morphological data were used to clarify taxonomic concepts for the Australian tribe Pomaderreae Reissek ex Endl. (Kellermann & al., 2005, 2006, 2007; Kellermann & Udovicic, 2008), and for the revision of the African tribe Phyliceae Reissek ex Endl. (Richardson & al., 2001; Weitz, 2003). In contrast, molecular analyses could not fully clarify the taxonomy of tribes Colletieae Reissek ex Endl. and Paliureae Reissek ex Endl. (Aagesen, 1999; Aagesen & al., 2005; Islam & Simmons, 2006; Tortosa, 2008; Medan & al., 2009). Notably, tribe Gouanieae Reissek ex Endl. as well as several genera incertae sedis have not been investigated molecularly yet. Parts of the rhamnoid and ampelozizyphoid groups have also been revised using molecular or morphological data, but

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the vast majority of these studies lacked sufficient molecular evidence to arrive at a sound taxonomic treatment (Bolmgren & Oxelman, 2004; Callmander & al., 2008). Nevertheless, within both rhamnoid and ziziphoid groups, molecular studies have contributed to assessing the validity of some controversial genera. A notable example is *Frangula* Mill., formerly included in *Rhamnus* L. (Pool, 2013). However, there are ongoing debates concerning the taxonomic treatment of *Condaliopsis* (Weberb.) Suess. and *Sarcomphalus* P.Browne, which are currently both included in *Ziziphus* Mill. (Johnston, 1963, 1964; Islam & Simmons, 2006).

Although the number of studies has increased during the past years, insufficient or unbalanced sampling of taxa still results in rather obscure phylogenetic relationships, for example in tribe Rhamneae Hook.f. Although notable taxonomic improvements have been made in *Gouania* Jacq. (Buerki & al., 2011; Pool, 2014) and *Ziziphus* (Johnston, 1963; Ara & al., 2008), most studies investigated these widespread taxa only regionally, resulting in taxonomic uncertainties outside their respective target regions. These uncertainties are sometimes simply overlooked, as it was the case in the latest phylogeny produced to date, of which purpose was admittedly not taxonomical (Onstein & al., 2015). Despite providing exciting results on diversification rates in Rhamnaceae across different Mediterranean-type ecosystems, the study of Onstein & al. (2015), as it is often the case for studies extracting sequence data from GenBank without prior taxonomical knowledge, are at risk to concatenate nonconspecific accessions, potentially obscuring their interpretations. Given the taxonomic complexity of this family, studies on Rhamnaceae are likely to be particularly prone to such limitations: efforts towards a family-wide taxonomic revision is therefore highly needed.

Table 1. Rhamnaceae tribes and genera incertae sedis after Richardson & al. (2000b), number of accepted genera per tribe and number of genera per tribe included in this study, accepted species in genera incertae sedis and number of included species.

Tribes	Accepted genera	Genera included
Ampelozizypheae	1	1
Bathiorhamneae	1	1
Colletieae	7	7
Doerpfeldieae	1	1
Gouanieae	8	5
Maesopsideae	1	1
Paliureae	3	3
Phyliceae	4	4
Pomaderreae	10	10
Rhamneae	17	17
Ventilagineae	2	1
Genera incertae sedis	**Accepted species**	**Species included**
Alphitonia Reissek ex Endl.	15	3
Araracuara Fern.Alonso	1	0
Ceanothus L.	53	49
Colubrina Rich. ex Brongn.	30+	4
Emmenosperma F.Muell.	5(3)	1
Granitites Rye	1	1
Lasiodiscus Hook.f.	12	1
Schistocarpaea F.Muell.	1	1

In the present study, we aim at overcoming these problems by including all major lineages and most genera, and by gathering data from samples adaequately reflecting the distribution of cosmopolitan groups. We also refer to the latest taxonomic treatments available, and do check the determination of vouchers used for sequencing for this study and in previous ones. In order to improve our current taxonomic knowledge regarding the tribal and generic classification within Rhamnaceae, we present the largest molecular dataset to date for this family, comprising more than 500 accessions, 11 tribes and 57 genera. By reconstructing the phylogeny of Rhamnaceae using genetic markers for which a vast number of sequences is available (Hardig & al., 2000; Richardson & al., 2000a; Fay & al., 2001; Bolmgren & Oxelman, 2004; Aagesen & al., 2005; Islam & Simmons, 2006; Onstein & al., 2015), we ask: (1) Is the most recent tribal classification by Richardson & al. (2000b) supported by our analyses? (2) Which are the remaining uncertainties concerning tribal phylogenetic relationships in Rhamnaceae? (3) Are all accepted Rhamnaceae genera monophyletic? (4) If not, which taxonomic treatments can be suggested to arrive at a sound classification of the buckthorn family?

■ MATERIALS AND METHODS

Data sampling. — Plant material of 258 species and subspecies was obtained from vouchers (listed in Appendix 1) deposited in the following herbaria: FL, FR, HAL, HUJ, JE, KUN, L, LZ, M, MEXU, MO, SING, ULM, and WU. The identification of each voucher specimen included in this study was checked prior to sequencing. Our sampling includes species of all Rhamnaceae tribes (Richardson & al., 2000b), and almost all of the currently recognized genera, with the exception of monotypic genera such as *Alvimiantha* Grey-Wilson, *Araracuara* Fernandez, *Johnstonalia* Tortosa, *Pleuranthodes* Weberb., and *Smythea* Seemann ex A.Grey (Table 1). Additional sequence data for 274 species and subspecies were retrieved from GenBank and, whenever possible, the determination of voucher specimens corresponding to these GenBank accessions was confirmed. GenBank sequence data from vouchers of uncertain identification were not included in the dataset.

DNA extraction, amplification and sequencing. — Total genomic DNA was extracted with the Qiagen DNeasy Plant Mini Kit (Hilden, Germany) and the NucleoSpin Plant II Kit (Macherey-Nagel, Dueren, Germany) from leaf fragments of ca. 1 cm² size, following the manufacturers' protocols. As shown in Bolmgren & Oxelman (2004), a well-supported topology of Rhamneae (particularly at the tribal and generic levels) can be obtained from a combination of nuclear and plastid markers. We therefore amplified the nuclear ribosomal internal transcribed spacer region (ITS) and the *trnL-trnF* gene and spacer region: for the former following the protocols by Grudinski & al. (2014), and for the latter following Taberlet & al. (1991). Internal primers were occasionally used to amplify ITS, following Matuszak & al. (2016). PCR products were cleaned using a NukleoSpin Gel and PCR clean up kit (Macherey-Nagel), and sequencing was performed on an ABI 3730xl capillary sequencer (Thermo Fisher Scientific, Waltham, U.S.A.), following the manufacturers' protocols.

Alignments. — Sequences were aligned using the MUSCLE and Geneious alignment algorithm as implemented in Geneious v.6.1.6 (Kearse & al., 2012), followed by minor manual corrections. We identified a hairpin inversion (Kelchner & Wendel, 1996) of 16 bases in three species (*Rhamnus lamprophylla* C.K.Schneid., *Rhamnus parvifolia* Bunge, *Ceanothus purpureus* Jeps.) for *trnL-trnF* sequences, using mfold v.3 (Zuker, 2003). Because hairpin inversions might bias evolutionary signal (Kelchner & Wendel, 1996), we removed these bases as advised in Lehtonen & al. (2009). We also removed ambigous positions in the alignment via gBlocks v.0.91b filter (Castresana, 2000). In the end, 69% of the original alignment remained in the analyses. The application of filters on alignments has been shown to drastically reduce alignment errors (Jordan & Goldman, 2012).

Phylogenetic reconstructions. — We used three methods for our phylogenetic analyses of Rhamnaceae: fasttree-like (FT, Stamatakis, 2014), maximum likelihood (ML, Stamatakis, 2006), and Bayesian analyses (B, Ronquist & Huelsenbeck, 2003). To test for incongruencies in phylogenetic topology between our two markers, fasttree-like analyses were performed on ITS (nuclear) and *trnL-trnF* (plastid) datasets independently as well as on the combined dataset. Fasttree-like searches and the corresponding Shimodaira-Hasegawa-like testing (SH, Shimodaira, 2001; Stamatakis, 2014) have only rarely been applied in phylogenetics, although they have been shown to give similar results as maximum likelihood estimates and Bayesian analyses in simulated and real data tests (Goldman & al., 2000; Buckley & al., 2001; Price & al., 2010; Liu & al., 2011). We did not observe any supported topological incongruencies, the topologies were very similar (Electr. Suppl.: Figs. S1–S4). This agrees with previous studies in Rhamnaceae, not observing major and supported conflicts when both markers are combined or compared (Bolmgren & Oxelman, 2004; Kellermann & Udovicic, 2008). Hence, we further analysed the combined dataset with two partitions (1-nuclear, 2-plastid) using maximum likelihood and Bayesian inference. All analyses were performed before and after removing ambigous positions with gBlocks (see above) to test for incongruencies. For all three datasets, mixed substitution model settings were used in Bayesian analyses. The GTR+G model was used in fasttree-like analyses and maximum likelihood analyses using raxmlGUI, as it is the most general model, and as it does not interfere with RAxML substitution likelihood functions, thus following the recommendations of the authors of raxmlGUI (Stamatakis, 2006; Silvestro & Michalak, 2012). Support of clades was estimated calculating bootstrap support (BS, set to autoMRE) in our maximum likelihood analyses, and applying Shimodaira-Hasegawa-like testing in our fasttree-like searches as implemented in RAxML (Shimodaira, 2001; Stamatakis, 2014). Bayesian analyses were run with MrBayes v.3.2.2 as implemented in the CIPRES platform (Ronquist & Huelsenbeck, 2003; Miller & al., 2010), with the following settings: four Markov chain Monte Carlo heuristic searches of 150 million generations were performed in four independent runs. Sampling frequency was set to 3000. A burn-in of 75 million generations was determined by Tracer v.1.6 (Drummond & Rambaut, 2007). Posterior probabilities (PP) were used to illustrate the support of nodes. The topologies of all analyses were rooted according to previous studies (Richardson & al., 2000a; Onstein & al., 2015), i.e., at the split between the ziziphoid group and the clades including the rhamnoid and the ampelozizyphoid groups. Throughout the manuscript, only values equal to or higher than 90 (SH), 85 (BS) and 0.90 (PP) are considered sufficiently supported.

■ RESULTS

Datasets. — In total, 258 newly sequenced taxa were included in this study. We generated a total of 231 ITS and 216 *trnL-trnF* sequences. A complete list of GenBank accessions used is provided in Appendix 1. The alignments had 1079 (ITS) and 1447 characters (*trnL-trnF*) including indels, and 782 (ITS) and 1023 (*trnL-trnF*) characters remained after treatment in gBlocks. Specific indels were recognized in both datasets for almost every tribe, most commonly in Ventilagineae Hook.f.

Phylogenetic reconstructions. — In all analyses, the exclusion of uncertain positions following gBlocks recommendations did not affect topologies significantly. Moreover, phylogenetic reconstructions were congruent among datasets (combined: Figs. S1, S2, S5; ITS: Fig. S3, *trnL-trnF*: Fig. S4; see Electr. Suppl.), type of analysis (B, ML, FT), and regardless of the model used (GTR+G, mixed). This shows the robustness of our approach despite including only two genetic regions. Nevertheless, BS values tended to be lower than the corresponding SH-like values. This might be due to the unbalanced nature of our dataset: some GenBank sequence data were only available for either ITS or *trnL-trnF*.

As shown in Fig. 1, the three main Rhamnaceae groups described by Richardson (the rhamnoid, the ampelozizyphoid, and the ziziphoid group) are clearly monophyletic in all analyses (SH: 100, BS: 100, PP: 1.00; SH: 100, BS: 100, PP: 1.00; SH: 98, BS: 98, PP: 1.00). The ampelozizyphoid group, including tribes Ampelozizypheae J.E.Richardson, Bathiorhamneae J.E.Richardson and Doerpfeldieae J.E.Richardson, is closely related to the rhamnoid group, and together they are sister clade to the ziziphoid group. The rhamnoid group comprises tribes Maesopsideae Weberb., Rhamneae and Ventilagineae, all with high support (SH: 100, BS: 100, PP: 1.00; SH: 96, BS: 87, PP: 1.00; SH: 98, BS: 100, PP: 1.00). The ziziphoid group includes the unattributed *Colubrina* Rich. ex Brongn. (SH: 100, BS: 100, PP: 1.00), tribes Gouanieae (SH: 100, BS: 100, PP: 1.00) and Paliureae (SH: 85, BS: 59, PP: 0.85), and a weakly

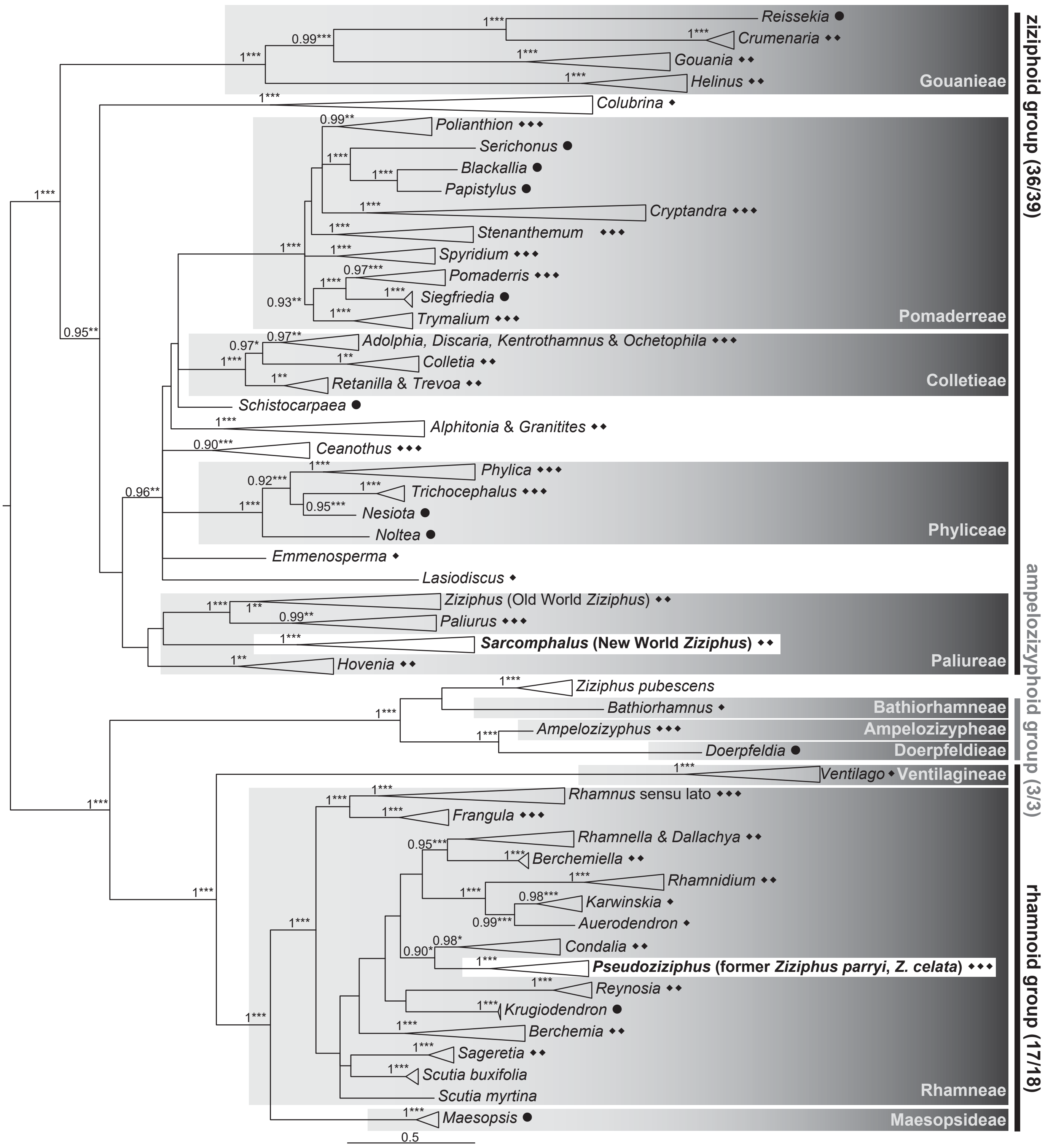

Fig. 1. Majority-rule consensus tree (Bayesian analysis) of Rhamnaceae based on combined nuclear ribosomal internal transcribed spacer (ITS) and *trnL-trnF* sequence data. The three major groups are named and outlined on the right (numbers in brackets: genera included in this study / genera attributed to this group). Numbers at nodes represent posterior probabilities. The stars after the numbers refer to fasttree-like searches and maximum likelihood estimates: * node present in all analyses (ITS, *trnL-trnF*, combined), but not supported; ** node present and supported in two of three analyses; *** node present and supported in all analyses. Tribes after Richardson & al. (2000b), genera newly recognised in this study highlighted. Species included in this study: ● genus monotypic, ◆ less than 20% included, ◆◆ 20%–49% included, ◆◆◆ 50% or more of species included.

supported clade (SH: 90, BS: 45, PP: 0.96) comprising tribes Colletieae (SH: 99, BS: 86, PP: 1.00), Phyliceae (SH: 100, BS: 100, PP: 1.00), Pomaderreae (SH: 100, BS: 93, PP: 1.00) as well as several unassigned genera including *Alphitonia* Reissek ex Endl. (not supported), *Ceanothus* (SH: 100, BS: 86, PP: 0.90), *Emmenosperma* F.Muell., *Granitites* Rye (not supported), *Lasiodiscus* Hook.f. and *Schistocarpaea* F.Muell. (monotypic tribe Paliureae is without support in any of our three analyses).

Ziziphus is polyphyletic in all analyses, and consists of four clearly distinct and supported entities. Two former *Ziziphus* clades are within the ziziphoid group: *Ziziphus* (95 SH, 79 BS, 1.00 PP) and the reattributed New World *Ziziphus* named *Sarcomphalus* (100 SH, 100 BS, 1.00 PP). The third clade including former *Ziziphus* taxa appears within the rhamnoid group and here is named *Pseudoziziphus* (100 SH, 100 BS, 1.00 PP), weakly supported as sister to *Condalia* Cav. (SH: 60, BS: 76, PP: 0.90). The fourth *Ziziphus* clade is nested within the ampelozizyphoid group, comprising two accessions of *Ziziphus pubescens* Oliv. (100 SH, 100 BS, 1.00 PP). Locally low support of the backbone of the phylogeny makes it difficult to uncover phylogenetic relationships between *Alphitonia* and *Granitites*, *Ceanothus*, *Colubrina*, *Emmenosperma*, *Lasiodiscus*, and *Schistocarpaea*, and the existing tribes. In Rhamneae, our analyses strongly support *Berchemia* Neck. ex DC. (SH: 95, BS: 94, PP: 1.00), *Berchemiella* T.Nakai (SH: 100, BS: 100, PP: 1.00), *Karwinskia* Zucc. (SH: 90, BS: 89, PP: 0.98), *Rhamnidium* Reissek (SH: 100, BS: 99, PP: 1.00), *Reynosia* Griseb. (SH: 100, BS: 100, PP: 1.00), and *Sageretia* Brongn. (SH: 100, BS: 100, PP: 1.00), and weakly support *Condalia* (SH: 74, BS: 37, PP: 0.98), whereas *Rhamnella* Miq. and *Scutia* (Comm. ex DC.) Brongn. are not supported in any analysis. *Rhamnella* includes *Dallachya* F.Muell. in our analyses, however this relationship is not supported (SH: 89, BS: 61, PP: 0.64). Finally, *Scutia* appears polyphyletic in our topologies.

■ DISCUSSION

Our phylogenetic reconstructions generally support the taxonomic treatment of Rhamnaceae by Richardson (2000b), which was based on molecular data and included additional morphological, anatomical, and geographical information. Furthermore, for the majority of nodes, our topology is fairly similar to that of Onstein & al. (2015). Nevertheless, our results highlight the need for a few taxonomic adjustments, for example, by transferring parts of genera to tribes different from their current attribution. Since all three phylogenetic methods applied yielded similar results (particularly for strongly supported nodes), we are confident that only minor modifications, if any, might occur with a further extended dataset (in terms of absolute species numbers and/or additional genetic regions).

Polyphyly of *Ziziphus*. — Paliureae, when including New and Old World taxa of the species-rich and widespread genus *Ziziphus*, has never been supported as a monophylum in any study to date, including ours. Despite limitations in resolving intertribal phylogenetic relationships, *Ziziphus* is clearly polyphyletic and consists of four clades in our phylogeny as well as in the phylogenies by Islam & Simmons (2006) and Onstein & al. (2015). To maintain the monophyly of *Ziziphus*, and to incorporate three of the four genetic entities into a taxonomic concept, our analyses suggest for *Ziziphus* that the previously described genus *Sarcomphalus* should be resurrected and a new genus should be described (hereafter named *Pseudoziziphus* gen. nov.). The relationships of the fourth genetic entity (*Z. pubescens*) cannot be sufficiently solved with our data.

First of all, American species of *Ziziphus* form a clade distinct from both *Paliurus* and Old World *Ziziphus* (Fig. 1). Grisebach already noticed discrepancies between American and Old World *Ziziphus* in 1859 and attributed *Rhamnus sarcomphalus* L. (≡ *Ziziphus sarcomphalus* (L.) M.C.Johnst.) to the genus *Sarcomphalus* P.Browne (Grisebach, 1859). In a later study, Grisebach added another American *Ziziphus* species (*Z. havanensis* Kunth) to *Sarcomphalus* (Grisebach, 1866). The generic concept separating *Sarcomphalus* from *Ziziphus* s.str. mirrors Schirarend's grouping in *Ziziphus*, which was primarily supported by wood anatomical structures (Schirarend, 1991; Islam & Simmons, 2006). The grouping observed in this study is consistent with the conclusions by Islam & Simmons (2006) who studied a different set of New and Old World *Ziziphus*. However, *Sarcomphalus* was merged with *Ziziphus* by Johnston (1963, 1964) in an attempt to separate *Condalia* from *Ziziphus* (Johnston, 1962, 1963, 1964). In this process, he excluded *Condalia* s.str. and merged *C.* sect. *Condaliopsis* as well as *Sarcomphalus* and *Ziziphus* in a single genus (*Ziziphus*). Our data clearly indicate that the description of *Sarcomphalus* as a genus was justified.

Two vouchers of another species of American *Ziziphus*, *Z. parryi* Torr., and two accessions of the Florida endemic *Z. celata* Judd & D.W.Hall, appear as members of Rhamneae (and not Paliureae) in our phylogeny (Fig. 1). *Ziziphus parryi*, also described as a species of *Condaliopsis* by Suessenguth (1953), shares a number of morphological traits with Rhamneae. These traits, absent in *Sarcomphalus* and the remaining *Ziziphus* species, are: *Z. parryi* has unpaired branches with terminal thorns while *Ziziphus* and *Sarcomphalus* possess paired spines, as common in Paliureae (Weberbauer, 1895; Johnston, 1962, 1964; Islam & Simmons, 2006). Furthermore, *Z. parryi* has pinnate leaf venation in constrast to the palmately 3-veined leaves of *Ziziphus*. Yet, *Condaliopsis* has pinnate as well as palmately 3-veined leaf venation whereas leaves in Paliureae are exclusively palmately 3-veined (Johnston, 1962; Medan & Schirarend, 2004). This variation in venation in *Condaliopsis* results from the fact that one species, the type of former *Condaliopsis* (now *Ziziphus obtusifolia*), is obscurely palmately 3-veined. Furthermore, our phylogenetic reconstructions clearly attribute *Z. obtusifolia* and subordinate taxa to *Sarcomphalus*. Hence, we attribute *Z. obtusifolia* to *Sarcomphalus* (New World *Ziziphus*). Consequently, as the type of *Condaliopsis* is included in *Sarcomphalus*, the name and genus *Condaliopsis* is not suited for *Z. parryi* and *Z. celata* after their exclusion from *Ziziphus*. We here describe the new genus *Pseudoziziphus* gen. nov. Along with these morphological traits, our genetic data support the inclusion of *Z. parryi* in tribe Rhamneae. Whether this species should be named under

Pseudoziziphus or *Condalia* is arguable because phylogenetic relationship among *Z. parryi*, *Z. celata* (*Condaliopsis*) and *Condalia* would allow both alternatives. Yet, *Z. parryi* and *Z. celata*, and *Condalia* differ morphologically in some easily identifiable characters (Table 2): petals are present in the former two but absent in *Condalia* and *Z. parryi*. Also, *Z. celata* never has unilocular pyrenes which are common in *Condalia*. However, *Z. celata* may display up to 4-locular putamina untypical for *Condalia* (Johnston, 1962; Medan & Schirarend, 2004). Hence, we rather describe *Z. parryi* and the morphologically very similar *Z. celata* as a new genus (*Pseudoziziphus*), than attribute both to *Condalia* which would render the latter morphologically very variable, blurring its circumscription. We provide a new taxonomic treatment for *Pseudoziziphus* below.

After the exclusion of *Pseudoziziphus* and *Sarcomphalus*, species of *Ziziphus* s.str. appear closely related to *Paliurus*, from which they differ in their fruit: *Paliurus* has a dry drupe with an unmistakable membranous ring, whereas *Ziziphus* s.str. produces fleshy drupes without membranous rings. To maintain this particular fruit as a key trait, we present the concept of five separate genera, among which *Pseudoziziphus* is justified primarily by morphological, and *Sarcomphalus* by geographical and molecular support. Maintaining *Sarcomphalus* in *Ziziphus* would result in one of the following scenarios: (1) a polyphyletic *Ziziphus* or (2) a drastically enlarged concept of *Paliurus*, encompassing *Paliurus*, *Ziziphus* s.str. and *Sarcomphalus* (the name *Paliurus* being older than *Ziziphus*), with *Paliurus* losing its key trait (dry drupe with membranous ring; Medan & Schirarend, 2004). In summary, we consider the concept of five separate genera (*Condalia*, *Paliurus*, *Pseudoziziphus* gen. nov., *Sarcomphalus*, and *Ziziphus*) as the most conservative strategy to maintain the current taxonomic understanding of this group on the one hand, and the morphological peculiarities of *Paliurus* on the other hand.

A persistent uncertainty in *Ziziphus* are the relationships of *Z. pubescens* Oliv. from eastern Africa. Our phylogenetic reconstructions placed two accessions of this species in the ampelozizyphoid group, as also shown in other studies (Islam & Simmons, 2006; Onstein & al., 2015). The accessions were produced for the study of Islam & Simmons (2006), and misidentifications and contaminations were ruled out. Beyond this statement no further investigations were done. Using these sequences, the most recent study of Rhamnaceae did not comment on this taxomomic issue (Onstein & al., 2015). *Ziziphus pubescens* and the morphologically similar *Z. robertsonia* Beentje are morphologically closely similar to the Madagascan endemic *Bathiorhamnus* Capuron. Even more, *Z. robertsonia* perfectly fits the description of *Bathiorhamnus*. Based on the latest circumscription of *Bathiorhamnus* (Callmander & al., 2008), there are no key traits to delimit *Bathiorhamnus* from *Z. pubescens* and *Z. robertsonia*. In order to determine if *Bathiorhamnus* should be extended to include species from the African mainland, or if the description of a new genus (based on *Z. robertsonia* and *Z. pubescens*) is preferable, additional studies involving more genetic data will be necessary.

Remaining uncertainties in the ziziphoid and rhamnoid groups. — In addition to the ziziphoid, rhamnoid and ampelozizyphoid groups, our analyses support a large number of tribes and genera throughout the phylogeny. However, as already illustrated by *Ziziphus*, the ziziphoid group is complex and only partially resolved phylogenetically and taxonomically. Phylogenetic relationships among its tribes are only poorly supported despite the fact that many genera of some tribes are clearly monophyletic. For example, all genera in Pomaderreae and Phyliceae are supported in our analysis, confirming previous morphological and/or genetic studies (Richardson & al., 2001; Kellermann & al., 2005; Kellermann & Udovicic, 2008). In contrast, in Colletieae some uncertainties remain concerning the phylogenetic placement of *Adolphia* Meisn., *Discaria* Hook., *Kentrothamnus* Suess. & Oberkott and *Ochetophila* Poepp. ex Endl., while *Colletia* and a clade comprising *Trevoa* Miers ex Hook. and *Retanilla* (DC.) Brongn. are in agreement with previous studies (Aagesen, 1999; Aagesen & al., 2005; Kellermann & al., 2005). Furthermore, the ziziphoid group includes several genera that have not been attributed to any tribe yet (Richardson & al., 2000b), and for these we could not resolve phylogenetic relationships. These genera are *Ceanothus*, *Colubrina*, *Emmenosperma*, *Lasiodiscus* and *Schistocarpaea*. For example, *Colubrina*, a cosmopolitan genus which was last revised by Johnston (1971), might be closely related to Paliureae or could represent an independent section, not yet described. Clearly, for *Colubrina* and other non-monotypic genera, more evidence is needed to resolve these phylogenetic uncertainties, probably by increasing both the number of species included and the quantity of genetic data (for *Colubrina*, for example, sequence data exist only for 4 of about 30 recognized species).

Table 2. Morphological comparison of *Condalia*, *Pseudoziziphus*, *Paliurus*, *Sarcomphalus* and *Ziziphus*.

Trait	*Condalia* Cav.	*Pseudoziziphus* gen. nov.	*Paliurus* Mill.	*Sarcomphalus* P.Browne	*Ziziphus* Mill. s.str.
Inflorescence	solitary or fascicled flowers	fascicled flowers	cymose	cymose	cymose
Petals	absent	present	present	present	present
Drupes	fleshy	fleshy	dry, with membranous ring	fleshy	fleshy
Putamen	1–2 pyrenes or 1–2-locular	2–4-locular	2–3-locular	2–3-locular	2–4-locular
Leaf venation	pinnate	pinnate	palmate	pinnate or palmate	palmate
Spines	terminal spines	terminal spines	paired spines	paired spines	paired spines

The assingnment to tribes of monotypic genera is more challenging, and increasing phylogenetic resolution will obviously require more genetic data.

The rhamnoid group comprises three morphologically and genetically distinct tribes: Maesopsideae, Rhamneae and Ventilagineae. The topology generated by our analyses broadly resembles previous taxonomic concepts (Richardson & al., 2000a). Within tribe Rhamneae, the alternately branched, unarmed *Berchemiella*, *Rhamnella* and *Dallachya* grouped together. Another group comprises *Auerodendron* Urb., *Karwinskia* and *Rhamnidium*. Until the last revision of Rhamnaceae, the generic limits among *Auerodendron*, *Karwinskia*, *Reynosia* and *Rhamnidium* remained unclear (Medan & Schirarend, 2004). In our study, the delimitation of *Auerodendron* and *Karwinskia* remains poorly resolved, as only one species of *Auerodendron* was included. Yet, *Rhamnidium* is clearly monophyletic in all our analyses, and *Reynosia* seems to be more closely related to *Krugiodendron* than to *Rhamnidium*. Hence, by providing additional support for the validity of *Rhamnidium* and *Reynosia*, only two genera (*Auerodendron*, *Karwinskia*) have delimitation problems. Also within Rhamneae, the delineation of *Frangula* and *Rhamnus* has repeatedly been modified. While the latest exclusion of *Frangula* has resulted in a clearly defined genus, the taxonomic concept of *Rhamnus* remains obscure (Pool, 2013). This latter problem has been investigated in another study (Hauenschild & al., 2016).

■ TAXONOMIC TREATMENT

Our molecular phylogenetic reconstructions revealed the polyphyly of *Ziziphus* s.l. resulting in a need for taxonomic adjustments: we move New World *Ziziphus*, except for two species (*Z. parryi*, *Z. celata*), to *Sarcomphalus* that we here re-instate, and place *Z. parryi* and *Z. celata* in the new genus *Pseudoziziphus* with only these two species which had previously been described either as *Ziziphus* or as section *Condaliopsis* of *Condalia*. Resurrecting *Condaliopsis* as a section or genus was not possible because the type (*Condaliopsis lycioides* (A.Gray) Suess. ≡ *Ziziphus obtusifolia* (Hook. ex Torr. & A.Gray) A.Gray) is more closely related to New World *Ziziphus* than to *Condalia*, *P. parryi* and *P. celata* (Islam & Simmons, 2006). The following descriptive and taxonomic information is based on Weberbauer (1895), Suessenguth (1953), Johnston (1962, 1964) and Judd & Hall (1984). Species of *Pseudoziziphus* and *Sarcomphalus* are transferred from *Ziziphus*, based on the above mentioned secondary publications, original publications, and type collections. We designated lectotypes when appropriate.

Pseudoziziphus

Pseudoziziphus Hauenschild, **gen. nov.** – Type: *Pseudoziziphus parryi* (Torr.) Hauenschild (≡ *Ziziphus parryi* Torr.).

Diagnosis. – *Pseudoziziphus* includes two species of the *Condaliopsis* group of *Ziziphus*: *P. parryi* and *P. celata*. *Pseudoziziphus* differs from *Ziziphus* and other related ziziphoid genera by the pinnate venation of its leaves (palmately 3-veined in *Ziziphus*), and branches transformed to terminal thorns (paired spines in *Ziziphus*). It differs from *Condalia* by the presence of petals (always absent in *Condalia*) and 2–4-locular putamina (1 or?—2 pyrenes or rarely 2-locular putamina in *Condalia*). Furthermore, molecular analyses of ITS and *trnL-trnF* sequence data support this genus as an independent monophyletic entity.

Description. – Shrubs, deciduous, 1–4 m tall, spinose. Branches alternate, flexuous, glabrous, terminating in small shoots. Secondary branchlets thorn-tipped (thorns 1.5–4 cm). Leaves alternate or fascicled, glabrous, elliptic to oblong or obovate, often mucronate. Leaf margin entire. Venation pinnate, conspicuous or obscure, with 7–15 pairs of lateral veins. Petioles short (0.1–0.5 cm), stipules present. Flowers 5-merous, fascicled (2–7), rarely solitary, on small, leaf-bearing shoots. Hypanthium tube 2–2.2 mm in diameter, sepals triangular (2–3 mm), glabrous, usually greenish. Petals present, surrounding antepetalous stamens, narrowly oblong, clawed, caducous. Disk fleshy, surrounding but free from ovary. Ovary globose, glabrous, 2–4-locular. Style 2–4-lobed. Pedicels glabrous, 2–8 mm long, elongating to 1–2 cm at fruit maturity. Drupe ovoid to ellipsoid, 1–3 cm. Putamen 2–3(–4) celled, containing 1–3 seeds.

Etymology. – The name *Pseudoziziphus* refers to the former attribution of these species to *Ziziphus* on the one hand, and to their morphological distinctiveness from this genus on the other hand.

Distribution. – Southwestern United States and northern Mexico and the Lake Wales Ridge on Florida Peninsula (Fig. 2A).

Habitat. – *Pseudoziziphus* grows on sandy soils in semiarid to arid habitats, from near sea level to above 2000 m.

IUCN conservation assessment. – *Pseudoziziphus parryi* is a rather widespread and common plant occupying arid areas unavailable for many plants. It may therefore be considered as Least Concern (LC) according to the *IUCN Red List Categories and Criteria* (v.3.1, 2nd ed.; IUCN, 2014). In contrast, *P. celata* is listed as vulnerable (VU, see http://www.iucnredlist.org/details/32102/0): it is endemic to Lake Wales Ridge on the Florida Peninsula. This shrub-land is surrounded by agriculture, the city of Orlando, sea, marsh and forest, and therefore isolated and substantially degraded. More recently, populations of *Pseudoziziphus celata* were shown to be highly clonal and extremely limited in numbers (Ellis & al., 2007). Because low genetic diversity might render small populations highly susceptible to global climate change, and because only 12 populations remained in 2007, we suggest that *P. celata* should rather be ranked as Critically Endangered (CR D).

Pseudoziziphus parryi (Torr.) Hauenschild, **comb. nov.** ≡ *Ziziphus parryi* Torr. in Emory, Rep. U.S. Mex. Bound. 2(1): 46. 1859 ≡ *Condalia parryi* (Torr.) Weberb. in Engler & Prantl, Nat. Pflanzenfam. III(5): 404. 1896 ≡ *Condaliopsis parryi* (Torr.) Suess. in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 20d: 135. 1953 – **Lectotype (designated here):** United States: *Parry s.n.* (GH barcode 00051527!).

Pseudoziziphus parryi var. ***microphylla*** (I.M.Johnst.) Hauenschild, **comb. nov.** ≡ *Condalia parryi* var. *microphylla* I.M.Johnst. in Univ. Calif. Publ. Bot. 7: 439. 1922 ≡ *Ziziphus parryi* var. *microphylla* (I.M.Johnst.) I.M.Johnst. in Brittonia 14: 368. 1962 – Lectotype (designated by Johnston in Amer. J. Bot. 50: 1027. 1963): *Brandegee s.n.* (UC barcode UC80099!).

Pseudoziziphus celata (Judd & D.W.Hall) Hauenschild, **comb. nov.** ≡ *Ziziphus celata* Judd & D.W.Hall in Rhodora 86: 382, f. 1. 1984 – Holotype: United States, *Garrett s.n.* (FLAS barcode FLAS 136888 n.v.).

Sarcomphalus

Sarcomphalus P.Browne, Civ. Nat. Hist. Jamaica: 179. 1756, **emend.** Hauenschild. – Type: *Sarcomphalus laurinus* Griseb.

Diagnosis. – *Sarcomphalus* includes New World species which were formerly placed in *Ziziphus* (except for two *Ziziphus* species transferred to *Pseudoziziphus*, see above). The species placed in *Sarcomphalus* are genetically clearly distinct from Old World *Ziziphus* (*Ziziphus* s.str.), *Paliurus*, and *Pseudoziziphus*. *Sarcomphalus* differs morphologically from *Paliurus* by having fleshy drupes (dry drupes with membranous rings in *Paliurus*), and from *Pseudoziziphus* by having palmate leaf venation (pinnate in *Pseudoziziphus*) and occasional stipular spines (always absent in *Pseudoziziphus*).

Description. – Shrubs or small to medium-sized trees, deciduous, 1–10(–15) m tall, spinose. Stems and primary branches with brownish or greyish bark, branchlets glabrous to pubescent, often with short, leaf-baring shoots. Plants armed by usually paired, non-leafy spines. Leaves deciduous, alternate to subopposite, usually fascicled on short shoots, glabrous to puberulent, simple but variable in shape. Leaf margin entire. Venation palmate, 3-veined: one midvein and two additional basal primary veins, but venation pinnate in *Sarcomphalus laurina*, secondary veins conspicuous or inconspicuous. Petioles usually short, non-transformed stipules present, caducous. Infloresence a cyme. Peduncles 0.2–4 cm long. Flowers 5-merous, small. Hypanthium tube hemispheric. Sepals triangular (1–3 mm), glabrous or pubescent, usually greenish or yellowish. Petals present, cucullate, about as long as the stamens, often equal in size to hemispheric sepals, clawed. Stamens 5, antepetalous. Disk usually thickened, surrounding but free from ovary. Ovary usually glabrous, 2(–3)-locular. Style 2(–3)-lobed. Pedicels glabrous or pubescent. Drupe with 2(–3)-celled putamen containing 1–3 seeds.

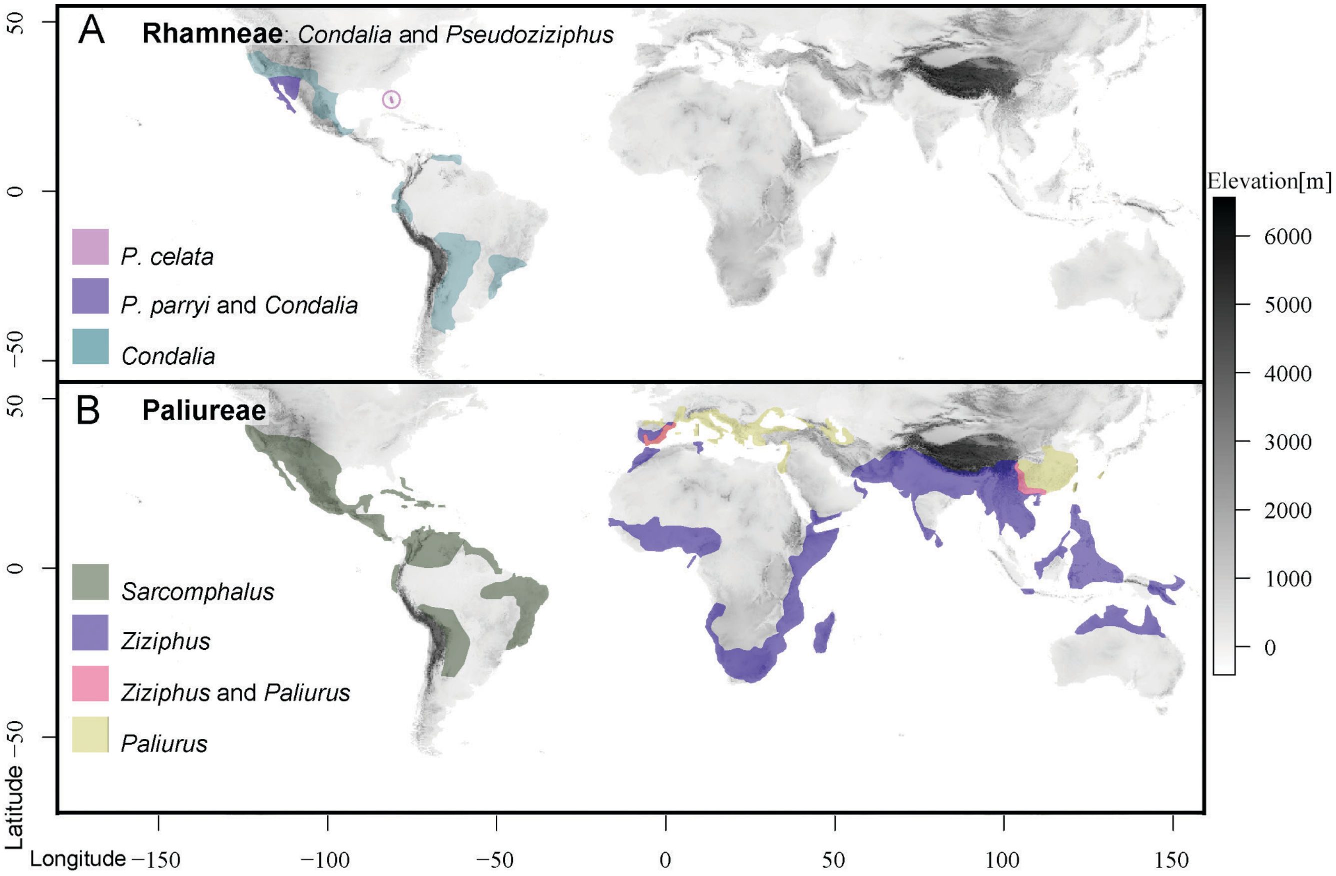

Fig. 2. Approximate range of: **A,** *Pseudoziziphus* and *Condalia*; **B,** *Sarcomphalus*, *Ziziphus* and *Paliurus*. — The introduced range of some species of *Ziziphus* and *Paliurus* (subcosmopolitan) is not shown. This map was downloaded from WORLDCLIM (Hijmans & al., 2005) and modified manually.

Distribution. – Taxa of this genus are distributed throughout subtropical and tropical America, including the Caribbean, and rarely in temperate North America and south of the Tropic of Capricorn (Fig. 2B).

Habitat. – *Sarcomphalus* species often grow in arid costal shrub-dominated areas. Some species occur on humid slopes in mountain ranges of South and Central America as well as on the Caribbean islands.

IUCN conservation assessment. – Some species of *Sarcomphalus* are widespread and common: *S. amole*, *S. cinnamomus*, *S. guatemalensis*, *S. jaozeiro*, *S. mexicanus*, *S. mistol*, *S. obtusifolius*, *S. platyphyllus*, and *S. strychnifolia*, *S. yucatanensis*. Hence, those species should be considered as Least Concern (LC). Some species (*S. glaziovii*, *S. lloydii*, *S. rhodoxylon*, *S. saeri*, *S. taylorii*) come close to threatened categories: these species face a threat by habitat size and quality (B1biii), by ruderalisation and commercial land use, and might be considered as Near Threatened (NT). Other species clearly should be assigned to threathened categories, in most cases because their potential habitat does not exceed 20,000 km² (or even 5000 km² for some endemics), and is undergoing strong ruderalisation or deforestation. This includes the Caribbean Island species *S. laurinus* (VU B1biii), *S. acutifolius* (VU B1biii), *S. chloroxylon* (EN B1biii+B2biii), *S. domingensis* (VU B1biii), *S. havanensis* (EN B1+2c), *S. obovatus* (EN B2a), *S. parvifolius* (VU B1biii), *S. reticulatus* (VU B1biii), as well as the mainland species *S. cyclocardius* (VU B1biii), *S. pendunculata* (VU B1biii), and *S. thyrsiflorus* (VU B1biii). The above habitat-based classifications quantify the minimum risk of threat, but as population data is lacking, the actual threat might be higher. Data deficiency (DD) is greatest in *S. bidens*, *S. crenatus*, *S. divaricatus*, and *S. microdictyus*: distributional data rely on historical vouchers and observations without vouchers to double-check, indicating major threats. Hence, studies on population size and dynamics should be encouraged for many species of *Sarcomphalus*.

Sarcomphalus laurinus Griseb, Fl. Brit. W. I.: 100. 1859 ≡ *Rhamnus sarcomphalus* L., Syst. Nat., ed. 10: 937. 1759 ≡ *Ceanothus sarcomphalus* (L.) DC., Prodr. 2: 30. 1825 ≡ *Sarcomphalus retusus* Raf. in Sylva Tellur. 29. 1838 ≡ *Sarcomphalus sarcomphalus* (L.) Britton ex Britton & Millsp. in Sci. Surv. Porto Rico & Virgin Islands 5: 535. 1924, nom. illeg. ≡ *Ziziphus sarcomphalus* (L.) M.C.Johnst. in Amer. J. Bot. 50: 1021. 1963 – Lectotype (designated by Johnston in Amer. J. Bot. 50: 1115. 1963): Jamaica, *Browne 179*, Herb. Linn.No. 262.11 (LINN!).

Sarcomphalus laurinus var. ***fawcettii*** Krug & Urb. in Notizbl. Königl. Bot. Gart. Berlin 1: 319: 1897 – **Lectotype (designated here):** Jamaica, *Harris 6677* (BM barcode BM000838626!; isolectotypes: F barcode F0068175F n.v., NY barcode 00084121 n.v., US barcode 00094365!).

Sarcomphalus acutifolius Griseb., Cat. Pl. Cub.: 32. 1866 ≡ *Ziziphus acutifolia* (Griseb.) M.C.Johnst. in Amer. J. Bot. 51: 1116. 1964 – **Lectotype (designated here):** Cuba, *Wright 2101* (GOET barcode GOET009872!; isolectotypes: GH barcode 00051506!, K barcode K000531800!, MO barcode MO-2049566!, P barcode P018185654!).

Sarcomphalus amole (Sessé & Moc.) Hauenschild, **comb. nov.**
≡ *Rhamnus amole* Sessé & Moc., Pl. Nov. Hisp.: 38. 1887 ≡ *Ziziphus amole* (Sessé & Moc.) M.C.Johnst in Amer. J. Bot. 50: 1021–1022, f. 1. 1963 – Lectotype (designated by Johnston in Amer. J. Bot. 50: 1022. 1963): Mexico, *Sessé & Mocino 817* (MA barcode MA 602482 n.v.).
= *Ziziphus sonorensis* S.Watson in Proc. Amer. Acad. Arts 24: 44. 1889 – **Lectotype (designated here):** Mexico, *Palmer 124* (E barcode E00296039!; isolectotypes: ARIZ barcode ARIZ-BOT-0005566 n.v., BM barcode BM000617453 n.v., GH barcode 00051534 n.v., K barcode K000531805 n.v.).

Sarcomphalus bidens Urb., Symb. Antill. 9(2): 229. 1924 ≡ *Ziziphus bidens* (Urb.) M.C.Johnst. in Amer. J. Bot. 51: 1116. 1964 – Holotype: Cuba, *Ekman 4039* (S No. S-R-7630 n.v.).

Sarcomphalus chloroxylon (L.) Hauenschild, **comb. nov.** ≡ *Laurus chloroxylon* L., Syst. Nat., ed. 10: 1010. 1759 ≡ *Ziziphus chloroxylon* (L.) Oliv. in Hooker's Icon. Pl. 19: t. 1862. 1889 – **Lectotype (designated here):** Jamaica, *Browne s.n.*, herb. Linn. 518-8 (LINN n.v.).

Sarcomphalus cinnamomum (Triana & Planch.) Hauenschild, **comb. nov.** ≡ *Ziziphus cinnamomum* Triana & Planch. in Ann. Sci. Nat., Bot., sér. 5, 16: 380. 1872 – Lectotype (designated by Lima & Giulietti in Acta Bot. Brasil. 28: 380. 2014): Venezuela, *Linden 1548* (P barcode P01818567; isolectotypes: BM barcodes BM000838623 & BM000838624, F barcodes F0068182F & F0068183F, G, K barcode K000529999, P barcode 01818568, W; all. n.v.).
= *Ziziphus itacaiunensis* Fróes in Bol. Tecn. Inst. Agron. N. 36: 151. 1958 – **Lectotype (designated here):** Brazil, *Fróes 26977* (K barcode K000531791!; isolectotypes: IAN, MG, U barcode U 0005785; all n.v.).

Sarcomphalus crenatus Urb., Symb. Antill. 3: 316. 1902 ≡ *Ziziphus crenata* (Urb.) M.C.Johnst. in Amer. J. Bot. 51: 1116. 1964 – Lectotype (designated by Johnston in Amer. J. Bot. 50: 1116. 1963): Haiti, *Buch 592* (GH barcode 00051507 n.v.).

Sarcomphalus cyclocardius (S.F.Blake) Hauenschild, **comb. nov.** ≡ *Ziziphus cyclocardia* S.F.Blake in Contr. Gray Herb. 53: 35. 1918 – Holotype: Venezuela, *Curran & Haman 560* (GH barcode 00056492!).

Sarcomphalus divaricatus Griseb., Cat. Pl. Cub.: 31. 1866 ≡ *Ziziphus grisebachiana* M.C.Johnst. in Amer. J. Bot. 51: 1117. 1964 – **Lectotype (designated here):** Cuba, *Wright 2102* (GOET barcode GOET009873!; isolectotypes: GH barcode 00051510!, K barcode K000531798!, MO barcode MO-2049565!).

Sarcomphalus domingensis (Spreng.) Krug & Urb. in Urban, Symb. Antill. 1: 357. 1899 ≡ *Cassine domingensis* Spreng., Syst. Nat., ed. 16, 1: 939. 1824 – **Lectotype (designated here):** Dominican Republic, *Eggers 1801* (KFTA barcode KFTA0000551 n.v.).

= *Ziziphus rignonii* Delponte in Mem. Reale Accad. Sci. Torino, ser. 2, 14: 405. 1854 – Lectotype (designated by Johnston in Amer. J. Bot. 51: 1116. 1964): [illustration] "*Ziziphus Rignonii*" in Mem. Reale Accad. Sci. Torino, ser. 2, 14: t. VII. 1854.

Sarcomphalus glaziovii (Warm.) Hauenschild, **comb. nov.** ≡ *Ziziphus glaziovii* Warm. in Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn, ser. 4, 1880: 373. 1880 – Lectotype (designated by Lima & Giulietti in Acta Bot. Brasil. 28: 381. 2014): Brazil, *Glaziou 864* (P barcode P01818574 n.v.; isolectotype: P barcode P01818573 n.v.).

Sarcomphalus guatemalensis (Hemsl.) Hauenschild, **comb. nov.** ≡ *Ziziphus guatemalensis* Hemsl., Diagn. Pl. Nov. Mexic. 1: 6. 1878 – Holotype: Guatemala, *Skinner s.n.* (K barcode K000531808 n.v.).

Sarcomphalus havanensis (Kunth) Griseb., Cat. Pl. Cub.: 31. 1866 ≡ *Ziziphus havanensis* Kunth in Humboldt & al., Nov. Gen. Sp. 7: 57. 1824 – Lectotype (designated by Johnston in Amer. J. Bot. 51: 1116. 1964): Cuba, *Bonplant 4520* (P barcode P00660065!).

Sarcomphalus havanensis var. ***bullatus*** (Urb.) Hauenschild, **comb. nov.** ≡ *Ziziphus havanensis* var. *bullata* (Urb.) M.C. Johnst. in Amer. J. Bot. 51: 1117. 1964 ≡ *Sarcomphalus bullatus* Urb., Symb. Antill. 9: 230. 1924 – Lectotype (designated by Johnston in Amer. J. Bot. 51: 1117. 1964): Cuba, *Ekman 6620* (S No. S-R-7628; isolectotype: NY barcode 00008190 n.v.).

Sarcomphalus joazeiro (Mart.) Hauenschild, **comb. nov.** ≡ *Ziziphus joazeiro* Mart. in Martius & Spix, Reise Bras.: 581. 1828 – Lectotype (designated by Lima & Giulietti in Acta Bot. Brasil. 28: 381. 2014): Brazil, *Martius 1812* (M barcode M-0211782 n.v.).

= *Ziziphus guaranitica* Malme in Bih. Kongl. Svenska Vetensk.-Akad. Handl. 27, Afd. 3, 11: 20, pl. 1, f. 1. 1901 – Lectotype (designated by Lima & Giulietti in Acta Bot. Brasil. 28: 381. 2014): Paraguay, *Malme 1108* (G n.v.; isolectotypes: BM barcode BM000817618 n.v., S Nos. S07-8501, S11-13177 n.v. & S11-13178 n.v., US barcodes 00094327! & 00094328!).

= *Ziziphus cotinifolia* Reissek in Martius, Fl. Bras. 11(1): 87. 1861 – Lectotype (designated by Lima & Giulietti in Acta Bot. Brasil. 28: 380. 2014): Brazil, *Gardner 1266* (W; isolectotypes: BM barcode BM000838625, F barcode F0068184F, GH barcode 00051535, K barcodes K000531794, K000531795 & K000531796, NY barcodes 00415089, 00415090 & 00415092, P barcodes P01818569, P01818570 & P01818571, SP barcode SP001517; all n.v.).

= *Ziziphus pseudojoazeiro* Mansf. in Notizbl. Bot. Gart. Berlin-Dahlem 9: 39. 1924 – **Lectotype (designated here):** *Luetzelburg 12396* (M barcode M-0211710 n.v.).

= *Ziziphus gardneri* Reissek in Endlicher, Nov. Stirp. Dec. 30. 1839 – **Lectotype (designated here):** *Gardner 959* (E barcode E00296038!; isolectotypes: BM barcode BM000838610!, K barcode K000530000!).

Sarcomphalus lloydii (Standl.) Hauenschild, **comb. nov.** ≡ *Condalia lloydii* Standl. in Contr. U.S. Natl. Herb. 23: 714. 1923 ≡ *Condaliopsis lloydii* (Standl.) Suess. in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 20d: 135. 1953 ≡ *Ziziphus lloydii* (Standl.) M.C.Johnst. in Brittonia 14: 367. 1962 – Holotype: Mexico, *Llyod 71* (US barcode 00094351 n.v.).

Sarcomphalus mexicanus (Rose) Hauenschild, **comb. nov.** ≡ *Ziziphus mexicana* Rose in Contr. U.S. Natl. Herb. 1(9): 315. 1895 – Holotype: Mexico, *Palmer 1278* (US barcode US00094335!).

Sarcomphalus microdictyus Urb. & Ekman in Ark. Bot. 20A(15): 72. 1926 ≡ *Ziziphus microdictya* (Urb. & Ekman) M.C.Johnst. in Amer. J. Bot. 51: 1116. 1964 – Lectotype (designated by Johnston in Amer. J. Bot. 51: 1116. 1964): Haiti, *Ekman H3749* (S No. S12-23577!).

Sarcomphalus mistol (Griseb.) Hauenschild, **comb. nov.** ≡ *Ziziphus mistol* Griseb. in Abh. Königl. Ges. Wiss. Göttingen 19: 99–100. 1874 – **Lectotype (designated here):** *Lorentz 515* (GOET barcode GOET009874!).

= *Ziziphus oblongifolius* S.Moore in Trans. Linn. Soc. London, Bot., ser. 2, 4: 339, pl. 24. 1895 – **Lectotype (designated here):** *Moore 1029* (BM barcode BM000838620!; isolectotypes: F (photo) barcode F0BN005836 n.v., NY barcode 00415091!).

= *Ziziphus weberbaueri* Pilg. in Bot. Jahrb. Syst. 54(1, Beibl. 117): 46. 1916 – **Lectotype (designated here):** *Weberbauer 5920* (GH barcode GH00051540!; isolectotypes: F barcode F0043057F!, US barcode 00094346!).

Sarcomphalus obovatus Urb. in Repert. Spec. Nov. Regni Veg. 19: 299. 1924 ≡ *Ziziphus obovata* (Urb.) M.C.Johnst. in Amer. J. Bot. 51: 1117. 1964 – **Lectotype (designated here):** Cuba, *Ekman 15480* (S No. S-R-7629!; isolectotype: NY barcode 00008187!).

Sarcomphalus obtusifolius (Hook. ex Torr. & A.Gray) Hauenschild, **comb.nov.** ≡ *Rhamnus obtusifolia* Hook. ex Torr. & A.Gray, Fl. N. Amer. 1: 685. 1840 ≡ *Ziziphus obtusifolia* (Hook. ex Torr. & A.Gray) A.Gray, Gen. Amer. Bor. 2: 170. 1849 ≡ *Condalia obtusifolia* (Hook. ex Torr. & A.Gray) Weberb. in Engler & Prantl, Nat. Pflanzenfam. III(5): 404. 1895 ≡ *Condaliopsis obtusifolia* (Hook. ex Torr. & A.Gray) Suess. in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 20d: 135. 1953 – **Lectotype (designated here):** United States, *Drummond 45* (K barcode K000723062!; isolectotype: GH barcode 00051526!).

= *Ziziphus lycioides* A.Gray in Boston J. Nat. Hist. 6(2): 168. 1850 ≡ *Condalia lycioides* (A.Gray) Weberb. in Engler & Prantl, Nat. Pflanzenfam. III(5): 404. 1895 ≡ *Condaliopsis lycioides* (A.Gray) Suess. in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 20d: 135. 1953 – **Lectotype (designated here):** *Wright 920* (K barcode K000723071!; isolectotype: BM barcode BM001024168!).

Sarcomphalus obtusifolius var. ***canescens*** (A.Gray) Hauenschild, **comb. nov.** ≡ *Ziziphus lycioides* var. *canescens* A.Gray in Rothrock, Rep. U.S. Geogr. Surv., Wheeler 6: 82. 1879 ≡ *Condalia obtusifolia* var. *canescens* (A.Gray) Trel. in Gray, Syn. Fl. N. Amer. 1: 403. 1897 ≡ *Condaliopsis obtusifolia* var. *canescens* (A.Gray) Suess. in Engler & Prantl, Nat. Pflanzenfam., ed. 2, 20d: 135. 1953 ≡ *Ziziphus obtusifolia* var. *canescens* (A.Gray) M.C.Johnst. in Brittonia 14: 367. 1962 – **Lectotype (designated here):** *Rothrock 331* (F barcode F0068179F!; isolectotypes: GH barcode 00051525!, US barcode 00094333!, YU barcode YU.068727!).
= *Condalia rigida* Wiggins in Contr. Dudley Herb. 4(2): 20, pl. 1, f. 5–8. 1950 ≡ *Condaliopsis rigida* (Wiggins) Wiggins, Veg. Fl. Sonoran Des. 2: 861. 1964 – Holotype: Mexico, *Johansen 601* (CAS barcode 0002105 n.v.).
= *Condalia divaricata* A.Nels. in Bot. Gaz. 47: 427. 1909 ≡ *Ziziphus divaricata* (A.Nels.) Davidson & Moxley, Fl. S. Calif.: 226. 1923 – **Lectotype (designated here):** United States, *Goodding 2300* (RM barcode RM0003669!; isolectotypes: GH barcode 00051304!, MO barcode MO-260879!).

Sarcomphalus parvifolius Urb. & Ekman in Ark. Bot. 20A(5): 18. 1926 ≡ *Ziziphus urbani* M.C.Johnst. in Amer. J. Bot. 51: 1117. 1964 – Lectotype (designated by Johnston in Amer. J. Bot. 51: 1117. 1964): Haiti, *Ekman 1004* (S No. S-R-7627 n.v.).

Sarcomphalus pedunculatus (Brandegee) Hauenschild, **comb. nov.** ≡ *Condalia pedunculata* Brandegee in Univ. Calif. Publ. Bot. 3: 384. 1909 ≡ *Ziziphus pedunculata* (Brandegee) Standl. in Contr. U.S. Natl. Herb. 23: 713. 1923 – **Lectotype (designated here):** Mexico, *Purpus 3173* (UC barcode UC 125752!; isolectotypes: F barcode F0068128F!, GH barcode 00051532!, NY barcode 00406592!, US barcode 00094352!).

Sarcomphalus piurensis (Pilger) Hauenschild, **comb. nov.** ≡ *Ziziphus piurensis* Pilger in Bot. Jahrb. Syst. 54(1, Beibl. 117): 46. 1916 – **Lectotype (designated here):** Mexico, *Weberbauer 5962* (F barcode F0075521F!; isolectotypes: S No. S-R-11086!, US barcode 00094339!).

Sarcomphalus platyphyllus (Reissek) Hauenschild, **comb. nov.** ≡ *Ziziphus platyphylla* Reissek in Martius, Fl. Bras. 11(1): 87. 1861 – Lectotype (designated by Lima & Giulietti in Acta Bot. Brasil. 28: 381. 2014): Brazil, *Sellow 1240* (W n.v.).

Sarcomphalus reticulatus (Vahl) Urb., Symb. Antill. 1: 357. 1899 ≡ *Paliurus reticulata* Vahl, Eclog. Amer. 3: 6. 1807 ≡ *Ziziphus reticulata* (Vahl) DC., Prodr. 2: 20. 1825 – Holotype: “America meridiolali”, *Rohr s.n.* (C No. LNR-5843 n.v.).
= *Rhamnus rotundifolia* Sessé & Moc., Fl. Mexic., ed. 2: 61. 1894, nom. illeg., non Pers. 1805 – **Lectotype (designated here):** Puerto Rico, *Sessé & Mocino 72* (BM barcode BM000838639!)

Sarcomphalus rhodoxylon (Urb.) Hauenschild, **comb. nov.** ≡ *Ziziphus rhodoxylon* Urb., Symb. Antill. 5: 407. 1908 – **Type collection:** Hispaniola, *Buch 653* (B†; no duplicate traceable).

Sarcomphalus saeri (Pittier) Hauenschild, **comb. nov.** ≡ *Ziziphus saeri* Pittier, Arb. Arbust. Venez.: 61. 1925 – **Lectotype (designated here):** Venezuela, *Saer 73* (VEN barcode VEN 7551!; isolectotypes: K barcode K000529994!, NY barcodes 00415093! & 00415094!, US barcodes 00094340! & 00094341!).

Sarcomphalus strychnifolius (Triana & Planch.) Hauenschild, **comb. nov.** ≡ *Ziziphus strychnifolia* Triana & Planch. in Ann. Sci. Nat., Bot., sér. 5, 16: 380. 1872 – **Lectotype (designated here):** Colombia, *Triana 3525* (BM barcode BM000838637!; isolectotypes: E barcode E00296037!, F barcode F0068185F!, K barcode K000529995!, P barcode P01818582!).

Sarcomphalus taylori Britton in Bull. New York Bot. Gard. 3: 445. 1905 ≡ *Ziziphus taylori* (Britton) M.C.Johnst. in Amer. J. Bot. 51: 1118. 1964 – **Lectotype (designated here):** Bahamas, *Nash & Taylor 961* (K barcode K000531797!; isolectotype: F barcode F0068176F!).

Sarcomphalus thyrsiflorus (Benth.) Hauenschild, **comb. nov.** ≡ *Ziziphus thyrsiflora* Benth., Bot. Voy. Sulphur: 78. 1844 – **Lectotype (designated here):** Ecuador, *Sinclair s.n.* (K barcode K000529993!).

Sarcomphalus undulatus (Reissek) Hauenschild, **comb. nov.** ≡ *Ziziphus undulata* Reissek. in Martius, Fl. Bras. 11(1): 88. 1861 – Lectotype (designated by Lima & Giulietti in Acta Bot. Brasil. 28: 381. 2014): Brazil, *Gardner 1522 s.n.* (W n.v.; isolectotypes: BM barcode BM00083611!, F barcode F0068186F!, GH barcodes 00051538! & 00051539!, K barcodes K000520001! & K000531790!, NY barcodes 00415095! & 00415096!, P barcodes P01818585!, P01818586! & P01818587!, SP barcode SP001519!).

Sarcomphalus yucatanensis (Standl.) Hauenschild, **comb. nov.** ≡ *Ziziphus yucatanensis* Standl. in Trop. Woods 32: 16–17. 1932 – Holotype: Mexico, *Flores s.n.* (F barcode F0068180F n.v.).

Excluded species

Sarcomphalus carolinianus (Walter) Raf., Sylva Tellur.: 29. 1838 ≡ *Rhamnus caroliniana* Walter, Fl. Carol.: 101. 1788 ≡ *Frangula caroliniana* (Walter) A.Gray, Gen. Amer. Bor. 2: 178. 1849.

Sarcomphalus cubensis Britton in Mem. Torrey Bot. Club 16: 79. 1920 ≡ *Doerpfeldia cubensis* (Britton) Urb., Symb. Antill. 9: 218. 1924.

Sarcomphalus graecus Raf., Sylva Tellur.: 29. 1838 ≡ *Rhamnus sibthorpiana* Roem. & Schult., Syst. Veg. 5: 286. 1819.

Sarcomphalus levigatus (Vahl) Raf., Sylva Tellur.: 29. 1838 ≡ *Rhamnus laevigata* Vahl, Symb. Bot. 3: 41. 1794 ≡ *Maytenus laevigata* (Vahl) Griseb. ex Eggers in Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn, ser. 3, 8: 109. 1876.
= *Maytenus elliptica* Krug & Urb. in Duss, Fl. Phan. Antill. Franç.: 145. 1897.

Sarcomphalus mauritianus (Lam.) Raf., Sylva Tellur.: 29. 1838 ≡ *Ziziphus mauritiana* Lam., Encycl. 3: 319. 1789.

Sarcomphalus punctatus Urb. & Ekman in Ark. Bot. 20A(5): 19. 1926 ≡ *Celtis punctata* (Urb. & Ekman) Urb. & Ekman in Ark. Bot. 22A: 14. 1929.
= *Celtis iguanaea* (Jacq.) Sarg. in Silva 7: 64. 1895.

Sarcomphalus shortianus (Nutt.) Raf., Sylva Tellur.: 29. 1838 ≡ *Rhamnus shortii* Nutt. in J. Acad. Nat. Sci. Philadelphia 7(1): 91. 1834.

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Appendix 1. Voucher information and GenBank accession numbers for ITS and *trnL-trnF* sequences used in this study.

Taxon, country, collector and number, or collection/accession number (herbarium code or accession location), GenBank accession number for ITS, *trnL-trnF*. Missing data is indicated by a dash (–). An asterisk (*) indicates newly sequenced specimens.

Adolphia infesta (Kunth) Meisn., cultivated, *858* (Rancho Santa Ana Botanic Garden), –, AY642142. ***Alphitonia excelsa*** (Fenzl) Reissek ex Endl., cultivated, *Kellermann 103* (MEL), HQ340157, HQ325600. ***Alphitonia incana*** (Roxb.) Teijsm. & Binn. ex Kurz, Australia, *Chase 2179* (K), AF328830, AJ390352. ***Alphitonia petrei*** Braid & C.T.White, cultivated, *Wells s.n.* (University of Queensland), –, KC428438. ***Alphitonia whitei*** Braid, cultivated, *Wells s.n.* (University of Queensland), –, KC428439. ***Ampelozizyphus amazonicus*** Ducke, Brazil, *Vilhena 1004* (K), –, AJ309341. ****Auerodendron acunae*** Borhidi & O.Muniz, Brazil, *Alvarez & al. 35559* (JE), KR083053, KR083104. ***Bathiorhamnus cryptophorus*** Capuron, Madagascar, *Labar 2044* (K), –, AJ306540. ****Berchemia flavescens*** Wall., India, *Chand 7505* (L), KR083054, KR083105. ****Berchemia floribunda*** (Wall.) Brongn., Thailand, *Hansen & Smitinand 12690* (L), KR083055, KR083106**.** ****Berchemia formosana*** C.K.Schneid., Taiwan, *Yang s.n.* (L), KR083059, KR083110. ****Berchemia huana*** Rehder, cultivated (Kunming Botanic Garden), KR083056, KR083107. ****Berchemia lineata*** (L.) DC., Japan, *Azuma s.n.* (FR), KR083058, KR083108. ****Berchemia lineata*** (L.) DC. (2), Japan, *Furuse 1569* (L), KR083057, KR083109. ***Berchemia*** **sp. 'racemosa'**, locality unknown, *Tsugaru 14944* (MO), JN900290, JN900314. ****Berchemia scandens*** (Hill) K.Koch, United States, *Leidolf 227* (FR), KR083060, –. ****Berchemiella berchemifolia*** (Makino) T.Nakai, Japan, *Togashi 599* (L), KR083061, KR083111. ***Berchemiella wilsonii*** (C.K.Schneid.) T.Nakai, locality unknown, *Deng 93007* (MO), JN900305, JN900321. ***Blackallia nudiflora*** (F.Muell.) Rye & Kellermann, Australia, *Kellermann 257* (MEL), AY911558, EF528505. ****Ceanothus americanus*** L., United States, *Leidolf 286* (FR), KR083062, KR083112. ***Ceanothus americanus*** L. (2), cultivated, *94-1526* (University of California Botanical Garden), AF048901, –. ***Ceanothus arboreus*** Greene, cultivated, *91-207* (Santa Barbara Botanical Garden), AF048902, –. ***Ceanothus buxifolius*** Willd., United States, *Hinton & al. 25295* (UTEX), AF048903, –. ***Ceanothus coeruleus*** Lag., United States, *Castillo 978* (UTEX), AF048904, –. ***Ceanothus coeruleus*** Lag. (2), United States, *Chase 2413* (K), AF328835, –. ***Ceanothus confusus*** J.T., cultivated, *Howell 88-1285* (University of California Botanical Garden), AF048933, –. ***Ceanothus cordulatus*** Kellogg, United States, *Bunge 9933a* (CAS), HQ325315, HQ325601. ***Ceanothus cordulatus*** Kellogg (2), cultivated, *93-1283* (University of California Botanical Garden), AF048905, –. ***Ceanothus crassifolius*** Torr., cultivated, *80-0116* (University of California Botanical Garden), AF048936, –. ***Ceanothus cuneatus*** (Hook.) Nutt., United States, *Hardig 694* (WS), AF048936, –. ***Ceanothus cuneatus*** (Hook.) Nutt. (2), United States, *Hardig 2395* (WS), AF048935, –. ***Ceanothus cuneatus*** (Hook.) Nutt. (3), United States, *Hardig 5195* (WS), AF048937, –. ***Ceanothus cuneatus*** (Hook.) Nutt. (4), cultivated, *87-1252* (University of California Botanical Garden), AF048938, –. ***Ceanothus cuneatus*** (Hook.) Nutt. (5), cultivated, *87-0292* (University of California Botanical Garden), AF048939, –. ****Ceanothus cuneatus*** (Hook.) Nutt. (6), United States, *Schrenk 11274* (FR), KR083063, KR083113. ***Ceanothus cyaneus*** Eastw., cultivated, *92-26* (Santa Barbara Botanical Garden), AF048906, –. ***Ceanothus dentatus*** Torr. & A.Gray, United States, *Michener & al. 4104* (RSA), AF048907, –. ***Ceanothus divergens*** Parry, United States, *Knight & Edwards 5186* (WS), AF048940, –. ***Ceanothus diversifolius*** Kellogg, cultivated, *81-0312* (University of California Botanical Garden), –, AF048908. ***Ceanothus fendleri*** A.Gray, United States, *Siplivinsky & Beck 4209* (WS), AF0408909, –. ***Ceanothus ferrisae*** McMinn, United States, *Hardig 2294* (WS), AF048941, –. ***Ceanothus foliosus*** McMinn, cultivated, *92-470* (Santa Barbara Botanical Garden), AF048910, –. ***Ceanothus foliosus*** McMinn (2), cultivated, *82-1584* (University of California Botanical Garden), AF048911, –. ***Ceanothus fresnensis*** Abrams, United States, *Michener & al. 4160b* (RSA), AF048942, –. ***Ceanothus gloriosus*** J.T.Howell, United States, *Hardig 296-4* (WS), AF048943, –. ***Ceanothus gloriosus*** J.T.Howell (2), cultivated, *7103* (Rancho Santa Ana Botanical Garden), AF048945, –. ***Ceanothus gloriosus*** J.T.Howell (3), cultivated, *7103-2*. (Rancho Santa Ana Botanical Garden), AF048944, –. ***Ceanothus greggi*** A.Grey, cultivated, *14269* (Rancho Santa Ana Botanical Garden), AF048946, –. ****Ceanothus greggi*** A.Grey (2), United States, *Schrenk 1893* (FR), –, KR083114. ***Ceanothus griseus*** (Trel.) McMinn, cultivated, *83-0664* (University of California Botanical Garden), AF048912, –. ***Ceanothus hearstiorum*** Hoover & Roof, cultivated, *72-54* (Santa Barbara Botanical Garden), AF048914, –. ***Ceanothus hearstiorum*** Hoover & Roof (2), cultivated, *82-1079* (University of California Botanical Garden), AF048915, –. ***Ceanothus herbaceous*** Raf., United States, *Kelso & Maentz 96-21* (UCO), AF048913, –. ****Ceanothus herbaceous*** Raf. (2), United States, *Schrenk 2679* (FR), –, KR083115. ***Ceanothus impressus*** Trel., cultivated, *82-269* (Santa Barbara Botanical Garden), AF048916, –. ***Ceanothus incanus*** Torr. & A.Grey, cultivated, *90-203* (Santa Barbara Botanical Garden), AF048917, –. ***Ceanothus integerrimus*** Hook.& Arn., cultivated, *90-1566* (University of California Botanical Garden), AF048918, –. ***Ceanothus jepsonii*** Greene, United States, *Hardig 794* (WS), AF048947, –. ***Ceanothus jepsonii*** Greene (2), United States, *Hardig 2194* (WS), AF048948, –. ***Ceanothus lanuginosus*** (Jones) Rose, United States, *Laferriere 1391* (UTEX), AF048919, –. ***Ceanothus lemmonii*** Parry, cultivated, *88-354* (Santa Barbara Botanical Garden), AF048919, –. ***Ceanothus leucodermis*** Greene, cultivated, *79-62* (Santa Barbara Botanical Garden), AF048920, –. ***Ceanothus maritimus*** Hoover, cultivated, *87-0006* (University of California Botanical Garden), AF048950, –. ***Ceanothus martini*** M.E.Jones, United States, *Tiem & Crisafulli 11802* (COLO), AF048921, –. ***Ceanothus masonii*** McMinn, United States, *Hardig 1196-26* (WS), AF048953, –. ***Ceanothus masonii*** McMinn (2), United States, *Hardig 1296-8* (WS), AF048954, –. ***Ceanothus masonii*** McMinn (3), United States, *Hardig 1496-22* (WS), AF048955, –. ***Ceanothus masonii*** McMinn (4), United States, *Hardig 3395* (WS), AF048951, –. ***Ceanothus masonii*** McMinn (5), United States, *Hardig 3795* (WS), AF048952, –. ***Ceanothus megacarpus*** Nutt., cultivated, *82-1653* (University of California Botanical Garden), AF048956, –. ***Ceanothus megacarpus*** Nutt. (2), cultivated, *87-1618* (University of California Botanical Garden), AF048957, –. ***Ceanothus ochracea*** Suess., United States, *Warnock & al. 2138* (UTEX), AF048922, –. ***Ceanothus oliganthus*** var. ***sorediatus*** (Hook. & Arn.) Hoover, cultivated, *92-368* (Santa Barbara Botanical Garden), AF048923, –. ***Ceanothus ophiochilus*** S.Boyd, T.S.Ross & Arnseth, cultivated, *15872* (Rancho Santa Ana Botanical Garden), AF048958, –. ***Ceanothus palmeri*** Trel., United States, *Carlquist 15868* (RSA), AF048924, –. ***Ceanothus papillosus*** McMinn, cultivated, *72-0344* (University of California Botanical Garden), AF048925, –. ***Ceanothus parryi*** Trel., cultivated, *88-348* (Santa Barbara Botanical Garden), AF048926, –. ***Ceanothus parvifolius*** (S.Watson) Trel., United States, *Michener & al. 4159* (RSA), AF048927, –. ***Ceanothus pinetorum*** Coville, United States, *Schmidt 1293* (WS), AF048960, –. ***Ceanothus prostratus*** Benth., cultivated, *82-1456* (University of California Botanical Garden), AF048960, –. ***Ceanothus pumilus*** Greene, United States, *Bunge 993a* (CAS), HQ340158, HQ325602. ***Ceanothus pumilus*** Greene (2), cultivated, *Hardig 194* (WS), AF048961, –. ***Ceanothus purpureus*** Jeps., United States, *Hardig 1694* (WS), AF048963, –. ***Ceanothus roderickii*** W.Knight, United States, *Ingle s.n.* (UC), AF048963, –. ***Ceanothus sanguineus*** Pursh, United States, *Halse 3793* (OSU), AF048928, –. ***Ceanothus sonomensis*** J.T.Howell, United States, *Hardig 1495* (WS), AF048966, –. ***Ceanothus sonomensis*** J.T.Howell (2), United States, *Hardig 1595* (WS), AF048965, –. ***Ceanothus sonomensis*** J.T.Howell (3), United States, *Hardig 1696-1* (WS), AF048969, –. ***Ceanothus sonomensis*** J.T.Howell (4), United States, *Hardig 1795* (WS), AF048971, –. ***Ceanothus sonomensis*** J.T.Howell (5), United States, *Hardig 1796-6* (WS), AF048970, –. ***Ceanothus sonomensis*** J.T.Howell (6), United States, *Hardig 1895* (WS), AF048964, –. ***Ceanothus sonomensis*** J.T.Howell (7), United States, *Hardig 1896-10* (WS), AF048968, –. ***Ceanothus sonomensis*** J.T.Howell (8), cultivated, *83-1515* (University of California Botanical Garden), AF048967, –. ***Ceanothus spinosus*** Torr. & A.Grey, cultivated, *81-17* (Santa Barbara Botanical Garden), AF048929, –. ***Ceanothus thyrsiflorus*** Eschsch., *Chase 3177* (K), AF328835, –. ***Ceanothus thyrsiflorus*** Eschsch. (2), cultivated, *82-1642* (University of California Botanical Garden), AF048930, –. ***Ceanothus tomentosus*** Parry, cultivated, *61-15* (Santa Barbara Botanical Garden), AF048931, –. ***Ceanothus***

Appendix 1. Continued.

velutinus Douglas, cultivated, *92-298* (Santa Barbara Botanical Garden), AF048932, –. **_Ceanothus verracosus_** Nutt., cultivated, *86-883* (University of California Botanical Garden), AF048972, –. **_Colletia hystrix_** Clos, Argentina, *Medan 775* (BAA), –, AY460409. **_Colletia paradoxa_** (Spreng.) Escal., Argentina, *Mantese s.n.* (BAA), –, AY642143. **_Colletia spinosissima_** Gmel., cultivated, *Living-607* (UBA), –, AY460411. **_Colletia ulcina_** Gillies & Hook., Chile, *Medan 791* (BAA), –, AY460412. **_Colletia ulcina_** Gillies & Hook. (2), Chile, *Swensen & al. s.n.* (n.a.), –, AY460412. **_Colubrina asiatica_** (L.) Brongn., Australia, *Chase 905* (K), AF328831, AJ390350. ***_Colubrina asiatica_** (L.) Brongn. (2), Australia, *Inkrot & Geyer s.n.* (LZ), KR083064, KR083116. **_Colubrina elliptica_** (Sw.) Brizicky & W.L.Stern, *Chase 2115* (K), AF328832, AJ390370. ***_Colubrina glandulosa_** Perkins, Brazil, *Bernacci & al. 21435* (FR), KR083065, KR083117. ***_Colubrina oppositifolia_** Brongn. ex H.Mann, United States, *Herbst & al. 5250* (FR), KR083066, KR083118. ***_Condalia henriquezii_** Bold., Curaçao, *de Wilde 9* (L), KR083067, KR083119. **_Condalia mexicana_** Schldl., Mexico, *Dziekanowski & al. 3312* (MO), JN900302, –. **_Condalia microphylla_** Cav., Argentina, *Kiesling & al. 5967* (K), AY626456, AJ390334. ***_Condalia microphylla_** Cav. (2), Argentina, *Pedersen 9901* (L), KR083068, KR083120. **_Condalia velutina_** I.M.Johnst., locality unkown, *Fernandez 719* (MO), JN900303, –. **_Crumenaria choretroides_** Reissek, Paraguay, *Zardini & Benitez 51053* (US), HQ325385, –. ***_Crumenaria polygaloides_** Reissek, Brazil, *Silva & Ribas 3489* (ULM), KR083069, KR083121. **_Cryptandra alpina_** Hook.f., cultivated, *Burns 98* (CANB), AY911540, EF528488. **_Cryptandra amara_** Sm., cultivated, *Walsh s.n.* (Walsh), AY911545, EF528489. **_Cryptandra arbutiflora_** Rye, Australia, *Kellermann 224* (MEL), AY911546, EF528491. **_Cryptandra connata_** C.A.Gardner, Australia, *Pringle 2494* (CANB), AY911561, EF528503. **_Cryptandra dielsii_** C.A.Gardner, Australia, *Kellermann 292* (MEL), AY911553, EF528500. **_Cryptandra ericoides_** Sm., Australia, *Albrecht 3989* (MEL), AY911541, EF528487. **_Cryptandra gemmata_** Bean, Australia, *Dunlop 4919* (MEL), AY911547, EF528494. **_Cryptandra hispidula_** Reissek & F.Muell., *Jackson 3187* (MEL), AY911542, EF528492. **_Cryptandra intratropica_** W.Fitzg., Australia, *Craven & Brubaker 9163* (CANB), AY911549, EF528492. **_Cryptandra lansiflora_** F.Muell., cultivated, *Fethers 8* (CANB), AY911543, EF528490. **_Cryptandra micrantha_** Rye, Australia, *Kellermann 278* (MEL), AY911544, EF528493. **_Cryptandra mutila_** Nees ex Reissek, *Keighery s.n.* (PERTH), AY911544, EF528493. **_Cryptandra nola_** Rye, Australia, *Rye 239044* (PERTH), AY911552, EF528499. **_Cryptandra pungens_** Steud., Australia, *Kellermann 375* (MEL), AY911551, EF528497. **_Dallachyia vitiensis_** (Benth.) F.Muell., locality unknown, *Forster & al. 27619* (NY), JN900300, JN900333. ***_Dallachyia vitiensis_** (Benth.) F.Muell. (2), Australia, *Inkrot & Geyer s.n.* (LZ), KR083084, KR083136. **_Discaria americana_** Gillies & Hook., cultivated, *48079210* (Botanischer Garten Berlin-Dahlem), –, AY642144. **_Discaria articulata_** (Phil.) Miers, cultivated, *Chaia s.n.* (n.a.), –, AY460414. **_Discaria articulata_** (Phil.) Miers (2), Argentina, *Hilger s.n.* (BSB), –, AY642145. **_Discaria chacaye_** (Don) Tortosa, Argentina, *Medan 775* (BAA), –, AY642146. **_Discaria chacaye_** (Don) Tortosa (2), Argentina, *Swensen & al. s.n.* (n.a.), –, AJ225797. **_Discaria nitida_** Tortosa, cultivated, *915497* (Royal Botanic Gardens Melbourne), –, AY642148. **_Discaria pubescens_** (Brongn.) Druce, cultivated, *Walsh s.n.* (Walsh), –, AY642149. **_Discaria toumatou_** Raoul, cultivated, *P19815496* (Botanic Garden of the University Copenhagen), –, AY642150. **_Doerpfeldia cubensis_** (Britton) Urb., Cuba, *Howard & al. 246* (K), –, AJ390342. **_Emmenosperma alphitonioides_** F.Muell., Australia, *Kellermann 419* (Australian National Botanic Gardens), HQ340159, – / *Clarkson 8826* (K), – AJ390351. **_Frangula alnus_** Mill., Sweden, *Bolmgren 104* (S), AY626431, AJ251691. **_Frangula alnus_** Mill. (2), locality unkown, *Gagnidze & Shetekauri 286* (MO), JN900292, JN900316. ***_Frangula alnus_** Mill. (3), Georgia, *Hilbig s.n.* (HAL), KR083198, KR083360. ***_Frangula alnus_** Mill. (4), Germany, *Krusche s.n.* (LZ), *KR083197, KR083359. **_Frangula alnus_** var. **_baetica_** (Reverchon & Willk.) Rivas, Goday ex Devesa, Spain, *Hampe s.n.* (S), AY626443, AY626429. ***_Frangula azorica_** Grubov, Portugal (Azores), *Reins s.n.* (FR), KR083227, KR083386. **_Frangula betulifolia_** (Greene) Grubov, North America, *Bolmgren 105* (S), AY626445, AY626445. ***_Frangula betulifolia_** (Greene) Grubov (2), United States, *Worthington 14109* (L), KR083168, KR083337. **_Frangula californica_** (Eschsch.) A.Grey, United States, *Holland 114* (S), AY626442, AY626421. ***_Frangula californica_** (Eschsch.) A.Grey (2), United States, *Schrenk 2044* (FR), KR083171, KR083339. ***_Frangula californica_** (Eschsch.) A.Grey (3), United States, *Scharsmith 4244* (L), KR083172, KR083340. ***_Frangula capreifolia_** (Schltdl.) Grubov, Mexico, *Nee 26582* (HAL), KR083173, KR083341. **_Frangula caroliniana_** (Walter) A.Grey, North America, *Schmidt 2559* (S), AY626444, AY626423. ***_Frangula crenata_** (Siebold & Zucc.) Miq., Japan, *Azuma s.n.* (FR), KR083180, –. **_Frangula crenata_** (Siebold & Zucc.) Miq. (2), China, *NieMinXiang 92169* (UPS), AY626443, AY626422. ***_Frangula crenata_** (Siebold & Zucc.) Miq. (3), cultivated, *KUN060934* (KUN), KR083177. ***_Frangula crenata_** (Siebold & Zucc.) Miq. (4), locality unknown, *L0550458* (L), KR083178, KR083345. ***_Frangula_** aff. **_crenata_** **'cambodiana'**, Thailand, *van Beusekom & al. 4585* (L), KR083179, KR083346. ***_Frangula_** aff. **_crenata_** **'styracifolia'**, China, *Renma 5436* (KUN), KR083306, –. ***_Frangula discolor_** (Donn. Sm.) Grubov, Guatemala, *Skutch 1901* (L), KR083187, KR083351. ***_Frangula henryi_** (C.K.Schneid.) Grubov, China, *Tibet Expedition 9405* (KUN), KR083206, KR083368. ***_Frangula hintonii_** (M.C.Johnst. & L.A.Johnst.) A.Pool, Mexico, *Ledezma & Corral 481* (MEXU), KR083207, KR083369. ***_Frangula longipes_** (Merr. & Chun) Grubov, China, *Zhiding 53038* (KUN), KR083233, KR083393. ***_Frangula longystyla_** (C.B.Wolf) A.Pool, Mexico, *Gallardo 3645* (MEXU), KR083234, KR083394. ***_Frangula macrocarpa_** (Standl.) Grubov, Mexico, *Cortes 33* (MEXU), KR083244, KR083401. ***_Frangula mcvaughii_** (L.A.Johnst. & M.C.Johnst.) A.Pool, Mexico, *Garcia 573* (MEXU), KR083245, KR083402. ***_Frangula microphylla_** (Humb. & Bonpl. ex Schult.) Grubov, Mexico, *Torres 17031* (MEXU), KR083246, KR083403**.** ***_Frangula mucronata_** (Schltdl.) Grubov, Mexico, *Cornejo 290* (MEXU), KR083250, KR083407. ***_Frangula mucronata_** (Schltdl.) Grubov (2), Mexico, *KUN0614324* (KUN), KR083249, KR083406. ***_Frangula oreodendron_** (L.O.Williams) A.Pool, Panama, *McPherson 9822* (L), KR083253, –. ***_Frangula palmeri_** (S.Watson) Grubov, Mexico, *Tellez 12749* (MEXU), KR083261, KR083414. ***_Frangula pinetorum_** (Standl.) Grubov, *Bye 34722* (MEXU), KR083270, KR083423. ***_Frangula pompana_** M.C.Johnst. & L.A.Johnst., Mexico, *Hernandez 251* (MEXU), KR083272, KR083425. ***_Frangula pompana_** M.C.Johnst. & L.A.Johnst. (2), Mexico, *Nee 26584* (HAL), KR083271, KR083424. ***_Frangula pringlei_** (Rose) Grubov, Mexico, *Calzada 22155* (MEXU), KR083273, KR083426. ***_Frangula purshiana_** (DC.) A.Grey, United States, *Gibson 1962.27* (L), KR150636, KR150649. ***_Frangula purshiana_** (DC.) A.Grey (2), cultivated, *XX-0-FRT-0000/3140* (Botanischer Garten Frankfurt am Main), KR083285, KR083437. **_Frangula purshiana_** (DC.) A.Grey (3), United States, *JEPS97840* (JEPS), AY626430, AY626411. ***_Frangula rubra_** var. **_obtusissima_** (Greene) Kartesz & Ghandi, United States, *Heller 8106* (L), KR083288, KR083440. ***_Frangula rubra_** (Greene) Grubov var. **_rubra_**, United States, *Sharsmith 4265* (L), KR083287, KR083439. ***_Frangula_** **sp. 'humboldtiana'**, Costa Rica, *Frounier 845* (ULM), KR083211, KR083373. ***_Frangula rupestris_** Schur, Croatia, *de Wilde-Duyfjies 158* (L), KR083292, KR083444. ***_Frangula rupestris_** Schur (2), Italy, *Gregor 2877* (FR), KR083293, KR083445. ***_Frangula rupestris_** Schur (3), cultivated, *XX-0-FRT-0000/1587* (Botanischer Garten Frankfurt am Main), KR083290, KR083442. ***_Frangula rupestris_** Schur (4), Serbia, *Stud. Biol. In Itinere s.n.* (L), KR083291, KR083443. ***_Frangula sphaerosperma_** (Sw.) Kartesz & Ghandi, Brazil, *Cordeiro & al. 1622* (ULM), KR083309, KR083459. ***_Frangula sphaerosperma_** (Sw.) Kartesz & Ghandi (2), Brazil, *Hatschbach 17401* (L), KR083299, KR083451. ***_Frangula sphaerosperma_** (Sw.) Kartesz & Ghandi (3), Brazil, *Hatschbach 54096* (ULM), –, KR083460. ***_Frangula sphaerosperma_** (Sw.) Kartesz & Ghandi (4), Costa Rica, *Poyeda 845* (LZ), KR083310, KR083461. ***_Frangula sphaerosperma_** (Sw.), Kartesz & Ghandi (5), Venezuela, *Schneider 968* (FR), KR083308, KR083458. ***_Gouania javanica_** Miq., Papua New Guinea, *Gabo 378* (L), KR083070, –. ***_Gouania latifolia_** Reissek, Paraguay, *Fiebrig 4470* (L), KR083071, KR083122. ***_Gouania leptostachya_** DC., Laos, *Newman & al. LAO401* (L), KR083072, KR083123. ***_Gouania longispicata_** Engl., Kenya, *Hindorf 640* (FR), KR083073, KR083124/ KR083125. ***_Gouania lupoloides_** (L.) Urb., Panama, *Croat 21902* (L), KR083074, KR083126. **_Gouania_** **sp. 'mauritiana'**, *Chase 904* (K), –, AJ390344. **_Granitites intagendus_** (F.Muell.) Rye, Australia, *Hooper 8486A* (K), –. AJ306540. **_Granitites intagendus_** (F.Muell.) Rye (2), Australia, *Hooper 8486A* (K), HQ340160. –. **_Helinus integrifolius_** (Lam.) Kuntze, South Africa, *Balsinhas 3112* (US), HQ325386, –. ***_Helinus integrifolius_** (Lam.) Kuntze (2), Kenya, *Schultka s.n.* (FR), KR083075, KR083127. **_Helinus integrifolius_** (Lam.) Kuntze (3), South Africa, *Tulin & Warfa 5865* (K), –, AJ390343. **_Hovenia dulcis_** Thunb., United States, *Bordelon s.n.* (MO), DQ146607, DQ146563. **_Hovenia dulcis_** Thunb. (2), South Korea, *Chase 968* (K), –, AJ390343. **_Hovenia dulcis_** Thunb. (3), locality unknown, *CW 16* (Onstein & al., 2015), –, KP299394. **_Hovenia dulcis_** Thunb. (4), Australia, *Goodwin 619814* (NSW), –, DQ146516. ***_Hovenia trichocarpa_** Chun & Tsiang, *Azuma s.n.* (FR), –, KR083128. **_Hovenia trichocarpa_** Chun & Tsiang (2), China, *Lai & Shan 5670* (MO), DQ146608, DQ156565. **_Karwinskia calderonii_** Standl., locality unknown, *Villacorta 2383* (MO), JN900297, JN900326. **_Karwinskia humboldtiana_** Zucc., locality unknown, *Tapia & Mai 1071* (MO), JN900297, JN900327. **_Kentrothamnus weddelianus_** (Miers) M.C.Johnst., Argentina, *Medan 777* (BAA), –, AY460422. **_Krugiodendron ferreum_** Urb., cultivated, *95598A* (Montgomery Botanical Center), JN900299, JN900332. **_Krugiodendron ferreum_** Urb. (2), cultivated, *9701323A* (Montgomery Botanical Center), JN900298, JN900331. **_Lasiodiscus midbreadii_** Engl., Sao Tomé, *Figueiredo & al. 29* (K), AF328833, AJ390353. **_Maesopsis eminii_** Engl., cultivated, *Chase 1338* (K), –,

Appendix 1. Continued.

AJ390336. *_**Maesopsis eminii**_ Engl. (2), cultivated, _Mogea 2461_ (L), –, KR083129. _**Nesiota elliptica**_ (Roxb.) Hook., St. Helena, _Chase 500_ (K), AF328823, AJ225803. _**Noltea Africana**_ (L.) Rchb.f., South Africa, _Richardson 48_ (UWC), AF328822, –._**Ochetophila nana**_ (Clos) Kellermann, Medan & Aagesen, Argentina, _Medan 840_ (BAA), –, AY642147. _**Ochetophila trinervis**_ (Gillies & Hook.) Poepp. ex Endl., Argentina, _Valla s.n._ (BAA), –, AY460421. _**Paliurus ramosissimus**_ (Loir.) Poir., China, _Bufford 23964_ (MO), DQ146611, DQ146568. _**Paliurus ramosissimus**_ (Loir.) Poir. (2), China, _Lua 0861_ (MO), DQ146612, DQ146569. *_**Paliurus spina-christi**_ Mill., Bulgaria, _Buttler 22478_ (FR), KR083077, –. _**Paliurus spina-christi**_ Mill. (2), locality unkown, _Chase 969_ (K), –. AJ390354. _**Paliurus spina-christi**_ Mill. (3), cultivated, _Christensen s.n._ (University of Copenhagen), DQ146613. DQ146570._**Papistylus grandiflora**_ (C.A.Gardner) Kellermann, Rye & Thiele, Australia, _Kellermann 274_ (MEL), AY911559, EF528504. _**Phylica arborea**_ A.Thoars, Tristan da Cunha, _Richardson 51_ (UWC), AF328803, AF327603. _**Phylica arborea**_ A.Thoars (2), Nightingale, _Richardson 114_ (UWC), AF328802, –. _**Phylica arborea**_ A.Thoars (3), New Amsterdam, _Richardson 166_ (UWC), AF328801, –. _**Phylica buxifolia**_ L., South Africa, _Richardson 1_ (UWC), AF328813, AF327614. _**Phylica cryptandroides**_ Sond., South Africa, _Richardson 28_ (UWC), AF328815, AF327615. _**Phylica emimensis**_ Pillans, Madagascar, _Goldblatt & Schatz 8972_ (K), –, AF327610. _**Phylica ericoides**_ L., South Africa, _Richardson 13_ (UWC), AF328817, AF327617. _**Phylica fruticosa**_ Schl., South Africa, _Richardson 22_ (UWC), AF328819, AF327619. _**Phylica montana**_ Sond., South Africa, _Richardson 42_ (UWC), AF328811, AF327612. _**Phylica natalensis**_ Pillans, South Africa, _Nicholson s.n._ (K), –, AF327609. _**Phylica nitida**_ Lam., Mauritius, _Soorer 645_ (MICH), AF328821, AJ390356. _**Phylica nitida**_ Lam. (2), Reunión, _Thébaud s.n._ (K), AF328820, AF327620. _**Phylica oleaefolia**_ Vent., _Richardson 25_ (UWC), AF328812, AF327613. _**Phylica paniculata**_ Wild., South Africa, _Chase 136_ (K), AF328808, AF327606. _**Phylica paniculata**_ Willd. (2), South Africa, _Richardson 162_ (UWC), AF328807, AF327605. _**Phylica paniculata**_ Willd. (3), South Africa, _van der Bank 1_ (UWC), AF328806, AF327604. _**Phylica paniculata**_ Wild. (4), South Africa, _Weitz 950_ (UWC), AF328809, AF327607. _**Phylica plumigera**_ Pillans, South Africa, _Richardson 26_ (UWC), AF328818, AF327618. _**Phylica polifolia**_ (Vahl) Pillans, St. Helena, _Chase 1751_ (K), AF328805 AJ390373. _**Phylica polifolia**_ (Vahl) Pillans (2), St. Helena, _Chase 2269_ (K), AF328804, –. _**Phylica pubescens**_ Ait., South Africa, _Chase 859_ (K), AF328814, Y16771. _**Phylica spicata**_ L., South Africa, _Richardson 46_ (UWC), AF328816, AF327616. _**Phylica thodei**_ E.Phillips, South Africa, _Hillard & Burtt 15379_ (K), AF328810, AF327611. _**Phylica tropica**_ Baker, Malawi, _Brass 16739_ (NY), –, AF327608. _**Polianthion bilucularis**_ (George) Kellermann, Australia, _Patrick 394_ (PERTH), AY911555, EF528502. _**Polianthion minutiflorum**_ (Ross) Thiele, Australia, _Bean & Turpin 9107_ (CANB), AY911557, EF528510. _**Polianthion wichurae**_ (Nees ex Reissek) Thiele, Australia, _Kellermann 183_ (MEL), AY911554, EF528501. _**Pomaderris angustifolia**_ Wakef., cultivated, _Walsh s.n._ (Walsh), AY911568, EF528518. _**Pomaderris brevifolia**_ Walsh, Australia, _Kellermann 388_ (MEL), AY911564, EF528513. _**Pomaderris elliptica**_ Labill., cultivated, _Walsh s.n._ (Walsh), AY911568, EF528519. _**Pomaderris flabellaris**_ (F.Muell. ex Reissek) Black, Australia, _Walker s.n._ (MEL), AY911572, –. _**Pomaderris forrestiana**_ F.Muell., Australia, _Archer 2271_ (MEL), AY911566, EF528514. _**Pomaderris grandis**_ F.Muell., Australia, _Walsh 2776_ (MEL), AY911567, EF528512. _**Pomaderris obcordata**_ Fenzl, Australia, _Walsh 3999_ (MEL), AY911563, EF528516. _**Pomaderris oraria**_ Walsh, _Walsh s.n._ (Walsh), AY911571, EF528515. _**Pomaderris phylicifolia**_ L.Moore, cultivated, _Walsh s.n._ (Walsh), AY911573, EF528520. _**Pomaderris prunifolia**_ var. _**edgerleyi**_ (Hook.f.) L.Moore, cultivated, _Walsh s.n._ (Walsh), AY911574, EF528521. _**Pomaderris rugosa**_ Cheesman, New Zealand, _Chase 857_ (K), AF328826, AJ390363. _**Pomaderris tropica**_ Wakef., Australia, _Telford 12045_ (CANB), AY911569, EF528517. _**Pomaderris rotundifolia**_ (F.Muell.) Rye, Australia, _Kellermann 379_ (MEL), AY911565, EF528550. _**Pseudoziziphus parryi**_ (Torr.) Hauenschild, North America, _Gregory 888_ (SD), JN900304, JN900315. _**Pseudoziziphus parryi**_ (Torr.) Hauenschild (2), North America, _Rebman 10045_ (SD), JN900303, JN900317. _**Reissekia smilacina**_ (Sm.) Endl., Brazil, _Arbo & al. 4921_ (K), –, AJ390345. _**Retanilla ephedra**_ (Vent.) Brongn., Argentina, _Medan s.n._ (BAA), –, AY460423. _**Retanilla patagonica**_ (Speg.) Tortosa, Argentina, _Medan 776_ (BAA), –, AY642153. *_**Retanilla**_ **sp.**, cultivated, _KUN640183_ (Kunming Botanic Garden), –, KR150652. _**Retanilla stricta**_ Hook. & Arn., Chile, _Medan 790_ (BAA), –, AY460425. _**Retanilla trinervia**_ (Gillies & Hook.) Hook. & Arn., Chile, _Medan s.n._ (BAA), –, AY642154. *_**Retanilla trinervia**_ (Gillies & Hook.) Hook. & Arn. (2), Chile, _Philippi s.n._ (HAL), –, KR083130. *_**Reynosia camagueyensis**_ Britton, Cuba, _Areces & al. 31191_ (JE), KR083078, KR083131. *_**Reynosia septemtrionalis**_ Urb., United States, _Hill 13393_ (JE), KR083079, –. _**Reynosia septemtrionalis**_ Urb. (2), locality unknown, _Seigler & Waterman 13779_ (MO), JN900288, JN900319. _**Reynosia uncinata**_ Urb., locality unknown, _Gentry & Zardini 50476_ (MO), JN900289, JN900320. *_**Rhamnella franguloides**_ (Maxim.) Weberb., China, _Liou 1720_ (M), KR083081, KR083133. _**Rhamnella franguloides**_ (Maxim.) Weberb. (2), locality unknown, _Miyagi 9151_ (NY), JN900306, JN900334. *_**Rhamnella franguloides**_ (Maxim.) Weberb. (3), Japan, _Walker 8402_ (L), KR083080, KR083132. *_**Rhamnella martini**_ (H.Lév.) C.K.Schneid., China, _Cenghong 101204_ (KUN), KR083083, KR083135. *_**Rhamnidium elaeocarpum**_ Reissek, Brazil, _Gottsberger 22-31186_ (ULM), KR083085, KR083137. _**Rhamnidium elaeocarpum**_ Reissek (2), Brazil, _Santos 693_ (K), –, AJ390332. _**Rhamnidium glabrum**_ Reissek, locality unknown, _Nee 50493_ (NY), JN900286, JN900324. _**Rhamnidium hasslerianum**_ Chodat, locality unknown, _Pastoreo 9515_ (NY), JN900287, JN900325. *_**Rhamnus alaternus**_ L., Spain, _Conert & al. 881_ (FR), KR083155, –. *_**Rhamnus alaternus**_ L. (2), Italy (Sicily), _de Wilde 11312_ (L), KR083154, KR083324. _**Rhamnus alaternus**_ L. (3), Europe, _Eriksson 988_ (S), AY626435, AY626416. *_**Rhamnus alaternus**_ L. (4), Maroc, _Lewalle 10833_ (L), KR083153, KR083323. _**Rhamnus alaternus**_ L. (5), Iraq, _Mati EM520_ (EM), FN870380, –. *_**Rhamnus alaternus**_ var. _**obovata**_ (Timb.-Lagr. & Fages) Rouy, Algeria, _Fevrier s.n._ (L), KR083156, KR083325. *_**Rhamnus alnifolia**_ L'Her., Canada, _Brisson 74017_ (JE), KR083157, KR083326. *_**Rhamnus**_ aff. _**alnifolia**_ L'Her. (2), United States, _Wofford & al. s.n._ (KUN), KR150624, KR150642. *_**Rhamnus alpina**_ L. subsp. _**alpina**_, Spain, _Buttler 19340_ (FR), KR083159, KR083328. _**Rhamnus alpina**_ L. subsp. _**alpina**_ (2), Europe, _Chase 8482_ (K), AY626438, AY626417. *_**Rhamnus alpina**_ L. subsp. _**alpina**_ (3), France, _Gutte s.n._ (LZ), KR083161, KR083330. *_**Rhamnus alpina**_ L. subsp. _**alpina**_ (4), cultivated, _FR-0-FRT-1993/395_ (Botanischer Garten Frankfurt am Main), KR083158, KR083327. *_**Rhamnus alpina**_ L. subsp. _**alpina**_ (5), Italy, _Schneeweiss & al. 5586_ (WU), KR083160, KR083329. *_**Rhamnus alpina**_ subsp. _**fallax**_ (Boiss.) Maire & Petitm., Austria, _Gutte s.n._ (LZ), KR083162, KR083332. *_**Rhamnus alpina**_ subsp. _**fallax**_ (Boiss.) Maire & Petitm. (2), cultivated, _XX-0-FRT-1993/500_ (Botanischer Garten Frankfurt am Main), KR083163, KR083331. *_**Rhamnus alpina**_ subsp. _**glaucophylla**_ (Sommier) Tutin, Italy, _Aldobrandi s.n._ (FL), KR083164, KR083333. *_**Rhamnus arguta**_ Maxim., China, _Meusel s.n._ (HAL), KR083165, KR083334. *_**Rhamnus aurea**_ Heppeler, China, _Bartolomew & al. 814_ (KUN), KR083167, KR083336. *_**Rhamnus bodinieri**_ H.Lév., China, _Hang SH042_ (KUN), KR083169, –. *_**Rhamnus borneensis**_ Steenis, Malaysia, _Ng 1992_ (L), KR083170, KR083338. _**Rhamnus cathartica**_ L., Europe, _Bolmgren 9_ (S), AY626436, –. *_**Rhamnus cathartica**_ L. (2), cultivated, _XX-0-FRT-0000/3138_ (Botanischer Garten Frankfurt am Main), KR083174, KR083342. *_**Rhamnus cathartica**_ L. (3), Bulgaria, _Vihodocevsky s.n._ (L), KR083175, KR083343. *_**Rhamnus costata**_ Maxim., Japan, _Hotta 2_ (L), KR083176, KR083344. *_**Rhamnus crenulata**_ Ait., Spain (Canaries), _Conert 369_ (FR), KR083181, KR083347. _**Rhamnus crenulata**_ Ait. (2), Spain (Canaries), _Eriksson s.n._ (S), AY626448, AY626428. *_**Rhamnus crocea**_ Nutt., United States, _Bartholomew & al. 2458_ (KUN). KR083183, –. *_**Rhamnus crocea**_ Nutt. (2), United States, _Bracelin 1214_ (L). KR083184, –. *_**Rhamnus crocea**_ Nutt. (3), United States, _Rose s.n._ (FR). KR083182, KR083348. _**Rhamnus davurica**_ Pall., China, _Bolmgren 102_ (S), AY626441, AY626420. *_**Rhamnus davurica**_ Pall. (2), Japan, _L997044696_ (L), KR083185, KR083349. *_**Rhamnus diamantiaca**_ Nakai, cultivated, _KUN0640377_ (Kunming Botanic Garden), KR083186, KR083350. *_**Rhamnus disperma**_ Ehrenb. & Boiss., Egypt, _Hilevy S4497-1_ (HUJ), KR083188, KR083352. *_**Rhamnus disperma**_ Ehrenb. & Boiss. (2), Israel, _Hilevy S4497-2_ (HUJ), KR083189, KR083353. *_**Rhamnus dumetorum**_ C.K.Schneid., China, _Dolongjiang Expedition 6229_ (KUN), KR083190, KR083354. *_**Rhamnus erythroxylon**_ Pall., Mongolia, _Hilbig 18581_ (HAL), KR083192, KR083356. *_**Rhamnus erythroxylon**_ Pall. (2), Mongolia, _Hilbig 26083_ (HAL), KR083191, KR083355. _**Rhamnus esquirolii**_ H.Lév., China, _Bell 1706_ (S), AY626440, AY626440. *_**Rhamnus esquirolii**_ H.Lév. (2), cultivated, _KUN0468940_ (Kunming Botanic Garden), KR083193, –. *_**Rhamnus flavescens**_ Chen & Chou, cultivated, _KUN0640092_ (Kunming Botanic Garden), KR083194, KR083357. *_**Rhamnus formosana**_ Mats., Taiwan, _Mizushima 10907_ (L), KR083196, KR083358. *_**Rhamnus formosana**_ Mats. (2), cultivated (Kunming Botanic Garden), KR083195, –. *_**Rhamnus fulvo-tincta**_ Metcalf, China, _Fan 482_ (KUN), –, KR150653. *_**Rhamnus gilgiana**_ Heppeler, cultivated (Kunming Botanic Garden), KR083200, KR083362. *_**Rhamnus gilgiana**_ Heppeler (2), cultivated, _Kunming Botanic Institute 85109_ (KUN), KR083199, KR083361. _**Rhamnus glandulosa**_ Ait., Spain (Canaries), _Eriksson s.n._ (S), AY626446, AY626425. *_**Rhamnus glandulosa**_ Ait. (2), Spain (Canaries), _Gutte s.n._ (LZ), KR083201, KR083363. *_**Rhamnus glandulosa**_ Ait. (3), Spain (Canaries), _L540762_ (L), KR083202, KR083364. *_**Rhamnus globosa**_ Bunge, China, _Shaobie & al. 81_ (KUN), KR083203, KR083365. *_**Rhamnus hainanensis**_ Merr. & Chou, Vietnam, _Poilane 30986_ (L), KR150625, KR150643. *_**Rhamnus heldreichii**_ Boiss., Cyprus, _FR0034044_ (FR), KR083204, KR083366. *_**Rhamnus hemsleyana**_ C.K.Schneid., China, _Northeast Yunnan Expedition 1207_ (KUN), KR083205,

Appendix 1. Continued.

KR083367. ***Rhamnus hirtella*** Boiss., Turkey, *Ellenberg 924* (JE), KR083208, KR083370. ***Rhamnus hirtella*** Boiss. (2), Turkey, *Schwarz 252* (JE), KR083209, KR083371. ***Rhamnus imeretina*** Booth, Petz. & Kirchn., locality unknown, *Bornmueller s.n.* (JE), KR083212, KR083374. ***Rhamnus imeretina*** Booth, Petz. & Kirchn. (2), cultivated, *XX-0-LZ-ZF 1339-1996* (Botanischer Garten Leipzig), KR083213, KR083375. ***Rhamus infectoria**** L., cultivated, *Endtmann a1* (JE), KR150627, KR150644. ***Rhamus infectoria**** L. (2), France, *van Ooststroom 19974* (L), KR150626, –. ***Rhamnus iranica*** Hausskn. & C.K.Schneid., Iran, *Strauss 126* (JE), KR083258, –. ***Rhamnus iranica*** Hausskn. & C.K.Schneid. (2), Iran, *Strauss 307* (JE), KR083257, –. ***Rhamnus integrifolia*** DC., Spain (Canaries), *Cahera s.n.* (L), KR083214, –. ***Rhamnus iteinophylla*** C.K.Schneid., China, *Bartholomew 1019* (KUN), KR083215, –. ***Rhamnus*** aff. ***iteinophylla*** C.K.Schneid., China, *Matuszak R188* (KUN), KR083216, KR083376. ***Rhamnus*** aff. ***iteinophylla*** C.K.Schneid. (2), China, *Matuszak R189* (KUN), KR083217, KR083377. ***Rhamnus*** aff. ***iteinophylla*** C.K.Schneid. (3), China, *Matuszak R190* (KUN), KR083218, KR083378. ***Rhamnus japonica*** Maxim., Japan, *L997061071* (L), KR083220, KR083380. ***Rhamnus japonica*** Maxim. (2), Japan, *Yamazaki 10986* (L), KR083219, KR083379. ***Rhamnus japonica*** var. ***microphylla*** H.Hara, Japan, *Murata 18048* (L), KR083221, KR083381. ***Rhamnus kanagusukii*** Makino, Japan, *Walker 5769* (L), KR083222, KR083382. ***Rhamnus kurdica*** Boiss. & Hohen., Iraq, *Bornmueller 1021* (JE), KR083224, –. ***Rhamnus kurdica*** Boiss. & Hohen., Turkey, *Buttler 16000* (FR), KR083223, KR083383. ***Rhamnus lamprophylla*** C.K.Schneid., China, *KUN0629144* (Kunming Botanic Garden), KR083225, KR083384. ***Rhamnus lanceolata*** Pursh, United States, *Leidolf 831* (FR), KR083226, KR083385. ***Rhamnus leptophylla*** C.K.Schneid., China, *Fan 95* (L), KR083229, KR083388. ***Rhamnus leptophylla*** C.K.Schneid. (2), China, *Yu & Bo 408* (KUN), KR083228, KR083387. ***Rhamnus libanotica*** Boiss., Turkey, *Buttler & Erben 17887* (FR), KR083230, KR083389. ***Rhamnus liukiuensis*** (E.H.Wilson) Koidz., cultivated (Kunming Botanic Garden), KR083232, KR083391. ***Rhamnus liukiuensis*** (E.H.Wilson) Koidz., Japan, *Walker & al. 5769* (L), KR083231, KR083390. ***Rhamnus lojaconoi*** Raimondo, Italy (Sicily), *di Nato s.n.* (FL), –, KR083392. ***Rhamnus ludovici-salvatoris*** Chodat, Spain (Baleares), *Botanische Excursie Spanje 1980 1004* (L), KR083235, KR083395. ***Rhamnus ludovici-salvatoris*** Chodat (2), Spain (Baleares), *Lewejohann Ma87053* (FR), KR083236, –. ***Rhamnus lycioides*** L., Georgia, *Hilbig s.n.* (HAL), KR083237, KR083396**.** ***Rhamnus lycioides*** subsp. ***graeca*** (Boiss. & Reuter) Tutin, Greece, *Meusel s.n.* (HAL), KR083238, KR083397. ***Rhamnus lycioides*** L. subsp. ***lycioides***, Spain, *Botanische Excursie Spanje 1980 162* (L), KR083239, –. ***Rhamnus lycioides*** L. subsp. ***lycioides*** (2), Europe, *Eriksson 784* (S), AY626437, AJ390374. ***Rhamnus lycioides*** subsp. ***oleoides*** (L.) Jahand. & Maire, Malta, *van Balgooy 2794* (L), KR083240, KR083398. ***Rhamnus lycioides*** subsp. ***oleoides*** **'microphylla'**, Cyprus, *de Wilde 21415* (L), KR083241, KR083399. ***Rhamnus lycioides*** subsp. ***velutina*** (Boiss.) Tutin, Spain, *Veldkamp 5092* (L), KR083242, –. ***Rhamnus lycioides*** subsp. ***velutina*** (Boiss.) Tutin (2), Spain, *Villaret s.n.* (HAL), KR083243, KR083400. ***Rhamus minuta*** Grubov, China, *Huang He Expedition 1180* (KUN), KR083248, KR083405. ***Rhamus minuta*** Grubov (2), Afghanistan, *Rodenburg 178* (L), KR083247, KR083404. ***Rhamnus nakaharae*** (Hayata) Hayata, Taiwan, *Lee & Kao K3938* (L), KR083251, KR083408. ***Rhamnus nigricans*** Hand.-Mazz., China, *Ende 1701* (KUN), KR083252, KR083409. ***Rhamnus orbiculata*** Bornm., Montenegro, *Bornmueller s.n.* (HAL), KR150628, KR150645. ***Rhamnus palaestina*** Boiss. (2), Palaestina, *Meyers 679* (L), KR150629, KR150646. ***Rhamnus palaestina*** Boiss. (3), Israel, *Liston s.n.* (HUJ), KR083254, KR083410. ***Rhamnus palaestina*** Boiss. (4), Jordan, *Shmida 13005* (HUJ), KR083255, KR083411. ***Rhamnus pallasii*** Fisch. & C.A.Mey., Iran, *Bornmueller 6551* (JE), KR083259, –. ***Rhamnus pallasii*** Fisch. & C.A.Mey. (2), Georgia, *Gutte s.n.* (LZ), KR083256, KR083412. ***Rhamnus pallasii*** Fisch. & C.A.Mey. (3), Iran, *Jacobs 6824* (L), KR150630, KR150647. ***Rhamnus papuana*** Lauterbach, Papua New Guinea, *Streitmann & Kairo 45496* (L), –, KR083415. ***Rhamnus parvifolia*** Bunge, South Korea, *Hyun s.n.* (KUN), KR083263, KR083417. ***Rhamnus parvifolia*** Bunge (2), China, *Meusel s.n.* (HAL), KR083166, KR083335. ***Rhamnus parvifolia*** Bunge (3), China, *Tibet Expedition 11306* (KUN), KR083262, KR083416. ***Rhamnus parvifolia*** Bunge (4), locality unknown, *Wang 1080* (MO), JN900291, JN900322. ***Rhamnus pendula*** Pamp., Libya, *Pampanini 4857* (L), KR083264, KR083418. ***Rhamnus pendula*** Pamp. (2), Libya, *Pampanini 4860* holotype (FL), KR083265, KR083419. ***Rhamnus persica*** Boiss., Iran, *Bornmueller 86* (JE), KR150632, –. ***Rhamnus persica*** Boiss. (2), Iran, *Jacobs 6661* (L), KR083266, KR083420. ***Rhamnus persica*** Boiss. (3), Iran, *Jacobs 6321* (L), KR150631, KR150648. ***Rhamnus persica*** Boiss. (4), Iran, *Zohary s.n.* (HUJ), KR083267, KR083421. ***Rhamnus petiolaris*** Boiss., Turkey, *Bunhard 126* (JE), KR083268, –. ***Rhamnus*** aff. ***petiolaris*** Boiss., Turkey, *Bornmueller 4259* (L), KR150633, –. ***Rhamnus pilushanensis*** Liu & Wang, Taiwan, *KUN0091551* (Kunming Botanic Garden), KR083269, KR083422. ***Rhamnus prinoides*** L'Hér., Africa, *Bolmgren 101* (S), AY626432, AY626413. ***Rhamnus prinoides*** L'Hér. (2), Ethiopia, *van Steenis 24093* (L), KR083274, –. ***Rhamnus procumbens*** Edgew., India, *Bhattacharyya 13036* (L), KR150634, –. ***Rhamnus procumbens*** Edgew. (2), Nepal, *Suzuki & al. 9455232* (KUN), KR083275, KR083427. ***Rhamnus prostrata*** Jacq., China, *Tibet Expedition 768500* (KUN), KR083276, KR083428. ***Rhamnus pulogensis*** Merr., Philippines, *Jacobs 7135* (L), KR083277, KR083429. ***Rhamnus pumila*** Turra, Europe, *Bolmgren 102* (S), AY626433, AY626414. ***Rhamnus pumila*** Turra (2), Switzerland, *Gregor 6448* (FR), KR083278, KR083430. ***Rhamnus pumila*** Turra (3), Switzerland, *Groep 11968* (L), KR083279, KR083431. ***Rhamnus pumila*** Turra (4), cultivated, *XX-0-FRT-0000/3139* (Botanischer Garten Frankfurt am Main), KR083280, KR083432. ***Rhamnus pumila*** **'hispanica'** W.Vent, Spain, *Sparovsky s.n.* (JE), KR083281, KR083433. ***Rhamnus pumila*** var. ***velutina*** Bornm., Austria, *Bornmueller s.n.* (JE), KR083282, KR083434. ***Rhamnus punctata*** Boiss., Lebanon, *Coffe s.n.* (L), KR150635, –. ***Rhamnus punctata*** Boiss. (2), Israel, *Meyers & Dinsmore 1861* (L), KR083283, KR083435. ***Rhamnus purpurea*** Edgew., East Asia, *Chase 8483* (K), AY626439, AY626418. ***Rhamnus purpurea*** Edgew. (2), Nepal, *Suzuki & al. 919337* (KUN), KR083284, KR083436. ***Rhamnus rhodopea*** Velen., Bulgaria, *Stribrny s.n.* (FR), KR150637, KR150650. ***Rhamnus rhodopea*** Velen. (2), Macedonia, *van Ooststroom 23923* (L), KR083286, KR083438. ***Rhamnus rugulosa*** Hemsl., China, *Northwest University 86* (KUN), KR083289, KR083441. ***Rhamnus sargentiana*** C.K.Schneid., China, *Peng & al. 8746* (KUN), KR083294, KR083446. ***Rhamnus sargorskii*** Bornm., Montenegro, *Sargorskii s.n.* (HAL), KR150638, KR150651. ***Rhamnus sargorskii*** Bornm. (2), Croatia, *Sargorskii s.n.* (JE), KR150639, –. ***Rhamnus saxatilis*** Jacq. subsp. ***saxatilis***, Germany, *Buttler 20898* (FR), KR083295, KR083447. ***Rhamnus saxatilis*** Jacq. subsp. ***saxatilis*** (2), Austria, *Till s.n.* (WU), KR083296, KR083448. ***Rhamnus saxatilis*** subsp. ***tinctoria*** Nyman, Romania, *Barabas 371* (L), KR083298, KR083450. ***Rhamnus saxatilis*** **'spicata'** Beck, Croatia, *Stud. Biol. In Itinere s.n.* (L), KR083297, KR083449. ***Rhamnus serrata*** Schult., Mexico, *Ventura & Lopez 9213* (HAL), KR083300, KR083452. ***Rhamnus*** aff. ***serrata*** Schult., Mexico, *Purpus 5330* (L), KR150640, –. ***Rhamnus sibthorpiana*** Schult., Greece, *Bornmueller 354* (JE), KR083301, KR083453. ***Rhamnus sibthorpiana*** Schult. (2), Greece, *Leonis 221* (JE), KR083302, –. ***Rhamnus sintenisii*** Rech.f., Azerbaijan, *Proskuriakova s.n.* (JE), KR083260, KR083413. ***Rhamnus songorica*** Gontsch., Kazakhstan, *Arystangaliev 4828b* (L), KR083303, KR083454. ***Rhamnus songorica*** Gontsch. (2), Kazakhstan, *Roldugin 4828a* (L), KR083304, KR083455. ***Rhamnus songorica*** Gontsch. (3), China, *Taiyi 650649* (KUN), KR083305, KR083456. ***Rhamnus* sp.**, China, *KUN0602432* (KUN), KR083082, KR083134. ***Rhamnus spathulifolia*** Fisch & C.A.Mey., Azerbaijan, *Gutte s.n.* (LZ), KR083307, KR083457. ***Rhamnus staddo*** A.Rich., Kenya, *Schultka s.n.* (FR), KR083311, KR083462. ***Rhamnus staddo*** A.Rich. (2), Somalia, *Thulin & Warfa 6053* (UPS), AU626449, AY626427. ***Rhamnus staddo*** **'holstii'**, Kenya, *Geesteranus 6176* (L), KR083210, KR083372. ***Rhamnus subpetala*** Merr., China, *Gongyuan & al. 101819* (KUN), KR083312, KR083463. ***Rhamnus subpetala*** Merr. (2), China, *KUN0614575* (Kunming Botanic Garden), KR083313, KR083464. ***Rhamnus tangutica*** J.J.Vassil., China, *Boufford 36251* (KUN), KR083314, KR083465. ***Rhamnus triquetra*** (Wall.) Brandis, India, *Venkarta 97875* (L), –, KR150654. ***Rhamnus ussuriensis*** J.J.Vassil., Russia, *Mueller s.n.* (LZ), KR083317, KR083468. ***Rhamnus ussuriensis*** J.J.Vassil. (2), China, *Northeast Agriculture University Plant Expedition 8070* (KUN), KR083316, KR083467. ***Rhamnus utilis*** Decne., China, *Bartholomew 1413* (KUN), KR083319, KR083470. ***Rhamnus utilis*** Decne. (2), cultivated, *XX-0-FRT-1994/1150* (Botanischer Garten Frankfurt am Main), KR083318, KR083469. ***Rhamnus utilis*** Decne. (3), China, *Bartgikinew 1419* (KUN), KR150641, KR150655. ***Rhamnus virgata*** Roxb., cultivated (Kunming Botanic Garden), KR083321, KR083472. ***Rhamnus virgata*** Roxb. (2), China, *Sino-American Botanical Expedition 1984 886* (KUN), KR083320, KR083471. ***Rhamnus wightii*** Wight & Arn., India, *Kostermans 26247* (L), –, KR150656. ***Rhamnus wilsonii*** C.K.Schneid., China, *Wulingshan Expedition 3565* (KUN), –, KR083473. ***Rhamnus xizangensis*** Chen & Chou, China, *Ende 397* (KUN), KR083315, KR083466. ***Rhamnus yoshinoi*** Makino, Japan, *Watanabe s.n.* (KUN), KR083322, KR083474. ***Sageretia hayatae*** Kaneh., Taiwan, *Liao 10264* (L), KR083086, KR083138. ***Sageretia minutiflora*** (Michx.) C.Mohr, United States, *MacDonald 8650* (FR), KR083087, KR083139. ***Sageretia thea*** (Osbeck) M.C.Johnst., locality unknown, *Thulin & al. s.n.* (n.a.), –, AJ225792. ***Sageretia thea*** (Osbeck) M.C.Johnst. (2), locality unknown, *Wang 439* (MO), JN900294, JN900329. ***Sageretia wrightii*** S.Watson, locality unknown, *Miller 7729* (MO), JN900295, JN900330. ***Sarcomphalus amole*** (Sessé & Moc.) Hauenschild, Mexico, *Dorado & al. 1585* (MO), DQ146579, DQ146535. ***Sarcomphalus***

Appendix 1. Continued.

guatemalensis (Hemsl.) Hauenschild, Costa Rica, *Morales 2906* (MO), DQ146585, DQ146541. ***Sarcomphalus guatemalensis*** (Hemsl.) Hauenschild (2), Nicaragua, *Stevens & al. 17116* (NY), DQ146584, DQ146540. ***Sarcomphalus lloydii*** (M.C.Johnst.) Hauenschild, locality unknown, *Chiang & al. 7983* (NY), JN900313, JN900336. ***Sarcomphalus lloydii*** (M.C.Johnst.) Hauenschild (2), locality unknown, *Johnston & al. 11202* (F), JN900312, JN900335. ***Sarcomphalus mexicana*** (Rose) Hauenschild, locality unknown, *Carrillo-Reyes 1683* (NY), JN900310, –. ***Sarcomphalus mexicana*** (Rose) Hauenschild (2), locality unknown, *Steinmann 3088* (NY), JN900309, JN900340. ***Sarcomphalus mistol*** (Griseb.) Hauenschild, cultivated, *Goodwin 619812* (NSW), DQ146591, DQ146548. ***Sarcomphalus mistol*** (Griseb.) Hauenschild (2), Bolivia, *Nee 51192* (NY), DQ146590, DQ146547. ***Sarcomphalus obtusifolius*** var. ***canescens*** (A.Gray) Hauenschild, cultivated, *Darmrel s.n.* (DES), DQ146595, DQ146552. ***Sarcomphalus obtusifolius*** (Hook. ex Torr. & A.Gray) Hauenschild var. ***obtusifolius***, cultivated, *Darmrel s.n.* (DES), DQ146594, DQ146551. ***Sarcomphalus obtusifolius*** (Hook. ex Torr. & A.Gray) Hauenschild var. ***obtusifolius*** (2), cultivated, *Darmrel s.n.* (DES), DQ146596, DQ146553. ***Sarcomphalus pendunculata*** (Brandg.) Standl., locality unknown, *Tenorio & Martinez 17366* (F), JN900311, JN900337. ***Sarcomphalus taylori*** Britton, Bahamas, *Correll 41621* (MO), DQ146605, DQ146561. ***Sarcomphalus thyrsiflora*** (Benth.) Hauenschild, *Cornejo & Bonifaz 7796* (GUAY), DQ146606, DQ146562. ***Sarcomphalus yucatanensis*** (Standl.) Hauenschild, locality unknown, *Sima 2106* (F), JN900307, JN900338. ***Sarcomphalus yucatanensis*** (Standl.) Hauenschild (2), locality unknown, *Sima & Duran 2127* (NY), JN900308, JN900339. ***Schistocarpaea johnstonii*** F.Muell., Australia, *Forster & al. 25158* (MEL), HQ325385, – / *Gray 1247* (K), –, AJ390349. ***Scutia buxifolia*** Reissek, Argentina, *Chase 858* (K), –, AJ390335. ***Scutia buxifolia*** Reissek (2), locality unknown, *Nee 50694* (MO), JN900293, JN900323. ****Scutia myrtina*** (Burm.f.) Kurz, Kenya, *Schultka 63* (FR), KR083088, KR083140. ***Siegfriedia darwinioides*** C.A.Gardner, Australia, *Chase 2181* (K), AF328827, AJ390375. ***Serichonus gracilipes*** (Diels) Thiele, Australia, *Kellermann 262* (MEL), AY911560, EF528506. ***Siegfriedia darwinioides*** C.A.Gardner (2), *Walsh s.n.* (Walsh), AY911575, EF528507. ***Spyridium burragorang*** Thiele, cultivated, *Donaldson 903* (CANB), AY911594, EF528536. ***Spyridium buxifolium*** (Fenzl) Thiele, Australia, *Hosking 1848* (MEL), AY911595, EF528508. ***Spyridium cordatum*** Benth., Australia, *Kellermann 370* (MEL), EF528508, EF528530. ***Spyridium daltonii*** (F.Muell.) Kellermann, Australia, *Read s.n.* (MEL), AY911598, EF528534. ***Spyridium eriocephalum*** Fenzl, cultivated, *Lyne 675* (CANB), AY911581, EF528522. ***Spyridium globulosum*** (Lab.) Bentham, Australia, *Archer 2255* (MEL), AY911590, EF528529. ***Spyridium globulosum*** (Lab.) Bentham (2), Australia, *Chase 2021* (K), AF328828, AJ390358. ****Spyridium globulosum*** (Lab.) Bentham (2), Australia, *Wilson 7956* (L), KR083089, –. ***Spyridium gunii*** (Hook.f.) Benth., cultivated, *Burns 76* (CANB), AY911593, EF528524. ***Spyridium halmaturinum*** (F.Muell.) F.Muell., cultivated, *Nightingale 143* (CANB), AY911582, EF528527. ***Spyridium mucronatum*** Rye, Australia, *Kellermann 367* (MEL), AY911589, EF528528. ***Spyridium nitidum*** Wakef., Australia, *Jackson 3253* (MEL), AY911584, EF528531. ***Spyridium parvifolium*** (Hook.) F.Muell, Australia, *Bayer 94-025* (NSW), AF048975, –. ***Spyridium parvifolium*** (Hook.) F.Muell. (2), cultivated, *Kellermann 112* (MEL), AY911588, EF528526. ****Spyridium parvifolium*** (Hook.) F.Muell. (3), Australia, *Schaarschmidt s.n.* (FR), KR083090, –. ***Spyridium ramosissimum*** (Audas) Kellermann, Australia, *Kellermann 122*, AY911597, EF528535. ***Spyridium scortechinii*** (F.Muell.) Thiele, cultivated, *Kellermann 409* (MEL), AY911596, EF528537. ***Spyridium subchreatum*** (F.Muell.) Reissek, cultivated, *McAuliffe 250* (CANB), AY911585, EF528532. ***Spyridium thymifolium*** Reissek, cultivated, *Nightingale 139* (CANB), AY911586, EF528533. ***Spyridium tricolor*** Barker & Rye, Australia, *Archer 329* (MEL), AY911591, –. ***Spyridium ulicinum*** (Hook.) Benth., Australia, *Buchanan 15952* (MEL), AY911592, EF528523. ****Spyridium vexiliferum*** Reissek, Australia, *Schaatschmidt s.n.* (FR), KR083092, –. ***Spyridium waterhousei*** F.Muell., cultivated, *Jackson 13* (CANB), AY911583, EF528538. ***Stenanthemum argentemum*** A.R.Bean, Australia, *Clarkson 8895* (CANB), AY911602, –. ***Stenanthemum centrale*** Thiele, Australia, *Matthews s.n.* (MEL), AY911605, EF528544. ***Stenanthemum complicatum*** (F.Muell.) Rye, Australia, *Kellermann 239* (MEL), AY911599, EF528539. ***Stenanthemum humile*** Benth. *Kellermann 194* (MEL), AY911600, EF528540. ***Stenanthemum leucopharctum*** (Schltdl.) Reissek, Australia, *Kellermann 136* (MEL), AY911604, EF528545. ***Stenanthemum petraeum*** Rye, Australia, *Collins s.n.* (MEL), AY911601, EF528541. ***Stenanthemum pimeleoides*** (Hook.f.) Benth., Australia, *Davies & al. 1238* (MEL), AY911606, –. ***Stenanthemum reissekii*** Rye, Australia, *Kellermann 197* (MEL), AY911603, EF528543. ***Trevoa quinquenervis*** Gillies & Hook., Chile, *Medan s.n.* (BAA), –, AY642155. ****Trevoa quinquenervis*** Gillies & Hook. (2), Chile, *Philippi s.n.* (HAL), –, KR083141. ***Trichocephalus stipularis*** (L.) Brongn., South Africa, *Richardson 4*, UWC, AF328825, AF327621. ***Trichocephalus stipularis*** (L.) Brongn. (2), South Africa, *Weitz 1080* (UWC), AF328824, –. ***Trymalium angustifolium*** Reissek, Australia, *Kellermann 302* (MEL), AY911580, EF528548. ***Trymalium elachophyllum*** Rye, Australia, *Kellermann 384* (MEL), AY911576, EF528547. ***Trymalium ledifolium*** Fenzl, Australia, *Chase 2184* (K), AF328829, AJ390361. ***Trymalium ledifolium*** Fenzl (2), Australia, *Kellermann 294* (MEL), AY911579, EF528551. ***Trymalium monospermum*** Rye, Australia, *Sage 1540* (MEL), AY911577, EF528546. ***Trymalium odoratissimum*** var. ***trifidum*** (Rye) Kellermann, Australia, *Kellermann 389* (MEL), AY911578, –. ****Trymalium spathulatum*** F.Muell., Australia, *Clifton 1425* (L), KR083091, –. ***Trymalium waye*** F.Muell., Australia, *Kraehenbuehl 5197* (CANB), AY911562, EF528509. ****Ventilago denticulata*** Willd., China, *Gouda & Xiwen 39689* (KUN), KR083093, KR083142. ****Ventilago denticulata*** Willd. (2), Laos, *Vanthanouvong 193* (L), KR083094, KR083143. ****Ventilago ecorollata*** F.Muell., Australia, *Irvine 210* (L), KR083095, KR083144. ****Ventilago harmandiana*** Pierre, Thailand, *van Beusekom & al. 4195* (L), KR083096, –. ****Ventilago kurzii*** Ridl., Thailand, *Kerr 13831* (L), KR083097, KR083145. ****Ventilago maingayii*** M.A.Lawson, Singapore, *Leong & al. SING2009-250* (SING), KR083098, KR083146. ****Ventilago neocaledonia*** Schltr., New Caledonia, *McKee 3594* (L), KR083099, KR083147. ****Ventilago pseudocalyculata*** Guillaumin, New Caledonia, *McPherson 4447* (L), KR083100, KR083148. ***Ziziphus acidojujuba*** C.Y.Cheng & M.J.Liu, China, no voucher (n/a), EU075089, –. ***Ziziphus acidojujuba*** C.Y.Cheng & M.J.Liu (2), cultivated, *Zhao s.n.* (RCCJ), DQ146572, DQ146528. ***Ziziphus acidojujuba*** C.Y.Cheng & M.J.Liu (3), cultivated, *Zhao s.n.* (RCCJ), DQ146571, DQ146527. ***Ziziphus apetala*** Hook.f., China, no voucher (n/a), EU075094, –. ***Ziziphus attopensis*** Pierre, China, no voucher (n/a), EU075099, –. ****Ziziphus brunoniana*** Clarke ex Brandis, Thailand, *Sands 5717* (L), KR083101, KR083149. ***Ziziphus calophylla*** Wall. ex Hook.f., cultivated, *Lee SL04* (CS), DQ146580, DQ146536. ***Ziziphus fungii*** Merr., China, no voucher (n/a), EU075095, –. ***Ziziphus glabrata*** B.Heyne, Saudi Arabia, *Collenette 5/93* (K), DQ146583, DQ146539. ***Ziziphus glabrata*** B.Heyne (2), locality unknown, *Thulin & al. s.n.* (K), –, AJ225799. ***Ziziphus horsfeldii*** Miq., Indonesia, *Astuti s.n.* (CS), DQ146586, DQ146542. ***Ziziphus jujuba*** Mill., cultivated, *MA48-915* (USNA), DQ146573, DQ146529. ***Ziziphus jujuba*** Mill. (2), cultivated, *NA65-890* (USNA), DQ146574, DQ146530. ***Ziziphus jujuba*** Mill. (3), cultivated, *NA96-22* (USNA), DQ146575, DQ146531. ***Ziziphus jujuba*** Mill. (4), cultivated, *E5717-0001* (UCBG), DQ146576, DQ146532. ***Ziziphus jujuba*** Mill. (5), cultivated, *682201016* (DBG), DQ146574, DQ146530. ***Ziziphus jujuba*** Mill. (6), cultivated, *Zhao s.n.* (RCCJ), DQ146578, DQ146534. ****Ziziphus jujuba*** Mill. (7), cultivated, *XX-0-LZ-AD 105-2001* (Botanischer Garten Leipzig), –, KR083150. ***Ziziphus jujuba*** Mill. (8), China, no voucher (n/a), EU075088, –. ***Ziziphus jujuba*** Mill. (9), locality unknown, *Daniel cdK110* (n/a), FJ593183, –. ***Ziziphus lotus*** (L.) Lam., Israel, *Ori s.n.* (n.a.), DQ146587, DQ146543. ***Ziziphus mairei*** Dode, China, no voucher (n/a), EU075092, –. ***Ziziphus mauritiana*** Lam., cultivated, *78591* (MBC), DQ146589, DQ146545. ***Ziziphus mauritiana*** Lam. (2), Bangladesh, *Islam 001* (CS), –, DQ146546. ***Ziziphus mauritiana*** Lam. (2), Myanmar, *Kress 03-7355* (US), DQ146588, DQ146544. ***Ziziphus montana*** W.W.Sm., China, no voucher (n/a), EU075091, –. ***Ziziphus mucronata*** Willd., cultivated, *Annable 3701* (NY), DQ146592, DQ146549. ***Ziziphus mucronata*** Willd. (2), cultivated, *Davidson s.n.* (n.a.), DQ146593, DQ146550. ****Ziziphus mucronata*** Willd. (3), Kenya, *Schultka s.n.* (FR), KR083102, KR083151. ***Ziziphus oenopolia*** (L.) Mill., cultivated, *Astuti s.n.* (CS), –, DQ146597. ***Ziziphus oenopolia*** (L.) Mill. (2), Sri Lanka, *Bernadi 16046* (NY), DQ146598, DQ146554. "***Ziziphus obtusifolia*** (Hook.f. ex A.Gray) A.Gray", is ***Condalia*** **sp.**, locality unknown, *Hardig & al. #062zizyobt* (WS), –, AF048974. ***Ziziphus ornata*** Lam., Indonesia, *Chase 2117* (K), –, AJ390355. ***Ziziphus pubescens*** Oliv., cultivated, *1997-0117* (UHBG), DQ146599, DQ146555. ***Ziziphus pubescens*** Oliv. (2), Tanzania, *Kindeketa & al. 332* (MO), DQ146600, DQ146556. ***Ziziphus pubisnervis*** Rehder, China, no voucher (n/a), EU075093, –. ***Ziziphus rugosa*** Lam., Myanmar, *Kress 03-7371* (US), DQ146601, DQ146557. ***Ziziphus rugosa*** Lam. (2), China, no voucher (n/a), EU075097, –. ***Ziziphus spina-christi*** (L.) Desf., cultivated, *S1972-1131* (UCBG), DQ146602, DQ146558. ***Ziziphus spina-christi*** (L.) Desf. (2), cultivated, *S1977-0415* (UCBG), DQ146603, DQ146559. ****Ziziphus spina-christi*** (L.) Desf. (3), Sudan, *Neumann 151* (FR), KR083103, KR083152. ***Ziziphus spina-christi*** (L.) Desf. (4), cultivated, *Ori s.n.* (The Jerusalem Botanical Garden), DQ146604, DQ146560. ***Ziziphus xizhangensis*** Y.L.Chen & P.K.Chou, China, no voucher (n/a), EU075090, –.

Supplement to:

Chapter 1: Phylogenetic relationships within the cosmopolitan buckthorn family (Rhamnaceae) support the resurrection of *Sarcomphalus* and the description of *Pseudoziziphus* gen. nov.

Electronic supplement.

http://www.ingentaconnect.com/content/iapt/tax/2016/00000065/00000001/art00005/supp-data/content-1500065es1

Alignment:

http://www.ingentaconnect.com/content/iapt/tax/2016/00000065/00000001/art00005/supp-data/content-1500065es2

Chapter 2: Analysis of the cosmopolitan buckthorn genera *Frangula* and *Rhamnus* s.l. supports the description of a new genus, *Ventia*

Analysis of the cosmopolitan buckthorn genera *Frangula* and *Rhamnus* s.l. supports the description of a new genus, *Ventia*

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Abstract The taxonomy of the species-rich and widespread *Rhamnus* s.l. is partially unresolved. While *Frangula* is morphologically similar to *Rhamnus* but separate according to previous molecular studies, the delimitation of sections within *Rhamnus* s.str., including the large *Rhamnus* sect. *Rhamnus*, remains unclear. In order to clarify the taxonomy of *Rhamnus*, we present the largest molecular dataset available to date, including ITS and *trnL-trnF* sequences of 103 species. fasttree-like searches, maximum likelihood estimates and Bayesian analyses were performed and compared. Our results are congruent across all analyses and show that the current subdivision of *Rhamnus* s.l. should be extended from two (*Frangula*, *Rhamnus*) to four distinct genera. First, we exclude six species from *Rhamnus* and attribute them to the new genus *Ventia*. Second, we raise the entire *Rhamnus* sect. *Oreoherzogia* to generic level. Finally, we confirm the monophyly of *Rhamnus* sect. *Alaternus* and *R.* sect. *Rhamnus* within *Rhamnus* s.str.

Keywords *Frangula*; internal transcribed spacer; molecular phylogenetics; *Oreoherzogia*; Rhamnaceae; *Rhamnus*, *trnL-trnF*; *Ventia*

Supplementary Material The Electronic Supplement (Figs. S1–S2) and alignment are available in the Supplementary Data section of the online version of this article at http://ingentaconnect.com/content/iapt/tax

■ INTRODUCTION

The taxonomy of species-rich genera often represents a challenge for scientists because reaching a sound classification requires extensive sampling, ideally of all putative lineages of lower taxonomic rank. For each of these lineages, data should reflect their entire distribution range, which is most challenging for cosmopolitan taxa (Van Welzen & al., 2009).

Rhamnus L. s.l. is an example of a cosmopolitan genus with a challenging taxonomic history, as is also the case for other genera of Rhamnaceae. The family is large and includes three major groups: the ampelozizyphoid, the ziziphoid, and the rhamnoid group (Richardson & al., 2000). The latter comprises three tribes: Ventilagineae Hook.f., Maesopsideae Weberb., and Rhamneae Hook.f., of which the last includes the type *Rhamnus* (Medan & Schirarend, 2004). Since its description in the 18th century, *Rhamnus* s.l. was revised several times, and its taxonomy went through various modifications (Linnaeus, 1753; Miller, 1754; Grubov, 1949; Johnston & Johnston, 1978; Chen & Shirarend, 2008). However, several of these studies had a regional focus and only dealt with a fraction of the entire distribution range of each taxon, leading to a global overestimation of species numbers. This artefact has most likely contributed to the tremendous number of described species in *Rhamnus*, which currently includes more than 700 published specific and subspecific names. Several of these names represent local variations of more broadly distributed taxa, as illustrated for *Rhamnus crenata* Siebold & Zucc. from China and *R. acuminatifolia* Hayata from Japan (Chen & Schirarend, 2008). In some instances, local populations of a single species were even described under different genera, e.g., *Frangula azorica* Grub. and *Rhamnus latifolia* L'Hér. (Grubov, 1949).

Apart from regional taxonomic studies, regional investigation of phylogenetic relationships within widespread taxa also limits taxonomic interpretation at higher taxonomic levels (Hohna, 2014). *Ziziphus* Mill. (Rhamnaceae), for example, was shown to be monophyletic in a study which included only Eurasian species (Richardson & al., 2000), whereas the inclusion of American species in subsequent studies (Islam & Simmons, 2006; Hauenschild & al., 2016) recovered two independent American lineages, rendering the genus paraphyletic.

Several genera described in the past were referred to as *Rhamnus* s.l., the four most important being *Alaternus* Mill. from northwestern Africa and southern Europe, *Frangula* Mill. which is almost cosmopolitan, *Oreoherzogia* W.Vent, occurring from Europe to Central Asia, and *Rhamnus* from Northern America, Eurasia and Africa (Linnaeus, 1753; Miller, 1754; Vent, 1962). Together, these genera form a monophyletic major

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lineage within tribe Rhamneae as shown in several molecular studies (Richardson & al., 2000; Onstein & al., 2015). Rhamneae currently encompasses 14 genera, and *Rhamnus* s.l., with more than 100 species, contributes almost half of the tribe's diversity (Medan & Schirarend, 2004). Based on molecular evidence, only *Rhamnus* and *Frangula* are currently considered valid genera, while *Alaternus* and *Oreoherzogia* are treated as sections within *Rhamnus* s.str. (Bolmgren & Oxelman, 2004; Pool, 2013). The study by Bolmgren & Oxelman (2004), which included only a limited sampling of *Rhamnus* s.l., indicated that *R.* sect. *Alaternus* and sect. *Oreoherzogia* (with only two species included for each), were monophyletic, nested within the polyphyletic *R.* sect. *Rhamnus* (not supported), whereas *Frangula* was sister to a likely monophyletic *Rhamnus* s.str. Hence, as only a small proportion of those sections was included, the status of *Alaternus*, *Oreoherzogia*, and *R.* sect. *Rhamnus* remained uncertain, as did their phylogenetic relationships, because of insufficient species coverage.

In order to revise the current systematics of *Rhamnus* s.l., we reconstructed the phylogeny of the genus using a broad-scale sampling of taxa, aiming at answering the following questions: (1) Is *Rhamnus* s.str. a monophyletic lineage sister to *Frangula* or comprising the latter when more taxa are added? (2) Are *Rhamnus* sect. *Alaternus* and sect. *Oreoherzogia* monophyletic? (3) Is *Rhamnus* sect. *Rhamnus* polyphyletic, and if so, (4) how would the above mentioned sections best be treated so that only monophyletic lineages are recognized?

■ MATERIALS AND METHODS

Sampling. — Recognised genera and sections within *Rhamnus* s.l. were represented by at least 50% of their extant species: 26 (out of ~40) taxa of *Frangula*, 6 (out of 6) of *Rhamnus* sect. *Alaternus*, 7 (out of 9) of *R.* sect. *Oreoherzogia*, and 70 (out of ~110) of *Rhamnus* sect. *Rhamnus*. We generated 167 new sequences for the internal transcribed spacers (ITS) of nuclear ribosomal DNA, and 154 for the plastid *trnL-trnF* region (which mostly consists of the type I intron of *trnL*, the *trnL-trnF* intergenic spacer, and short exon portions), and retrieved additional data from GenBank. Our study therefore presents the largest dataset available to date for *Rhamnus* s.l., and, in contrast to other studies, from across the entire distribution range of the genus (Appendix 1). The identity of all GenBank sequences and vouchers used in the present study was checked; GenBank sequences from vouchers of uncertain determination were not included. A total of 196 DNA accessions were included in our study (Appendix 1).

DNA extraction, PCR amplification and sequencing. — We extracted genomic DNA with the Qiagen DNeasy Plant Mini Kit (Hilden, Germany) and the NucleoSpin Plant II Kit (Macherey-Nagel, Dueren, Germany) from leaf fragments of 1 cm² size, as advised by the manufacturers' protocols. Some nuclear and plastid markers were already shown to include sufficient information to reconstruct well-supported topologies in *Rhamnus* (Bolmgren & Oxelman, 2004). Hence, we amplified the *trnL-trnF* gene and spacer region, following Taberlet & al. (1991), and the nuclear ribosomal ITS region, following the protocols by Grudinski & al. (2014). We occasionally used internal primers to amplify ITS, following Matuszak & al. (2016). NucleoSpin Gel and PCR clean up kits (Macherey-Nagel) were used to clean PCR products, and the markers were sequenced using an ABI 3730xl capillary sequencer (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.), following the manufacturers' protocols.

Sequence alignment. — MUSCLE and Geneious alignment algorithms as implemented in Geneious v.6.1.6 (Kearse & al., 2012) were used to align sequences. Nuclear and plastid marker were treated as two partitions. We used gBlocks 0.91b (Castresana, 2000) to test for (and exclude) ambiguous sites, which resulted in an alignment of 88% of the size of the original matrix. Furthermore, we cropped the alignment to eliminate rogue taxa using the rogue taxon detection tool as implemented in raxmlGUI v.1.3 (Stamatakis, 2006, 2014; Silvestro & Michalak, 2012).

Congruence testing. — We performed fasttree-like searches in raxmlGUI (FT, Stamatakis, 2008, 2014; Silvestro & Michalak, 2012) on both plastid and nuclear datasets separately in order to test for incongruencies. Shimodaira-Hasegawa-like tests (SH) were used to estimate node support (Shimodaira, 2001; Stamatakis, 2014). Both methods are known to gather similar results as maximum likelihood approaches, whether on simulated or real datasets (Buckley & al., 2001; Price & al., 2010; Liu & al., 2011). Consequently, fasttree-like searches in raxmlGUI are an ideal time-efficient way to test for incongruencies, producing similar phylogenies in comparison to other, more time consuming maximum likelihood approaches.

Phylogeny reconstructions. — We performed fasttree-like (FT), maximum likelihood (ML) and Bayesian (B) analyses on the combined dataset of both markers after congruence checking following Pirie (2015). We performed maximum likelihood and fasttree-like analyses and did not identify supported incongruencies (Bootstrap >70, Shimodaira-Hasegawa >90) in the topologies derived from either ITS (Electr. Suppl.: Fig. S1) or *trnL-trnF* (Electr. Suppl.: Fig. S2). We used the GTR+G substitution model, as it is the most general model. Moreover, GTR+G is recommended by the authors of raxmlGUI (for example over GTR+G+I) as it does not interfere with either RAxML 8 substitution likelihood functions (FT and ML), and the reconstructions can easily be compared with phylogenies reconstructed with mixed model settings (B). fasttree-like searches and maximum likelihood estimates were performed using raxmlGUI (Stamatakis, 2006; Stamatakis, 2008, 2014; Silvestro & Michalak, 2012), and Bayesian analyses were run with MrBayes v.3.2.2 as implemented on the CIPRES platform (Ronquist & Huelsenbeck, 2003; Miller & al., 2010; Ronquist & al., 2012). We used Shimodaira-Hasegawa-like tests (SH) in fasttree-like searches (Shimodaira, 2001; Stamatakis, 2006), and rapid bootstrapping (Stamatakis, 2008; bootstrap support, BS) in the maximum likelihood approach to estimate clade support. Bootstrapping was set to autoMRE (ML, Stamatakis, 2014), and the determination of the best tree was performed as described by Stamatakis (2008). Parameters of the Bayesian analyses were set to four Markov chain Monte Carlo heuristic searches of 30 million generations each. We performed this analysis four times at a sampling frequency of 3000 with a burn-in of 3 million generations based on Tracer v.1.6 statistics (Drummond & Rambaut, 2007). Posterior probabilities (PP) below 0.90, and bootstrap support and Shimodaira-Hasegawa-like values below 80% were not treated as strongly supported throughout this study. *Berchemia floribunda* (Wall.) Brongn. was used as the outgroup to *Frangula* and *Rhamnus* (ingroup), based on previous studies (Richardson & al., 2000; Onstein & al., 2015).

■ RESULTS

For ITS, our dataset included 191 sequences, 172 of which were newly produced for this study. Likewise, 155 of 173 *trnL-trnF* sequences were newly obtained. Altogether our dataset represents 103 ingroup species. A complete list of accessions and respective GenBank numbers is presented in Appendix 1.

Phylogenetic reconstructions. — There were no topological incongruencies among the reconstructed phylogenies from fasttree-like analyses, maximum likelihood estimates and Bayesian analyses. Our analyses of the combined sequence data identified several major clades within *Rhamnus* s.l. (Fig. 1) and largely resolved the phylogenetic relationships among these clades (Figs. 1, 2): *Frangula* (SH: 100, BS: 100, PP: 1.00) is sister to both *Rhamnus* s.str. (SH: 84, BS: 71, PP: 1.00), including the monophyletic *R.* sect. *Rhamnus* (SH: 76, BS: 61, PP: 0.98) and *R.* sect. *Alaternus* (SH: 97, BS: 78, PP: 0.98), and a clade comprising North American and Eurasian rhamnoid taxa (SH: 97, BS: 92, PP: 1.00). The latter clade is divided into monophyletic *Oreoherzogia* (SH: 98, BS: 87, PP: 1.00) and a monophyletic group comprising all native American *Rhamnus* s.str. (SH: 93, BS: 87, PP: 1.00, see Fig. 1). Within *R.* sect. *Rhamnus* relationships among major clades are unresolved (Fig. 2). Our phylogenetic reconstructions support a clade comprising *R. formosana*, *R. nigricans*, *R. prinoides* and *R. subpetala* (SH: 100, BS: 79, PP: 0.99), and weakly support a clade comprising *R. hemsleyana*, *R. pulogensis* and *R. sargentiana* (SH: 66, BS: 55, PP: 0.93). Those two clades, some additional taxa (*R. bodinieri*, *R. papuana*, *R. procumbens*, *R. purpurea*, *R. xizangensis*), and a clade comprising all remaining *Rhamnus* species, with support values of 100 (SH), 79 (BS) and 0.99 (PP), form *R.* sect. *Rhamnus*. Within *Frangula*, five major clades were identified, hereafter referred to as the Californian clade (SH: 100, BS: 67, PP: 1.00), the Central and South American clade (SH: 100, BS: 51, PP: 0.97), the East Asian clade (SH: 100, BS: 98, PP: 1.00), the Southeast Asian clade (SH: 100, BS: 64, PP: 0.95), and the *Frangula alnus* clade (SH: 100, BS: 95, PP: 1.00, see Fig. 1).

■ DISCUSSION

Several back-and-forth taxonomic modifications have occurred in *Rhamnus* s.l. The recent exclusion of *Frangula* based on molecular and morphological evidence (Pool, 2013) is strongly supported by previous (Bolmgren & Oxelman, 2004; Onstein & al., 2015) and our (Fig. 1) phylogenetic reconstructions. However, because previous molecular studies did not include a sufficient number of taxa of North American *Rhamnus* (Richardson & al., 2000; Bolmgren & Oxelman, 2004; Onstein & al., 2015), two regional clades morphologically intermediate between *Frangula* and *Rhamnus* could not be delimited. Bolmgren & Oxelman (2004) observed the monophyly of *R.* sect. *Oreoherzogia*, and its close phylogenetic relationships to *R. crocea* Nutt., yet only based on three species. Hence, as we included more taxa, both *R.* sect. *Oreoherzogia* and the clade including *R. crocea* require taxonomic adjustments: we here resurrect the genus *Oreoherzogia*, and describe the new genus *Ventia* (Table 2). Because our sampling design largely covers the distribution range of these genera and because all phylogenetic analyses we performed recovered highly congruent topologies, we are confident that the inclusion of further data (either in terms of species or gene regions) will not affect the stability of those groups. This study also shows that taxonomic revision of species-rich cosmopolitan taxa should be undertaken only if sufficient coverage of their distribution is achieved.

Sections and genera. — The taxonomy of *Rhamnus* s.l. has undergone multiple changes since the 20th century (Grubov, 1949; Vent, 1962; Johnston & Johnston, 1978; Medan & Schirarend, 2004; Pool, 2013). In particular, the phylogenetic relationships and taxonomic status of *Frangula*, *Alaternus*, *Oreoherzogia*, and *R.* sect. *Rhamnus* have been of focal concern (Bolmgren & Oxelman, 2004). Our analyses confirm the monophyly of five major clades, corresponding to existing sectional or generic names, i.e., *Alaternus*, *Frangula*, *Oreoherzogia*, and *Rhamnus*, plus a clade composed of native North American rhamni (i.e., the new genus *Ventia* described below). *Frangula* is the sister group to all others, thus supporting previous studies suggesting that *Frangula* and *Rhamnus* are distinct entities (Richardson & al., 2000; Bolmgren & Oxelman, 2004). Although molecular data clearly separate *Frangula* from *Rhamnus*, morphological differences between these two taxa are difficult to identify, especially when evergreen taxa are considered. Probably the best distinctive traits are the winter buds covered by scales (usually present in *Rhamnus* and absent in *Frangula*), and a furrow on the seeds in *Rhamnus* (Medan & Schirarend, 2004). The former trait, however, is absent in some evergreen *Rhamnus* species. Up to date, the difficulty in identifying unequivocal diagnostic traits to differentiate *Rhamnus* from *Frangula* has most likely been due to the inclusion of *Oreoherzogia* and *Ventia* in *Rhamnus*, the latter including *R.* sect. *Alaternus* and sect. *Rhamnus*. In fact, *Oreoherzogia* and *Ventia* could be seen as morphologically intermediate between *Frangula* and the monophyletic *Rhamnus* s.str. (including *R.* sect. *Alaternus* and sect. *Rhamnus*), sharing a series of traits with either one or the other genus. On the one hand, *Oreoherzogia* and *Ventia* resemble *Rhamnus* s.str. by their 4-merous flowers and scaled winterbuds (both always absent in *Frangula*). On the other hand, *Oreoherzogia* and *Ventia* are more similar to *Frangula* in their broader and more densely veined leaves. Furthermore, *Oreoherzogia* is similar to *Frangula* because of its bisexual flowers and its sometimes

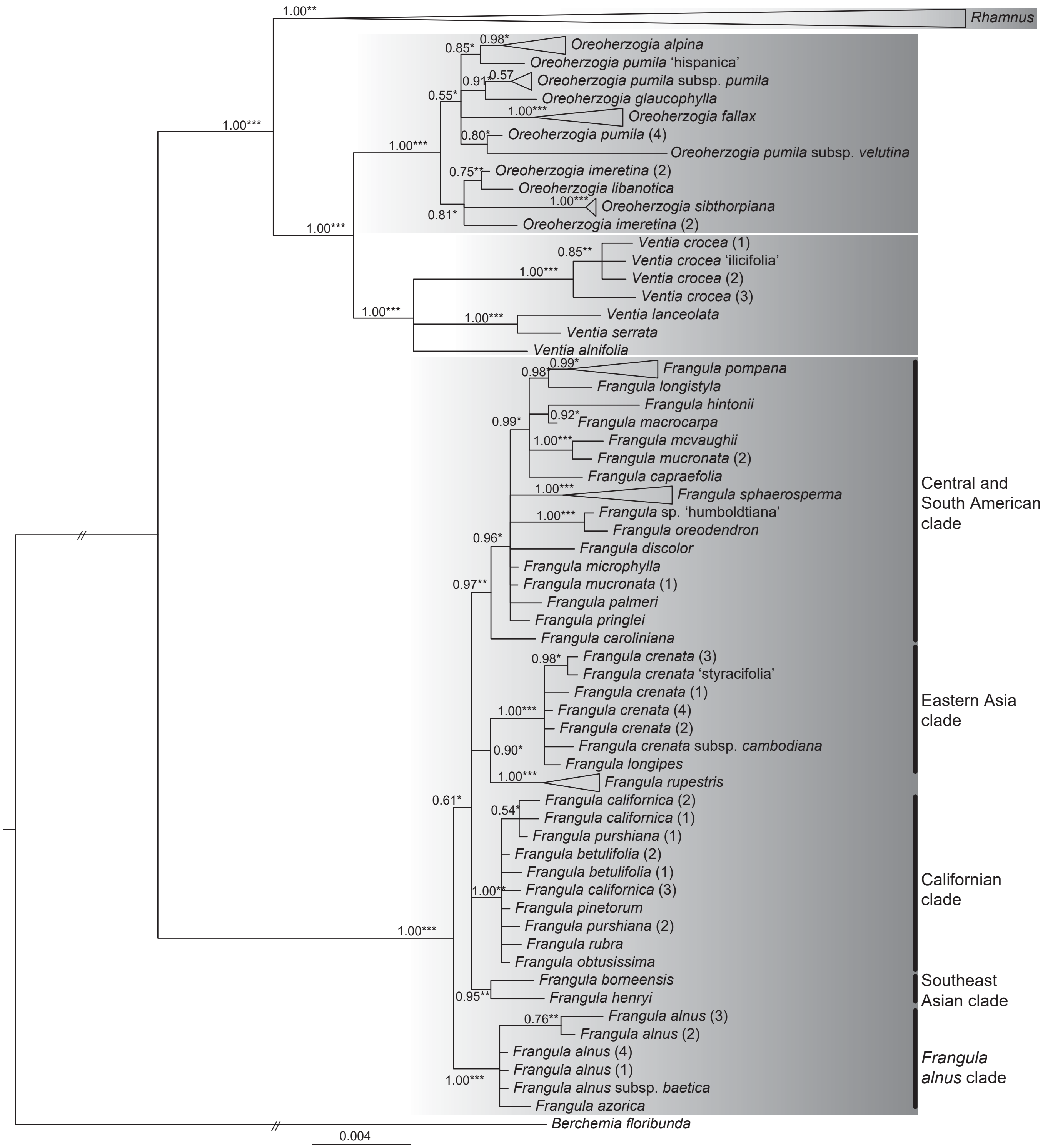
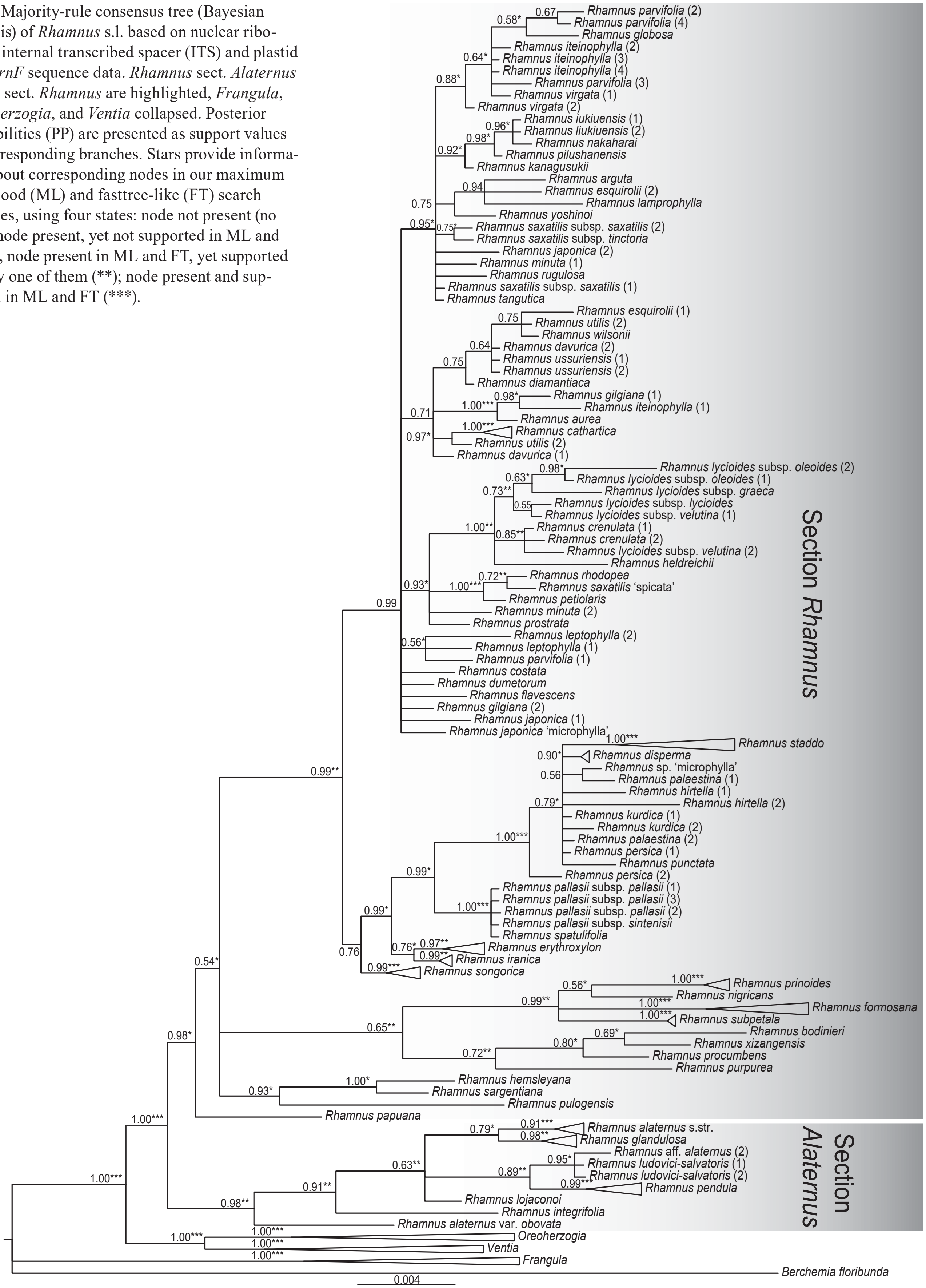


Fig. 1. Majority-rule consensus tree (Bayesian analysis) of *Rhamnus* s.l. based on combined nuclear ribosomal internal transcribed spacer (ITS) and plastid *trnL-trnF* sequence data. The four genera *Frangula*, *Oreoherzogia*, *Rhamnus* (collapsed) and *Ventia* are highlighted. Posterior probabilities are presented as support values on corresponding branches. Stars provide information about corresponding nodes in our maximum likelihood (ML) and fasttree-like (FT) search analyses using four states: node not present (no star); node present, yet not supported in ML and FT (*); node present in ML and FT, yet supported in only one of them (**); node present and supported in ML and FT (***). Bars and labels represent clades in *Frangula* and *Oreoherzogia*.

Fig. 2. Majority-rule consensus tree (Bayesian analysis) of *Rhamnus* s.l. based on nuclear ribosomal internal transcribed spacer (ITS) and plastid *trnL-trnF* sequence data. *Rhamnus* sect. *Alaternus* and *R.* sect. *Rhamnus* are highlighted, *Frangula*, *Oreoherzogia*, and *Ventia* collapsed. Posterior probabilities (PP) are presented as support values on corresponding branches. Stars provide information about corresponding nodes in our maximum likelihood (ML) and fasttree-like (FT) search analyses, using four states: node not present (no star); node present, yet not supported in ML and FT (*), node present in ML and FT, yet supported in only one of them (**); node present and supported in ML and FT (***).



tree-like habit (Grubov, 1949; Vent, 1962; Johnston, 1975). In contrast, *Ventia* is more similar to *Rhamnus* because of its unisexual flowers and a mostly shrubby habit. In addition, *Oreoherzogia* displays alternate branching, while the branching of *Ventia* is often subopposite (Table 1).

We argue, based on molecular and morphological evidence, that the recognition of four distinct genera within *Rhamnus* s.l. (*Frangula*, *Oreoherzogia*, *Rhamnus*, *Ventia*) is the most reasonable taxonomic treatment, because (1) the delimitation between *Rhamnus* and *Frangula* is then unambiguous, (2) the identification of the two genera is now also possible for non-fruiting evergreen plants (the identification key now includes both vegetative and reproductive traits), (3) *Ventia* shares more traits with *Rhamnus*, while *Oreoherzogia* is more similar to *Frangula*, and (4) *Ventia* and *Oreoherzogia* are easily distinguishable from either *Frangula* or *Rhamnus* by a combination of diagnostic vegetative and reproductive traits (see key to *Rhamnus* and close allies).

Alternative taxonomic concepts. — In the following, we will discuss previously applied (but in our view less appropriate) alternative taxonomic concepts, and argue why ours makes more sense. These former taxonomic treatments for the buckthorn clade we study here included either one genus (*Rhamnus* s.l.), two genera (*Frangula*, *Rhamnus*), three genera (*Oreoherzogia* W.Vent, *Frangula*, *Rhamnus*), or four genera (*Alaternus*, *Frangula*, *Oreoherzogia*, *Rhamnus*). All these treatments failed to reflect the morphology of at least one or the other taxon they included. Clearly, the one-genus concept (*Rhamnus* s.l.) is too broad and fails to be supported by key traits because of the morphological diversity within this clade. The two-genera concept (Grubov, 1949; Johnston & Johnston, 1978; Pool, 2013), distinguishing *Frangula* from other species of *Rhamnus* s.l., partly solved this problem by reflecting better the obvious phylogenetic separation between these taxa. Yet, it did not provide sufficient morphological evidence for these clades because some species of *R*. sect. *Oreoherzogia* (in this case belonging to the remaining species of *Rhamnus* s.l.) are more similar to *Frangula* than to the vast majority of other *Rhamnus* species. Hence, morphological evidence hardly justifies *Frangula* alone as a genus distinct from *Rhamnus*, and further distinctions had to be provided. This was done by the three-genus concept (Vent, 1960, 1962). However, this latter concept, separating *Oreoherzogia* from *Frangula* and *Rhamnus*, requires the recognition of *Ventia* gen. nov. to reflect the evolutionary lineages, shown by our phylogenetic analyses (Fig. 1). A combination including *Oreoherzogia* and *Ventia* faces similar limitations (no known synapomorphies) as the two-genera concepts, because some species are more similar to *Frangula*, and others (corresponding to the *Ventia* clade) to *Rhamnus*. This highlights the need to separate *Ventia* gen. nov. from *Oreoherzogia*, as we suggest in this study (based on morphological evidence and strong phylogenetic support in our molecular phylogenetic trees), establishing the number of genera in former *Rhamnus* s.l. to at least four.

As suggested by other studies, a fifth well-supported clade could be considered as potential candidate for generic rank: *Rhamnus* sect. *Alaternus* could, based on molecular reconstructions, also be excluded from *Rhamnus* (Miller, 1754). In our study, the phylogenetic separation between *R*. sect. *Alaternus* and the remaining species of *R*. sect. *Rhamnus* is clearly visible (Fig. 2). Both *R*. sect. *Alaternus* and sect. *Rhamnus* are also clearly supported by the unique possession of lateral-medial seed furrows, a characteristic set of trait combinations (Table 1), allowing the one (*Rhamnus*) and two (*Alaternus*, *Rhamnus*) genera concept within this lineage. Yet, raising *R*. sect. *Alaternus* to generic level is questionable, because a few species of *R*. sect. *Rhamnus* (e.g., the African *R. prinoides* and the Asian *R. nigricans*) are very similar to species of *R*. sect. *Alaternus*, such as *R. alaternus* L., *R. ludovici-salvatoris* Chodat and *R. pendula* Pamp. (e.g., evergreen habit and lacking spines). Summing up, we consider the four-genus concept, including *Frangula*, *Oreoherzogia*, *Rhamnus* and *Ventia*, and two sections within *Rhamnus* s.str. (*R*. sect. *Alaternus*, *R*. sect. *Rhamnus*), as preferable. This approach provides easily distinguishable traits (or a combination of traits) for generic identification and recognizes strongly supported evolutionary lineages recovered by our phylogenetic analyses.

Table 1. Comparative table of selected morphological traits of *Frangula*, *Oreoherzogia*, *Ventia* and *Rhamnus.*

	Frangula Mill.	*Oreoherzogia* W.Vent	*Ventia* gen. nov.	*Rhamnus* L.
Inflorescence	flowers solitary	flowers solitary or fascicled	flowers solitary or fascicled	flowers fascicled or cymose
Flowers	bisexual 5-merous	±bisexual ±4-merous	±unisexual 4-merous	unisexual 4–5-merous
Winterbuds	naked	scaled	scaled	scaled
Furrow on seeds	absent	dorso-medial	dorso-medial	lateral-medial
Leaf venation	pinnate, 6–15+ pairs of lateral veins	pinnate, 6–20 pairs of lateral veins	pinnate, 6–15 pairs of lateral veins	pinnate, usually 3–5(–6) pairs of lateral veins
Spines	none	none	none	present or absent
Branching	alternate	alternate	alternate to subopposite	alternate to opposite
Habit	trees	trees, few shrubs	±shrubs	±shrubs
Native distribution	cosmopolitan (excl. Australia)	northern Africa, Europe, central Asia	North America	Africa, Eurasia

■ TAXONOMIC TREATMENT

In this study, we recognise five monophyletic taxa in former *Rhamnus* s.l.: the genera *Frangula* (as suggested most recently by Pool, 2013), *Oreoherzogia*, *Ventia* and *Rhamnus*, the latter including two monophyletic sections, *R.* sect. *Alaternus* and sect. *Rhamnus*. Here, we also slightly expand *Frangula* by assigning another species to it. Additionally, we exclude all six North American species from *R.* sect. *Rhamnus* to maintain the monophyly of this section: these six species are now placed in the new genus *Ventia*, which is sister to *Oreoherzogia*. The exclusion of this latter section from *Rhamnus* had already been suggested by Vent (1960). Here, we reinstall *Oreoherzogia* as a genus, as proposed and described by the same author. Furthermore, we transfer *Rhamnus pubescens* Sibth. & Sm. 1806, nom. illeg. (non Poir. 1796) and *R. guicciardii* Heldr. & Sart. ex Boiss. (*R. sibthorpiana* Roem. & Schult.) to *Oreoherzogia* (Table 2). Finally, we provide an identification key to these genera below, based on the key to Rhamnaceae by Medan & Schirarend (2004). The descriptive and taxonomic information hereafter is based on Vent (1960, 1962) and Johnston (1975).

Key for *Rhamnus* and close allies

1. Flowers 5-merous, winterbuds naked, plants always bisexual …………………………………………… ***Frangula***
1. Flowers ±4-merous, winterbuds with scales, plants unisexual or bisexual ……………………………………… 2
2. Leaves with 3–5(–6) pairs of lateral veins, seeds lateral-medially furrowed, plants armed or unarmed, inflorescence often cymose ………………………… ***Rhamnus***
2. Leaves with 6 or more pairs of lateral veins, seeds dorso-medially furrowed, inflorescence never cymose …….. 3
3. Leaves always alternate with 6–20 pairs of lateral veins, flowers bisexual (rarely unisexual), Eurasia and Northwest Africa …………………………………… ***Oreoherzogia***
3. Leaves subopposite (rarely alternate), flowers unisexual (rarely bisexual), endemic to North America ….. ***Ventia***

Oreoherzogia

Oreoherzogia W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 44. 1962 ≡ *Rhamnus* subg. *Oreoherzogia* (W.Vent) Yu.E.Alexeev & Tzvelev in Federov & al., Fl. Vostochnoĭ Evropy 9: 396. 1996 – Type: *Oreoherzogia fallax* (Boiss.) W.Vent (≡ *Rhamnus fallax* Boiss.).

Diagnosis. – Species of *Oreoherzogia* resemble either *Frangula* or *Rhamnus*, but are clearly distinguishable from both by a combination of traits. *Oreoherzogia* clearly differs from *Frangula* by its scaled winterbuds, 4-merous flowers, and furrowed seeds (which are all traits present in *Rhamnus*), and from *Rhamnus* by its broad leaves with more than five pairs of lateral veins, and its dorso-medial furrowed seeds (lateral-medial in *Rhamnus*). Furthermore, *Oreoherzogia* is phylogenetically sister to the North American *Ventia*, which can be distinguished from *Oreoherzogia* by its unisexual flowers and subopposite branching.

Description. – Translated and slightly modified (in square brackets) from the description published by W. Vent (1960): [Procumbent or erect] shrubs or small [to medium-sized trees], deciduous, 0.1–3.5 m [(–20 m)] tall, unarmed. Plants bisexual or dioecious. [Stems and primary branches with reddish, brownish

Table 2. List of recognised species *of Oreoherzogia* and *Ventia*, including basionyms. Synonyms are presented after slashes. Taxa in grey colour were not sequenced in our study.

Oreoherzogia W.Vent	Basionym // Synonyms
Oreoherzogia alpina (L.) W.Vent	*Rhamnus alpina* L. subsp. *alpina*
Oreoherzogia depressa (Grubov) W.Vent	*Rhamnus depressa* Grubov
Oreoherzogia fallax (Boiss.) W.Vent	*Rhamnus fallax* Boiss.
Oreoherzogia glaucophylla (Sommier) W.Vent	*Rhamnus glaucophylla* Sommier
Oreoherzogia imeretina (Booth. & al.) W.Vent	*Rhamnus imeretina* Booth & al.
Oreoherzogia libanotica (Boiss.) W.Vent	*Rhamnus libanotica* Boiss.
Oreoherzogia microcarpa (Boiss.) W.Vent	*Rhamnus microcarpa* Boiss.
Oreoherzogia pumila (Turra) W.Vent	*Rhamnus pumila* Turra
Oreoherzogia sibthorpiana (Roem. & Schult.) Hauenschild, comb. nov.	*Rhamnus sibthorpiana* Roem. & Schult. // *Oreoherzogia guicciardii* (Boiss.) W.Vent, *Oreoherzogia pubescens* (Sibth. & Sm.) W.Vent
Ventia **gen. nov.**	**Basionym**
Ventia alnifolia (L'Hér.) Hauenschild, comb. nov.	*Rhamnus alnifolia* L'Hér.
Ventia crocea (Nutt.) Hauenschild, comb. nov.	*Rhamnus crocea* Nutt.
Ventia lanceolata (Pursh) Hauenschild, comb. nov.	*Rhamnus lanceolata* Pursh
Ventia serrata (Schult.) Hauenschild, comb. nov.	*Rhamnus serrata* Schult.
Ventia smithii (Pursh) Hauenschild, comb. nov.	*Rhamnus smithii* Pursh
Ventia standleyana (C.B.Wolf) Hauenschild, comb. nov.	*Rhamnus standleyana* C.B.Wolf

or greyish bark, branchlets glabrous to pubescent], rarely with short, leaf-bearing shoots. Primary branches with phyllopodia opposing a leaf. Buds in leaf-axils, with scales, glabrous or pubescent, vernation conduplicate. Bud scales ciliate. Leaves alternate, glabrous to puberulent, 4–30 cm long. Leaf margin crenulate or serrulate, rarely entire. Venation pinnate, 6–20 pairs of lateral veins. Petioles 0.2–3 cm. Stipules caducous. Pedicels glabrous or pubescent. Flowers solitary or fascicled in leaf axils. Flowers 4(–5)-merous, small. Floral cup hemispheric, sepals triangular (1–4 mm), glabrous or pubescent, [greenish or yellowish]. Petals present in bisexual and male flowers, surrounding stamens, obcordate to oblong, less than twice as long as wide, distally notched, yellowish to whitish. Stamens epipetalous, present in bisexual and male flowers, rudimentary in female flowers. Filaments curved inwards. Anthers opening laterally-longitudinally. Pollen 15.0–24.0 µm in diameter. Pollen exine structure reticulate. Disk thin, surrounding but free from ovary, glabrous. Ovary 2(–3)-locular. Drupe 3–10 mm, glabrous, subglobose to pyriform, blackish to blackish-blue. Pulp thin, without secretory capsules. Putamina with 3–4 seeds. Seeds with dorso-medial furrow, yellowish to brownish, obovate. Embryos with flattened (laterally incurved) cotyledons.

Etymology. – Walter Vent named this genus after the German-Bolivian botanist Theodor Carl Julius Herzog: "*Herzogia*" nom. inval. The name was invalid because it was already in use (K. Schum., Rutaceae). Hence, the prefix Oreo- was added by Vent as reference to the genus' montane habitats.

Distribution. – The genus comprises species distributed from the Mediterranean basin (northwest Africa, southern Europe, Anatolia, and the Middle East) to central Asia and central Europe.

Habitat. – *Oreoherzogia* species grow on rocky slopes, on rocks, in open forest and shrublands, usually between 1000 m and 2000 m elevation.

IUCN conservation assessment. – Most species of *Oreoherzogia* are fairly common and occupy a large distribution range, and are only under marginal threat. Therefore, we assume that their populations are stable. Hence, we suggest *O. fallax*, *O. alpina* (L.) W.Vent, *O. pumila* (Turra) W.Vent and *O. sibthorpiana* (Roem. & Schult.) Hauenschild to be considered as Least Concern (LC). Although none of the criteria for threatened categories are met for *O. imeretina* (Booth & al.) W.Vent, *O. libanotica* (Boiss.) W.Vent, and *O. microcarpa* (Boiss.) W.Vent, their natural habitat is comparatively less common and their distributions are more restricted. Because population data are missing, we suggest these species to be considered between Least Concern and Near Threatened, and encourage population studies to be performed. *Oreoherzogia* also includes narrow endemics: the western Tuscanian *O. glaucophylla* (Sommier) W.Vent is clearly Vulnerable (VU B1biii), whereas the Caucasian endemic *O. depressa* (Grubov) W.Vent (Eastern Azerbaijan, Armenia) lacks sufficient accounts about its range, the trend of its population, and the threat they encounter (DD). For *O. depressa*, too, we strongly encourage population studies to be performed.

Oreoherzogia fallax (Boiss.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 49, 52. 1962 ≡ *Rhamnus fallax* Boiss., Diagn. Pl. Orient., ser. 2, 5: 73. 1856 ≡ *Rhamnus alpina* subsp. *fallax* (Boiss.) Maire & Petitm., Etude Pl. Vasc. Grèce [Matér. Étude Fl. Géogr. Bot. Orient. 4]: 60 1908: 60 1908 – Lectotype (designated by Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 53. 1962): Greece, *Orphanides 444* (G n.v.; isolectotype: BM barcode BM000641888!).

Oreoherzogia alpina (L.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 48, 64. 1962 ≡ *Rhamnus alpina* L. subsp. *alpina*, Sp. Pl.: 193. 1753 ≡ *Alaternus alpinus* (L.) Moench., Methodus: 344. 1794 – Lectotype (designated by Baldini in Taxon 51: 378. 2002): France, "An *Alnus nigra polycarpus* Bauh. Monspelii in horto Regio" Burser Herb. XXIII: 14 (UPS No. UPS:BOT:V-175699).

= *Rhamnus alpina* var. *elongata* W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 65. 1962 – Holotype: France, *Briquet 5328* (G).

Oreoherzogia depressa (Grubov) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 49, 101. 1962 ≡ *Rhamnus depressa* Grubov in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 12: 126. 1950 ≡ *Rhamnus microphylla* var. *acutifolia* Medw., nom. illeg. ≡ *Rhamnus microcarpa* var. *acutifolia* Medw. in Vestn. Tiflissk. Bot. Sada 25: 3. 1912 – Lectotype (designated by Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 53. 1962): Azerbaijan, *Schelkonikow 134* (LE; isolectotypes: JE barcode JE00003939!, K barcode K000723101!, TBI barcode TBI1016949!).

= *Rhamnus microcarpa* var. *microphylla* Trautv. in Trudy Imp. S.-Peterburgsk. Bot. Sada 4: 123. 1876 – Type collection: Georgia, *Radde s.n.* (LE).

Oreoherzogia glaucophylla (Sommier) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 51, 105. 1962 ≡ *Rhamnus glaucophylla* Sommier in Nuovo Giorn. Bot. Ital., n.s., 1: 19. 1894 ≡ *Rhamnus alpina* var. *glaucophylla* (Sommier) Paol., Fiori & Bég. in Fiori & Paoletti, Fl. Italia 2: 215. 1900 ≡ *Rhamnus alpina* subsp. *glaucophylla* (Sommier) Tutin in Feddes Repert. 74: 26. 1967 – Lectotype (designated by Bechi & al. in Webbia 51: 36. 1996): Italy, *Sommier s.n.* (FI barcode FI001508!; isolectotypes: FI barcode FI001850!, M barcode M-0211836!).

Oreoherzogia imeretina (Booth, Petz. & Kirchn.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 49, 62. 1962 ≡ *Rhamnus imeretina* Booth, Petz. & Kirchn. in Petzold & Kirchner, Arbor. Muscav.: 344. 1864 – **Neotype (designated here):** Georgia, *Formin s.n.* (E barcode E00405833!; isoneotype: E barcode E00405834!).

= *Rhamnus alpina* var. *colchica* Kusnez. in Bull. Acad. Imp. Sci. Saint-Pétersbourg 34: 417, t. 1. 1892 ≡ *Rhamnus colchica* (Kusnez.) Sommier & Levier in Trudy Imp. S.-Peterburgsk. Bot. Sada 16: 107. 1900 ≡ *Rhamnus colchica* (Kusnez.) Medw. in Trudy Tiflissk. Bot. Sada 18(1, Pril. 1): 88. 1915 ≡ *Rhamnus imeretina* Koehne, Deut. Dendrol.: 393. 1893, nom. illeg., non Booth & al. 1864 – Lectotype (designated by Kusnezow in Bull. Acad. Imp. Sci.

Saint-Pétersbourg 34: 418. 1892): Georgia, *Radde s.n.* (LE).

= *Rhamnus grandifolia* var. *brachypus* Boiss., Fl. Orient. 2: 22. 1872 ≡ *Rhamnus alpina* var. *grandifolia* Dippel in Handb. Laubholzk. 2: 524–525. 1891 ≡ *Rhamnus libanotica* Hook. in Bot. Mag.: t. 6721. 1883, nom. illeg., non Boiss. 1854 ≡ *Rhamnus imeretina* hort. ex Dippel, Handb. Laubholzk. 2: 525. 1891, nom. illeg., non Booth & al. 1864 – Lectotype (designated by Kusnezow in Bull. Acad. Imp. Sci. Saint-Pétersbourg 34: 418. 1892): Georgia, *Frick 997* (LE).

Oreoherzogia libanotica (Boiss.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 48, 76. 1962 ≡ *Rhamnus libanotica* Boiss., Diagn. Pl. Orient., ser. 2, 1: 119. 1854 – Lectotype (designated by Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 53. 1962): Libanon: *Aucher-Eloy 2055* (G; isolectotype: K barcode K000723108!).

Oreoherzogia microcarpa (Boiss.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 52, 103. 1962 ≡ *Rhamnus microcarpa* Boiss., Fl. Orient. 2: 20. 1872 ≡ *Rhamnus cordata* Medw. in Vestn. Tiflissk. Bot. Sada 25: 3. 1912 – Lectotype (designated by Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 55. 1962): Libanon: *Balansa 458* (G; isolectotypes: JE, K barcode K000723097!).

Oreoherzogia pumila (Turra) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 50, 87. 1962 ≡ *Rhamnus pumila* Turra in Giorn. Italia Sci. Nat. 1: 120. 1764 ("*pumilus*") ≡ *Rhamnus alpina* var. *pumila* (Turra) Dippel, Handb. Laubholzk. 2: 524. 1891 – Neotype (designated by Baldini in Taxon 51: 378. 2002): "*Rhamnus carthaticus*" Herb. Linn. No. 262.17 (LINN!).

= *Rhamnus pumila* L., Mant. Pl.: 49. 1767, nom. illeg., non Turra 1764 – Type: unknown.

= *Rhamnus pumila* var. *velutina* Bornm. in Repert Spec. Nov. Regni Veg. 25: 191. 1928 ≡ *Oreoherzogia pumila* subsp. *velutina* (Bornm.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 87. 1962 – Lectotype (designated by Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 87. 1962): Austria, *Bornmueller s.n.* (JE!)

= *Rhamnus pumila* Wulfen in Jacquin, Collectanea 2: 141, t. 11. 1788, nom. illeg., non Turra 1764 ≡ *Rhamnus wulfenii* Spreng., Syst. Veg. 1: 768. 1824 ≡ *Rhamnus pumila* var. *wulfenii* DC., Prodr. 2: 25. 1825 – Lectotype (designated by Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 87. 1962): [illustration in] Jacquin, Collectanea 2: t. 11. 1788.

Oreoherzogia pumila subsp. ***hispanica*** W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 87. 1962 – Holotype: *Gandoger s.n.* (PRC).

Oreoherzogia pumila subsp. ***legionensis*** (Rothm.) Hauenschild, **comb. nov.** ≡ *Rhamnus legionensis* Rothm. in Bol. Soc. Esp. Hist. Nat. 34(2–3): 152. 1934 ≡ *Oreoherzogia legionensis* (Rothm.) W.Vent in Feddes Repert Spec. Nov. Regni Veg. 65: 48, 102. 1962 – Lectotype (designated by Vent in Feddes Repert Spec. Nov. Regni Veg. 65: 102. 1962): *Rothmaler 384* (JE: Herb. Rothmaler, Tafel 44, Abb. 15, n.v.).

Oreoherzogia sibthorpiana (Roem. & Schult.) Hauenschild, **comb. nov.** ≡ *Rhamnus sibthorpiana* Roem. & Schult., Syst. Veg. 5: 286. 1819 ≡ *Rhamnus pubescens* Sibth. & Sm., Fl. Graec. Prodr. 1: 158. 1806, nom. illeg., non Poiret 1798 ≡ *Oreoherzogia pubescens* (Sibth. & Sm.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 84. 1962 ≡ *Sarcomphalus graecus* Raf., Sylva Tellur.: 29 1838 ("*grecus*") – Lectotype (designated by Vent in Feddes Repert Spec. Nov. Regni Veg. 65: 87. 1962): [illustration] "Rhamnus pubescens" in Sibthorp & Smith, Fl. Graeca: t. 239. 1819.

= *Rhamnus fallax* var. *guicciardii* Boiss., Fl. Orient. 2: 20. Dec 1872 or Jan 1873 ≡ *Rhamnus libanotica* Heldr., Nutzpfl. Griechenl.: 57. 1862, nom. illeg., non Boiss. 1854 ≡ *Rhamnus guicciardii* Heldr. & Sartori ex Boiss., Fl. Orient. 2: 20. Dec 1872 or Jan 1873 ≡ *Oreoherzogia guicciardii* (Boiss.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg 65: 48, 86. 1962 (basionym attributed to "Heldr. & Sartori ex Halácsy") – Lectotype (designated by Vent in Feddes Repert Spec. Nov. Regni Veg. 65: 86. 1962): Heldreich, Fl. Graeca Exsicc., *Guiccardi 2979* (MPU barcode MPU018438).

Ventia

Ventia Hauenschild, **gen. nov.** – Type: *Ventia serrata* (Schult.) Hauenschild ≡ *Rhamnus serrata* Schult. in Roemer & Schultes, Syst. Veg. 5: 295. 1819.

Diagnosis. – The new genus *Ventia* comprises all native North American *Rhamnus* species. *Ventia* can be distinguished from *Frangula* by its unisexual (bisexual in *Frangula*) 4-merous (5-merous in *Frangula*) flowers, the presence of scales on winterbuds (naked in *Frangula*), and the presence of longitudinal furrowed seeds (without furrow in *Frangula*). All these traits are shared with *Rhamnus*, but it is clearly distinguishable from *Rhamnus* by its broader leaves with more than five pairs of lateral veins and its dorso-medially furrowed seeds. Both traits are shared with *Oreoherzogia*, the phylogenetic sister to *Ventia*, from which it can be distinguished by its unisexual flowers and its subopposite branching. Another notable difference between *Oreoherzogia* and *Ventia* is their distribution; they occur disjunctly from each other (Fig. 3).

Description. – Erect shrubs or small trees, deciduous or evergreen, 2–10 m tall, unarmed. Winterbuds scaled. Primary branches with reddish, brownish or greyish bark, branchlets glabrous or pubescent, rarely with short, leaf-bearing shoots. Leaves subopposite or alternate, simple, glabrous to puberulent, 4–15 cm long, lanceolate, elliptic to obovate. Leaf margin entire, crenulate or serrulate. Venation pinnate, 6–9(–15) pairs of lateral veins. Petioles 0.2–2 cm. Stipules present or caducous. Pedicels glabrous or pubescent. Flowers solitary or in fascicles in leaf axils. Flowers 4-merous, unisexual, rarely bisexual, small. Floral cup hemispheric, sepals triangular (1–4 mm), glabrous or pubescent, usually greenish or yellowish. Petals present, fully enclosing reduced anthers in female flowers, partly enclosing anthers in male flowers. Pollen exine structure reticulate. Disk surrounding but free from ovary. Ovary 2–3-locular. Drupe globose, 3–10 mm, with a 2(–3)-celled putamen. Seeds with dorso-medial furrow.

Etymology. – This new genus is named after Walter Vent (1920–2008), a German botanist who first recognised and described the sister genus of *Ventia* (i.e., *Oreoherzogia* W.Vent).

Distribution. – The genus comprises species distributed throughout North America.

Habitat – *Ventia* species grow in various habitats, including arid and subtropical montane limestone habitats in Mexico, shrublands of central North America and fringes of temperate forests in Canada.

IUCN conservation assessment. – None of the six species of *Ventia* are currently listed in the IUCN Red List. These species have large distribution areas and occur in fairly common habitats. According to the *IUCN Red List Categories and Criteria* (v.3.1, 2nd ed; IUCN, 2014) they may therefore be considered as Least Concern (LC).

Ventia serrata (Schult.) Hauenschild, **comb. nov.** ≡ *Rhamnus serrata* Schult. in Roemer & Schultes, Syst. Veg. 5: 295. 1819 ≡ *Rhamnus serrulata* Kunth in Humboldt & al., Nov. Gen. Sp. 7, ed. qu.: 51, t. 617. 1824, nom. superfl. – Holotype: Mexico, *Friedrich Wilhelm Heinrich Alexander von Humboldt 4126* (B).

= *Rhamnus serrata* var. *guatemalensis* L.A.Johnst. in Sida 6(2): 74. 1976 – Lectotype (designated by Johnston in Sida 6(2): 75. 1975): Guatemala, *Steyermark 48377* (A barcode 00051409!; isolectotype: F barcode F0068167F!)

= *Rhamnus fasciculata* Greene in Leafl. Bot. Observ. Crit. 1: 63. 1904 ≡ *Rhamnus smithii* subsp. *fasciculata* (Greene) C.B.Wolf in Rancho Santa Ana Bot. Gard. Monogr., Bot. Ser. 1: 58. 1938 – **Lectotype (designated here):** U.S.A., *Wooton 203* (NDG barcode NDG30289!; isolectotype: US barcode 00094410!)

Ventia alnifolia (L'Hér.) Hauenschild, **comb. nov.** ≡ *Rhamnus alnifolia* L'Hér., Sert. Angl.: 3–4. 1789 ("1788") ≡ *Apetlorhamnus alnifolia* (L'Hér.) Nieuwl. in Amer. Midl. Naturalist 4(3): 90. 1915 – **Neotype (designated here):** U.S.A., *Wied s.n.* (BR barcode 000005576210!)

= *Rhamnus franguloides* Michx., Fl. Bor.-Amer. 1: 153. 1803 – Holotype: U.S.A., New York, Champlain, *Michaux s.n.* (type lost, no duplicates found).

Ventia crocea (Nutt.) Hauenschild, **comb. nov.** ≡ *Rhamnus crocea* Nutt. in Torrey & Gray, Fl. N. Amer. 1: 261. 1838 – **Lectotype (designated here):** U.S.A., *Thomas Nuttall s.n* (GH barcode 00051382!; isolectotypes: GH barcode 00051381!, PH barcode 00023962!).

Ventia crocea subsp. ***ilicifolia*** (Kellogg) Hauenschild, **comb. nov.** ≡ *Rhamnus ilicifolia* Kellog in Proc. Calif. Acad. Sci. 2: 37. 1863 ≡ *Rhamnus crocea* var. *ilicifolia* (Kellogg) Greene, Fl. Francisc.: 79. 1891 ≡ *Rhamnus crocea* subsp. *ilicifolia* (Kellogg) C.B.Wolf in Rancho Santa Ana Bot. Gard. Monogr., Bot. Ser. 1: 39. 1938 – **Neotype (designated here):** U.S.A., *Rattan 228* (US barcode 01122406!).

Ventia crocea subsp. ***insula*** (Kellogg) Hauenschild, **comb. nov.** ≡ *Rhamnus insula* Kellogg in Proc. Calif. Acad. Sci. 1: 36. 1861 ≡ *Rhamnus insularis* Greene in Bull. Calif.

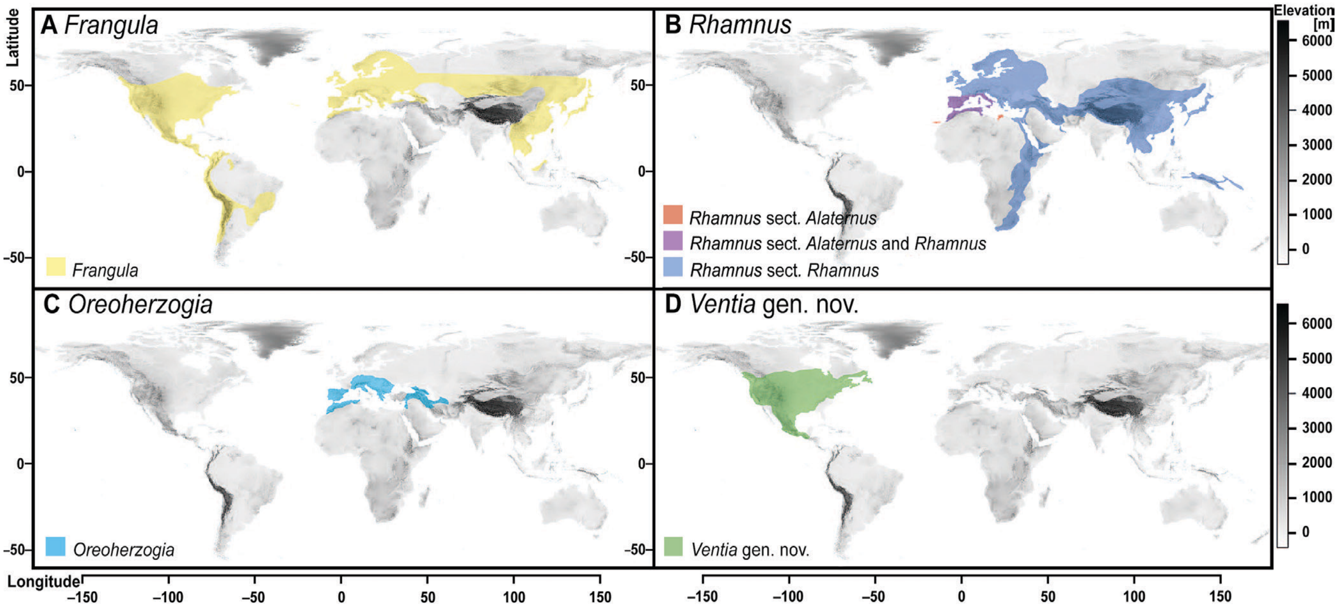


Fig. 3. Approximate natural distribution ranges of: **A,** *Frangula*; **B,** *Rhamnus*; **C,** *Oreoherzogia*; **D** *Ventia.* — Human-introduced range of *Rhamnus* (North and South America, West Africa, Australia) and *Frangula* (cosmopolitan) is not shown on the map. This map was downloaded from WORLDCLIM (Hijmans & al., 2005).

Acad. Sci. 2(7C): 392–393. 1887 ≡ *Rhamnus crocea* subsp. *insula* (Kellogg) C.B.Wolf in Rancho Santa Ana Bot. Gard. Monogr., Bot. Ser. 1: 36–38. 1938 – **Neotype (designated here):** U.S.A., *Palmer 738* (US barcode 0086925!).

Ventia crocea subsp. ***pilosa*** (Trel. ex Curran) Hauenschild, **comb. nov.** ≡ *Rhamnus crocea* var. *pilosa* Trel. ex Curran in Proc. Calif. Acad. Sci, ser. 2, 1(13): 251. 1888 ≡ *Rhamnus pilosa* (Trel. ex Curran) Abrams in Bull. Torrey Bot. Club 37(3): 153. 1910 ≡ *Rhamnus crocea* subsp. *pilosa* (Trel. ex Curran) C.B.Wolf in Rancho Santa Ana Bot. Gard. Monogr., Bot. Ser. 1: 38. 1938 – **Lectotype (designated here):** U.S.A., *Curran s.n.* (CAS barcode 0006198!; isolectotypes: MO barcode MO-260862, GH barcode 00051383).

Ventia crocea subsp. ***pirifolia*** (Greene) Hauenschild, **comb. nov.** ≡ *Rhamnus crocea* var. *insularis* Sarg. in Gard. & Forest 2: 364. 1889 ≡ *Rhamnus pirifolia* Greene in Pittonia 3(13): 15–16. 1896 ≡ *Rhamnus crocea* subsp. *pirifolia* (Greene) C.B.Wolf in Rancho Santa Ana Bot. Gard. Monogr., Bot. Ser. 1: 45. 1938 ≡ *Rhamnus crocea* var. *pirifolia* (Greene) Little in Amer. Midl. Naturalist 33: 496. 1945 – **Lectotype (designated here):** U.S.A., *Greene s.n.* (NDG barcode NDG30348!; isolectotype: PH barcode 00023977!).

Ventia lanceolata (Pursh) Hauenschild, **comb. nov.** ≡ *Rhamnus lanceolata* Pursh, Fl. Amer. Sept.: 166. 1813 (“1814”) ≡ *Cardiolepis rubra* Raf., Neogenyton: 1. 1825 ≡ *Sageretia lanceolata* (Pursh) G.Don, Gen. Syst. 2: 29. 1832 – Holotype: United States, Tennessee: *Lyon s.n.* (PH barcode 00008130!).

= *Rhamnus parvifolius* Torr. & Gray, Fl. North Amer. 1: 262. 1838, nom. illeg. – Holotype: *Pursh 31a* (PH barcode 00026900!).

= *Rhamnus shortii* Nutt. in J. Acad. Nat. Sci. Philadelphia 7: 91. 1834 ≡ *Sarcomphalus shortianus* Raf., Sylva Tellur.: 29. 1838 – Holotype: U.S.A., *Short s.n.* (PH barcodes 00023983! & 00008131!; isolectotype: PH barcode 00039290!).

Ventia lanceolata subsp. ***glabrata*** (Gleason) Hauenschild, **comb. nov.** ≡ *Cardiolepis nigra* Raf., Neogenyton: 1. 1825 ≡ *Rhamnus lanceolata* var. *glabrata* Gleason in Phytologia 2: 288. 1947 ≡ *Rhamnus lanceolata* subsp. *glabrata* (Gleason) Kartesz & Gandhi in Phytologia 76: 451. 1994 – Holotype: *Deam 787* (NY barcode 00415014!).

Ventia smithii (Greene) Hauenschild, **comb. nov.** ≡ *Rhamnus smithii* Greene in Pittonia 3(13): 17. 1896 – Lectotype (designated by Johnston in Sida 6(2): 72. 1975): U.S.A., *Smith s.n.* (PH barcode 00023986; isolectotypes: A barcode 00051392!, PH barcode 00023988!).

Ventia standleyana (C.B.Wolf) Hauenschild, **comb. nov.** ≡ *Rhamnus standleyana* C.B.Wolf in Rancho Santa Ana Bot. Gar. Monogr., Bot. Ser. 1: 51. 1938 – **Lectotype (designated here):** Mexico: *Purpus 4968* (UC barcode UC 153290!; isolectotypes: GH barcode 00051411!, MO barcode MO-194245!, US barcode 00094443!).

Frangula

Frangula borneensis (Steenis) Hauenschild, **comb. nov.** ≡ *Rhamnus borneensis* Steenis in J. Bot. 72: 6. 1934 – **Lectotype (designated here):** Malaysia, *Clemens & Clemens 27876* (L barcode L 0652564!, isolectotypes: A barcode 00051483!, K barcode K000723160!).

= *Rhamnus lancifolia* Steenis in J. Bot. 72: 7. 1934 – Holotype: Indonesia,*Steenis bb6419* (L barcode L 0652563).

= *Oreorhamnus serrulatus* Ridl. in J. Fed. Malay States Mus. 10: 132. 1920 – Holotype: Malaysia, *native collector s.n.* (K barcode K000723159!).

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Appendix 1. Voucher information and GenBank accession numbers of ITS and *trnL-trnF* sequences used in this study.

Taxon, country, collector and number, or collection/accession number (herbarium code or accession location), GenBank accession number for ITS, *trnL-trnF*. Missing data is indicated by a dash (–). An asterisk (*) indicates newly sequenced specimens.

Berchemia floribunda (Wall.) Brongn., Thailand, *Hansen & Smitinand 12690* (L), KR083055, KR083106. ***Frangula alnus*** Mill., Sweden, *Bolmgren 104* (S), AY626431, AJ251691. ***Frangula alnus*** Mill. (2), locality unknown, *Gagnidze & Shetekauri 286* (MO), JN900292, JN900316. ****Frangula alnus*** Mill. (3), Georgia, *Hilbig s.n.* (HAL), KR083198, KR083360. ****Frangula alnus*** Mill. (4), Germany, *Krusche s.n.* (LZ), KR083197, KR083359. ***Frangula alnus*** var. ***baetica*** (Reverchon & Willk.) Rivas, Goday ex Devesa, Spain, *Hampe s.n.* (S), AY626443, AY626429. ****Frangula azorica*** Grubov, Portugal (Azores) *Reins s.n.* (FR), KR083227, KR083386. ***Frangula betulifolia*** (Greene) Grubov, North America, *Bolmgren 105* (S), AY626445, AY626445. ****Frangula betulifolia*** (Greene) Grubov (2), United States, *Worthington 14109* (L), KR083168, KR083337. ****Frangula borneensis*** (Steenis) Hauenschild, Malaysia, *Ng*

Appendix 1. Continued.

1992 (L), KR083170. KR083338. ***Frangula californica*** (Eschsch.) A.Grey, United States, *Holland 114* (S), AY626442, AY626421. *___Frangula californica___ (Eschsch.) A.Grey (2), United States, *Schrenk 2044* (FR), KR083171, KR083339. *___Frangula californica___ (Eschsch.) A.Grey (3), United States, *Scharsmith 4244* (L), KR083172, KR083340. *___Frangula capreifolia___ (Schltdl.) Grubov, Mexico, *Nee 26582* (HAL), KR083173, KR083341. ***Frangula caroliniana*** (Walter) A.Grey, North America, *Schmidt 2559* (S), AY626444, AY626423. *___Frangula crenata___ (Siebold & Zucc.) Miq., Japan, *Azuma s.n.* (FR), KR083180, –. ***Frangula crenata*** (Siebold & Zucc.) Miq. (2), China, *NieMinXiang 92169* (UPS), AY626443, AY626422. *___Frangula crenata___ (Siebold & Zucc.) Miq. (3), cultivated, *KUN060934* (KUN), KR083177, –. *___Frangula crenata___ (Siebold & Zucc.) Miq. (4), locality unknown, *L0550458* (L), KR083178, KR083345. *___Frangula___ aff. ***crenata*** **'cambodiana'**, Thailand, *van Beusekom & al. 4585* (L), KR083179, KR083346. *___Frangula___ aff. ***crenata*** **'styracifolia'**, China, *Renma 5436* (KUN), KR083306, –. *___Frangula discolor___ (Donn. Sm.) Grubov, Guatemala, *Skutch 1901* (L), KR083187, KR083351. *___Frangula henryi___ (C.K.Schneid.) Grubov, China, *Tibet Expedition 9405* (KUN), KR083206, KR083368. *___Frangula hintonii___ (M.C.Johnst. & L.A.Johnst.) A.Pool, Mexico, *Ledezma & Corral 481* (MEXU), KR083207, KR083369. *___Frangula longipes___ (Merr. & Chun) Grubov, China, *Zhiding 53038* (KUN), KR083233, KR083393. *___Frangula longystyla___ (C.B.Wolf) A.Pool, Mexico, *Gallardo 3645* (MEXU), KR083234, KR083394. *___Frangula macrocarpa___ (Standl.) Grubov, Mexico, *Cortes 33* (MEXU), KR083244, KR083401. *___Frangula mcvaughii___ (L.A.Johnst. & M.C.Johnst.) A.Pool, Mexico, *Garcia 573* (MEXU), KR083245, KR083402. *___Frangula microphylla___ (Humb. & Bonpl. ex Schult.) Grubov, Mexico, *Torres 17031* (MEXU), KR083246, KR083403**.** *___Frangula mucronata___ (Schltdl.) Grubov, Mexico, *Cornejo 290* (MEXU), KR083250, KR083407. *___Frangula mucronata___ (Schltdl.) Grubov (2), Mexico, *KUN0614324* (KUN), KR083249, KR083406. *___Frangula oreodendron___ (L.O.Williams) A.Pool, Panama, *McPherson 9822* (L), KR083253, –. *___Frangula palmeri___ (S.Watson) Grubov, Mexico, *Tellez 12749* (MEXU), KR083261, KR083414. *___Frangula pinetorum___ (Standl.) Grubov, Mexico, *Bye 34722* (MEXU), KR083270, KR083423. *___Frangula pompana___ M.C.Johnst. & L.A.Johnst., Mexico, *Hernandez 251* (MEXU), KR083272, KR083425. *___Frangula pompana___ M.C.Johnst. & L.A.Johnst. (2), Mexico, *Nee 26584* (HAL), KR083271, KR083424. *___Frangula pringlei___ (Rose) Grubov, Mexico, *Calzada 22155* (MEXU), KR083273, KR083426. *___Frangula purshiana___ (DC.) A.Grey, United States, *Gibson 1962.27* (L), KR150636, KR150649. ***Frangula purshiana*** (DC.) A.Grey (2), cultivated, *XX-0-FRT-0000/3140* (Botanischer Garten Frankfurt am Main), KR083285, KR083437. ***Frangula purshiana*** (DC.) A.Grey (3), United States, *JEPS97840* (JEPS), AY626430, AY626411. *___Frangula rubra___ var. ***obtusissima*** (Greene) Kartesz & Ghandi, United States, *Heller 8106* (L), KR083288, KR083440. *___Frangula rubra___ (Greene) Grubov var. ***rubra***, United States, *Sharsmith 4265* (L), KR083287, KR083439. *___Frangula___ **sp. 'humboldtiana'**, Costa Rica, *Frounier 845* (ULM), KR083211, KR083373. *___Frangula rupestris___ Schur, Croatia, *de Wilde-Duyfjies 158* (L), KR083292, KR083444. *___Frangula rupestris___ Schur (2), Italy, *Gregor 2877* (FR), KR083293, KR083445. *___Frangula rupestris___ Schur (3), cultivated, *XX-0-FRT-0000/1587* (Botanischer Garten Frankfurt am Main), KR083290, KR083442. *___Frangula rupestris___ Schur (4), Serbia, *Stud. Biol. In Itinere s.n.* (L), KR083291, KR083443. *___Frangula sphaerosperma___ (Sw.) Kartesz & Ghandi, Brazil, *Cordeiro & al. 1622* (ULM), KR083309, KR083459. *___Frangula sphaerosperma___ (Sw.) Kartesz & Ghandi (2), Brazil, *Hatschbach 17401* (L), KR083299, KR083451. *___Frangula sphaerosperma___ (Sw.) Kartesz & Ghandi (3), Brazil, *Hatschbach 54096* (ULM), –, KR083460. *___Frangula sphaerosperma___ (Sw.) Kartesz & Ghandi (4), Costa Rica, *Poyeda 845* (LZ), KR083310, KR083461. *___Frangula sphaerosperma___ (Sw.) Kartesz & Ghandi (5), Venezuela, *Schneider 968* (FR), KR083308, KR083458. *___Oreoherzogia alpina___ L., Spain, *Buttler 19340* (FR), KR083159, KR083328. *___Oreoherzogia alpina___ L. (2), France, *Gutte s.n.* (LZ), KR083161, KR083330. *___Oreoherzogia alpina___ L. (3), cultivated, *FR-0-FRT-1993/495* (Botanischer Garten Frankfurt am Main), KR083158, KR083327. *___Oreoherzogia alpina___ L. (4), Italy, *Schneeweiss & al. 5586* (WU), KR083160, KR083329. *___Oreoherzogia fallax___ L., Austria, *Gutte s.n.* (LZ), KR083162, KR083331. ***Oreoherzogia fallax*** L. (2), cultivated, *XX-0-FRT-1993/500* (Botanischer Garten Frankfurt am Main), KR083163, KR083332. ***Oreoherzogia glaucophylla*** (Sommier) W.Vent, Italy, *Aldobrandi s.n.* (FL), KR083164, KR083333. *___Oreoherzogia libanotica___ (Boiss.) W.Vent, Turkey, *Buttler & Erben 17887* (FR), KR083230, KR083389. *___Oreoherzogia imeretina___ (Booth, Petz. & Kirchn.) W.Vent, locality unknown, *Bornmueller s.n.* (JE), KR083212, KR083374. *___Oreoherzogia imeretina___ (Booth, Petz. & Kirchn.) W.Vent (2), cultivated, *XX-0-LZ-ZF 1339-1996* (Botanischer Garten Leipzig), KR083213, KR083375. ***Oreoherzogia pumila*** (Turra) W.Vent, Europe, *Bolmgren 102* (S), AY626433, AY626414. *___Oreoherzogia pumila___ (Turra) W.Vent (2), Switzerland, *Gregor 6448* (FR), KR083278, KR083430. *___Oreoherzogia pumila___ (Turra) W.Vent (3), Switzerland, *Groep 11968* (L), KR083279, KR083431. *___Oreoherzogia pumila___ (Turra) W.Vent (4), cultivated, *XX-0-FRT-0000/3139* (Botanischer Garten Frankfurt am Main), KR083280, KR083432. *___Oreoherzogia pumila___ subsp. ***hispanica*** W.Vent, Spain, *Sparovsky s.n.* (JE), KR083281, KR083433. *___Oreoherzogia pumila___ subsp. ***velutina*** (Bornm.) W.Vent, Austria, *Bornmueller s.n.* (JE), KR083282, KR083434. *___Oreoherzogia sibthorpiana___ (Roem. & Schult.) W.Vent, Greece, *Bornmueller 354* (JE), KR083301, KR083453. *___Oreoherzogia sibthorpiana___ (Roem. & Schult.) W.Vent (2), Greece, *Leonis 221* (JE), KR083302, –. *___Rhamnus alaternus___ L., Spain, *Conert & al. 881* (FR), KR083153, –. ***Rhamnus alaternus*** L. (2), Europe, *Eriksson 988* (S), AY626435, AY626416. *___Rhamnus alaternus___ L. (3), Maroc, *Lewalle 10833* (L), KR083154, KR083323. ***Rhamnus alaternus*** L. (4), Iraq, *Mati EM520* (EM), FN870380, –. *___Rhamnus alaternus___ var. ***obovata*** (Timb.-Lagr. & Fages) Rouy, Algeria, *Fevrier s.n.* (L), KR083156, KR083325. *___Rhamnus___ aff. ***alaternus*** L., Italy, *de Wilde 11312* (L), KR083155, KR083324. *___Rhamnus arguta___ Maxim., China, *Meusel s.n.* (HAL), KR083165, KR083334. *___Rhamnus aurea___ Heppeler, China, *Bartolomew & al. 814* (KUN), KR083167, KR083336. *___Rhamnus bodinieri___ H.Lév., China, *Hang SH042* (KUN), KR083169, –. *___Rhamnus cathartica___ L., cultivated, *XX-0-FRT-0000/3138* (Botanischer Garten Frankfurt am Main), KR083174, KR083342. *___Rhamnus cathartica___ L. (2), Bulgaria, *Vihodocevsky s.n.* (L), KR083175, KR083343. *___Rhamnus costata___ Maxim., Japan, *Hotta 2* (L), KR083176, KR083344. *___Rhamnus crenulata___ Ait., Spain (Canaries), *Conert 369* (FR), KR083181, KR083347. ***Rhamnus crenulata*** Ait. (2), Spain (Canaries), *Eriksson s.n.* (S), AY626448, AY626428. ***Rhamnus davurica*** Pall., China, *Bolmgren 102* (S), AY626441, AY626420. *___Rhamnus davurica___ Pall. (2), Japan, *L997044696* (L), KR083185, KR083349. *___Rhamnus diamantiaca___ Nakai, cultivated, *KUN0640377* (Kunming Botanic Garden), KR083186, KR083350. *___Rhamnus disperma___ Ehrenb. & Boiss., Egypt, *Hilevy S4497-1* (HUJ), KR083188, KR083352. *___Rhamnus disperma___ Ehrenb. & Boiss. (2), Israel, *Hilevy S4497-2* (HUJ), KR083189, KR083353. *___Rhamnus dumetorum___ C.K.Schneid., China, *Dolongjiang Expedition 6229* (KUN), KR083190, KR083354. *___Rhamnus erythroxylon___ Pall., Mongolia, *Hilbig 18581* (HAL), KR083192, KR083356. *___Rhamnus erythroxylon___ Pall. (2), Mongolia, *Hilbig 26083* (HAL), KR083191, KR083355. ***Rhamnus esquirolii*** H.Lév., China, *Bell 1706* (S), AY626440, AY626440. *___Rhamnus esquirolii___ H.Lév. (2), cultivated, *KUN0468940* (Kunming Botanic Garden), KR083193, –. *___Rhamnus flavescens___ Chen & Chou, cultivated, *KUN0640092* (Kunming Botanic Garden), KR083194, KR083357. *___Rhamnus formosana___ Mats., Taiwan, *Mizushima 10907* (L), KR083196, KR083358. *___Rhamnus formosana___ Mats. (2), cultivated, *Matuszak s.n.* (Kunming Botanic Garden), KR083195, –. *___Rhamnus fulvo-tincta___ Metcalf, China, *Fan 482* (KUN), –, KR150653. *___Rhamnus gilgiana___ Heppeler, cultivated, *Matuszak s.n.* (Kunming Botanic Garden), KR083200, KR083362. *___Rhamnus gilgiana___ Heppeler (2), cultivated, *Kunming Botanic Institute 85109* (KUN), KR083199, KR083361. ***Rhamnus glandulosa*** Ait., Spain (Canaries), *Eriksson s.n.* (S), AY626446, AY626425. *___Rhamnus glandulosa___ Ait. (2), Spain (Canaries), *Gutte s.n.* (LZ), KR083201, KR083363. *___Rhamnus glandulosa___ Ait. (3), Spain (Canaries), *L540762* (L), KR083202, KR083364. *___Rhamnus globosa___ Bunge, China, *Shaobie & al. 81* (KUN), KR083203, KR083365. *___Rhamnus hainanensis___ Merr. & Chou, Vietnam, *Poilane 30986* (L), KR150625, KR150643. *___Rhamnus heldreichii___ Boiss., Cyprus, *FR0034044* (FR), KR083204, KR083366. *___Rhamnus hemsleyana___ C.K.Schneid., China, *Northeast Yunnan Expedition 1207* (KUN), KR083205, KR083367. *___Rhamnus hirtella___ Boiss., Turkey, *Ellenberg 924* (JE), KR083208, KR083370. *___Rhamnus hirtella___ Boiss. (2), Turkey, *Schwarz 252* (JE), KR083209, KR083371. *___Rhamnus iranica___ Hausskn. & C.K.Schneid., Iran, *Strauss 126* (JE), KR083258, –. *___Rhamnus iranica___ Hausskn. & C.K.Schneid. (2), Iran, *Strauss 307* (JE), KR083257, –. *___Rhamnus integrifolia___ DC., Spain (Canaries), *Cahera s.n.* (L), KR083214, –. *___Rhamnus iteinophylla___ C.K.Schneid., China, *Bartholomew 1019* (KUN), KR083215, –. *___Rhamnus___ aff. ***iteinophylla*** C.K.Schneid., China, *Matuszak R188* (KUN), KR083216, KR083376. *___Rhamnus___ aff. ***iteinophylla*** C.K.Schneid. (2), China, *Matuszak R189* (KUN), KR083217, KR083377. *___Rhamnus___ aff. ***iteinophylla*** C.K.Schneid. (3), China, *Matuszak R190* (KUN), KR083218, KR083378. *___Rhamnus japonica___ Maxim., Japan, *L997061071* (L), KR083220, KR083380. *___Rhamnus japonica___ Maxim. (2), Japan, *Yamazaki 10986* (L), KR083219, KR083379. *___Rhamnus japonica___ var. ***microphylla*** Hara, Japan, *Murata 18048* (L), KR083221, KR083381. *___Rhamnus kanagusukii___ Makino, Japan, *Walker 5769* (L), KR083222, KR083382. *___Rhamnus kurdica___ Boiss. & Hohen., Iraq, *Bornmueller 1021* (JE), KR083224, –. *___Rhamnus kurdica___ Boiss. & Hohen., Turkey, *Buttler 16000* (FR), KR083223, KR083383. *___Rhamnus lamprophylla___ C.K.Schneid., China, *KUN0629144*

Appendix 1. Continued.

(Kunming Botanic Garden), KR083225, KR083384. *Rhamnus leptophylla* C.K.Schneid., China, *Fan 95* (L), KR083229, KR083388. ****Rhamnus leptophylla*** C.K.Schneid. (2), China, *Yu & Bo 408* (KUN), KR083228, KR083387. ****Rhamnus liukiuensis*** (E.H.Wilson) Koidz., cultivated, *Matuszak s.n.* (Kunming Botanic Garden), KR083232, KR083391. ****Rhamnus liukiuensis*** (E.H.Wilson) Koidz., Japan, *Walker & al. 5769* (L), KR083231, KR083390. ****Rhamnus lojaconoi*** Raimondo, Italy (Sicily), *di Nato s.n.* (FL), –, KR083392. ****Rhamnus ludovici-salvatoris*** Chodat, Spain (Baleares), *Botanische Excursie Spanje 1980 1004* (L), KR083235, KR083395. ****Rhamnus ludovici-salvatoris*** Chodat (2), Spain (Baleares), *Lewejohann Ma87053* (FR), KR083236, –. ****Rhamnus lycioides*** L., Georgia, *Hilbig s.n.* (HAL), KR083237, KR083396. ****Rhamnus lycioides*** subsp. ***graeca*** (Boiss. & Reuter) Tutin, Greece, *Meusel s.n.* (HAL), KR083238, KR083397. ****Rhamnus lycioides*** L. subsp. ***lycioides***, Spain, *Botanische Excursie Spanje 1980 162* (L), KR083239, –. ***Rhamnus lycioides*** L. subsp. ***lycioides*** (2), Europe, *Eriksson 784* (S), AY626437, AJ390374. ****Rhamnus lycioides*** subsp. ***oleoides*** (L.) Jahand. & Maire, Malta, *van Balgooy 2794* (L), KR083240, KR083398. ****Rhamnus lycioides*** subsp. ***oleoides*** **'microphylla'**, Cyprus, *de Wilde 21415* (L), KR083241, KR083399. ****Rhamnus lycioides*** subsp. ***velutina*** (Boiss.) Tutin, Spain, *Veldkamp 5092* (L), KR083242, –. ****Rhamnus lycioides*** subsp. ***velutina*** (Boiss.) Tutin (2), Spain, *Villaret s.n.* (HAL), KR083243, KR083400. ****Rhamus minuta*** Grubov, China, *Huang He Expedition 1180* (KUN), KR083248, KR083405. ****Rhamus minuta*** Grubov (2), Afghanistan, *Rodenburg 178* (L), KR083247, KR083404. ****Rhamnus nakaharae*** (Hayata) Hayata, Taiwan, *Lee & Kao K3938* (L), KR083251, KR083408. ****Rhamnus nigricans*** Hand.-Mazz., China, *Ende 1701* (KUN), KR083252, KR083409. ****Rhamnus palaestina*** Boiss., Israel, *Liston s.n.* (HUJ), KR083254, KR083410. ****Rhamnus palaestina*** Boiss. (2), Jordan, *Shmida 13005* (HUJ), KR083255, KR083411. ****Rhamnus pallasii*** Fisch. & C.A.Mey., Iran, *Bornmueller 6551* (JE), KR083259, –. ****Rhamnus pallasii*** Fisch. & C.A.Mey. (2), Georgia, *Gutte s.n.* (LZ), KR083256, KR083412. ****Rhamnus pallasii*** Fisch. & C.A.Mey. (3), Iran, *Jacobs 6824* (L), KR150630, KR150647. ****Rhamnus papuana*** Lauterbach, Papua New Guinea, *Streitmann & Kairo 45496* (L), –, KR083415. ****Rhamnus parvifolia*** Bunge, South Korea, *Hyun s.n.* (KUN), KR083263, KR083417. ****Rhamnus parvifolia*** Bunge (2), China, *Meusel s.n.* (HAL), KR083166, KR083335. ****Rhamnus parvifolia*** Bunge (3), China, *Tibet Expedition 11306* (KUN), KR083262, KR083416. ***Rhamnus parvifolia*** Bunge (4), locality unknown, *Wang 1080* (MO), JN900291, JN900322. ****Rhamnus pendula*** Pamp., Libya, *Pampanini 4857* (L), KR083264, KR083418. ****Rhamnus pendula*** Pamp. (2), Libya, *Pampanini 4860* Holotype (FL), KR083265, KR083419. ****Rhamnus persica*** Boiss., Iran, *Jacobs 6661* (L), KR083266, KR083420. ****Rhamnus persica*** Boiss. (2), Iran, *Zohary s.n.* (HUJ), KR083267, KR083421. ****Rhamnus petiolaris*** Boiss., Turkey, *Bunhard 126* (JE), KR083268, –. ****Rhamnus pilushanensis*** Liu & Wang, Taiwan, *KUN0091551* (Kunming Botanic Garden), KR083269, KR083422. ***Rhamnus prinoides*** L'Hér., Africa, *Bolmgren 101* (S), AY626432, AY626413. ****Rhamnus prinoides*** L'Hér. (2), Ethiopia, *van Steenis 24093* (L), KR083274, –. ****Rhamnus procumbens*** Edgew., Nepal, *Suzuki & al. 9455232* (KUN), KR083275, KR083427. ****Rhamnus prostrata*** Jacq., China, *Tibet Expedition 768500* (KUN), KR083276, KR083428. ***Rhamnus pulogensis*** Merr. *Jacobs 7135* (L), KR083277, KR083429. ***Rhamnus punctata*** Boiss., Lebanon, *Coffe s.n.* (L), KR150635, –. ****Rhamnus punctata*** Boiss. (2), Israel, *Meyers & Dinsmore 1861* (L), KR083283, KR083435. ****Rhamnus purpurea*** Edgew., Nepal, *Suzuki & al. 919337* (KUN), KR083284, KR083436. ****Rhamnus rhodopea*** Velen., Bulgaria, *Stribrny s.n.* (FR), KR150637, KR150650. ****Rhamnus rhodopea*** Velen. (2), Macedonia, *van Ooststroom 23923* (L), KR083286, KR083438. ****Rhamnus rugulosa*** Hemsl., China, *Northwest University 86* (KUN), KR083289, KR083441. ****Rhamnus sargentiana*** C.K.Schneid., China, *Peng & al. 8746* (KUN), KR083294, KR083446. ****Rhamnus saxatilis*** Jacq. subsp. ***saxatilis***, Germany, *Buttler 20898* (FR), KR083295, KR083447. ****Rhamnus saxatilis*** Jacq. subsp. ***saxatilis*** (2), Austria, *Till s.n.* (WU), KR083296, KR083448. ****Rhamnus saxatilis*** subsp. ***tinctoria*** Nyman, Romania, *Barabas 371* (L), KR083298, KR083450. ****Rhamnus saxatilis*** **'spicata'** Beck, Croatia, *Stud. Biol. In Itinere s.n.* (L), KR083297, KR083449. ****Rhamnus sintenisii*** Rech.f., Azerbaijan, *Proskuriakova s.n.* (JE), KR083260, KR083413. ****Rhamnus songorica*** Gontsch., Kazakhstan, *Arystangaliev 4828b* (L), KR083303, KR083454. ****Rhamnus songorica*** Gontsch. (2), Kazakhstan, *Roldugin 4828a* (L), KR083304, KR083455. ****Rhamnus songorica*** Gontsch. (3), China, *Taiyi 650649* (KUN), KR083305, KR083456. ****Rhamnus spathulifolia*** Fisch & C.A.Mey., Azerbaijan, *Gutte s.n.* (LZ), KR083307, KR083457. ****Rhamnus staddo*** A.Rich., Kenya, *Schultka s.n.* (FR), KR083311, KR083462. ***Rhamnus staddo*** A.Rich. (2), Somalia, *Thulin & Warfa 6053* (UPS), AU626449, AY626427. ****Rhamnus staddo*** **'holstii'**, Kenya, *Geesteranus 6176* (L), KR083210, KR083372. ****Rhamnus subapetala*** Merr., China, *Gongyuan & al. 101819* (KUN), KR083312, KR083463. ****Rhamnus subapetala*** Merr. (2), China, *KUN0614575* (Kunming Botanic Garden), KR083313, KR083464. ****Rhamnus tangutica*** J.J.Vassil., China, *Boufford 36251* (KUN), KR083314, KR083465. ****Rhamnus triquetra*** (Wall.) Brandis, India, *Venkarta 97875* (L), –, KR150654. ****Rhamnus ussuriensis*** J.J.Vassil., Russia, *Mueller s.n.* (LZ), KR083317, KR083468. ****Rhamnus ussuriensis*** J.J.Vassil. (2), China, *Northeast Agriculture University Plant Expedition 8070* (KUN), KR083316, KR083467. ****Rhamnus utilis*** Decne., China, *Bartholomew 1413* (KUN), KR083319, KR083470. ****Rhamnus utilis*** Decne. (2), cultivated, *XX-0-FRT-1994/1150* (Botanischer Garten Frankfurt am Main), KR083318, KR083469. ****Rhamnus utilis*** Decne. (3), China, *Bartgikinew 1419* (KUN), KR150641, KR150655. ****Rhamnus virgata*** Roxb., cultivated, *Matuszak s.n.* (Kunming Botanic Garden), KR083321, KR083472. ****Rhamnus virgata*** Roxb. (2), China, *Sino-American Botanical Expedition 1984 886* (KUN), KR083320, KR083471. ****Rhamnus wightii*** Wight & Arn., locality unknown, *Kostermans 26247* (L), –, KR150656. ****Rhamnus wilsonii*** C.K.Schneid., China, *Wulingshan Expedition 3565* (KUN), –, KR083473. ****Rhamnus xizangensis*** Chen & Chou, China, *Ende 397* (KUN), KR083315, KR083466. ****Rhamnus yoshinoi*** Makino, Japan, *Watanabe s.n.* (KUN), KR083322, KR083474. ****Ventia alnifolia*** (L'Her) Hauenschild, Canada, *Brisson 74017* (JE), KR083157, KR083326. ****Ventia crocea*** (Nutt.) Hauenschild, United States, *Bartholomew & al. 2458* (KUN), KR083183, –. ****Ventia crocea*** (Nutt.) Hauenschild (2), United States, *Bracelin 1214* (KUN), KR083184, –. ****Ventia crocea*** (Nutt.) Hauenschild (3), United States, *Rose s.n.* (FR), KR083182, KR083348. ****Ventia lanceolata*** (Pursh) Hauenschild, United States, *Leidolf 831* (FR), KR083226, KR083385. ****Ventia serrata*** (Schult.) Hauenschild, Mexico, *Ventura & Lopez 9213* (HAL), KR083300, KR083452.

Supplement to:

Chapter 2: Analysis of the cosmopolitan buckthorn genera *Frangula* and *Rhamnus* s.l. supports the description of a new genus, *Ventia*

Electronic supplement.

http://www.ingentaconnect.com/content/iapt/tax/2016/00000065/00000001/art00006/supp-data/content-1500066es1

Alignment:

http://www.ingentaconnect.com/content/iapt/tax/2016/00000065/00000001/art00006/supp-data/content-1500066es2

ERRATUM

Corrigendum to **Hauenschild, F. & al.,** Analysis of the cosmopolitan buckthorn genera *Frangula* and *Rhamnus* s.l. supports the description of a new genus, *Ventia*. *Taxon* 65: 65–78.

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Ventia Hauenschild is superfluous, as it is antedated by *Endotropis* Raf. (and *Apetlorhamnus* Niewl. as well as *Girtanneria* Neck. ex Raf.); moreover, *Atadinus* Raf. antedates *Oreoherzogia* W.Vent, so that the correct names for the taxa recognized in those genera referred to in *Taxon* 65: 65–78 (names there asterisked below) are as follows, including the new combinations: ***Atadinus depressus*****,** ***A. fallax*****,** ***A. glaucophyllus*****,** ***A. imeretinus*****,** ***A. libanoticus*****,** ***A. microcarpus*****,** ***A. pumilus*****,** ***A. pumilus*** subsp. ***hispanicus*****,** ***A. pumilus*** subsp. ***legionensis*****,** ***A. sibthorpianus*****,** ***Endotropis alnifolia*****,** ***E. crocea*****,** ***E. crocea*** subsp. ***ilicifolia*****,** ***E. crocea*** subsp. ***insula*****,** ***E. crocea*** subsp. ***pilosa*****,** ***E. crocea*** subsp. ***pirifolia*****,** ***E. lanceolata*****,** ***E. lanceolata*** subsp. ***glabrata*****,** ***Endotropis serrata*****,** ***Endotropis smithii*****,** and ***E. standleyana*****.** In this corrigendum, we merely list basionyms and homotypic, antedated combinations. For synonyms, please see Hauenschild & al. (in Taxon 65: 65–78. 2016).

Atadinus

Atadinus Raf., Sylva Tellur. 30: 30. 1838, emend. Hauenschild. = *Oreoherzogia* W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 44. 1962 ≡ *Rhamnus* subg. *Oreoherzogia* (W.Vent) Yu.E.Alexeev & Tzvelev in Federov & al., Fl. Vostochnoĭ Evropy 9: 396. 1996.

Atadinus alpinus (L.) Raf., Sylva Tellur. 30: 30. 1838 ≡ *Rhamnus alpina* L., Sp. Pl.: 193. 1753 ≡ *Alaternus alpinus* (L.) Moench., Methodus: 344. 1794 ≡ *Oreoherzogia alpina* (L.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 48, 64. 1962.

Atadinus depressus (Grubov) Hauenschild, **comb. nov.** ≡ *Rhamnus depressa* Grubov in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 12: 126. 1950 ≡ *Oreoherzogia depressa* (Grubov) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 49, 101. 1962.

Atadinus fallax (Boiss.) Hauenschild, **comb. nov.** ≡ *Rhamnus fallax* Boiss., Diagn. Pl. Orient., ser. 2, 5: 73. 1856 ≡ *Oreoherzogia fallax* (Boiss.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 49, 52. 1962 ≡ *Rhamnus alpina* subsp. *fallax* (Boiss.) Maire & Petitm., Etude Pl. Vasc. Grèce [Matér. Étude Fl. Géogr. Bot. Orient. 4]: 60 1908: 60 1908.

Atadinus glaucophyllus (Sommier) Hauenschild, **comb. nov.** ≡ *Rhamnus glaucophylla* Sommier in Nuovo Giorn. Bot. Ital., n.s., 1: 19. 1894 ≡ *Oreoherzogia glaucophylla* (Sommier) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 51, 105. 1962 ≡ *Rhamnus alpina* var. *glaucophylla* (Sommier) Paol., Fiori & Bég. in Fiori & Paoletti, Fl. Italia 2: 215. 1900 ≡ *Rhamnus alpina* subsp. *glaucophylla* (Sommier) Tutin in Feddes Repert. 74: 26. 1967.

Atadinus imeretinus (Booth, Petz. & Kirchn.) Hauenschild, **comb. nov.** ≡ *Rhamnus imeretina* Booth, Petz. & Kirchn. in Petzold & Kirchner, Arbor. Muscav.: 344. 1864. ≡ *Oreoherzogia imeretina* (Booth, Petz. & Kirchn.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 49, 62. 1962.

Atadinus libanoticus (Boiss.) Hauenschild, **comb. nov.** ≡ *Rhamnus libanotica* Boiss., Diagn. Pl. Orient., ser. 2, 1: 119. 1854 ≡ *Oreoherzogia libanotica* (Boiss.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 48, 76. 1962.

Atadinus microcarpus (Boiss.) Hauenschild, **comb. nov.** ≡ *Rhamnus microcarpa* Boiss., Fl. Orient. 2: 20. 1872 ≡ *Oreoherzogia microcarpa* (Boiss.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 52, 103. 1962 ≡ *Rhamnus cordata* Medw. in Vestn. Tiflissk. Bot. Sada 25: 3. 1912.

Atadinus pumilus (Turra) Hauenschild, **comb. nov.** ≡ *Rhamnus pumila* Turra in Giorn. Italia Sci. Nat. 1: 120. 1764 (“pumilus”) ≡ *Oreoherzogia pumila* (Turra) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 50, 87. 1962 ≡ *Rhamnus alpina* var. *pumila* (Turra) Dippel, Handb. Laubholzk. 2: 524. 1891.

Atadinus pumilus subsp. ***hispanicus*** (W.Vent) Hauenschild, **comb. nov.** ≡ *Oreoherzogia pumila* subsp. *hispanica* W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 87. 1962.

Atadinus pumilus subsp. ***legionensis*** (Rothm.) Hauenschild, **comb. nov.** ≡ *Rhamnus legionensis* Rothm. in Bol. Soc. Esp. Hist. Nat. 34(2–3): 152. 1934 ≡ *Oreoherzogia legionensis* (Rothm.) W.Vent in Feddes Repert Spec. Nov. Regni Veg. 65: 48, 102. 1962.

Atadinus sibthorpianus (Roem. & Schult.) Hauenschild, **comb. nov.** ≡ *Rhamnus sibthorpiana* Roem. & Schult., Syst. Veg. 5: 286. 1819 ≡ *Rhamnus pubescens* Sm., Fl. Graec. Prodr. 1: 158. 1806, nom. illeg., non Poiret 1798 ≡ *Oreoherzogia pubescens* (Sm.) W.Vent in Feddes Repert. Spec. Nov. Regni Veg. 65: 84. 1962 ≡ *Sarcomphalus graecus* Raf., Sylva Tellur.: 29 1838 (“*grecus*”).

Endotropis

Endotropis Raf. in Neogenyton: 1. 1825, emend. Hauenschild ≡ *Cardiolepis* Raf., nom illeg., non Wallr. in Neogenyton 1: 1825.

= *Apetlorhamnus* Niewl. in Amer. Midl. Naturalist 4(3): 90. 1915 ≡ *Girtanneria* Neck. ex Raf., Silva Tellur.: 28. 1838. = *Ventia* Hauenschild in Taxon 65: 73–74, nom. superfl.

Endotropis alnifolia (L'Hér.) Hauenschild, **comb. nov.** ≡ *Rhamnus alnifolia* L'Hér., Sert. Angl.: 3–4. 1789 ("1788") ≡ *Apetlorhamnus alnifolia* (L'Hér.) Nieuwl. in Amer. Midl. Naturalist 4(3): 90. 1915, nom. superfl. ≡ *Girtanneria lineata* Neck., Elem. Bot. 2: 121. 1790, nom. inval.; Raf., Sylva Tellur. 28. 1838 ≡ **Ventia alnifolia* (L'Hér.) Hauenschild, Taxon 65: 74. 2016, nom. superfl.

Endotropis crocea (Nutt.) Hauenschild, **comb. nov.** ≡ *Rhamnus crocea* Nutt. in Torrey & Gray, Fl. N. Amer. 1: 261. 1838 ≡ **Ventia crocea* (Nutt.) Hauenschild in Taxon 65: 74. 2016.

Endotropis crocea subsp. ***ilicifolia*** (Kellogg) Hauenschild, **comb. nov.** ≡ *Rhamnus ilicifolia* Kellog in Proc. Calif. Acad. Sci. 2: 37. 1863 ≡ *Rhamnus crocea* var. *ilicifolia* (Kellogg) Greene, Fl. Francisc.: 79. 1891 ≡ *Rhamnus crocea* subsp. *ilicifolia* (Kellogg) C.B.Wolf in Rancho Santa Ana Bot. Gard. Monogr., Bot. Ser. 1: 39. 1938 ≡ **Ventia crocea* subsp. *ilicifolia* (Kellogg) Hauenschild in Taxon 65: 74. 2016, nom. superfl.

Endotropis crocea subsp. ***insula*** (Kellogg) Hauenschild, **comb. nov.** ≡ *Rhamnus insula* Kellogg in Proc. Calif. Acad. Sci. 1: 36. 1861 ≡ *Rhamnus insularis* Greene in Bull. Calif. Acad. Sci. 2(7C): 392–393. 1887 ≡ *Rhamnus crocea* subsp. *insula* (Kellogg) C.B.Wolf in Rancho Santa Ana Bot. Gard. Monogr., Bot. Ser. 1: 36–38. 1938 ≡ **Ventia crocea* subsp. *insula* (Kellogg) Hauenschild in Taxon 65: 74. 2106, nom. superfl.

Endotropis crocea subsp. ***pilosa*** (Trel. ex Curran) Hauenschild, **comb. nov.** ≡ *Rhamnus crocea* var. *pilosa* Trel. ex Curran in Proc. Calif. Acad. Sci, ser. 2, 1(13): 251. 1888 ≡ *Rhamnus pilosa* (Trel. ex Curran) Abrams in Bull. Torrey Bot. Club 37(3): 153. 1910 ≡ *Rhamnus crocea* subsp. *pilosa* (Trel. ex Curran) C.B.Wolf in Rancho Santa Ana Bot. Gard. Monogr., Bot. Ser. 1: 38. 1938 ≡ **Ventia crocea* subsp. *pilosa* (Trel. ex Curran) Hauenschild, TAXON 65: 74. 2016, nom. superfl.

Endotropis crocea subsp. ***pirifolia*** (Greene) Hauenschild, **comb. nov.** ≡ *Rhamnus crocea* var. *insularis* Sarg. in Gard. & Forest 2: 364. 1889 ≡ *Rhamnus pirifolia* Greene in Pittonia 3(13): 15–16. 1896 ≡ *Rhamnus crocea* subsp. *pirifolia* (Greene) C.B.Wolf in Rancho Santa Ana Bot. Gard. Monogr., Bot. Ser. 1: 45. 1938 ≡ *Rhamnus crocea* var. *pirifolia* (Greene) Little in Amer. Midl. Naturalist 33: 496. 1945 ≡ **Ventia crocea* subsp. *pirifolia* (Greene) Hauenschild in Taxon 65: 75. 2016, nom. superfl.

Endotropis lanceolata (Pursh) Hauenschild, **comb. nov.** ≡ *Rhamnus lanceolata* Pursh, Fl. Amer. Sept.: 166. 1813 ("1814") ≡ *Sageretia lanceolata* (Pursh) G.Don, Gen. Syst. 2: 29. 1832 ≡ *Cardiolepis rubra* Raf., Neogenyton: 1. 1825 ≡ **Ventia lanceolata* (Pursh) Hauenschild in Taxon 65: 75. 2016, nom. superfl.

Endotropis lanceolata subsp. ***glabrata*** (Gleason) Hauenschild, **comb. nov.** ≡ *Rhamnus lanceolata* var. *glabrata* Gleason in Phytologia 2: 288. 1947 ≡ *Rhamnus lanceolata* subsp. *glabrata* (Gleason) Kartesz & Gandhi in Phytologia 76: 451. 1994 ≡ *Cardiolepis nigra* Raf., Neogenyton: 1. 1825 ≡ **Ventia lanceolata* subsp. *glabrata* (Gleason) Hauenschild in Taxon 65: 75. 2016, nom. superfl.

Endotropis serrata (Schult.) Hauenschild, **comb. nov.** ≡ *Rhamnus serrata* Schult. in Roemer & Schultes, Syst. Veg. 5: 295. 1819 ≡ *Rhamnus serrulata* Kunth in Humboldt & al., Nov. Gen. Sp., ed. qu., 7: 51, t. 617. 1824, nom. superfl. ≡ **Ventia serrata* (Schult.) Hauenschild in Taxon 65: 74. 2016, nom. superfl.

Endotropis smithii (Greene) Hauenschild, **comb. nov.** ≡ *Rhamnus smithii* Greene in Pittonia 3(13): 17. 1896 ≡ **Ventia smithii* (Greene) Hauenschild in Taxon 65: 75. 2016, nom. superfl.

Endotropis standleyana (C.B.Wolf) Hauenschild, **comb. nov.** ≡ *Rhamnus standleyana* C.B.Wolf in Rancho Santa Ana Bot. Gar. Monogr., Bot. Ser. 1: 51. 1938 ≡ **Ventia standleyana* (C.B.Wolf) Hauenschild in Taxon 65: 75. 2016, nom. superfl.

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Chapter 3: The influence of the Gondwanan break-up on the biogeographic history of the ziziphoids (Rhamnaceae)

(*submitted to Journal of Biogeography*)

The influence of the Gondwanan break-up on the biogeographic history of the ziziphoids (Rhamnaceae)

Biogeographic history of the ziziphoids

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ABSTRACT

Aim

We investigated whether the fossil-rich and cosmopolitan buckthorn family (Rhamnaceae, dating back to the Cretaceous) was influenced by vicariance events following the Gondwanan breakup. To answer this question, we focused on the ziziphoid lineage of the buckthorn family, because extant ziziphoid taxa comprise tribes and genera exclusively or at least predominantly distributed in the southern hemisphere (Australia, Africa, and South America).

Location

World-wide.

Methods

We generated a DNA alignment of 26989 bp (from plastid, mitochondrial, and nuclear genomes), comprising 575 taxa of Rhamnaceae and related families, including all major lineages within Rhamnaceae and closely related families. We used nine internal fossils to set constraints in our molecular dating analyses. We used BioGeoBEARS in R to reconstruct ancestral areas in order to infer the impact of vicariance events on the ziziphoids caused by Gondwanan fragmentation.

Results

Our biogeographic analyses illustrate that the ziziphoid lineage was strongly influenced by Gondwanan break-up vicariance events, although those events cannot explain all divergence events at the backbone of this lineage.

Main conclusions

Our study highlights that a taxon distributed throughout the Northern Hemisphere can be the result of a vicariance, but this process will be obliterated by the taxon's more recent biogeographic history, such as LDD. Our study also highlights that taxa disjunctly distributed on Southern Hemisphere continents might be unfitting models to investigate the impact of Gondwana-driven vicariance, if the group is too young and its distribution may only explained by more recent LDD events.

Keywords BEAST 1.8.4., BioGeoBEARS, Gondwanan breakup, molecular dating, Rhamnaceae, vicariance, ziziphoids.

Whether distribution patterns of closely related taxa that occur on different, distant land masses of former Gondwana are a result of old vicariance events or more recent long-distance dispersal (LDD) events, is a baseline question asked for many animal and plant groups (Mao et al., 2012; Nauheimer, Metzler & Renner, 2012; Buerki, Devey, Callmander, Phillipson & Forest, 2013; Armstrong et al., 2014; Luebert et al., 2017; Toussaint, Bloom & Short, 2017). The vicariance scenario assumes a common ancestor distributed across the supercontinent Gondwana, before fragmentation would prevent (or at least severely impair) gene flow among populations on the drifting continental fragments, resulting in allopatric speciation. However, despite ongoing discussion on the details of its dislocation, scientists agree that Gondwana´s fragmentation occurred gradually, following a rather well accepted sequence of events. These events started in the Middle Jurassic, with Antarctica separating from Africa, followed by Cretaceous ruptures of Africa from South America and simultaneously of India and Madagascar from the Antarctic Gondwanan remnants. Hence, during the Cretaceous, the former supercontinent transformed into a corridor of connected (Zealandia, Australia, Antarctica, South America, and Africa) fragments. The isolation of Australia and Zealandia, and South America from the already cooled-down Antarctica was completed in the Middle to Late Eocene (McLoughlin, 2001; Seton et al., 2012; Matthews et al., 2015). This gradual process of fragmentation spanned more than 140 million years and molecular dating for such deep time frames is usually associated with large confidence intervals. Hence, determination of a temporal correlation of a taxon’s biogeographic history with supercontinent’s breakup may be hard to accomplish. Moreover, more recent LDD over (large) water bodies after the break-up of Gondwana could also have resulted in closely related taxa distributed on disjunct land masses, further obscuring biogeographic patterns. The reconstruction of ancestral areas and modelling of dispersal events are nowadays often used to increase precision and favour one dispersal scenario over the other, while vicariance is often ruled out *a priori* in case reconstructed age estimates fall outside the time-frame of fragmentation (Richardson, Chatrou, Mols, Erkens & Pirie, 2004; Särkinen et al., 2007; Nie et al., 2012; Martín-Bravo & Daniel, 2016). However, this may also be due to overly small highest posterior density intervals of the time estimated based on too narrowly set priors, and vicariance thereby may be erroneously ruled out (Beaulieu, Tank & Donoghue, 2013). If the importance of vicariance in the course of the Gondwanan breakup is to be evaluated via the pragmatic criterion of mismatch between age estimates and geological history, the priors defining the molecular dating analyses have to be solid. As those are predominantly influenced by fossil attribution (Magallón, 2014; Pirie, Litsios, Bellstedt, Salamin & Kissling, 2015; Shen et al., 2016), taxonomy and phylogeny of the study group are of crucial importance. Consequently, taxonomically resolved groups with a rich fossil record should constitute ideal model groups, because they are more likely to uncover accurate age estimates (Favre et al., 2016).

Such an ideal taxon is the buckthorn family (Rhamnaceae Juss.). The family was recently revised, and many sequences were produced (Richardson, Fay, Cronk, Bowman & Chase, 2000; Richardson, Fay, Cronk & Chase, 2000; Kellermann & Udovicic, 2008; Hauenschild, Matuszak, Muellner-Riehl & Favre, 2016; Hauenschild, Salazar, Muellner-Riehl & Favre, 2016a, b). More than 25 fossil taxa are described within the family, rather evenly distributed through time and across main lineages, e.g. in Paliureae and Rhamneae

(Correa, Jaramillo, Manchester & Gutierrez, 2010; Chen et al., 2017). The family consists of three major clades, the large rhamnoid (300+ species) and ziziphoid (600+ species) clades, as well as the species-poor ampelozizyphoid (10+ species) clade (Richardson, Fay, Cronk & Chase, 2000). The ziziphoid clade, comprising the tribes Colletieae Reissek ex Endl., Gouanieae Jacq., Paliureae Reissek ex Endl., Pomaderreae Reissek ex Endl., and Phyliceae Reissek ex Endl., and the genera incertae sedis *Alphitonia* Reissek ex Endl., *Ceanothus* L., *Colubrina* Rich. ex Brongn., *Emmenosperma* F. Muell., *Granitites* Rye, *Lasiodiscus* Hook. f., and *Schistocarpaea* F. Muell., is of special interest to test for the effect of Gondwanan breakup. This clade is predominantly distributed in the Southern Hemisphere, with the exception of *Ceanothus* (North America) (Fig. 1). Colletieae occur in South America, while *Alphitonia*, *Granitites*, Pomaderreae and *Schistocarpaea* are almost exclusively distributed in Australia and the South Pacific Islands. Finally, *Lasiodiscus* and Phyliceae occur in Africa and neighbouring islands (Richardson et al., 2001; Aagesen, Medan, Kellermann & Hilger, 2005; Ladiges, Kellermann, Nelson, Humphries & Udovicic, 2005; Onstein & Linder, 2016). The occurrence of vicariance events caused by the Gondwanan breakup in the ziziphoids has already been tested and rejected, because the reconstructed age estimates were considered to be too young (Richardson et al., 2004) for a temporal coincidence, hence favouring a post-Gondwanan dispersal scenario among distant and individualised continents. However, Richardson et al. (2004) did not perform molecular dating by direct fossil calibration but by secondary calibration, a method nowadays criticized (Graur & Martin, 2004; Schenk, 2016). In addition, it is worth noting that Richardson et al. (2004) reconstructed the age of the Rhamnaceae crown and of multiple internal nodes as being younger than the existing fossil record (Calvillo-Canadell &

Cevallos-Ferriz, 2007; Burge & Manchester, 2008; Onstein, Carter, Xing, Richardson & Linder, 2015). The latter indicates at least a Late Cretaceous origin of Rhamnaceae. In contrast, the ages recovered by more recent studies would favour the Gondwanan breakup scenarios at least partially (Magallón, Gomez-Acevedo, Sanchez-Reyes & Hernandez-Hernandez, 2015; Onstein et al., 2015).

To test if, and to what extent, the Gondwanan breakup shaped the evolution of inner ziziphoids, we therefore used the biggest data set on Rhamnaceae so far, comprising all major lineages of the buckthorn family and its related families, using plastid, mitochondrial, and nuclear markers, for a final alignment of over 26000 bp in length. With this approach, we aimed to reconstruct the spatio-temporal history of Rhamnaceae, with a focus on the ziziphoid clade. We asked if vicariance events during the break-up of the Gondwanan supercontinent, or younger LDD events, influenced the current distribution of ziziphoid clades.

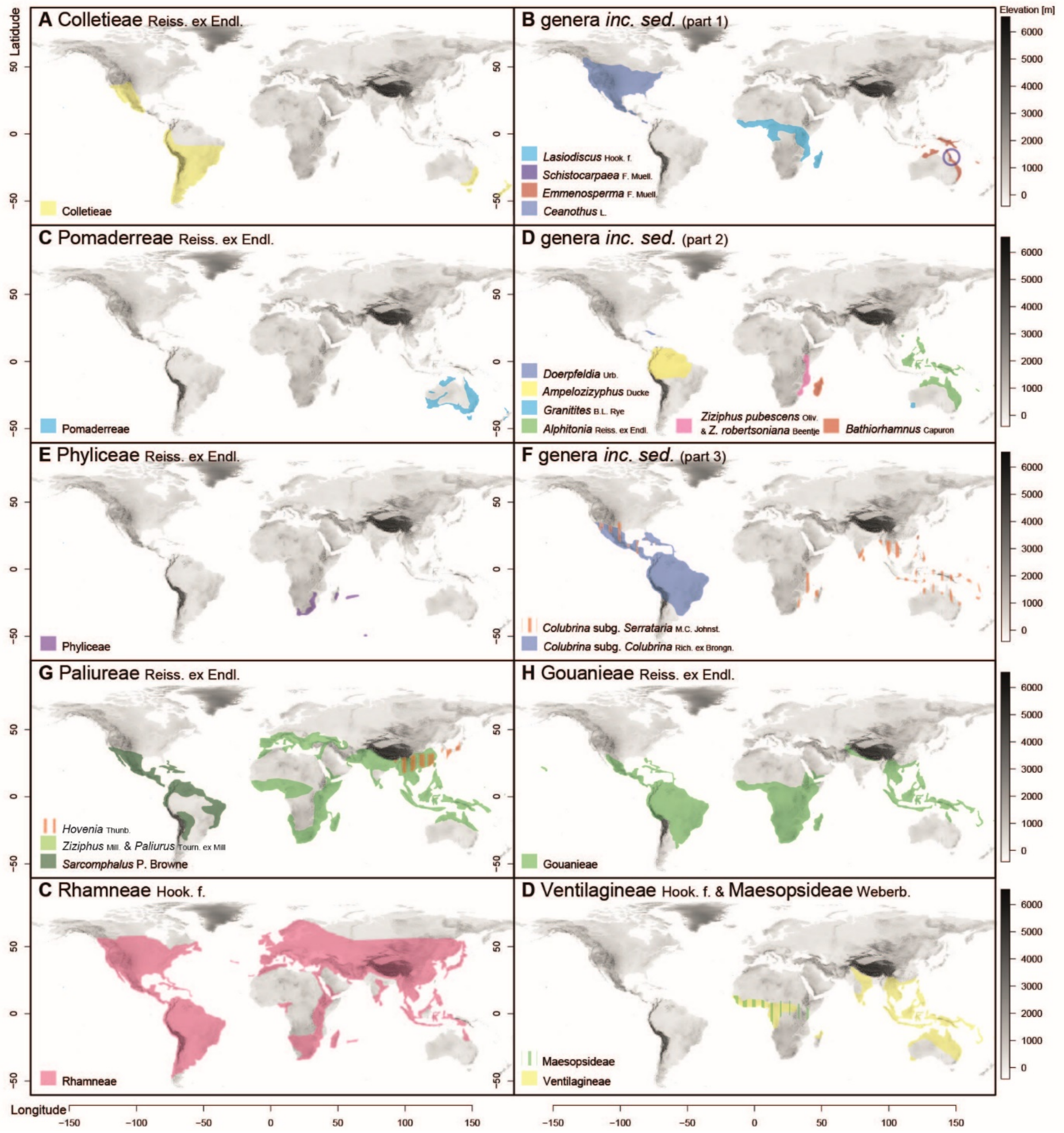
A Colletieae Reiss. ex Endl.
Colletieae
B genera inc. sed. (part 1)
Lasiodiscus Hook. f.
Schistocarpaea F. Muell.
Emmenosperma F. Muell.
Ceanothus L.
Elevation [m]
6000
5000
4000
3000
2000
1000
0
C Pomaderreae Reiss. ex Endl.
Pomaderreae
D genera inc. sed. (part 2)
Doerpfeldia Urb.
Ampelozizyphus Ducke
Granitites B.L. Rye
Alphitonia Reiss. ex Endl.
Ziziphus pubescens Oliv. & Z. robertsoniana Beentje
Bathiorhamnus Capuron
E Phyliceae Reiss. ex Endl.
Phyliceae
F genera inc. sed. (part 3)
Colubrina subg. Serrataria M.C. Johnst.
Colubrina subg. Colubrina Rich. ex Brongn.
G Paliureae Reiss. ex Endl.
Hovenia Thunb.
Ziziphus Mill. & Paliurus Tourn. ex Mill
Sarcomphalus P. Browne
H Gouanieae Reiss. ex Endl.
Gouanieae
C Rhamneae Hook. f.
Rhamneae
D Ventilagineae Hook. f. & Maesopsideae Weberb.
Maesopsideae
Ventilagineae
Latitude
50
0
-50
Longitude
-150
-100
-50
0
50
100
150

Fig. 1. Approximate distribution ranges (excluding introduced ranges) of all recognized Rhamnaceae taxa, including tribes (if monophyletic), genera (if not attributed to a tribe or genera incertae sedis), or lower rank (*Ziziphus pubescens* & *Z. robertsoniana*). The distribution data was collected from literature (cf. App. 1). The map was downloaded from WORLDCLIM (Hijmans & al., 2005), and modified manually.

MATERIALS AND METHODS

Data sampling

In total, 10 plastid, four nuclear, and one mitochondrial (cytochrome oxidase subunit 1, cox1) markers have been incorporated in our study, resulting in a set of 575 taxa and 26989 bp, covering all major phylogenetic lineages, tribes and all but two monophyletic genera. For the included markers, we used all genetic data available in GenBank up to April 2017. Plastid markers included the ATP synthase beta subunit (*atpB*), the ribulose bisphosphate carboxylase oxygenase (*rbcL*), the maturase K gene (*matK*), the NADH dehydrogenase gene F (*ndhF*), the photosystem II CP47 chlorophyll apoprotein gene to photosystem II phosphoprotein partial gene regions and intergenic spacers (*psbB-psbT*), the plastid RNA-polymerase subunit beta, the ribosomal protein 2 to photosystem II protein D1 partial gene region and intergenic spacers (*rpl2-psbA*), the ribosomal protein L16 (*rpl16*), tRNA-Leu gene (*trnL*), the tRNA-Leu to tRNA-Phe intergenic spacer (*trnL-trnF*), and the tRNA-Gln to the ribosomal protein S16 intergenic spacer (*trnQ-rps16*). Nuclear markers included the 18S and 26S ribosomal RNA gene (18S, 26S), the internal transcribed spacer region (ITS), the putative Mg-protoporphyrin IX monomethyl ester cyclase gene (at103). GenBank contains additional markers present in Rhamnaceae, however, we excluded all markers either present for less than 10 taxa or distributed among less than 3 major lineages. Problematic taxonomic attributions within *Ziziphus* have already been addressed by Hauenschild *et al*. (2016a, 2016b), and a robust phylogenetic reconstruction was achieved in multiple studies (Richardson, Fay, Cronk, Bowman, et al., 2000; Onstein et al., 2015; Hauenschild, Matuszak, et al., 2016; Hauenschild, Salazar, et al., 2016a; Onstein & Linder, 2016). In contrast to some of these some studies, the present data set was reduced to include only one accession per taxon, by the approach described in Hauenschild et al. (2017) for *Allium*. Monophyly of strongly supported clades from our previous studies was constrained in the molecular dating analyses (Hauenschild, Matuszak, et al., 2016).

Molecular dating

We used macrofossils to calibrate a maximum of seven internal nodes, evenly distributed on rhamnoid and ziziphoid lineages (App. 1). Whenever possible, we used more than one fossil taxon of the same age to constrain a single node. For example, *Rhamnus salicifolious* Lesq. ex Hayen leaf fossils and *Coahuilanthus belindeae* Calvillo-Canadell & Cevallos-Ferriz flower fossils constrained the same node to the same age. We used *Frangula alnus* Mill. and *Frangula californica* (Eschsch.) A. Grey. to set a minimum age for the *Frangula* Mill. crown node, using a lognormal distribution with an offset of 5 Ma. We used *Frangula hordwellensis* Chandler to calibrate the stem of *Frangula*, at the last common ancestor of *Frangula* and *Rhamnus*, using a lognormal function with an offset of 34 Ma (Eocene – Oligocene boundary).

We calibrated the Reynosia-Karwinskia complex using *Karwinskia californica* Axelrod / *Karwinskia axamilpense* Velasco de Leon with a lognormal function with an offset of 34 Ma (Eocene – Oligocene boundary). We placed *Rhamnus salicifolius* Lesq. ex Hayen and *Coahuilanthus belindae* Calvillo-Canadell & Cevallos-Ferriz at the combined crown of Rhamneae and Maesopsideae and use its age as a lognormal function with an offset of 70 Ma. We used *Archeopaliurus boyacensis* Correa, Manchester, Jaramillo & Gutierrez and *Paliurus* sp. to set a log normal prior with an offset of 66 Ma to the most recent common ancestor of all extant species of *Paliurus* and *Ziziphus* s.s. We calibrated the crown of *Ceanothus* using *Ceanothus leichti* Axelrod and *Ceanothus precuneatus* Axelrod as a lognormal function with an offset at 12 Ma. We used *C. spiraeafolia* (Lesquereux) MacGinitie to calibrate the minimum age of the *Colubrina* crown to 34 Ma. Last, we used the Albian-Aptian boundary (113 Ma) as hard bound maximum age for our temporal reconstruction (truncated), and the Cenomanian-Albian boundary (approximately 100 Ma), where the fossil record still includes fossils with affinities to buckthorns, as soft maximum constraint (lognormal prior to the stem, truncated to 113 Ma) (Appendix 1).

All molecular age reconstructions were performed in BEAST 1.8.4 (Drummond & Rambaut, 2007). After rejecting a clock-like behaviour of the data, and testing the sensitivity of the underlying molecular data by a comparison with a prior-only analysis, we used an uncorrelated lognormal clock (ucln) model with a Birth-Death (incomplete sampling) tree prior, and GTR+Γ as substitution model. Two runs were performed, with 100,000,000 generations each, sampling every 10,000th generation. Convergence of the parameters and burn-in proportions were checked in Tracer 1.6 by ESS scores. Resampling and combinations of runs were performed in LogCombiner 1.8.4 (Drummond & Rambaut, 2007). Identification of the most credible tree and tree annotation were done in

TreeAnnotator 1.8.4 (Drummond & Rambaut, 2007) and R (R core Team, 2015) with the ape package (Paradis, Claude & Strimmer, 2004).

Ancestral area reconstructions

The annotated most credible tree was used as input for our ancestral area reconstructions (App. 2) to infer the role of the Gondwanan breakup on the biogeographical history of the inner ziziphoids. We defined seven areas as follows: 1) North America (including Central America), 2) South America, 3) Europe (to the Ural Mountains, the Caucasus, and the Bosporus) and Northwestern Africa, 4) Africa (excluding Northwestern Africa), 5) Australia and Oceanic Islands, 6) Asia, and 7) India (delineated northward by the Indus and the Himalaya and eastward by the Naga Hills) (Fig. 2). We chose the unconventional coding of Africa because the majority of North African taxa are present in Europe or endemic to the Canary Islands. Except for widespread taxa, the African ziziphoid clade does not occur north of the Western Sahara. Furthermore, we coded India separately, as this Gondwanan fragment drifted northward in absolute isolation, and might have acted as dispersal vector within the ziziphoids, as previously hypothesised by Chen et al. (2017). We allowed a maximum combination of six ancestral areas (as this is the minimum given by the pantropical distribution of *Colubrina asiatica* (L.) Brongn. BioGeoBEARS (Matzke, 2012; Matzke, 2014) in R 3.3.3 was used for all biogeographic reconstructions. Dispersal-Extinction-Cladogenesis (DEC) analysis and a likelihood variant of the Dispersal-Vicariance-Analysis (DIVA-like) were performed with and without founder events (+J). We did not perform BayArea-like analyses, as the model does not allow vicariance-driven events (Matzke, 2014). We used five time slices defined as follows: Pliocene to present (<5

Ma), closure of the Central American Seaway and rise of the Isthmus of Panama (5 to <20 Ma), isolation of Australia from Antarctica (20 to <40 Ma), isolation of India from Gondwanan fragments (40 to <85 Ma), Cretaceous American interchange (85 to <100 Ma), and older (Seton et al., 2012; Barat et al., 2014; Bacon et al., 2015; Matthews et al., 2015). For all time slices, we allowed dispersal among all areas, including long-distance dispersal. Adjacent areas were set as preferred, using a dispersal multiplier of 1; non-adjacent areas were penalized by using a dispersal multiplier of 0.1. Combination of areas was allowed after the continental boarder reconstructions by Seton et al. (2014). Following the analyses, a likelihood ratio test, followed by using the AIC, was performed to uncover the best-fitting biogeographic model. Finally, cumulative ancestral area reconstructions were displayed for the best-fitting biogeographic model for the ziziphoid lineages (Ebersbach et al., 2016; Hauenschild et al., 2017).

RESULTS

Molecular dating

Our alignment comprised 5881 nuclear, 2341 mitochondrial, and 18767 plastid positions (total: 26989). In total, 7510 distinct alignment patterns have been detected, ranging from 102 (18S) to 1187 (*trnL-trnF*). The final alignment included 91% gaps. Substitution rates across the partitions were normally distributed and varied between 0.31±0.01 A-T and 0.94±0.04 A-G (plastid), <0.01 (mitochondrial), and 0.11±0.00 C-G and 0.37±0.02 A-G (nuclear) substitutions per site per million years.

The age of Rhamnaceae was recovered between 91.4 and 102.6 Ma (95% highest posterior density, HPD, mean: 101.0 Ma), and of the ziziphoid clade between 74.6 and 93.2 Ma (HPD, mean: 89.8 Ma). The stem of tribes Gouanieae and Paliureae (excluding *Sarcomphalus*) was reconstructed between 70.6 and 89.5 Ma (HPD, mean: 77.8 Ma). Paliureae was placed between 68.4 and 82.6 Ma (HPD, mean: 75.8 Ma). Gouanieae was estimated to be between 21.5 and 56.3 Ma (HPD, mean: 46.9 Ma). The crown of the clade comprising all other ziziphoid taxa was placed as between 43.1 and 90.3 Ma (HPD, mean: 81.6 Ma). The crown of the clade comprising *Alphitonia* sensu lato (*Alphitonia*, *Emmenosperma*, *Granitites*, and *Jaffrea*, 18.1 to 40.8 Ma, mean: 29.9 Ma), *Ceanothus* (12.0 to 15.7 Ma, HPD, mean: 12.5 Ma), Colletieae (8.6 to 24.6 Ma, HPD, mean: 10.3 Ma), Phyliceae (14.7 to 32.0 Ma, HPD, mean: 20.3 Ma), Pomaderreae (16.8 to 28.4 Ma, HPD, mean: 21.8 Ma), and *Schistocarpaea* was estimated to date back to between 32.1 and 49.6 Ma (HPD, mean: 40.2 Ma). The latter clade was sister to a clade comprising *Colubrina* and *Sarcomphalus* (38.1 to 85.4 Ma, HPD, mean: 78.4 Ma). The full chronogram is given in Appendix 2.

Biogeographic analyses

DEC+J was selected using the Akaike Information criterion (weighted AIC 1.00 vs <0.0001, against DEC, DIVA, and DIVA+J) to best represent our data. A full tree with plotted ancestral age reconstructions on the corresponding nodes is given in Appendix 2. In the following, we will focus on the inner ziziphoid lineage, which comprises extant taxa predominantly distributed in the Southern Hemisphere (except for *Ceanothus*, and tribe Paliureae, Fig. 1).

Our ancestral area reconstructions suggested that the inner ziziphoids' ancestor was distributed in the Late Cretaceous Southern Hemisphere, potentially including Africa (34.6 %)and South America (37.5 %, Fig. 2, App. 2). A full list of probabilities for each node is presented in App. S.3, and node numbers are illustrated in App. S4. The reconstructions suggested dispersal from Africa into Asia for tribe Paliureae (excluding the genus *Sarcomphalus*) and tribe Gouanieae (except *Helinus*), while all other ziziphoid taxa shared a common ancestor distributed in Cretaceous South America (68.2 %). While *Colubrina* and *Sarcomphalus* remained only in South America (80.2 %), the ancestral area of most other ziziphoids extended westwards, including Australia or Australia and South America during the early Paleogene (Australia: 40.0 % to 43.6 %, South America: 40.9 % to 43.5 %). *Alphitonia* s.l. became extinct in South America, reducing its ancestral area to Australia (84.1 %, Eocene).. The common ancestor of all remaining taxa (tribes Colletieae, Phyliceae, and Pomaderreae, as well as the genus inc. sed. *Schistocarpaea*) remained on the Eocene landmasses of Australia (79.1 %), followed by a dispersal from Australia into South America (57.5 %, Colletieae) and Africa and the Antarctic Islands (57.2 %, Phyliceae) throughout the Late Eocene, Oligocene and Miocene (Fig. 2).

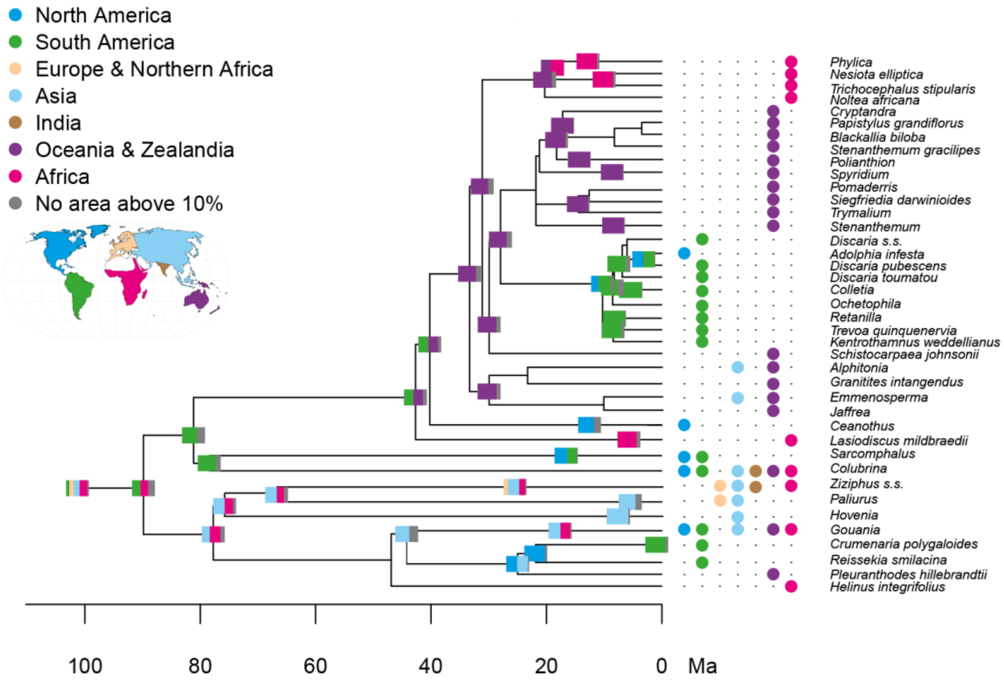

Fig. 2. Summary of the ancestral area analysis. The full reconstruction of the ziziphoids is illustrated in App. 1. Below the time scale, a trace of the biogeographic history of all ziziphoid taxa is given. Colours of the areas are as follows: North America: darker blue, South America: green, Africa: pink, Asia: light blue, India: brown, Europe: white, and Australia: purple. *Alphitonia* s.l. refers to the genera *Alphitonia*, *Emmenosperma*, *Granitites*, and *Jaffrea*. Mixed colours refer to potentially combined areas. Grey refers to an extant distribution in more than two areas.

DISCUSSION

Our biogeographic reconstructions, based upon the largest Rhamnaceae data set available to date, allowed us to infer the spatio-temporal history of the buckthorn family. The topology produced by our analyses (App. 2) was in accordance with previously reconstructed phylogenies (Richardson, Fay, Cronk, Bowman, et al., 2000; Richardson et al., 2004; Hauenschild, Matuszak, et al., 2016). Our reconstructions showed minor (within genera) and major (among genera, in tribe Rhamneae) differences to the topology by Onstein et al. (2015; 2016), as already discussed by Hauenschild et al. (2016a).. However, this is because we used topological constraints whenever nodes were consistently supported in previous studies. Our molecular dating approach did not rely on age estimates from previous dating approaches, but was based on setting internal fossil constraints points. Despite this, the age estimates recovered in our study are only slightly older than those of Onstein et al. (2015, 2016). We consider the early Late Cretaceous as likely temporal origin of the common ancestor of the buckthorns, which is supported by the fossil record. Indeed, the Albian epoch includes fossils with affinities to Rhamnaceae (Basinger & Dilcher, 1984; Spicer et al., 2002). Hence, we consider our temporal reconstructions, as well as the underlying topology, as robust enough to deduct a reasonable biogeographic scenario for the ziziphoid clade.

Likely scenarios of the inner ziziphoids’ biogeography across the fragments of Gondwana

The most robust and compelling interpretation supports the ancestral area for the common ancestor of all inner ziziphoids to be located in Africa and/or South America during the late Cretaceous (Fig. 2, App. 2). During this epoch, dispersal between Australia and South America was possible via Antarctica, while the African continent was already divided by a southern oceanic gap, and only accessible via eastern South America (Seton et al., 2012; Matthews et al., 2015). The opening of the sea between South America and Africa might be the reason for the establishment of two separate lineages of ziziphoid taxa: tribes Gouanieae and Paliureae (excluding *Sarcomphalus*) distributed in Africa on the one hand, and all other ziziphoid taxa distributed in the South American fragment of Gondwana on the other hand.

Our reconstructions date this split to the Late Cretaceous and suggest the African continent as vector for the Paliureae into Asia, where most of the diversity within this lineage is distributed nowadays. However, Chen et al. (2017) proposed an "out of India" hypothesis for *Paliurus*, claiming that *Paliurus* (and potentially the whole tribe) migrated with the Indian plate from Gondwana to Asia. This is additionally supported by the presence of a fossil closely related to extant *Paliurus* from the Paleogene/Cretaceous boundary in India. Yet, †*Archaeopaliurus*, also displaying close affinities to *Paliurus*, was found in northern South America. The latter, however, might be a distant remnant of a once wider (African and South American) distribution of a common ancestor in the ziziphoids (as potentially allowed by our reconstructions) or the result of an LDD event during the Palaeogene-Late Cretaceous. Furthermore, our analyses do not allow us to elucidate whether the fossil leading to the "out of India" hypothesis is an extinct basal lineage of

Paliureae (suggesting that the migration of the tribe could have been via Africa), or, if our data from India is simply to sparse to allow for drifting India to be reconstructed as area of origin for tribe Paliureae. Nonetheless, our reconstructions clearly illustrate that the extant pantropical distribution of tribe Gouanieae, sister to tribe Paliureae, is the result of Neogene LDD events rather than Gondwanan vicariance events.

The ancestors of all other ziziphoid lineages, however, prevailed on the southern Gondwanan fragment (Australia, Antarctica, South America) during the Paleogene. Only the distribution of *Lasiodiscus* was reconstructed as being the result of a dispersal event back into Africa. The subsequent break up of Australia and South America from Antarctica, and the consequent cooling of that continent most likely resulted in the isolation of lineages during the early Paleogene. . Two lineages, comprising *Ceanothus*, and *Sarcomphalus* and *Colubrina*, prevailed only in South America, and subsequently recolonised Africa and Australia, and dispersed into North America from the Oligocene onwards. Other lineages remained on the drifting Australia, and comprised Australian, Oceanic and Southeast Asian extant taxa, such as *Alphitonia*, *Emmenosperma*, *Granitites*, and *Jaffrea*, which will be more closely be investigated in a separate study (Hauenschild et al., *in prep*.). The Australian lineages during the Paleogene also comprised tribes Colletieae, Phyliceae, and Pomaderreae, as well as the *Schistocarpaea*. Later during the Neogene, LDD events of a common ancestor of tribes Colletieae and Phyliceae resulted in their current distribution predominantly in South America and Africa, respectively.

As shown above, our study clearly identified a strong influence of Gondwanan break-up events resulting in the evolution of different lineages, such as *Alphitonia* and its related genera, *Colubrina* and *Sarcomphalus*, Paliureae, and Pomaderreae. Hence, the biogeographic history of the ziziphoids (between 72.0 and 88.4 Ma) correlates with Late Cretaceous, Palaeogene and Early Eocene Gondwanan break-up events and the current distribution of the group was strongly affected by vicariance events. This finding is in line with the fossil record of the ziziphoid lineage: The oldest fossils attributed to the ziziphoids are sparse, but are from the Southern Hemisphere of the Cretaceous, such as *Paliurus* sp. and *Ziziphus* sp. from India, and †*Archeopaliurus* from South America (Correa et al., 2010; Singh, Prasad, Kumar, Rana & Singh, 2010; Chen et al., 2017). Younger fossils of the ziziphoids are, however, also distributed in the Northern hemisphere, such as multiple Eocene (and younger) taxa of *Ziziphus* and *Paliurus* (Burge & Manchester, 2008; Li et al., 2014), and the Late Miocene fossils of *Ceanothus* and *Distigouania* (Axelrod, 1985, 1995; Chambers & Poinar, 2014). This supports a subsequent dispersal of ziziphoids from South to North. The oldest fossils related to whole Rhamnaceae, however, are from the Northern Hemisphere, such as the Rose Creek flower from Cretaceous North America, or the leaf of "*Ziziphus*" sp. from northeastern Russia (Basinger & Dilcher, 1984; Spicer et al., 2002), indicating that Gondwana might be the origin of the ziziphoids, but not the origin of the entire family.

However, other tribes predominantly distributed in the Southern Hemisphere, such as Colletieae, Gouanieae, or Phyliceae, evolved following Oligocene and Neogene LDD events, and were not driven by vicariance. In line with multiple other studies (Nauheimer et al., 2012; Bechteler et al., 2017; Kayaalp, Stevens & Schwarz, 2017; Ye, Zhen, Zhou & Bu, 2017), our reconstructions highlight that the intuitive idea that a taxon with tribes distributed on the three Southern Hemisphere continents would necessarily be the result of vicariance might be wrong, even if the group was old enough. Support for Gondwanan vicariance as a biogeographic driver, however, is rare in taxa from the Late Cretaceous, but has also been documented (Luebert et al., 2017; Toussaint et al., 2017). In our study, the strongest evidence for vicariance events was found in the case of ancestors of Paliureae migrating on a Gondwanan fragment (Africa or potentially India) towards the Northern Hemisphere, where the tribe is most diverse nowadays. Another strong support for vicariance was found in the case of the common ancestors of *Colubrina* remaining on the Australian fragment, yet resulting in the pantropical genus *Colubrina* by subsequent LDD events. Consequently, many biogeographic studies investigating a possible impact of Gondwanan fragmentation processes might have been too focused on the “best fitting” groups, i.e. sister clades endemic to separate fragments of the former supercontinent. Instead, such studies should investigate widespread taxa that are old enough to have experienced parts of the Gondwanan break-up, prior to their dispersal into other parts of the world (Waters & Craw, 2006). Naturally, those processes are likely to be blurred by more recent events, as they are dating back to the Cretaceous (Waters & Craw, 2006). Hence, by focussing on “best fitting” groups which then turn out as being too young for Gondwana-driven vicariance, biogeographers sometimes might have jumped too quickly to the conclusion that LDD events dominated over vicariance, simultaneously underestimating the impact of vicariance. Rather, vicariance events in a taxon’s early history are as vital to its evolution as the subsequent dispersals and a taxon radiation in a secondary centre of diversification.

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Journal of Biogeography

SUPPORTING INFORMATION

The influence of the Gondwanan break-up on the biogeographic history of the ziziphoids (Rhamnaceae)

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Appendix 1

Fossils included for setting constraints in the molecular dating analysis.

Frangula alnus Mill. and *Frangula californica* (Eschsch.) A. Grey. – Both fossil taxa are attributed to extant species. While *Frangula alnus* is widely distributed in the northern hemisphere, *Frangula californica* is restricted to western North America. The fossils are described in various publications, and found in numerous sites in North America and Europe. Their age ranges from the Early Pliocene to the Quaternary, with the oldest records dating back to approximately 5 Ma ago (Axelrod, 1958; Menke, 1976; Gregor, 1977). Both species form species complexes, including for example *F. azorica* Grubov (*F. alnus* group) or *Frangula betulifolia* (Greene) Grubov (*F. californica* group). The fossil record includes fruits, parts of fruits, seeds and pollen. Although the fossils are attributed to a single species, they rather represent two separate lineages within *Frangula* Mill. We used these fossils to set a minimum age for the *Frangula* Mill. crown node using a lognormal distribution with an offset of 5 Ma, as both fossil taxa are sufficiently different from other lineages included in this clade, e.g. *Frangula crenata* (Siebold & Zucc.) Miq. and *Frangula rupestris* Schur. The *Frangula* crown has not yet been used as a calibration point in previous dated phylogenies.

Frangula hordwellensis Chandler – This is a fossil taxon dated to the Late Eocene from the Lower Headon Beds, England (Chandler, 1962; Gregor, 1977). The recovered seeds and pyrenes show affinities to extant *Frangula* taxa, and differ from *Rhamnus* L. especially by the longitudinal furrow present on the seeds, a trait still used to delimit *Frangula* from *Rhamnus sensu lato* (i.e. including *Atadinus* Raf. and

Endotropis Raf, see Hauenschild et al. 2016a, b). Besides *Frangula hordwellensis*, other fossil taxa attributable to *Frangula* were described from the Oligocene and Miocene, among them *Frangula solitaria* Gregor (Gregor, 1977). As those fossils cannot be distinguished from extant lineages of *Frangula*, we used them to calibrate the stem of *Frangula*, at the last common ancestor of *Frangula* and *Rhamnus*, using a lognormal function with an offset of 34 Ma (Eocene – Oligocene boundary). The *Frangula* stem has not been used as a calibration point in previous dated phylogenies. A possible reason is that *Frangula* appeared to be polyphyletic in earlier topologies ("*Rhamnus sphaerospermus*" ≡ *Frangula sphaerosperma* (Sw.) Kartesz & Gandhi was nested within *Rhamnus*). This finding was neither corroborated by our phylogenetic reconstructions, nor in previous ones (Bolmgren & Oxelman, 2004; Hauenschild, Salazar, Muellner-Riehl & Favre, 2016b, a).

Karwinskia californica Axelrod / *Karwinskia axamilpense* Velasco de Leon – These leaf fossils from the Eocene – Oligocene boundary (North America) and the middle to late Oligocene (North America), respectively, were attributed to the extant genus *Karwinskia* Zucc. However, the clear attribution of extant taxa to either *Karwinskia*, *Rhamnidium* Reissek, or *Auerodendron* Urb., can only be done when flowers and/or seeds are present. The fossilized leaves show affinities to the complex including *Auerodendron*, *Condalia* Cav., *Karwinskia*, *Krugiodendron* Urb., *Pseudoziziphus* Hauenschild, *Reynosia* Griseb., and *Rhamnidium*, and delimiting traits to *Berchemia*, *Frangula*, *Rhamnus*, and ziziphoid taxa. We therefore calibrated this Reynosia-Karwinskia complex using a lognormal function with an offset of 34 Ma (Eocene – Oligocene boundary). A *Karwinskia* fossil, "*Karwinskia paucicostata* (Reid & Reid, 1915), from the Netherlands was used by Onstein *et al.* (2015) to calibrate *Karwinskia* stem (1 accession). Yet, the authors did not give any justification for the use of this fossil. The fossil might be problematic, because this fossil, in our view, it lacks a clear attribution to the above presented complex, and was found in the Netherlands, quite distant from the extant distribution of *Karwinskia* (northern and central Americas).

Rhamnus salicifolius Lesq. ex Hayen and *Coahuilanthus belindae* Calvillo-Canadell & Cevallos-Ferriz – Both fossils are of Late Cretaceous origin from North America. *Rhamnus salicifolius* was already described in 1868, but a more recent revision included new fossil material (Peppe, Erickson & Hickey, 2007). Although the authors present seven informative characters present in all 130 examined fossils, historic literature, and show affinities to extant *Frangula* (Peppe *et al.* refer to "*Rhamnus*", yet they compare the fossil to *Frangula* species only), only, a few traits (if at all) seem to distinguish the fossil from *Berchemia* Neck. ex DC., *Sageretia* Brongn., and *Scutia* (Comm. ex DC) Brongn. Moreover, only leaf-margin traits delimit *R. salicifolius* from *Maesopsis eminii* Engl. As the latter represents a monotypic genus and tribe (Maesopsideae A. Weberb.), we can hardly speculate about extinct variation within this lineage. However, when the fossil taxon is compared to Ventilagineae Hook. f., and Gouanieae Reissek ex Endl., venation traits also separate *R. salicifolius*, Maesopsideae and Rhamneae from those tribes. Colletieae Reissek ex Endl., Paliureae Reissek ex Endl., Phyliceae Reissek ex Endl., and *Ceanothus* L. are clearly distinct from the trait combination in *Rhamnus salicifolius*. Hence, we place this fossil at the combined crown of Rhamneae and Maesopsideae and use its age as a lognormal function with an offset of 70 Ma. Additionally, this node constrain is supported by *Coahuilanthus belindae*, represented by Mexican fossilized flowers from the Campanian – Maastrichtian boundary (Calvillo-Canadell & Cevallos-Ferriz, 2007). The authors describe close similarities to extant members of tribes Rhamneae and "Zizipheae", namely *Rhamnus* (including *Frangula*), *Sageretia* (both Rhamneae), and *Berchemia* ("Zizipheae"). Although Zizipheae has been renamed Paliureae, *Berchemia* is no longer attributed to this tribe. It is attributed to Rhamneae, based on morphological and phylogenetic support. Thus, when corrected, the authors' opinion on the placement of *Coahuilanthus belindae* is near Rhamneae. We agree with this concept.

Archeopaliurus boyacensis Correa, Manchester, Jaramillo & Gutierrez and *Palirus* sp. (Chen et al., 2017) – *Archeopaliurus* is represented by a single fossilized fruit from the Late Maastrichtian in Colombia, and shows undoubtfully affinities with the fruits of extant *Paliurus* species, except for its larger size (Correa, Jaramillo, Manchester & Gutierrez, 2010).Yet, this age for the stem of Paliurus is additionally supported by another fossil described as *Paliurus* sp. from India (Chen et al., 2017). We used both fossils to set a log normal prior with an offset of 66 Ma to the most recent common ancestor of all extant species of *Paliurus* and *Ziziphus* s.s..

Ceanothus leichti Axelrod and *Ceanothus precuneatus* Axelrod – Both leaves from the mid to late Miocene of North America show close affinities to *Ceanothus* (Axelrod, 1958, 1985, 1995). In fact, both fossil taxa were attributed to extant taxa (which might be questionable since the fossils are represented by leaves only). However, as also concluded by Onstein *et al.* (2015), attribution to a subgenus of *Ceanothus* (and thus distinction from another) is clearly supported. Hence, we calibrated the crown of *Ceanothus* using a lognormal function with an offset of 12 Ma ago. Onstein *et al.* (2015) used a more offensive calibration (compared to our conservative setup): They used *C. precuneatus* to calibrate the crown of *Ceanothus*, and thus *C. leichti* (Serravallian – Tortonian boundary) to calibrate the crown of *C.* subg. *Ceanothus*. However, the age of *C. precuneatus* (16 to 20 Ma old) is not sufficiently documented, and might be overestimated.

Colubrina spiraeafolia (Lesquereux) MacGinitie – These fossilized leaves were dated back to the Late Eocene of Northern America, and were attributed to *Colubrina* Rich. ex Brongn. by MacGinitie (1953) and reviewed by Manchester (2001). The fossil record is completed by younger fossil taxa attributed to *Colubrina*, e.g. *Colubrina lanceolata* Axelrod (MacGinitie, 1953; Axelrod, 1958; Manchester, 2001) from the early Miocene. A previous study used the fossil to calibrate the crown of *Colubrina*, as it “shows similarities with two extant species *C. arbora* and *C. glomerata*” (Onstein *et al.* 2015). Both given species are heterotypic synonyms of *C. triflora* Brongn. ex G.Don. We agree on the similarities between *C. triflora* and the fossil leaves of *C. spiraeafolia*. Additionally the delimitation of *Colubrina* leaves towards rhamnoid leaf-forms, other ziziphoid leaf-types, e.g. in *Ziziphus*, *Paliurus*, and *Ceanothus*, is quite distinct. Yet, similarities to one species are not sufficient evidence to calibrate the crown group the species belongs to. Furthermore, *C. triflora* is not included in our data set. We use *C. spiraeafolia* to calibrate the minimum age of the *Colubrina* crown, yet with a slightly different justification. *Colubrina* comprises two morphologically well supported subgenera (*C.* subg. *Colubrina* and *C.* subg. *Serrataria* M.C. Johnst.) which are both represented in our data set. Major traits to delimit both subgenera are leaf margin traits, allowing to attribute *C. spiraeafolia* to *C.* subg. *Colubrina*. This is additionally supported by the younger *C. lanceolata* which may be attributed to *C.* subg. *Colubrina*, based on the same justification (MacGinitie, 1953; Axelrod, 1958; Johnston, 1971).

Maximum tree height – We used fossils with affinities to Rhamnaceae, such as the Rose Creek Flower (Basinger & Dilcher, 1984; Onstein, Carter, Xing, Richardson & Linder, 2015), from the Cenomanian of North America, and *Ziziphus* sp. from the Albian of North East Russia only indirectly. They are the oldest fossils, and the fossil record of the Aptian epoch does not include fossils with closer affinities to Rhamnaceae. Hence, we used the Albian-Aptian boundary (113 Ma) as hard bound maximum age for our temporal reconstruction (truncated), and the Cenomanian-Albian boundary (approximately 100 Ma), where the fossil record still includes fossils with affinities to buckthorns, as soft maximum (lognormal prior to the stem, truncated to 113 Ma).

To Fig. 1. Approximate distribution ranges (excluding introduced ranges) of all recognized Rhamnaceae taxa, including tribes (if monophyletic), genera (if not attributed to a tribe or genera incertae sedis), or lower rank (*Ziziphus pubescens* & *Z. robertsoniana*). The distribution data was collected from literature (Grubov, 1949; Johnston, 1971, 1973, 1974; Grey-Wilson, 1978; Johnston & Johnston, 1978; Moe, 1984; Tortosa, 1992; Schirarend & Olabi, 1994; Press & Short, 1997; Aagesen, 1999; Richardson, Fay, Cronk & Chase, 2000; Medan & Schirarend, 2004; Ladiges, Kellermann, Nelson, Humphries & Udovicic, 2005; Zhao & Zhao, 2006; Ellis, Weekley & Menges, 2007; Kellermann, Rye & Thiele, 2007; Burge & Manchester, 2008; Chen & Shirarend, 2008; Meier & Berry, 2008; Buerki, Phillipson & Callmander, 2011; Burge et al., 2011; Pool, 2014; Hauenschild, Matuszak, Muellner-Riehl & Favre, 2016). The map was downloaded from WORLDCLIM (Hijmans & al., 2005), and modified manually.

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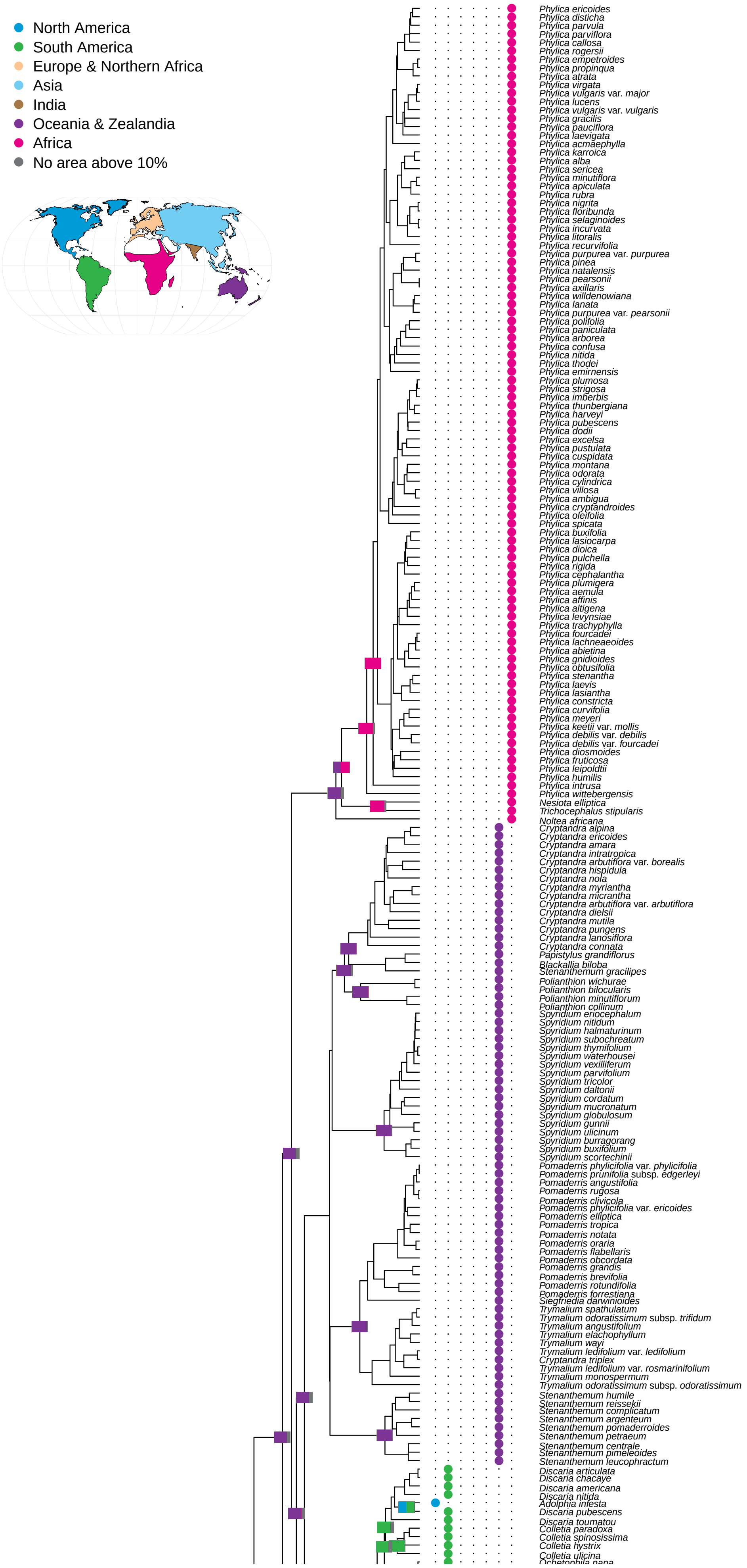
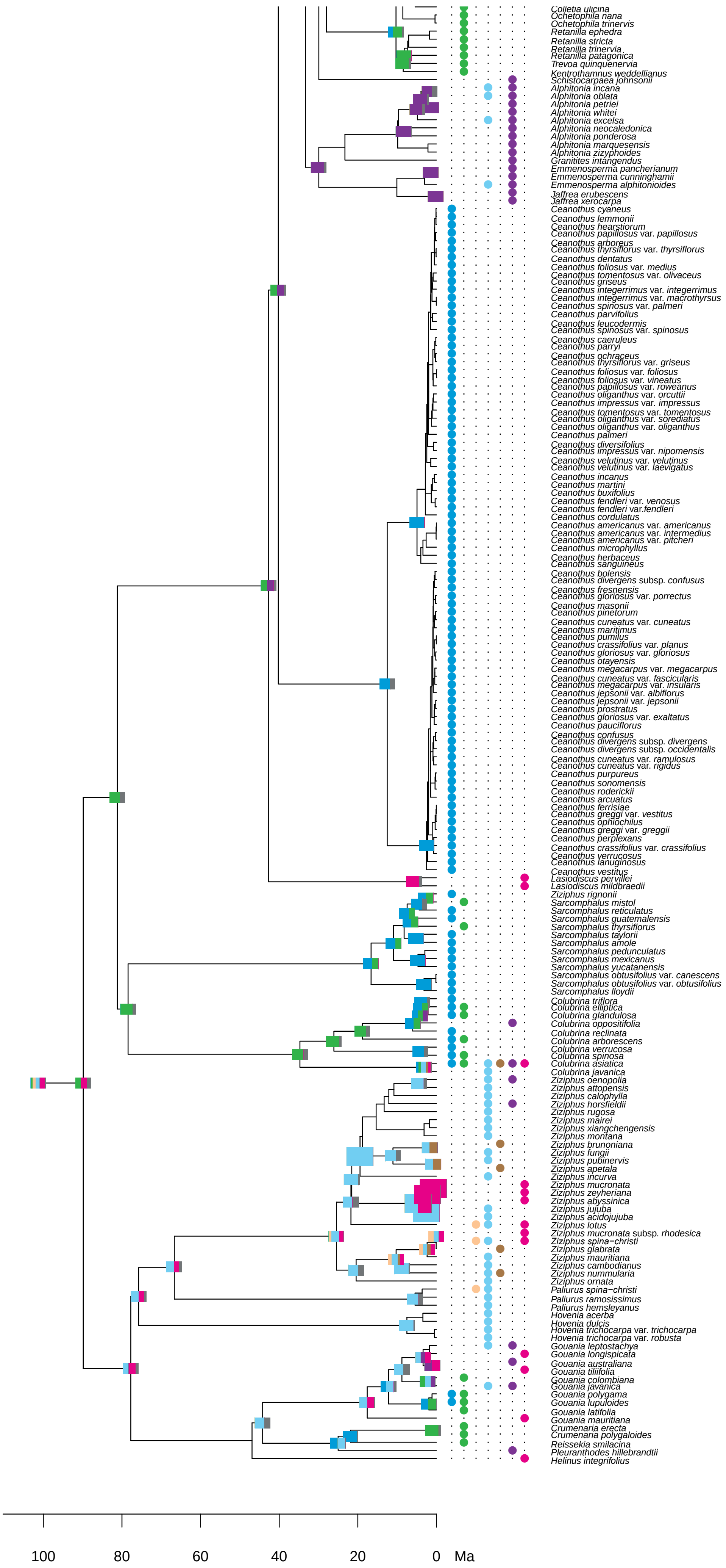
SUPPORTING INFORMATION

The influence of the Gondwanan break-up on the biogeographic history of the ziziphoids (Rhamnaceae)

Frank Hauenschild, Adrien Favre, Ingo Michalak, Alexandra N. Muellner-Riehl

Appendix 2




Colletia ulicina
Ochetophila nana
Ochetophila trinervis
Retanilla ephedra
Retanilla stricta
Retanilla trinervia
Retanilla patagonica
Trevoa quinquenervia
Kentrothamnus weddellianus
Schistocarpaea johnsonii
Alphitonia incana
Alphitonia oblata
Alphitonia petriei
Alphitonia whitei
Alphitonia excelsa
Alphitonia neocaledonica
Alphitonia ponderosa
Alphitonia marquesensis
Alphitonia zizyphoides
Granitites intangendus
Emmenosperma pancherianum
Emmenosperma cunninghamii
Emmenosperma alphitonioides
Jaffrea erubescens
Jaffrea xerocarpa
Ceanothus cyaneus
Ceanothus lemmonii
Ceanothus hearstiorum
Ceanothus papillosus var. papillosus
Ceanothus arboreus
Ceanothus thyrsiflorus var. thyrsiflorus
Ceanothus dentatus
Ceanothus foliosus var. medius
Ceanothus tomentosus var. olivaceus
Ceanothus griseus
Ceanothus integerrimus var. integerrimus
Ceanothus integerrimus var. macrothyrsus
Ceanothus spinosus var. palmeri
Ceanothus parvifolius
Ceanothus leucodermis
Ceanothus spinosus var. spinosus
Ceanothus caeruleus
Ceanothus parryi
Ceanothus ochraceus
Ceanothus thyrsiflorus var. griseus
Ceanothus foliosus var. foliosus
Ceanothus foliosus var. vineatus
Ceanothus papillosus var. roweanus
Ceanothus oliganthus var. orcuttii
Ceanothus impressus var. impressus
Ceanothus tomentosus var. tomentosus
Ceanothus oliganthus var. sorediatus
Ceanothus oliganthus var. oliganthus
Ceanothus palmeri
Ceanothus diversifolius
Ceanothus impressus var. nipomensis
Ceanothus velutinus var. velutinus
Ceanothus velutinus var. laevigatus
Ceanothus incanus
Ceanothus martini
Ceanothus buxifolius
Ceanothus fendleri var. venosus
Ceanothus fendleri var.fendleri
Ceanothus cordulatus
Ceanothus americanus var. americanus
Ceanothus americanus var. intermedius
Ceanothus americanus var. pitcheri
Ceanothus microphyllus
Ceanothus herbaceus
Ceanothus sanguineus
Ceanothus bolensis
Ceanothus divergens subsp. confusus
Ceanothus fresnensis
Ceanothus gloriosus var. porrectus
Ceanothus masonii
Ceanothus pinetorum
Ceanothus cuneatus var. cuneatus
Ceanothus maritimus
Ceanothus pumilus
Ceanothus crassifolius var. planus
Ceanothus gloriosus var. gloriosus
Ceanothus otayensis
Ceanothus megacarpus var. megacarpus
Ceanothus cuneatus var. fascicularis
Ceanothus megacarpus var. insularis
Ceanothus jepsonii var. albiflorus
Ceanothus jepsonii var. jepsonii
Ceanothus prostratus
Ceanothus gloriosus var. exaltatus
Ceanothus pauciflorus
Ceanothus confusus
Ceanothus divergens subsp. divergens
Ceanothus divergens subsp. occidentalis
Ceanothus cuneatus var. ramulosus
Ceanothus cuneatus var. rigidus
Ceanothus purpureus
Ceanothus sonomensis
Ceanothus roderickii
Ceanothus arcuatus
Ceanothus ferrisiae
Ceanothus greggi var. vestitus
Ceanothus ophiochilus
Ceanothus greggi var. greggii
Ceanothus perplexans
Ceanothus crassifolius var. crassifolius
Ceanothus verrucosus
Ceanothus lanuginosus
Ceanothus vestitus
Lasiodiscus pervillei
Lasiodiscus mildbraedii
Ziziphus rignonii
Sarcomphalus mistol
Sarcomphalus reticulatus
Sarcomphalus guatemalensis
Sarcomphalus thyrsiflorus
Sarcomphalus taylorii
Sarcomphalus amole
Sarcomphalus pedunculatus
Sarcomphalus mexicanus
Sarcomphalus yucatanensis
Sarcomphalus obtusifolius var. canescens
Sarcomphalus obtusifolius var. obtusifolius
Sarcomphalus lloydii
Colubrina triflora
Colubrina elliptica
Colubrina glandulosa
Colubrina oppositifolia
Colubrina reclinata
Colubrina arborescens
Colubrina verrucosa
Colubrina spinosa
Colubrina asiatica
Colubrina javanica
Ziziphus oenopolia
Ziziphus attopensis
Ziziphus calophylla
Ziziphus horsfieldii
Ziziphus rugosa
Ziziphus mairei
Ziziphus xiangchengensis
Ziziphus montana
Ziziphus brunoniana
Ziziphus fungii
Ziziphus pubinervis
Ziziphus apetala
Ziziphus incurva
Ziziphus mucronata
Ziziphus zeyheriana
Ziziphus abyssinica
Ziziphus jujuba
Ziziphus acidojujuba
Ziziphus lotus
Ziziphus mucronata subsp. rhodesica
Ziziphus spina−christi
Ziziphus glabrata
Ziziphus mauritiana
Ziziphus cambodianus
Ziziphus nummularia
Ziziphus ornata
Paliurus spina−christi
Paliurus ramosissimus
Paliurus hemsleyanus
Hovenia acerba
Hovenia dulcis
Hovenia trichocarpa var. trichocarpa
Hovenia trichocarpa var. robusta
Gouania leptostachya
Gouania longispicata
Gouania australiana
Gouania tiliifolia
Gouania colombiana
Gouania javanica
Gouania polygama
Gouania lupuloides
Gouania latifolia
Gouania mauritiana
Crumenaria erecta
Crumenaria polygaloides
Reissekia smilacina
Pleuranthodes hillebrandtii
Helinus integrifolius
100
80
60
40
20
0
Ma

SUPPLEMENTS

Suppl. 1. Chronogram used for the biogeographic analyses. Diamonds indicate calibrated constraints (App. 1). Posterior probabilities are given above branches; the frames of the 95% confidence interval (height) are given next to the corresponding nodes.

Suppl. 2. List of all sequences used after the initial reduction in our study. Taxa and Genbank accession numbers are given. Accession numbers separated by comma refer to the same marker.

Suppl. 3. List of area reconstructions per node. Cumulative probability of an area is given per node. To infer the position of a node, please refer to Suppl. 4.

Suppl. 4. Phylogenetic tree with node numbers plotted.

Suppl. 5. Phylogenetic reconstruction based on the alignment used for molecular dating analyses. No topologic constraints were set. The reconstruction is based on the Fasttree-like algorithm as implemented in RAxML8 (Stamatakis, 2014). Shimodaira-Hasegawa support values are given next to the corresponding nodes.

Chapter 4: Biogeographic analyses support an Australian origin for the Indomalesian-Australasian wet forest-adapted tropical tree and shrub genus Alphitonia and its close allies (Rhamnaceae)

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Biogeographic analyses support an Australian origin for the Indomalesian-Australasian wet forest-adapted tropical tree and shrub genus *Alphitonia* and its close allies (Rhamnaceae)

Biogeography of *Alphitonia*

Accepted at Botanical Journal of the Linnean Society

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Abstract

The “out of Australia” hypothesis describes taxa with ancestors present on the Australian continent before the contact of the Australian plate with Southeast Asia and the subsequent biological interchange between these regions across Wallacea. Our study supports the “out of Australia” hypothesis for the tropical tree genus *Alphitonia*, and its most closely related genera. A common ancestor of the entire clade inhabited Australia in the Miocene, while westward dispersal into Wallacea, the Philippines and the Asian continent was reconstructed to be of Quaternary origin. Furthermore, our study supports the currently applied taxonomy within *Alphitonia* and related genera, although it highlights potentially underestimated diversity. Finally, our study highlights the need for further investigations within *Alphitonia* and related genera and for including fossil-rich related taxa. The need for these investigations also arises from a potential human-mediated dispersal of *Alphitonia* species in Polynesia, which cannot be clearly proven by our approach.

Key words: *Alphitonia*, BioGeoBEARS, *Emmenosperma*, *Granitites*, ITS, *Jaffrea*, molecular dating, Rhamnaceae, *rbcL*, *trnL-trnF*.

Introduction

The Indo-Australian Archipelago (IAA) and the neighboring Pacific regions harbor organisms that have some of the most complex biogeographic histories on Earth. This is mainly because of the intricated distribution of land and sea (Lohman *et al.*, 2011). First of all, among other particularities, biotas on any Pacific island are the result of multiple long-distance dispersal events, resulting in an amalgamation of elements of heterogeneous origins, e.g., Asian, Australian, American, or even Polynesian (Crayn *et al.*, 2015; Taylor & Kumar, 2016; Whittaker *et al.*, 2016). Then, the convergence of the Asian and Australian tectonic plates since the Miocene (Hall, 2009; Seton *et al.*, 2012; Hall, 2017) has led to the formation of the Wallacean transition zone between the Sunda and the Sahul shelves, allowing some taxa to disperse from either side to the other, thus shaping an area characterized by a particularly high biodiversity (Richardson *et al.*, 2012). It has been shown that overall wet forest-adapted Asian taxa expanded their range preferably from West to East into the Wallacean transition zone (Richardson *et al.*, 2012; Richardson et al., 2014; Matuszak *et al.*, 2016), and even beyond to New Guinea and Australia (Muellner *et al.*, 2008; Muellner *et al.*, 2009; Nauheimer *et al.*, 2012; Thomas *et al.*, 2012; Grudinski *et al.*, 2014; Favre *et al.*, 2016; Matuszak *et al.*, 2016; Mezger & Moreau, 2016), whereas overall dry forest-adapted taxa would have found it more difficult to disperse from Australasia westwards (Cruaud *et al.*, 2011; Richardson *et al.*, 2012; Chen *et al.*, 2014; Johnson *et al.*, 2017; Jønsson *et al.*, 2017). The latter dispersal pattern, from Australia into the IAA and/or eastwards into Polynesia, can be seen as contrast to the “out of India” hypothesis (Karanth, 2006; Chen *et al.*, 2017). The “out of India” hypothesis refers to Asian lineages with ancestral areas in Paleocene India that used the Indian continent as a raft from Gondwana to Laurasia. Like India, Australia provided a raft for multiple Gondwanan taxa northwards (Weston & Jordan, 2017), and those lineages that subsequently and successfully dispersed into other continents may be best viewed in line with the “out of Australia” hypothesis, as done for Halograceae R. Br. by Chen et al. (2014). Unlike the earlier Indian raft (with a timing of the India-Eurasia collision at ca. 55 +/-10 Ma; Eocene;Karanth, 2006, and references therein; Hall, 2009; Deng & Ding, 2016), no physical terrestrial contact was made between the Australian raft and the Eurasian plate. An approximation of both landmasses was established only at ca. 20 to 10 Ma (Early to Late Miocene), hence the temporal criterion on the most recent common ancestor (mrca) to be of Australian origin would be an endemic distribution in Australia prior to the close approximation of both landmasses, i.e., in the Oligocene to Mid Miocene. In addition to the increased possibility of biotic exchange aided by the physical approximation of both landmasses, anoverall preference for drieer or wetter habitats has been suggested as important factor for dispersal asymmetries for multiple plant lineages(Richardson et al., 2012, and references therein). Corridors and tracks for wet forest-adapted and dry forest-adapted plants were not evenly accessible throughout the Neogene and Quaternary. Especially during the Quaternary, Sundania acted as a source area for broad eastward dispersal for multiple montane and lowland taxa into Australasia, westward dispersal was dominant via Steenis' Mountain Flora Tracks for montane taxa, and limited for wet forest-adapted lowland taxa from Australia via southern New Guinea into Southeast Asia (Richardson *et al.*, 2012). Taking a look at biogeographic analyses on a variety of organismic groups occurring at both ends of the IAA (and in between), it appears that the "out of Australia" hypothesis has been less often postulated than a general origin or intermediate presence of an mrca on the Sahul shelf, independent if the lineage originated in Gondwana/pre-contact Australia, the Pacific islands, or if its ancestors dispersed from Asia into Sahul in the course of a previous colonization event. Furthermore, east of the IAA, only a tiny fraction of the tremendous diversity occurring in Micronesia and Polynesia has been thoroughly investigated biogeographically to date. In this study, we will investigate the spatio-temporal evolution of the overall wet forest-adapted tropical tree and shrub genus *Alphitonia* Reissek ex. Endl. and its related genera, taxonomically placed within the ziziphoid Rhamnaceae (Braid, 1925; Suessenguth, 1952; Richardson *et al.*, 2000b;

Bean, 2010). This group is an interesting and suitable test model for biogeographic investigations in this part of the world, as it comprises widespread species in the West and East of its entire distribution, with distributional ranges from Southeast Asia into Australia, and throughout Polynesia, respectively. Apart from these widespread species, narrow endemics in Southwest Australia, Queensland, New Caledonia and Hawaii can also be observed.

As pointed out by Hopkins et al. (2015) and earlier studies, taxonomic uncertainties persist with regard to the delineation of *Alphitonia* and related genera on species level. Originally, *Alphitonia* was raised to generic rank in 1840 by the exclusion of *Alphitonia excelsa* (Fenzl) Reissek ex Benth. from *Colubrina* Rich. ex. Brongn. (Endlicher, 1840), and it included up to 23 species and two sections in the following years. These sections were *A.* sect. *Alphitonia* (≡ *A.* sect. *Tomentosae* Braid, nom. inval.), and *A.* sect. *Glabratae* Braid). However, the genus was recently split into several entities (Hopkins *et al.*, 2015), and currently, *Alphitonia* has three related genera: *Emmenosperma* F. Muell., *Granitites* Rye, and *Jaffrea* H.C. Hopkins & Pillon (see Table 1). The latter genus represents the former *A.* sect. *Glabratae*. *Alphitonia* and *Emmenosperma* are widespread across Southeast Asia and Australia (excluding Tasmania, and Victoria), expanding their range into the Pacific islands (Braid, 1925; Schirarend, 1995; Fay *et al.*, 2001; Chen & Schirarend, 2008; Bean, 2010; Hopkins *et al.*, 2015;). In contrast, *Granitites* and *Jaffrea* have a narrower distribution range, and include local endemics in Southwestern Australia, and New Caledonia. Although *Granitites* and *Jaffrea* include well-circumscribed species, taxonomic delineation within *Alphitonia* remains unsatisfactory, reflected by very contrasting species concepts (Bean, 2010). Basically, two schools co-exist with regard to the species-level taxonomy within *Alphitonia*. One school supports widespread species, for example, spreading from Australia to Malaysia, or across distant Pacific islands, whereas the other one proposes narrow endemic species within the formerly widespread species instead, for example with distribution ranges restricted to just one or a few neighboring islands in the Pacific. A succession of authors, including Braid (1925), followed by Bean (2010) and Hopkins et al. (2015), revised *Alphitonia* and its related genera, usually focusing on either a group of species or a geographical region. Their studies sum up to a total of 13 to 15 species for *Alphitonia*, some of which appear relatively well circumscribed, including: *A. macrocarpa* Mansf., *A. neocaledonica* (Schltr.) Guillaumin, *A. oblata* Bean, *A. petriei* Braid & C.T. White, *A. pomaderroides* (Fenzl) A.R. Bean, and *A. whitei* Braid. Yet, some issues still persist within the distribution range of *Alphitonia*, particularly in the IAA and the Pacific islands (see a complete taxonomic report in Appendix 3). For example, it remains unknown whether the latter region might contain one widespread species (*A. zizyphoides* A. Gray) or, alternatively, multiple local taxa (*A. marquesensis* F. Br., *A. franguloide*s A. Gray, *A. ponderosa* Hillebr.).

Table 1. List of species numbers described in the four closely genera, *Alphitonia*, *Emmenosperma*, *Granitites* , and *Jaffrea*, and how many species names are currently accepted (Bean, 2010; Braid, 1925; Hopkins *et al.*, 2015; Suessenguth, 1952), unresolved, and rejected (either as synonyms, or because they have been transferred to other genera; nom. illeg. are not included). Last, the table shows how many species names we included in our study.

Genus	**Species**				
	described	**accepted**	**unresolved**	**rejected**	**included**
Alphitonia Reissek ex. Endl.	22	15	2	7	12
Emmenosperma F. Muell.	5	5	0	0	4
Granitites Rye	1	1	0	0	1
Jaffrea H.C. Hopkins & Pillon	2	2	0	0	2

As for many other taxa in Rhamnaceae, straightforward diagnostic morphological traits are relatively scarce. Hence, the use of molecular tools is a necessary complement to morphological investigations, and has already provided crucial insights in some studies dealing with the taxonomy of this family (Richardson *et al.*, 2000a ; Fay *et al.*, 2001; Aagesen *et al.*, 2005; Kellermann *et al.*, 2005a; Kellermann *et al.*, 2005b; Kellermann *et al.*, 2007; Hauenschild *et al.*, 2016a; Hauenschild *et al.*, 2016b; Hauenschild *et al.*, 2016c). For this reason, our study will firstly attempt to gather a better understanding of phylogenetic relationships among species of *Alphitonia*, *Emmenosperma*, *Granitites*, and *Jaffrea*. We used samples from five herbaria, including specimens from 16 accepted species (plus accessions of three heterotypic synonyms), representing the entire distribution range of these genera. By doing so, we wanted to compare the currently accepted taxonomy with the patterns found in the molecular phylogeny, and test whether different accessions (preferably from different geographic areas) of the same morphological entity (species) would cluster together. Secondly, we reduced conspecific genetic clusters to one terminal and estimated the approximate age as well as the spatio-temporal history of *Alphitonia* and closely related genera. Ultimately, we aimed to test whether the "out of Australia" hypothesis was supported for *Alphitonia* and related genera.

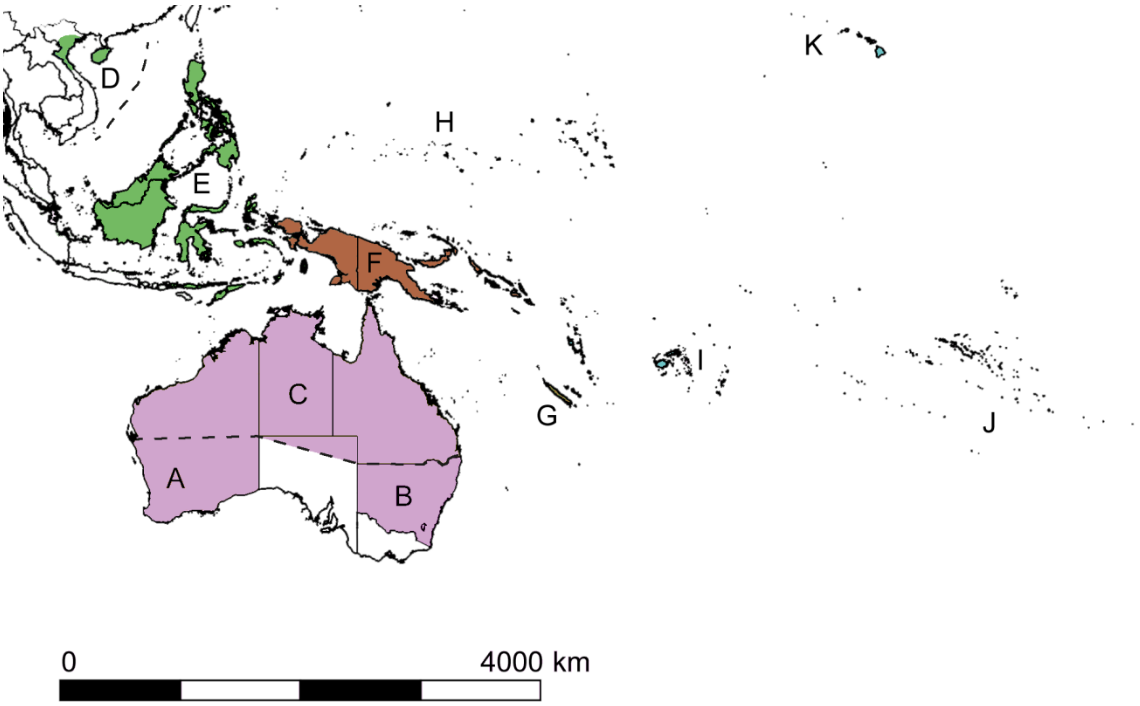

Figure 1. Areas used for the ancestral area reconstructions. Australia was subdivided into three parts: the Southwest (A), the temperate Southeast (B), and the North (C). Southeast Asia was subdivided into two parts: Mainland and Hainan (D), and Malesia and the Philippines (E). New Guinea and the Solomon Islands (F). New Caledonia (G). The Pacific islands region is dived into four parts: Micronesia, Palau, and adjacent Islands (H), western Polynesia (I), eastern Polynesia (J), and Hawaii and the Marshall Islands (K).

Material and Methods

Taxon sampling, DNA extraction, amplification and sequencing — We obtained plant material from 18 vouchers (Appendix 1) from the herbaria of ATH, CANB, MEL, NSW, and L. We extracted total genomic DNA with the NucleoSpin Plant II Kit (Macherey-Nagel, Dueren, Germany) as advised by the manufacturer's protocol. We then amplified three DNA markers, the nuclear ribosomal internal transcribed spacer region (ITS), the ribulose-1,5-bisphosphate carboxylase/oxygenase gene (partial, *rbcLa*), and the *trnL-trnF* spacer region, following the protocols published by Grudinski et al. (2014), Levin et al. (2003), and Taberlet (1991), respectively. We purified the PCR products with the NucleoSpin® Gel and PCR clean up kit (Macherey-Nagel GmbH & Co. KG, Dueren, Germany). Sequencing was performed on an ABI 3730xl capillary sequencer (Thermo Fisher Scientific, Waltham, U.S.A.). Our sequences were then combined with all existing sequences published on GenBank for the three markers of *Alphitonia* and its related genera, comprising *Alphitonia* s.s., *Granitites*, *Emmenosperma* and *Jaffrea*. All newly generated sequences were deposited in GenBank (MF787296 to MF787367, Appendix 1). Our final data set comprised 55 accessions (Appendix 1) of four genera, and 19 species, respectively.

Phylogenetic analyses — We used a two-step approach to reconstruct a phylogenetic hypothesis for *Alphitonia* and its related genera (1) All sequences were aligned using the MUSCLE alignment algorithm as implemented in Geneious 6.1. (Kearse *et al.*, 2012), and corrected by hand. We used maximum likelihood in raxmlGUI (Silvestro & Michalak, 2012; Stamatakis, 2014), and Bayesian analyses in MrBayes 3.2.2 (Ronquist *et al.*, 2012) to reconstruct phylogenetic hypotheses, based on a nuclear, a plastid (RAxML, Appendix 2), and a concatenated DNA data set (three partitions), to test for incongruencies (RAxML, MrBayes, see gene trees in Appendix 2). We used GTR+Γ as substitution model in all analyses to ensure comparability among raxmlGUI, MrBayes, and BEAST analyses. In raxmlGUI, we used the rapid bootstrap approach (with autoMRE); in MrBayes, we set four runs with four chains each and 30 million generations. Burn-in was set to 25%, after ESS values as given by Tracer 1.6 (Rambaut *et al.*, 2014) were stable and above 200 for all parameters. The topologies of all analyses were rooted according to previous studies (Richardson *et al.*, 2000a; Onstein *et al.*, 2015; Hauenschild *et al.*, 2016a), i.e., separating *Alphitonia* and *Granitites* from *Emmenosperma* and *Jaffrea*. These phylogenies place *Alphitonia* and its related genera within the ziziphoids, aside the tribes Colletieae, Gouanieae, Paliureae, Phyliceae, and Pomaderreae. The phylogenetic relation between the lineages (*Alphitonia* and *Granitites* versus *Jaffrea* and *Emmenosperma*) remains unsupported. Hence, one half of our data served as outgroup for the other half. Furthermore, we used this approach to identify potentially misidentified specimens on GenBank, for which no vouchers were accessible to us. Throughout the manuscript, we will treat support values as follows: Posterior probabilities below 0.90 and bootstrap values below 75 are considered as not supported. Posterior probabilities above 0.98 and bootstrap values above 90 are treated as strongly supported.

(2) In a second step, we reduced the concatenated alignment to one accession per genetic cluster by using consensus sequences of all valid accessions, and used this smaller alignment as input matrix for performing a molecular dating analysis. Based on the phylogenetic reconstructions obtained in the previous step (1), we generated consensus sequences for all valid species (corresponding to genetic clusters). As Bean (2010) had highlighted that *Alphitonia oblata* and *A. incana* might be conspecific in Australia, we performed a phylogenetic analysis on raxml8 (Appendix 2G) to infer if this still applies when individuals outside of Australia are taken into account. We combined our accessions of both species for the reduced dataset, as we did not find genetic differences between them. Furthermore, we combined *A. zizyphoides* 3 (Appendix 1) and *A. marquesensis*, because this *A. zizyphoides* voucher was from the Marquesas, and genetically more similar to *A. marquesensis*

than to the other accessions (1 & 2) of *A. zizyphoides* (Appendix 2 A-D). The specimens of *A. marquesensis*, which we re-determined and sequenced for our study (1 & 2), did not cluster with the Marquesas-complex from previous studies in our phylogeny (*A. ziziphoides* 3 and *A. marquesensis* 3, Appendix 2), yet we considered this to be a phylogenetic reconstruction bias, based on the fact that two accessions were only represented by *rbcL*, while the other two lacked *rbcL*. We therefore combined all *A. marquesensis* sequences (including *A. ziziphoides* 3 = *A. marquesensis*) into a consensus sequence, which better depicted taxonomic similarities within this group. Additionally, we kept the heterotypic synonym of *A. excelsa*, *A. philippinensis*, in our analysis, as both were reconstructed as separate genetic entities (Appendix 2 A-D). We excluded *Alphitonia* sp. 1 and sp. 2 from our analyses, as they were only represented by short fragments of the *trnL-trnF* region, which were possibly not sufficient to ascertain their phylogenetic position, although some affinities to *A. oblata* could be hypothesized (Appendix 2 D). We also excluded *A. excelsa* 6 (potential sequencing errors: unique substitutions to thymine in the *rbcL* sequence) and *Emmenosperma alphitonioides* 2 (GenBank accession), which was most likely a misdetermination of *E. cunninghamii*, as they clustered together in a strongly supported clade. As there are no attributable macrofossils available in *Alphitonia* and related genera, we used two sets of fossil evidence: The continuous presence of fossils with affinities to *Emmenosperma* before the Miocene-Oligocene boundary (23 Ma) in Australia might represent a minimum age estimate for the mrca of the group. In the fossil records of the Australian mid-Chattian (approximately 25 Ma), Rhamnaceae-type pollen seems entirely absent, which might also represent the potential absence of *Alphitonia* in Australia (Hill, 1994). Hence, we set a normal prior at the stem node of both, *Alphitonia* and *Emmenosperma*, at the Miocene-Oligocene boundary, and added a variance to the mid-Chattian to account for the absence of fossils (23 ±2 Ma). These ages are younger than the reconstructed mean ages of previous studies dating Rhamnaceae as a whole (Onstein *et al.*, 2015; Onstein & Linder, 2016). We used Beast 1.8.4. to reconstruct the node ages (Drummond & Rambaut, 2007) in six runs with 10 million generations each. We used the birth-death tree prior and a relaxed lognormal clock after testing and rejecting a strict clock behavior. Furthermore, we reconstructed a chronogram based only on prior data (Appendix 2 E).

Biogeographic analyses — We used the package BioGeoBEARS (Matzke, 2013) in R to perform DEC and DIVA-like analyses. Eleven areas were defined as shown in Fig. 1: Australia (southwestern, tropical and subtropical northern Australia, and temperate southeastern Australia); Southeast Asia (Vietnam and Hainan, and Malesia, including Borneo, Java, the lesser Sunda Islands, the Moluccas, and Sulawesi, and the Philippines); New Guinea and the Solomon Islands, New Caledonia, and the Pacific (Micronesia, West Polynesia (excluding Cook Islands and eastwards), East Polynesia (Cook Islands and eastwards), and Hawaii and Marshall Islands). We modified our dispersal multipliers according to the distance of two areas, rounded to integer numbers. To delineate areas, we referred to the currently known distribution of species: For example, there is not a single species in *Alphitonia* and related genera occurring in Sulawesi that would not also occur on Borneo and/or the Philippines – and vice versa. Therefore, we decided to lump these islands into one area, despite the fact that Wallace´s line (e.g., including the Makassar Strait) may be regarded as a strong barrier to dispersal for other organisms, such as Arecaceae Bercht. & J. Presl (Bacon *et al.*, 2013). The best-fitting model was tested by the AIC criterion, and DEC (including founder effect) was selected.

Results

Phylogenetic analyses – Our alignment of 55 accessions had 3284 positions, divided into three partitions: ITS (848 bp), *rbcL* (1408), and *trnL-trnF* (1028). We did not detect any incongruencies among the different gene trees and the analysis of the concatenated data set. (see Appendix 2). All four genera (*Alphitonia*, *Granitites*, *Emmenosperma* and *Jaffrea*) were reconstructed as monophyletic, yet not all of them were supported in each analysis (Appendix 2 A and B: *Alphitonia*: pp 0.69 / BS: 52, *Granitites*: pp: 0.83 / BS: 65, *Emmenosperma*: pp: 0.99 / BS: 84, and *Jaffrea*: pp: 0.99 / BS: 75). Only the ITS analysis supported *Alphitonia* s.s. (Appendix 2 C: BS: 85). Our phylogenetic analyses on the conspecific pattern of *A. incana* and *A. oblata* show a clustering of both sequences and no substitutions between them (Appendix 2 G).

The reduced alignment comprised 17 accessions. The phylogenetic analyses based on the reduced data set reconstructed all four genera as monophyletic (Fig. 1; *Alphitonia*: pp: 1.00 / BS: 87; *Emmenosperma*: pp: 1.00 / BS: 100; *Granitites*: monotypic, and *Jaffrea*: pp: 1.00 / BS: 100). Within *Alphitonia*, we reconstructed three supported lineages: The first comprised only *A. whitei* and thus was sister to a clade comprising all other *Alphitonia* species (pp: 1.00 / BS: 97). Within the latter clade, two sister lineages were reconstructed, the first comprising *A. ziziphoides* and *A. marquesensis* (pp: 1.00 / BS: 100), and the second comprising all remaining species of *Alphitonia* (pp: 1.00 / BS: 89). This clade lacked internal support, concerning the position of *A. ponderosa* and *A. neocaledonica* in relation to a clade comprising the remaining species of *Alphitonia*. Within the latter group, we observed a clade comprising *A. excelsa*, the heterotypic synonym *A. philippinensis*, and *A. pomaderroides* (pp: 1.00 / BS: 77). We reconstructed 1 MA (95% CI: 0-9 MA) as median age for crown *Alphitonia*, 1.7 MA (95% CI: 0-16 MA) for stem *Alphitonia*, 1.2 MA (95% CI: 0-5.5 MA) for crown *Emmenosperma*, and 0.1 MA (95% CI: 0-2 MA) for crown *Jaffrea* (Appendix 2 F). The reconstructed node ages included uncertainties as derived from the 95% confidence interval of more than 700 % (Appendix 2 F). As the estimated ages of ancestral nodes did not match those obtained from the scaling with the “prior only” approach, we can confidently say that the ages we uncovered are also sensitive to the underlying molecular data and not only to the calibration scheme (Appendix 2 E-F).

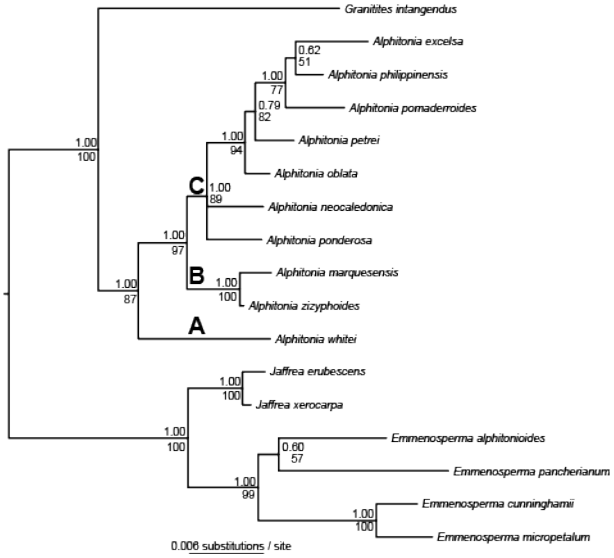

Fig. 2. Majority rule consensus tree of *Alphitonia*, *Emmenosperma*, *Granitites* and *Jaffrea* based on the reduced data set and three markers (ITS, *rbcL*, *trnL-trnF*). Posterior probabilities (above) and bootstrap values (maximum likelihood analyses on the same data; below) are given near the corresponding nodes. The three discussed lineages (A, B, and C) are labelled above the corresponding branches.

Biogeographic analyses – Australia was reconstructed as the most likely ancestral area for *Alphitonia* and *Granitites* (56.6%, Fig. 3A), whereas up to five areas (Australia: 42.4%, New Guinea: 8.7%, New Caledonia: 20.2%, the Asian region: 16.8%, and Polynesia: 12.0%) were reconstructed as having been part of the ancestral range of *Emmenosperma* and *Jaffrea* (see Fig. 3B). Australia was (weakly) supported as ancestral area (Fig. 3A) throughout the backbone of *Alphitonia* (38.8% to 57.7%). Furthermore, Australia was reconstructed as ancestral area with a likelihood above 50% for the clade comprising *A. excelsa*, *A. oblata*, *A. petrei*, *A. philippinensis*, and *A. pomaderroides*, some of which have a wide distribution from Australia to Asia. Southeast Asian areas did not contribute more than 35.6% (most recent common ancestor of *A. excelsa* and *A. philippinensis*) to any reconstructed ancestral node in *Alphitonia* and related genera (Fig. 3A). There were two westward range expansions within *Alphitonia*, including a) *A. oblata* and b) *A. excelsa* and *A. philippinensis*. To the east (New Caledonia, and Pacific islands), two range expansions were reconstructed. First, *A. marquesensis* and *A. zizyphoides* originated from an area potentially including Australia (35.1%), New Guinea (10.4%), Southeast Asian regions (13.9%), and/or New Caledonia (16.9%), or they were already present in the Pacific regions (23.6%) (Fig. 3A). Second, *A. neocaledonica* and *A. ponderosa* (clade not supported, cf. Fig. 2) evolved from an ancestral area potentially including Australia (41.3%), New Caledonia (19.4%), Southeast Asian regions (20.1%), and New Guinea (14.9%). New Caledonia (100.0%) was reconstructed as ancestral area of the most recent common ancestor of *Jaffrea* (crown). Australia (43.4%), Southeast Asia (excluding New Guinea) (18.4%), New Caledonia (15.7%), New Guinea (9.7%), and/or Polynesia (12.6%) were reconstructed as having been part of the ancestral area reconstructed for *Emmenosperma* (crown) (Fig. 3B).

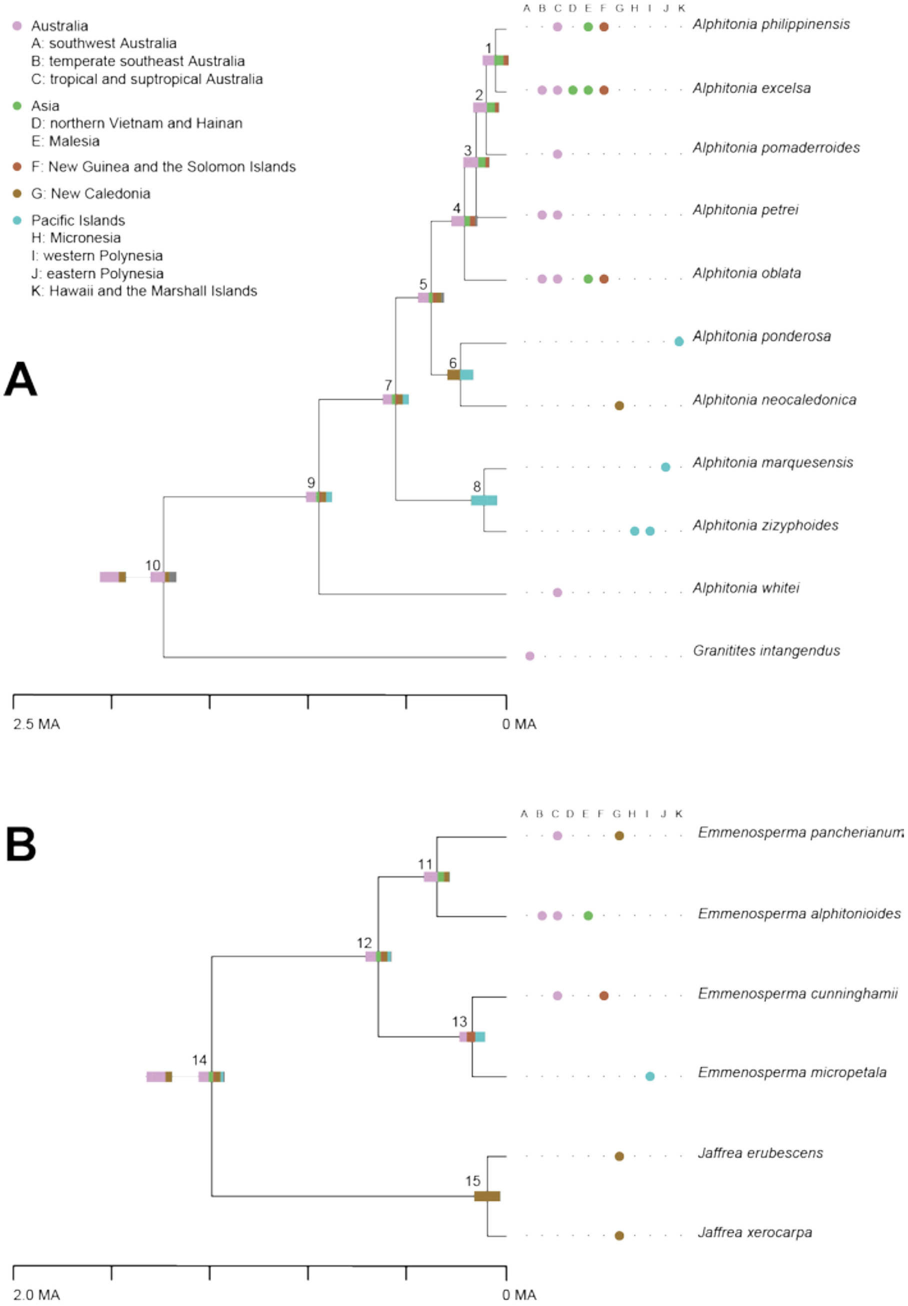
Australia
A: southwest Australia
B: temperate southeast Australia
C: tropical and suptropical Australia
Asia
D: northern Vietnam and Hainan
E: Malesia
F: New Guinea and the Solomon Islands
G: New Caledonia
Pacific Islands
H: Micronesia
I: western Polynesia
J: eastern Polynesia
K: Hawaii and the Marshall Islands
A
A B C D E F G H I J K
Alphitonia philippinensis
Alphitonia excelsa
Alphitonia pomaderroides
Alphitonia petrei
Alphitonia oblata
Alphitonia ponderosa
Alphitonia neocaledonica
Alphitonia marquesensis
Alphitonia zizyphoides
Alphitonia whitei
Granitites intangendus
1
2
3
4
5
6
7
8
9
10
2.5 MA
0 MA
B
A B C D E F G H I J K
Emmenosperma pancherianum
Emmenosperma alphitonioides
Emmenosperma cunninghamii
Emmenosperma micropetala
Jaffrea erubescens
Jaffrea xerocarpa
11
12
13
14
15
2.0 MA
0 MA

Fig. 3. Most credible tree from BEAST, section comprising *Alphitonia* and *Granitites* (A) and *Emmenosperma* and *Jaffrea* (B). Cumulative proportions of the ancestral areas reconstructed by DEC+J (as implemented in BioGeoBEARS) are plotted as bars at the corresponding nodes. The extant distribution of the included species is given next to the chronogram. Area delimitations are illustrated in Fig. 1. Internal nodes are numbered above the nodes.

Discussion

Phylogenetic reconstructions and taxonomic considerations

Overall, our analyses confirmed the current classification of *Alphitonia* and its close allies, i.e. the four genera (*Alphitonia*, *Emmenosperma*, *Granitites*, and *Jaffrea*) as depicted by Hopkins et al. (2015), as well as most of the currently accepted species within those genera (Braid, 1925; Bean, 2010; Hopkins *et al.*, 2015). Furthermore, we recovered phylogenetic support for the recently described *E. pancherianum* in Australia, which had previously been reported based on morphology alone (Bean, 2013). Yet, the potential inclusion of *A. philippinensis* into *A. incana* is not supported by our phylogenetic results, and thus is likely to lead to an underestimation of the species diversity. We suggest that these two species should therefore not be synonymized. Concerning *A. incana* and *A. oblata*, our analyses strengthen the idea of a conspecific pattern of both species also outside of Australia. However, both accessions of *A. incana* used in our studies are of New Guinean origin, and we thus miss accessions from the western areas of distribution of *A. incana*. Further analyses, then including *A. incana* from its western edge of distribution (e.g., Hainan, China), are needed to re-circumscribe the taxon. Aside the rank of species, the sectional division within *Alphitonia* is absent, as *A.* sect. *Glabratae* has been excluded from the genus. The former *A.* sect. *Alphitonia* corresponds exactly to the currently accepted genus *Alphitonia* (*sensu* Hopkins et al., 2015), within which we identified three supported lineages (Fig. 2). In our opinion, these further subdivisions of the genus (A, B, and C) reflect the intrageneric genetic and morphological structuring of *Alphitonia* better. Lineage A comprises *A. whitei*, an Australian endemic restricted to the rainforest of Queensland. We share the opinion of Bean (2010) that *A. whitei*, alongside the New Guinean *A. macrocarpa* (of which specimens were examined, however we were unable to extract suitable DNA material for sequencing), greatly differs from all other *Alphitonia* species. Those differences are predominantly found in fruit characters, such as the absence of a mesocarp and shorter (<4 mm) pedicels (Bean, 2010; Hopkins *et al.*, 2015). Lineage B comprises *A. ziziphoides* and *A. marquesensis*, distributed from Micronesia (and adjacent islands of Indonesia) to the Polynesian region. Finally, lineage C comprises the remaining Australian and Malesian species (*A. excelsa*, *A. pomaderroides*, *A. oblata*, *A. petrei*, and potentially *A. philippinensis*), as well as *A. neocaledonica* (New Caledonia) and *A. ponderosa* (Hawaii). It might appear tempting to describe sections based upon these three lineages, yet, since some species delineations remains unclear (e.g. *A. philippinensis*), and our phylogenetic analyses still lack *A. macrocarpa* Mansf. and A. *carolinensis* Hosok., we recommend that more populations should be molecularly investigated before further taxonomic efforts are taken.

Molecular dating and biogeography

We reconstructed 1 Ma as median ancestral crown age of *Alphitonia*, 1.2 Ma for *Emmenosperma*, and 100,000 years ago for *Jaffrea*. However, we want to point out that the reconstructed ages are based on incomplete fossil records and should be taken with care. Although our 95% confidence intervals are large, we consider our dating approach as the currently best possible. Any alternative outgroup calibration (e.g. including the fossil-rich genus *Paliurus* Mill.) would be prone to errors, as the topology within in the ziziphoid group is not sufficiently resolved (Onstein *et al.*, 2015; Hauenschild *et al.*, 2016a). The age of the four genera is relevant for our biogeographic reconstructions, as some islands have only emerged recently, such as New Guinea (<12 Ma) and the Society Islands (<4.5 Ma) (Neall & Trewick, 2008; Baldwin *et al.*, 2012). As the reconstructed ages of intrageneric nodes (max: *Alphitonia* crown, 95% confidence interval: <10 Ma) are not older than the geological age of any area, we consider our reconstructions to be young, yet realistic.

One of our main findings is that Miocene Australia is the most likely area of origin of *Alphitonia* and related genera (Fig. 3A). The Miocene stem age is younger than expected (Onstein *et al.*, 2015; Onstein & Linder, 2016), but our estimate is supported by two lines of evidence, which, taken together, give some credit to the "out of Australia" hypothesis (Chen *et al.*, 2014). First, for all nodes at the backbone of *Alphitonia* and related genera, Australia was suggested as ancestral area, with a probability varying between 38.8% and 57.7% (Fig 3A, nodes 3, 4, 5, 7, 9, and 10). Furthermore, fossils with affinities to *Emmenosperma* were described from Miocene Australia (Hill, 1994). *Alphitonia* and related genera could have originated from a mrca nested within the ziziphoid clade of Rhamnaceae (Richardson *et al.*, 2000a; Onstein *et al.*, 2015; Hauenschild *et al.*, 2016a), migrating into Australia, followed by an early diversification in situ (split between the two lineages: *Jaffrea*/*Emmenosperma* and *Alphitonia*/*Granitites*). Dispersal out of Australia would have promoted allopatric speciation within both lineages, for example, favoring the split between *Emmenosperma* (Australia) and *Jaffrea* (New Caledonia) (Fig 3B, nodes 12, 14, and 15). Simultaneously, in *Alphitonia*, or later on in *Emmenosperma*, several species (extant, or common ancestors of extant species) might have extended their range towards neighboring islands, and onwards to more distant areas. This range expansion occurred either towards the Pacific islands or into Asia. This biogeographic hypothesis is in line with geological evidence and our molecular dating analyses. Even if a very conservative interpretation of molecular dating results was adopted, considering the minimum and maximum values of the 95% confidence intervals, the ages we uncovered for these divergence and dispersal events would all be younger than the initial contact of the Australian plate with Southeast Asia between 20 and 15 Ma (Hall, 2009; Hall, 2017). For example, the mrca of *Alphitonia* and *Granitites* dates back to about 1.7 Ma (Appendix 2 F), and did not occur in Southeast Asia (Fig. 3). Hence, continental contact might have played a minor role compared to other factors, one being climatic changes. The aridification of Australia caused the fragmentation of wetter zones within the continent, culminating in small tropical rainforest refugia in Queensland, for example. Moreover, divergence and dispersal events within *Alphitonia* and its related genera, and the whole group's western expansion towards Asia, are in line with the timing of land masses emerging in Wallacea, Micronesia, and New Guinea during the last few million years (Hall, 2009; Seton *et al.*, 2012; Rehman *et al.*, 2013; Hall, 2017).

While the westward dispersal of *Alphitonia* and related genera is supported, for the eastward dispersals into New Caledonia, Polynesia and Hawaii our data do not provide clear evidence for the routes used. Our reconstructions suggest that eastward dispersal and establishment across large water bodies occurred at least twice within *Alphitonia* and up to two times in *Emmenosperma*. Yet, uncertainties in the ancestral area reconstructions at the backbone nodes within *Alphitonia* do not allow us to entirely exclude the presence of ancestral lineages in New Guinea, New Caledonia, or in the Pacific region (incl. Hawaii, Micronesia, and Polynesia) prior to the intrageneric divergence events. This is because for nodes 5, 7, and 9 (Fig. 3A) Australia is favoured as ancestral area (>38 %), with New Guinea, New Caledonia and the Pacific islands (<17%) as potential alternatives. In contrast to a continuous presence of the ancestral taxa in the Pacific region, followed by allopatric speciation, several back and forth dispersal events between Australia and the Pacific islands could have obscured the biogeographic scenario. The support for a single ancestral area is, in general, relatively low (<70%), compared to other biogeographic studies (Ebersbach *et al.*, 2016; Favre *et al.*, 2016). In *Emmenosperma*, the initial expansion (Fig. 3B, nodes 12 and 13) was reconstructed from Australia to New Guinea and potentially New Caledonia), following dispersal into Polynesia.

Within *Alphitonia*, we could not resolve the phylogenetic relationships among *A. neocaledonia* (New Caledonia), *A. ponderosa* (Hawaii), and the remaining taxa of lineage C. Consequently, it remains unknown whether only one dispersal event, followed by subsequent speciation, occurred (if *A. neocaledonia* and *ponderosa* were sister species), or these two species were the result of two independent range expansions (if they were no sister species). However, we are fairly certain that the colonization of Hawaii was the result of a dispersal event different from the one inferred for *A. zizyphoides* and *A. marquesensis* (Lineage B; throughout Polynesia). As already recognized by Hopkins et al. (2015), the Hawaiian *A. ponderosa* is not closely related to the Polynesian *A. zizyphoides* and *A. marquesensis*, and thus does not share the most recent biogeographic history (Fig. 3A). Whether the genus reached Hawaii via New Caledonia or directly from a Australia (and potentially New Guinea) remains uncertain, yet, a dispersal event of particularly long distance (over more than 6000 km of Pacific Ocean) was certainly involved. This event was dated to the Quaternary, between the present and 2.5 Ma (Appendix 2E), i.e., when most Pacific islands had already emerged(Neall & Trewick, 2008). In general, when taxa extend their distributional range from Australia to the Pacific islands, we would expect that dispersal to New Caledonia and Polynesia is limited due to large oceanic gaps. Dispersal within Polynesia may be regarded as more likely than multiple dispersal events from Australia into distant Polynesian island groups without intermediate steps. This assumption is supported by the fact that floral and faunal compositions are more similar between the western Pacific islands and New Guinea than between Australia and any of the Pacific regions (Ung *et al.*, 2017). However, our reconstructions favor multiple direct colonization events from Australia to the Pacific islands and Southeast Asia, as has also been shown for butterflies, *Melicope*, and sandalwoods (Braby & Pierce, 2007; Harbaugh & Baldwin, 2007; Appelhans *et al.*, 2018;), rather than a few expansions followed by in situ radiation in the West Pacific (Chen *et al.*, 2014). A reason for this discrepancy might be that the studies used for the analyses of Ung *et al.* (2017) target epochs older than the few thousands and millions of years relevant for our study. Our reconstructions illustrate the importance of long-distance dispersal events from Australia potentially to Eastern Polynesia and Hawaii within the last 1-1.5 Ma. As *Alphitonia* species are fed upon by birds (Barker & Vestjens, 1989), similar to what was found in sandalwoods (Harbaugh and Baldwin, 2007) these events might, at least in parts, be bird-mediated.

Possible impact of human migrations

Some dispersal events of *Alphitonia* may, however, not result from natural range expansion. Our temporal reconstruction for most intrageneric nodes within *Alphitonia* included the present (Appendix 2 F), and one might thus suspect repeated human intervention. For example, *A. ponderosa* could have been introduced to Hawaii by humans, and, given the phylogenetic relationships, probably be derived from an ancestor originating from New Caledonia and/or the Solomon Islands and Vanuatu. In fact, *Alphitonia* trees were used and cultivated by Polynesians, as for example documented for Hawaii (Abbott, 1992; Wagner *et al.*, 1999). The wood, bark, and leaves of *Alphitonia* were used by Polynesians for the production of small tools and weapons (Thomson & Thaman, 2008). Hence, the morphological variations described within *A. ponderosa* (St. John, 1977) may result from cultivation on this island. The strongest indication of human introduction and breeding of *A. ponderosa* was shown by a study on population genetics by Kwon & Morden (2002) who detected that recruitment of *A. ponderosa* was entirely absent from Hawaii. The authors associated this lack of recruitment with the co-occurrence of invasive species and grazing by herbivores, but it could also indicate that the species cannot persist on Hawaii without human management. Other studies have shown that Polynesians are likely to have acted as dispersal vector for several plant groups, e.g., gourds (Clarke *et al.*, 2006), breadfruit (Zerega *et al.*, 2004), and paper mulberries (González-Lorca *et al.*, 2015). Further indepth genetic studies should be performed to answer the question of when and how *A. ponderosa* arrived on Hawaii, and to shed light on dispersal routes of other species across the Pacific islands, to be able to better assess the potential of human influence on *Alphitonia*´s current distribution.

Conclusions

Are we confronted with an “out of Australia” pattern? Apart from human impact on the distribution of *Alphitonia ponderosa*, we find an “out of Australia” dispersal pattern in *Alphitonia* and related genera. Given the young age estimates and a manageable size of the group with only 20+ species, three approaches will help to unravel the fine-scale biogeographic history of *Alphitonia* and related genera. To be able to reconstruct more age estimates with smaller 95% HPD intervals will require a strongly supported and fully resolved topology within the fossil-rich ziziphoid lineage of Rhamnaceae. Second, the use of high-throughput sequencing methods in *Alphitonia* and related genera at populational level will allow the investigation of gene flow and dispersal routes, natural as well as human-mediated, which may not have been recovered by our current approach. Third, our study illustrates one of the rarely shown cases of dispersal of wet forest-adapted plants lineages from Australia into Southeast Asia. Fourth, our study confirms the currently accepted taxonomy within *Alphitonia* and closely related genera, but it could not fully clarify the relationship of the widespread species *A. zizyphoides* and *A. excelsa*. Increased geographical sampling density, combined with high-throughput sequencing approaches should, besides addressing biogeographical questions, allow a better estimation of the current species richness of *Alphitonia* and related genera. The proposed analyses are vital to identify fine-scale dispersal routes in *Alphitonia*, to detect possible hybridization events, and to compare the genetic diversity found within *A. ponderosa* to other species. This could add further evidence for answering the question whether this Hawaiian endemic was a valid species, or, alternatively, merely the result of human-mediated selective pressure.

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Appendix 1. Voucher information and GenBank accession numbers for sequences used in this study. Taxon, country and region of origin, collector and number, herbarium code, GenBank accession number for ITS, *rbcL*, *trnL-trnF*. Missing data is indicated by a dash (-). An asterisk (*) indicates newly sequenced specimens.

Species	**Country of origin, locality**	**Collector, number, location**	**ITS**	***rbcL***	***trnL-trnF***
***Alphitonia excelsa* 1 (Fenzl) Reissek ex Benth.**	Australia, Queensland	*Collins 314* (CANB)	*MF787296	*MF787345	*MF787321
***Alphitonia excelsa* 2 (Fenzl) Reissek ex Benth.**	Australia, Queensland	*Purdie 8990* (CANB)	*MF787297	*MF787346	*MF787322
***Alphitonia excelsa* 3 (Fenzl) Reissek ex Benth.**	Australia, Queensland	*Nelder & Clarkson 2982* (L)			*MF787323
***Alphitonia excelsa* 4 (Fenzl) Reissek ex Benth.**	Australia, Queensland	*Kellermann 103* (MEL)	HQ340157		HQ325600
***Alphitonia excelsa* 5 (Fenzl) Reissek ex Benth.**	Australia, Queensland	*Costion 1657 (no voucher)*		KF496294	
***Alphitonia excelsa* 6 (Fenzl) Reissek ex**	Australia, Queensland	*Dufourq KFNP23*		KM896142	

Benth.		(BRI)			
Alphitonia incana **1 (Roxb.) Teijsm. & Binn. ex Kurz =** ***Alphitonia oblata*** **A.R. Bean**	Indonesia, West Papua	*Kumekawa s.n.* (no voucher)			AB981758
Alphitonia incana **2 (Roxb.) Teijsm. & Binn. ex Kurz =** ***Alphitonia oblata*** **A.R. Bean**	Papua New Guinea	*James 938* (BISH)	KJ630930		KJ630943
Alphitonia marquesensis **1 F. Br.**	French Polynesia, Marquesas Islands	*Sachet 1902* (NSW)		*MF787347	
Alphitonia marquesensis **2 F. Br.**	French Polynesia, Marquesas Islands	*Oliver & Schäfer 3184* (NSW)		*MF787348	
Alphitonia marquesensis **3 F. Br.**	French Polynesia, Marquesas Islands	*Taputuarai 814* (PAP)	KP185119		KP185118
Alphitonia	New Caledonia	*Wilson*	*MF787298	*MF787349	*MF787324

***neocaledonica* 1 (Schltr.) Guillaumin**		*7045* (NSW)			
***Alphitonia neocaledonica* 2 (Schltr.) Guillaumin**	New Caledonia	*Abell 621* (NSW)	*MF787299	*MF787350	*MF787325
***Alphitonia neocaledonica* 3 (Schltr.) Guillaumin**	New Caledonia	*Barrabé 1163* (NOU)	KJ630932	KJ630939	KJ630944
***Alphitonia oblata* 1 A.R. Bean**	Australia, Queensland	*Bean 5784* (NSW)	*MF787300		
***Alphitonia oblata* 2 A.R. Bean**	Australia, Queensland	*Chase 2179* (K)	KJ630931	AJ390049	AJ390352
***Alphitonia obtusifolia* 1 Braid = *Alphitonia pomaderroides* (Fenzl) A.R. Bean**	Australia, Queensland	*Purdie 5758* (CANB)	*MF787301	*MF787351	*MF787326
***Alphitonia obtusifolia* 2 Braid = *Alphitonia pomaderroides* (Fenzl) A.R. Bean**	Australia, Queensland	*Purdie 5934* (CANB)	*MF787302	*MF787352	*MF787327
***Alphitonia petrei* 1 Braid & C.T. White**	Australia, New South Wales	*Purdie 8762* (CANB)	*MF787303	*MF787353	*MF787328
***Alphitonia petrei* 2**	Australia,	*Purdie*	*MF787304	*MF787354	*MF787329

Braid & C.T. White	Queensland	*6768* (CANB)			
***Alphitonia petrei* 3 Braid & C.T. White**	Australia, Queensland	*Wells s.n.* (QRS)			KC428438
***Alphitonia philippinensis* Braid = *Alphitonia excelsa* (Fenzl) Reissek ex Benth.**	Australia, Moa Island (Queensland, Torres Strait)	*Wannan 3010* (NSW)	*MF787305		
***Alphitonia pomaderroides* 1 (Fenzl) A.R. Bean**	Australia, Queensland	*Wannan 5260* (NSW)	*MF787306		*MF787330
***Alphitonia pomaderroides* 2 (Fenzl) A.R. Bean**	Australia, Queensland	*Wannan 5261* (NSW)	*MF787307		
***Alphitonia pomaderroides* 3 (Fenzl) A.R. Bean**	Australia, Queensland	*Crayn 1292* (ATH)	*MF787308	*MF787355	*MF787331
***Alphitonia ponderosa* 1 Hillebr.**	United States of America, Hawaii	*Degener s.n.* (NSW)	*MF787309	*MF787356	*MF787332
***Alphitonia ponderosa* 2 Hillebr.**	United States of America, Hawaii	*Pillon 1431* (NSW)	KJ630933		KJ630945

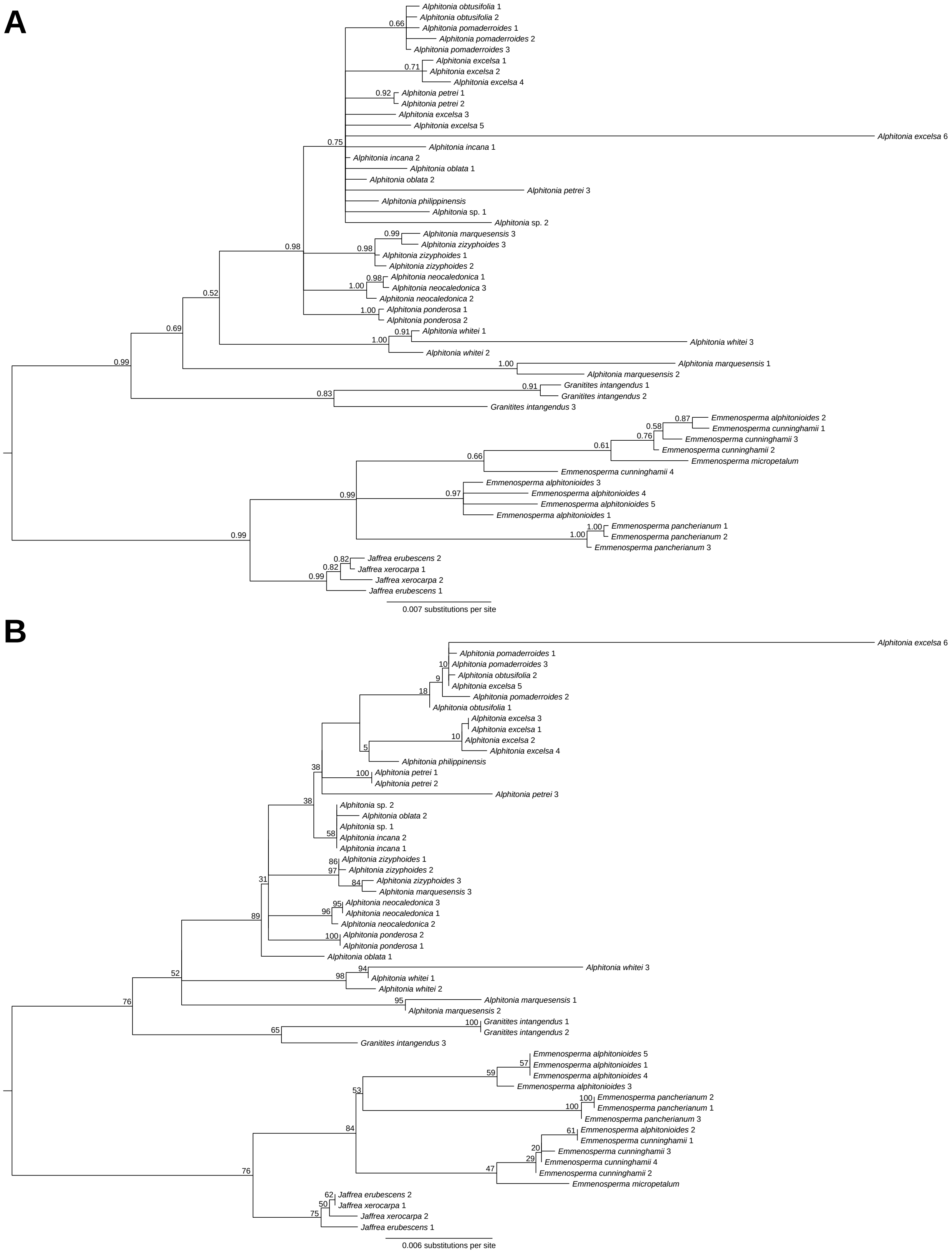
Alphitonia sp. 1	Indonesia, West Papua	*Kumekawa s.n. (no voucher)*			AB889446
Alphitonia sp. 2	Indonesia, West Papua	*Kumekawa s.n. (no voucher)*			AB889447
Alphitonia whitei 1 Braid	Australia, Queensland	*Grey 8771* (CANB)	*MF787310	*MF787357	*MF787333
Alphitonia whitei 2 Braid	Australia, Queensland	*Ford 6273* (ATH)	*MF787311	*MF787358	*MF787334
Alphitonia whitei 3 Braid	Australia, Queensland	*Wells s.n.* (QRS)			KC428439
Alphitonia zizyphoides 1 (Sol. ex Forst.) A. Gray	Vanuatu	*Munzinger 3891* (NOU)	KJ630936		KJ630948
Alphitonia zizyphoides 2 (Sol. ex Forst.) A. Gray	Wallis and Futuna, Wallis	*Pillon 845* (NOU)	KJ630934		KJ630946
Alphitonia zizyphoides 3 (Sol. ex Forst.) A. Gray	French Polynesia, Marquesas Islands	*Meyer 3086* (PAP)	KJ630935		KJ630947
Emmenosperma alphitonioides 1	Australia, Queensland	*Ford 6122* (ATH)	*MF787312	*MF787360	*MF787336

F.Muell.						
***Emmenosperma alphitonioides* F.Muell.**	**2**	Australia	*Clarkson 8826* (K)		AJ390048	AJ390351
***Emmenosperma alphitonioides* F.Muell.**	**3**	Australia, cultivated	*Kellermann 419* (MEL)	HQ340159		
***Emmenosperma alphitonioides* F.Muell.**	**4**	Australia, Queensland	*Gray 3325 (no voucher)*		KF496412	
***Emmenosperma alphitonioides* F.Muell.**	**5**	Australia, Queensland	*Shaw SS4 (no voucher)*		KM895701	
***Emmenosperma cunninghamii* Benth.**	**1**	Australia, Northern Territory	*Byrnes 2596* (L)	*MF787313	*MF787361	*MF787337
***Emmenosperma cunninghamii* Benth.**	**2**	Australia, Western Australia	*Kenneally 11185* (CANB)	*MF787314	*MF787362	*MF787338
***Emmenosperma cunninghamii* Benth.**	**3**	Australia, Queensland	*Costion 2153* (ATH)	*MF787315	*MF787363	*MF787339
Emmenosperma cunninghamii	**4**	Australia, Queensland	*Smyrell GS13-1*		KM895747	

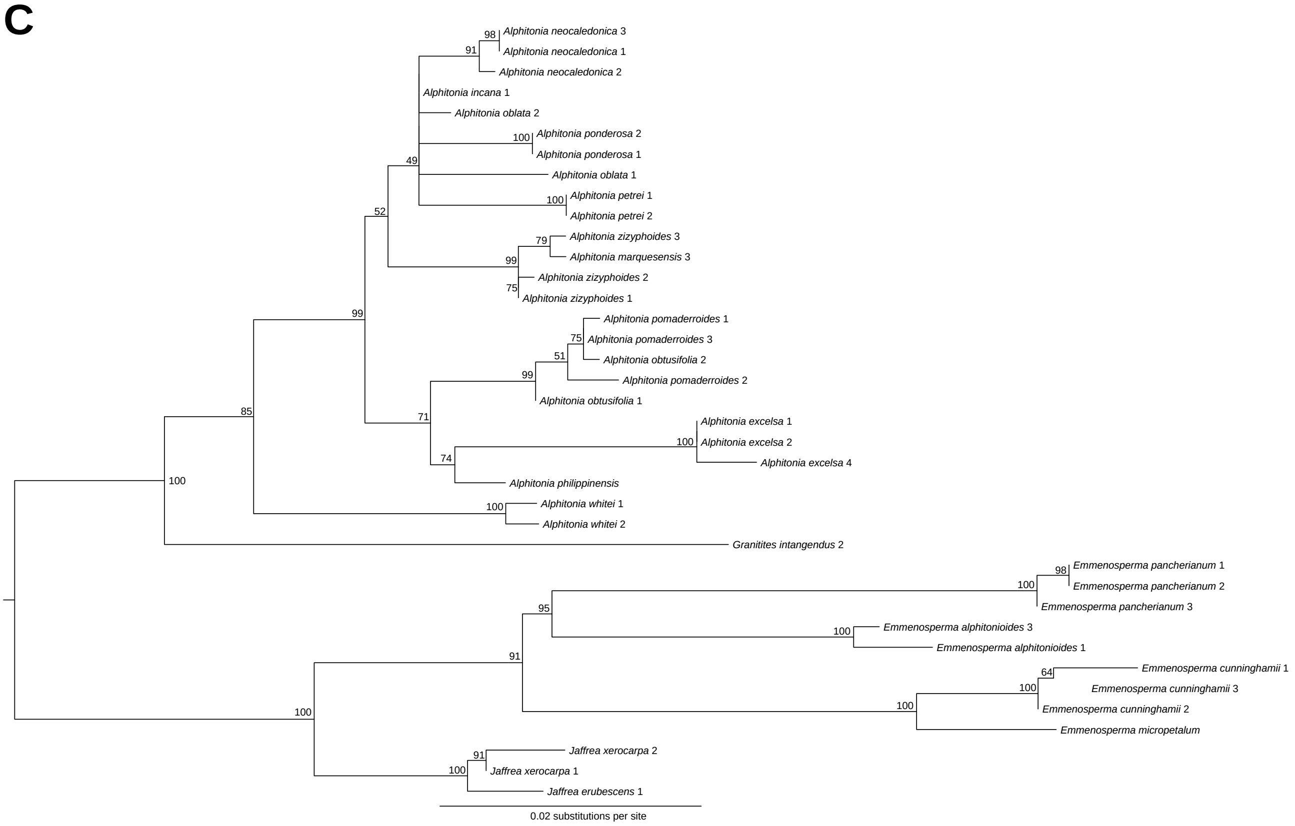
Benth.		(BRI)			
Emmenosperma micropetala (A.C. Sm.) M.C. Johnst.	Fiji, Naitasiri	*Tulavu K265* (L)	*MF787316	*MF787364	*MF787340
***Emmenosperma pancherianum* 1 Baill.**	Australia, Queensland	*Bean 28864* (CANB)	*MF787317	*MF787365	*MF787341
***Emmenosperma pancherianum* 2 Baill.**	Australia, Queensland	*Bean 28865* (ATH)	*MF787318	*MF787366	*MF787342
***Emmenosperma pancherianum* 3 Baill.**	New Caledonia	*Coulerie 3* (NOU)	KJ630938	KJ630942	KJ630951
***Granitites intangendus* 1 Baill.**	Australia, Western Australia	*Keighery & Keighery 1175* (MEL)		*MF787359	*MF787335
***Granitites intangendus* 2 Baill.**	Australia, Western Australia	*Mole 417* (MEL)	HQ340160		HQ325603
***Granitites intangendus* 3 Baill.**	Australia, Western Australia	*Hopper 8486A* (MEL)		AJ306539	
***Jaffrea erubescens* 1**	New Caledonia	*McPherson*	*MF787319		*MF787343

(Baill.) H.C. Hopkins & Pillon		*2047* (NSW)			
***Jaffrea erubescens* 2 (Baill.) H.C. Hopkins & Pillon**	New Caledonia	*Dagostini 1107* (NSW)		KJ630941	KJ630949
***Jaffrea xerocarpa* 1 (Baill.) H.C. Hopkins & Pillon**	New Caledonia		*MF787320	*MF787367	*MF787344
***Jaffrea xerocarpa* 2 (Baill.) H.C. Hopkins & Pillon**	New Caledonia	*Pillon 1221* (MEL)	KJ630940	KJ630937	KJ630950

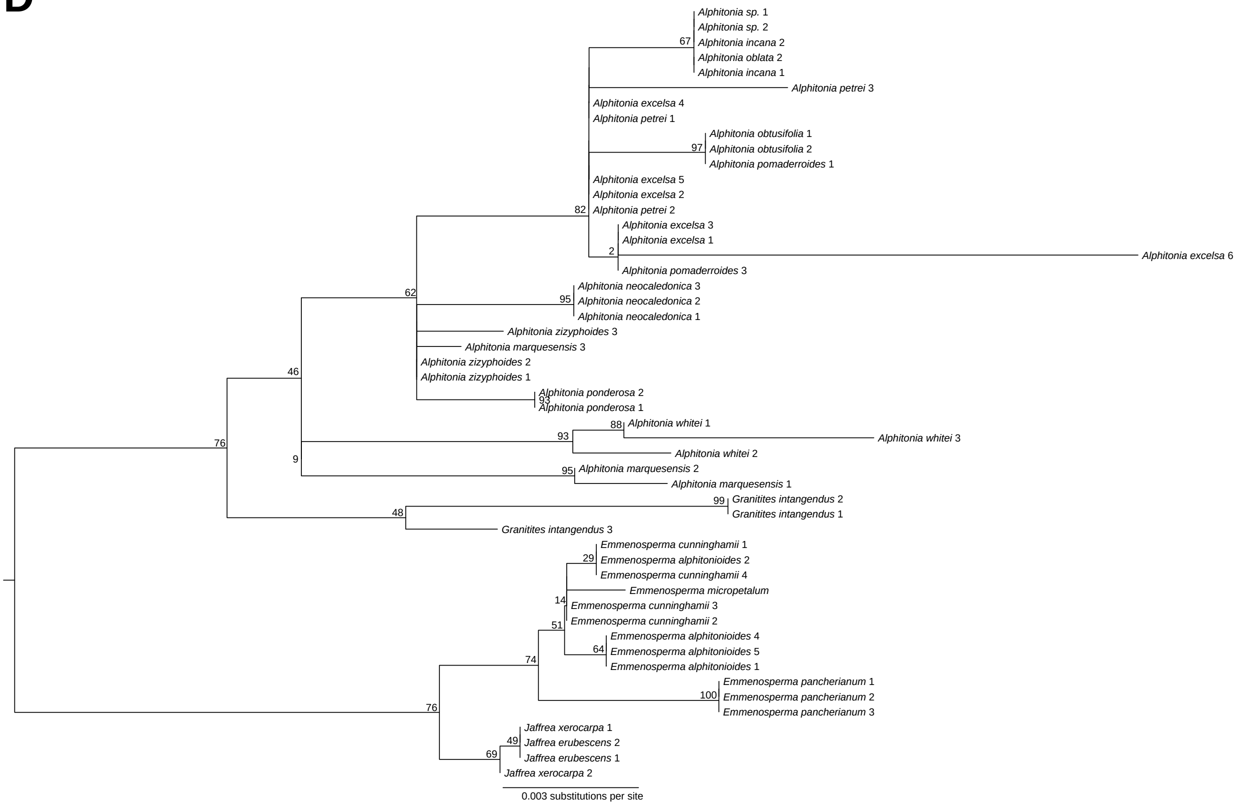
Appendix 2. Phylogenetic reconstructions based on the full data set (A-D), and temporal reconstructions based on the reduced data set (E-X). Appendix 2 A shows the majority rule consensus tree from the Bayesian analysis on the concatenated data set in three (ITS, *rbcL*, *trnL-trnF*) partitions. Posterior probabilities are given left of the corresponding node. Appendix 2 B shows the best tree from the concatenated maximum likelihood analysis. Bootstrap values are given left of nodes (left out if branch length is 0 and support is below 70 to enhance visualization). Appendix 2 C illustrates the best tree from the ITS-based maximum likelihood analysis. Bootstrap values are given left of nodes (left out if branch length is 0 and support is below 70 to enhance visualization), and Appendix 2 D shows the best tree from the plastid-based (in two partitions: *rbcL* and *trnL-trnF*) maximum likelihood analyses. Bootstrap values are given left of nodes (left out if branch length is 0 and support is below 70 to enhance visualization). Appendix 2 E illustrates the chronogram reconstructed excluding the molecular data (sample from priors only). Node age (above) and posterior probabilities (below) are given next to the corresponding nodes. Appendix 2 F illustrates the most credible tree reconstructed including the molecular data from our BEAST analyses. Median node ages are plotted next to the corresponding nodes. Bars indicate the 95% confidence intervals.


A
Alphitonia obtusifolia 1
Alphitonia obtusifolia 2
0.66
Alphitonia pomaderroides 1
Alphitonia pomaderroides 2
Alphitonia pomaderroides 3
Alphitonia excelsa 1
0.71
Alphitonia excelsa 2
Alphitonia excelsa 4
0.92
Alphitonia petrei 1
Alphitonia petrei 2
Alphitonia excelsa 3
Alphitonia excelsa 5
0.75
Alphitonia excelsa 6
Alphitonia incana 1
Alphitonia incana 2
Alphitonia oblata 1
Alphitonia oblata 2
Alphitonia petrei 3
Alphitonia philippinensis
Alphitonia sp. 1
Alphitonia sp. 2
0.99
Alphitonia marquesensis 3
Alphitonia zizyphoides 3
0.98
0.98
Alphitonia zizyphoides 1
Alphitonia zizyphoides 2
0.98
Alphitonia neocaledonica 1
1.00
Alphitonia neocaledonica 3
Alphitonia neocaledonica 2
0.52
1.00
Alphitonia ponderosa 1
Alphitonia ponderosa 2
0.91
Alphitonia whitei 1
1.00
Alphitonia whitei 3
0.69
Alphitonia whitei 2
1.00
Alphitonia marquesensis 1
0.99
Alphitonia marquesensis 2
0.91
Granitites intangendus 1
0.83
Granitites intangendus 2
Granitites intangendus 3
0.87
Emmenosperma alphitonioides 2
0.58
Emmenosperma cunninghamii 1
0.76
Emmenosperma cunninghamii 3
0.61
Emmenosperma cunninghamii 2
0.66
Emmenosperma micropetalum
Emmenosperma cunninghamii 4
Emmenosperma alphitonioides 3
0.97
Emmenosperma alphitonioides 4
0.99
Emmenosperma alphitonioides 5
Emmenosperma alphitonioides 1
0.99
1.00
Emmenosperma pancherianum 1
1.00
Emmenosperma pancherianum 2
Emmenosperma pancherianum 3
0.82
Jaffrea erubescens 2
0.82
Jaffrea xerocarpa 1
0.99
Jaffrea xerocarpa 2
Jaffrea erubescens 1
0.007 substitutions per site
B
Alphitonia excelsa 6
Alphitonia pomaderroides 1
10
Alphitonia pomaderroides 3
9
Alphitonia obtusifolia 2
Alphitonia excelsa 5
18
Alphitonia pomaderroides 2
Alphitonia obtusifolia 1
Alphitonia excelsa 3
Alphitonia excelsa 1
10
Alphitonia excelsa 2
Alphitonia excelsa 4
5
Alphitonia philippinensis
38
100
Alphitonia petrei 1
Alphitonia petrei 2
Alphitonia petrei 3
38
Alphitonia sp. 2
Alphitonia oblata 2
Alphitonia sp. 1
58
Alphitonia incana 2
Alphitonia incana 1
86
Alphitonia zizyphoides 1
97
Alphitonia zizyphoides 2
31
84
Alphitonia zizyphoides 3
Alphitonia marquesensis 3
95
Alphitonia neocaledonica 3
96
Alphitonia neocaledonica 1
89
Alphitonia neocaledonica 2
100
Alphitonia ponderosa 2
Alphitonia ponderosa 1
Alphitonia oblata 1
52
Alphitonia whitei 3
94
98
Alphitonia whitei 1
Alphitonia whitei 2
95
Alphitonia marquesensis 1
76
Alphitonia marquesensis 2
100
Granitites intangendus 1
65
Granitites intangendus 2
Granitites intangendus 3
Emmenosperma alphitonioides 5
57
Emmenosperma alphitonioides 1
59
Emmenosperma alphitonioides 4
53
Emmenosperma alphitonioides 3
100
Emmenosperma pancherianum 2
100
Emmenosperma pancherianum 1
Emmenosperma pancherianum 3
84
61
Emmenosperma alphitonioides 2
Emmenosperma cunninghamii 1
20
Emmenosperma cunninghamii 3
29
Emmenosperma cunninghamii 4
47
Emmenosperma cunninghamii 2
76
Emmenosperma micropetalum
62
Jaffrea erubescens 2
50
Jaffrea xerocarpa 1
75
Jaffrea xerocarpa 2
Jaffrea erubescens 1
0.006 substitutions per site

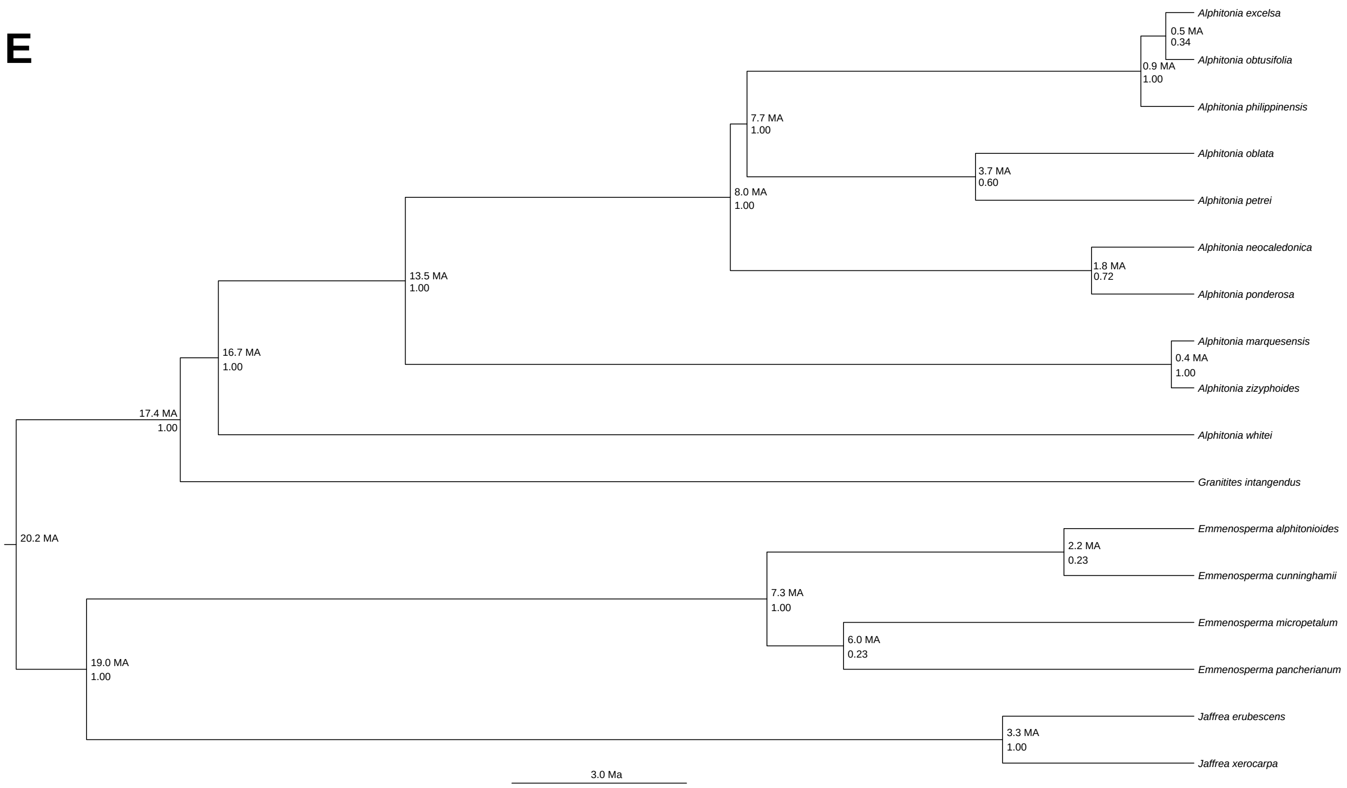
C


98
Alphitonia neocaledonica 3
91
Alphitonia neocaledonica 1
Alphitonia neocaledonica 2
Alphitonia incana 1
Alphitonia oblata 2
100
Alphitonia ponderosa 2
Alphitonia ponderosa 1
49
Alphitonia oblata 1
100
Alphitonia petrei 1
52
Alphitonia petrei 2
79
Alphitonia zizyphoides 3
99
Alphitonia marquesensis 3
Alphitonia zizyphoides 2
75
Alphitonia zizyphoides 1
99
Alphitonia pomaderroides 1
75
Alphitonia pomaderroides 3
51
Alphitonia obtusifolia 2
Alphitonia pomaderroides 2
99
Alphitonia obtusifolia 1
85
71
Alphitonia excelsa 1
100
Alphitonia excelsa 2
Alphitonia excelsa 4
74
Alphitonia philippinensis
100
100
Alphitonia whitei 1
Alphitonia whitei 2
Granitites intangendus 2
98
Emmenosperma pancherianum 1
100
Emmenosperma pancherianum 2
95
Emmenosperma pancherianum 3
100
Emmenosperma alphitonioides 3
Emmenosperma alphitonioides 1
91
64
Emmenosperma cunninghamii 1
100
Emmenosperma cunninghamii 3
100
Emmenosperma cunninghamii 2
100
Emmenosperma micropetalum
91
Jaffrea xerocarpa 2
100
Jaffrea xerocarpa 1
Jaffrea erubescens 1
0.02 substitutions per site

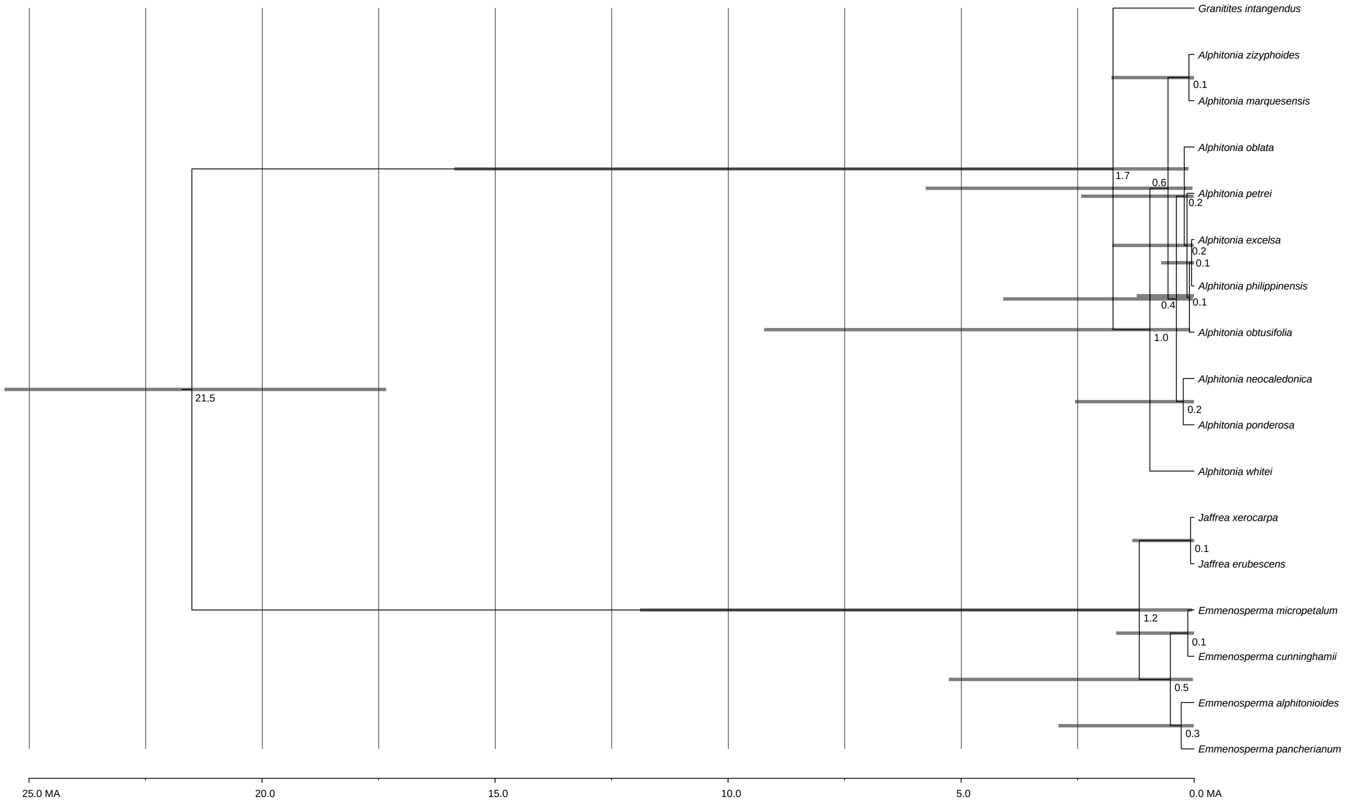
D

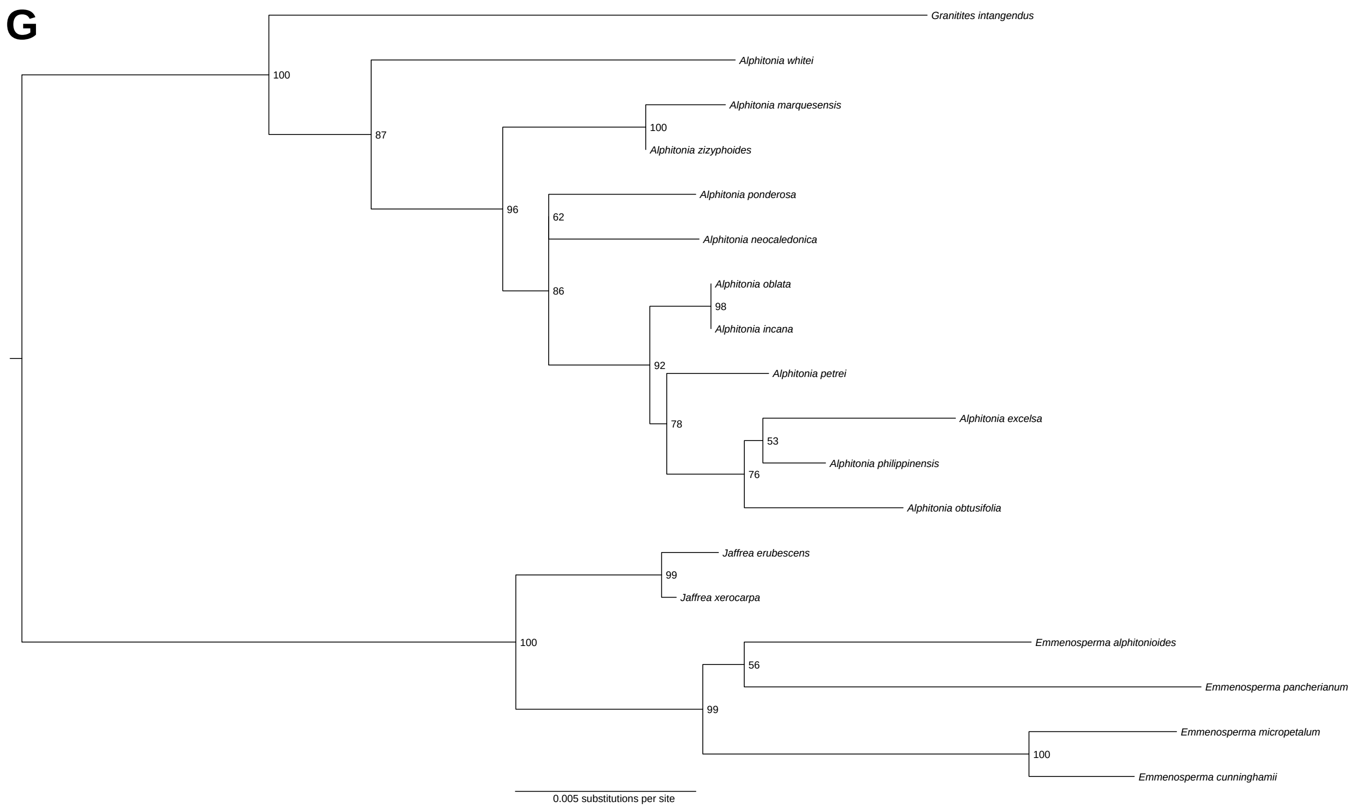

Alphitonia sp. 1
Alphitonia sp. 2
67
Alphitonia incana 2
Alphitonia oblata 2
Alphitonia incana 1
Alphitonia petrei 3
Alphitonia excelsa 4
Alphitonia petrei 1
Alphitonia obtusifolia 1
97
Alphitonia obtusifolia 2
Alphitonia pomaderroides 1
Alphitonia excelsa 5
Alphitonia excelsa 2
82
Alphitonia petrei 2
Alphitonia excelsa 3
Alphitonia excelsa 1
2
Alphitonia excelsa 6
Alphitonia pomaderroides 3
62
Alphitonia neocaledonica 3
95
Alphitonia neocaledonica 2
Alphitonia neocaledonica 1
Alphitonia zizyphoides 3
Alphitonia marquesensis 3
46
Alphitonia zizyphoides 2
Alphitonia zizyphoides 1
Alphitonia ponderosa 2
93
Alphitonia ponderosa 1
88
Alphitonia whitei 1
93
Alphitonia whitei 3
76
Alphitonia whitei 2
9
95
Alphitonia marquesensis 2
Alphitonia marquesensis 1
99
Granitites intangendus 2
48
Granitites intangendus 1
Granitites intangendus 3
Emmenosperma cunninghamii 1
29
Emmenosperma alphitonioides 2
Emmenosperma cunninghamii 4
Emmenosperma micropetalum
14
Emmenosperma cunninghamii 3
51
Emmenosperma cunninghamii 2
Emmenosperma alphitonioides 4
64
Emmenosperma alphitonioides 5
74
Emmenosperma alphitonioides 1
Emmenosperma pancherianum 1
100
Emmenosperma pancherianum 2
76
Emmenosperma pancherianum 3
Jaffrea xerocarpa 1
49
Jaffrea erubescens 2
69
Jaffrea erubescens 1
Jaffrea xerocarpa 2
0.003 substitutions per site

E


Alphitonia excelsa
0.5 MA
0.34
Alphitonia obtusifolia
0.9 MA
1.00
Alphitonia philippinensis
7.7 MA
1.00
Alphitonia oblata
3.7 MA
0.60
Alphitonia petrei
8.0 MA
1.00
Alphitonia neocaledonica
1.8 MA
0.72
Alphitonia ponderosa
13.5 MA
1.00
Alphitonia marquesensis
0.4 MA
1.00
Alphitonia zizyphoides
16.7 MA
1.00
17.4 MA
1.00
Alphitonia whitei
Granitites intangendus
20.2 MA
Emmenosperma alphitonioides
2.2 MA
0.23
Emmenosperma cunninghamii
7.3 MA
1.00
Emmenosperma micropetalum
6.0 MA
0.23
Emmenosperma pancherianum
19.0 MA
1.00
Jaffrea erubescens
3.3 MA
1.00
Jaffrea xerocarpa
3.0 Ma

F


Granitites intangendus
Alphitonia zizyphoides
0.1
Alphitonia marquesensis
Alphitonia oblata
1.7
0.6
Alphitonia petrei
0.2
Alphitonia excelsa
0.2
0.1
Alphitonia philippinensis
0.4
0.1
Alphitonia obtusifolia
1.0
Alphitonia neocaledonica
0.2
Alphitonia ponderosa
Alphitonia whitei
21.5
Jaffrea xerocarpa
0.1
Jaffrea erubescens
Emmenosperma micropetalum
1.2
0.1
Emmenosperma cunninghamii
0.5
Emmenosperma alphitonioides
0.3
Emmenosperma pancherianum
25.0 MA
20.0
15.0
10.0
5.0
0.0 MA


G
Granitites intangendus
100
Alphitonia whitei
87
Alphitonia marquesensis
100
Alphitonia zizyphoides
96
Alphitonia ponderosa
62
Alphitonia neocaledonica
86
Alphitonia oblata
98
Alphitonia incana
92
Alphitonia petrei
78
Alphitonia excelsa
53
Alphitonia philippinensis
76
Alphitonia obtusifolia
Jaffrea erubescens
99
Jaffrea xerocarpa
100
Emmenosperma alphitonioides
56
Emmenosperma pancherianum
99
Emmenosperma micropetalum
100
Emmenosperma cunninghamii
0.005 substitutions per site

Appendix 3. Taxonomic summary of *Alphitonia* and related genera. We do not suggest taxonomic changes of specific and generic circumscriptions, yet we consider a summary necessary, as multiple data bases use different concepts, and some commonly used synonyms are not in accordance with the current classification. All information is derived from previous revisions and lectotypifications (Bean, 2010; Bean, 2006; Braid, 1925; Chen & Schirarend, 2008; Fay *et al.*, 2001; Hopkins *et al.*, 2015; Hyland *et al.*, 2003; Kellermann & Thiele, 2008; Rye, 1996; Thomson & Thaman, 2008) and type vouchers.

Alphitonia* Reissek ex Endl.**, Gen. Pl.: 1098, 1840 ≡ ***Alphitonia* Reissek ex Endl. sect. *Alphitonia, Gen. Pl.: 1098, 1840. ≡ ***Alphitonia* sect. *Tomentosae* Braid**, Bull. Misc. Inform. Kew 1925: 173, 1925. Type: *Alphitonia excelsa* (Fenzl) Reissek ex Benth.≡ *Colubrina excelsa* Fenzl. Type: *Alphitonia excelsa* (Fenzl) Reissek ex Benth.≡ *Colubrina excelsa* Fenzl.

1) ***Alphitonia excelsa* (Fenzl) Reissek ex Benth.** Fl. Austral. 1: 414, 1863 ≡ *Colubrina excelsa* Fenzl in Enum. Pl. 20, 1837 ≡ *Alphitonia excelsa* (Fenzl) Reissek ex Endl., Gen. Pl.: 1098, 1840, nom. inval. ≡ *Ceanothus excelsus* (Fenzl) Steud. Nomencl. Bot. ed. 2: 313 1840.Lectotype designated by Bean 2010: Queensland, *Cunningham 21/1825* (W).

> = *Alphitonia excelsa* var. *acutifolia* Braid, Bull. Misc. Inform., Kew 1925: 177, 1925. Holotype: Queensland, *Hall 9* (K).

2) ***Alphitonia philippinensis* Braid**, Bull. Misc. Inform. Kew 1925: 183--184, 1925. Types: Philippines, Dumaquate, *Elmer 9432* (K, L, CANB, MEL), *Elmer 10335* (K, L, CANB, MEL).

3) ***Alphitonia carolinensis* Hosok.**, Trans. Nat. Hist. Soc. Taiwan 32: 288, 1942. Types: Palau, *Hosokawa 7107* (A), Carolines, *Hosokawa 6993* (L).

4) ***Alphitonia ferruginea*** **Merr. & L.M. Perry**, J. Arnold Arbor. 20: 338, 1939. Types: New Guinea, *Brass 4935* (BRI, A).

5) ***Alphitonia franguloides*** **A. Gray**, U.S. Expl. Exped., Phan. 1: 280, 1854 ≡ *Alphitonia excelsa* var. *franguloides* (A. Gray) F.M. Bailey, Compr. Cat. Queensland Pl. 837, 1913. Types: Fiji, *Wilkes Expedition s.n.* (GH, US, P, NY, K). Drawing: U.S. Expl. Exped., Phan.:Botany Plate 22, 1856.

> **=** ***Alphitonia franguloides*** **var.** ***obtusa*** **A. Gray**, U.S. Expl. Exped., Phan. 1: 280, 1854. Holotype: Tonga, *Wilkes s.n.* (GH).

6) ***Alphitonia incana*** **(Roxb.) Teijsm. & Binn. ex Kurz**, J. Bot. 11: 208, 1873 ≡ *Rhamnus incana* Roxb., Fl. Ind., ed. 1820, 2: 350, 1824. Lectotype designated by Bean 2010: [India, Kolkata - cultivated from seed from Moluccas], *Roxburgh s.n.* (BR).

> = ***Alphitonia moluccana*** **Teijsm. & Binn. ex. Braid**, Bull. Misc. Inform. Kew 1925: 184, 1925. Type collection: Types: Indonesia, Moluccas, *Teijsmann s.n.* (K, L, U).

7) ***Alphitonia macrocarpa*** **Mansf.**, Notizbl. Bot. Gart. Berlin–Dahlem 10: 222, 1928. Type collection. Papua New Guinea, New Ireland, *Peekel 945* (B, destroyed).

8) ***Alphitonia marquesensis*** **F. Br.**, Bernice P. Bishop Mus. Bull. 130: 166, 1935. Type collection: French Polynesia, Fatu Hiva, Brown 890 (K, P, NY).

9) ***Alphitonia neocaledonica*** **(Schltr.) Guillaumin**, Notul. Syst. (Paris) 2: 99, 1911 ≡ *Pomaderris neocaledonica* Schltr., Bot. Jahrb. Syst. 40: 27, 1908. Original type collection: New Caledonia, *Le Rat 322* (B, destroyed), Neotype designated by Hopkins et al. 2015: *Le Rat & Le Rat 2768* (P).

> = *Alphitonia vieillardii* Lenorm. ex Braid, Bull. Misc. Inform. Kew 1925: 179, 1925 ≡ *Alphitonia vieillardii* Lenorm. ex Guillaumin in Ann. Mus. Colon. Marseille ser. 2, 9:

121. 1911, nom. inval. Lectotype designated by Hopkins et al. 2010: New Caledonia, *Vieillard 2488* (K).

10) ***Alphitonia oblata*** **Bean**, Muelleria 28: 13—14, 2010. Holotype: Australia, Queensland, *Bean 5324* (BRI).

Alphitonia incana auct. non (Roxb.) Teijsm. & Binn ex Kurz: Hyland et al. (2003); Bean (2010).

11) ***Alphitonia petriei*** **Braid & C.T. White**, Bull. Misc. Inform. Kew 1925: 178, 1925. Lectotype designated by Bean 2010: Australia, Queensland: *Ladbrook 46* (K).

12) ***Alphitonia pomaderroides*** **(Fenzl) A.R. Bean**, Austrobaileya 7: 377. 2006 ≡ *Ziziphus pomaderroides* Fenzl, Enum. Pl. 20: 1837. Holotype: Australia, Queensland, *Bauer s.n.* (W).

= *Alphitonia obtusifolia* Braid, Bull. Misc. Inform. Kew 1925: 182, 1925 ≡ *Caenothoides obtusifolia* R. Br. ex Braid, Misc. Inform. Kew 1925: 182, 1925, nom. nud. Holotype: Australia, Queensland, *Brown* [Bennett no. 5364] (K).

= *Alphitonia obtusifolia* var. *tenuis* Braid in Bull. Misc. Inform. Kew 1925: 183, 1925. Type: Australia, Queensland, *Brown s.n.* (K).

13) ***Alphitonia ponderosa*** **Hillebr.**, Fl. Hawaiian Isl.: 81--82, 1888. Lectotype designated by St. John (1977): *Hillebrand s.n.* (BISH).

Alphitonia ponderosa var. *auwahiensis* H. St. John, Phytologia 35: 180, 1977. Holotype: United States, Hawaii, St. John 26873 (BISH).

Alphitonia ponderosa var. *costata* H. St. John, Phytologia 35: 180, 1977. Holotype: United States, Hawaii, *Oblata 76-305* (BISH).

Alphitonia ponderosa var. *grandifolia* H. St. John, Phytologia 35: 181, 1977. Holotype: United States, Hawaii, *Carlquist 1956* (BISH).

Alphitonia ponderosa var. *kauila* H. St. John, Phytologia 35: 181, 1977. Holotype: United States, Hawaii, *Degener 3344* (BISH).

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14) ***Alphitonia whitei*** **Braid**, Bull. Misc. Inform. Kew 1925: 181 1925. Lectotype designated by Bean 2010: Australia, Queensland: *Mocatta 23* (K).

= *Alphitonia excelsa* var. *franguloides* auct. non (A. Gray) F.M. Bailey: Bailey (1913).

15) ***Alphitonia zizyphoides*** **(Biehler) A. Gray**, U.S. Expl. Exped., Phan. 1: 278, 1854 ≡ *Rhamnus ziziphoides* Sol. ex Forst., Fl. Ins. Austr.: 90, 1786, nom. nud. ≡ *Rhamnus ziziphoides* Biehler, Pl. Nov. Herb. Spreng.: 15, 1807≡ *Pomaderris zizyphoides* (Biehler) Hook. & Arn., Bot. Beechey Voy. 61, 1832. Type: French Polynesia, *Forster & Sparrman s.n.* (C).

Excluded: *Alphitonia cinerascens* (Miq.) Hoogland ≡ *Berchemia cinerascens* (Blume) Blume ex Miq.; *Alphitonia emmenosperma* F.Muell. ≡ *Emmenosperma alphitonoides* F. Muell.; *Alphitonia erubescens* Baill. ≡ *Jaffrea erubescens* (Baill.) H.C. Hopkins & Pillon; *Alphitonia xerocarpa* Baill. ≡ *Jaffrea xerocarpa* (Baill.) H.C. Hopkins & Pillon; *Alphitonia xerocarpa* f. *arborea* Hürl. ex Guillaumin = *Jaffrea xerocarpa* (Baill.) H.C. Hopkins & Pillon; *Alphitonia lucida* Vieill. ex Guillaumin, nom. nud. = *Jaffrea xerocarpa* (Baill.) H.C. Hopkins & Pillon.

***Emmenosperma* F. Muell.**, Fragm. 3: 62. 1862. Type: *Emmenosperma alphitonioides* F. Muell.≡ *Alphitonia emmenosperma* F. Muell.

1) ***Emmenosperma alphitonioides* F. Muell.** in Fragm. 3: 63. 1862, as 'alphitonioides', ≡ *Alphitonia emmenosperma* F. Muell., Fragm. 3: 63. 1862, nom. inval. pro syn.. Lectotype designated by Kellermann & Thiele (2008): Australia, New South Wales, *J.S. Wilcox s.n.* (MEL).

2) ***Emmenosperma cunninghamii* Benth.,** Fl. Austral. 1: 415, 1863. Types: Australia, Western Australia, *Cunningham* (K, BM).

3) ***Emmenosperma micropetalum* (A.C. Sm.) M.C. Johnst.**, Brittonia 23: 50, 1971 ≡ *Colubrina micropetala* A.C. Sm., J. Arnold Arbor. 31:302—303, 1950. Holotype: Fiji, Vanua Levu, *Smith 6736* (A).

4) ***Emmenosperma pancherianum* Baill.**, Adansonia 11: 269, 1874. Lectotype designated by Bean (2013): New Caledonia, *Pancher s.n.* (P).

5) ***Emmenosperma papuanum* (Merr. & L.M. Perry) M.C. Johnst.**, Brittonia 23: 50, 1971 ≡ *Colubrina papuana* Merr. & L.M. Perry, J. Arnold Arbor. 22: 264, 1941. Holotype: Indonesia, Papua, *Brass 8986* (A).

***Granitites* Rye**, Nuytsia 10: 451--452, 1996. Type: *Granitites intangendus* (F. Muell.) Rye ≡ *Pomaderris intangenda* F. Muell.

1) ***Granitites intangendus* (F. Muell.) Rye** in Nuytsia 10: 451--452, 1996 ≡ *Pomaderris intangendus* F. Muell. in Fragm. 10: 52, 1876. Lectotype designated by Rye 1996: Australia, Western Australia, *Dempster s.n.* (MEL).

> **= *Cryptandra petraea* S. Moore**, J. Linn. Soc., Bot 34: 184 (1899). Holotype: Western Australia, *S. Moore s.n. June 1895* (BM).

Jaffrea **H.C. Hopkins & Pillon**, Kew. Bull. 70: 15, 2015. Type: *Jaffrea xerocarpa* (Baill.) H.C. Hopkins & Pillon ≡ *Alphitonia xerocarpa* Baill.

1) ***Jaffrea xerocarpa*** **(Baill.) H.C. Hopkins & Pillon**, Kew. Bull. 70: 15, 2015 ≡ *Alphitonia xerocarpa* Baill., Adansonia 11: 270, 1874. Lectotype designated by Hopkins et al. 2015: New Caledonia, *Pancher Herb. Mus. Néocal. No. 608* (P).

= *Alphitonia xerocarpa* f. *arborea* Hürl. ex Guillaumin, Mém. Mus. Natl. Hist. Nat. B, Bot. 15: 1 – 93, 1964. Type: New Caledonia, *Hürlimann 1572* (P).

= *Alphitonia lucida* Vieill. ex Guillaumin, Ann. Mus. Colon. Marseille, sér. 2, 9: 77 – 290, 1911, nom. nud.

2) ***Jaffrea erubescens*** **(Baill.) H.C. Hopkins & Pillon**, Kew. Bull. 70: 15, 2015 ≡ *Alphitonia erubescens* Baill., Adansonia 11: 271, 1874. Lectotype designated by Hopkins et al. 2015: New Caledonia, *Balansa 3491* (P).

GENERAL CONCLUSIONS

In my thesis, I provided a major contribution towards understanding the evolutionary processes that led to the extant biodiversity in buckthorns (Rhamnaceae Juss.). In a first step, I provided a substantial taxonomic and phylogenetic framework and improved our understanding of species diversity and lineage diversity within the group. In a second step, I unravelled the biogeographic history of the ziziphoid lineages, highlighting the contributions of Gondwanan vicariance and long-distance dispersal (LDD). Furthermore, I highlighted the information gained from the fossil record beyond its age, as for example the incorporation of fossil localities into the discussion in chapter three. Last but not least, I illustrated that the younger a taxon might be, the potential of human impact may be of interest, as shown in chapter 4.

Phylogenetic reconstructions and taxonomic revisions - This study focused on one aspect of biodiversity: diversity within phylogenetic lineages. Hence, before I assessed the biodiversity within Rhamnaceae, I collected taxonomic, distributional, fossil, and genetic data. Then, I performed robust phylogenetic analyses and revisions on the largest molecular data set to date in chapter 1 and 2 (Hauenschild et al., 2016a; Hauenschild et al., 2016b, 2016c). Both studies provided the basic foundation for chapters 3 and 4 to stand upon. This taxonomic foundation was robust concerning my follow-up analyses, yet it was neither complete, nor carved in stone. In my approach, I combined molecular data of every major lineage, evenly represented within those lineages, taxonomic framework provided by type specimens and literature, and morphology. The necessity for complete (in terms of extant lineages) and evenness within the groups was shown in multiple previous studies, which reanalysed molecular genetic phylogenies with a data set that meets those conditions (Favre et al., 2016; Renner and Schaefer, 2016). This evenly distributed sampling approach and the hereby generated phylogenetic reconstructions led to the split of the polyphyletic genus *Ziziphus* Mill. into *Ziziphus* s.s., the resurrected *Sarcomphalus* P. Browne, and the new genus *Pseudoziziphus* Hauenschild. This revision was of special importance when it came to place fossil constraints, as there were multiple fossil taxa of †*Ziziphus*, and the hereby polyphyletic tribe Paliureae Reissek ex Endl. As highlighted in my thesis, a correct placement of topological constraints was vital, and *Ziziphus*, before my studies, as well as tribe Paliureae sensu Richardson 2000, were not suitable for setting fossil constraints. Similarly, the resurrection of *Atadinus* Raf. and *Endotropis* Raf., and the corresponding delimitation of *Frangula* Mill. and *Rhamnus* L. provided not only a clearer taxonomic framework for extant taxa, but also helped to provide correct fossil attributions in this group, as already done for †*Atadinus*, †*Endotropis* and †*Frangula* (Doweld, 2017). Yet, a few genera were not present in the phylogeny, such as the monotypic *Alvimiantha* Grey-Wilson and *Smythea* Seem. Ex A. Gray. Furthermore, a section of the still polyphyletic genus *Ziziphus* was highlighted as waiting to be revised (Hauenschild et al., *submitted;* Islam and Guralnick, 2015; Islam and Simmons, 2006). This comprised the African *Z. pubescens* Oliv. and related species, which were phylogenetically related to *Bathiorhamnus* Capuron. The latter ampelozizyphoid genus is endemic to Madagascar and comprises a handful species, of which only a few were sequenced so far (Callmander et al., 2008). However, it was vital to include most *Bathiorhamnus* and *Ziziphus* species into a robust phylogeny to assess if *Z. pubescens* s.l. should be attributed to a new genus or incorporated in *Bathiorhamnus*. A good example for the dynamics in botanical nomenclature was given by the newly described *Pseudoziziphus* (Hauenschild et al., 2016a; Judd and Hall, 1984). I provided arguments for a new genus sister to *Condalia* Cav. to avoid conflicts with the current circumscription of the latter genus. However, as the phylogeny would allow *Pseudoziziphus* as a genus as well as a section within *Condalia*, a re-ranking might seem appropriate or not, when including all species of *Condalia,* as raised by Walter Judd (personal communication). Most important however remained the attribution of *Pseudoziziphus* to tribe Rhamneae Hook f. within the rhamnoid lineage sister to *Condalia* instead of the former placement within genus *Ziziphus* in the ziziphoid lineage sister to *Paliurus* Mill.

Temporal reconstructions - Now that a robust phylogeny was reconstructed I included all suitable genetic markers available from GenBank for the spatio-temporal analyses. This resulted again in the largest molecular data set of Rhamnaceae and close allies to date. By this approach, I provided reliable age estimates for all major lineages, tribes and genera (except the monotypic *Alvimiantha* Grey-Wilson and *Smythea* Seem. Ex A. Gray) in Rhamnaceae. In general, my reconstructions were in accordance with the results from Renske Onstein and colleagues (Onstein et al., 2015; Onstein and Linder, 2016), who worked on a smaller data set and different questions, yet with minor alterations. Those differences arose from several points: while the working group around Renske Onstein relied on the existing genetic data, combined with further addition of African samples (tribe Phyliceae), I increased the taxonomic sampling. Tribes Rhamneae and Ventilagineae were drastically undersampled in previous studies: Less than 10% of all species of the rhamnoids were covered by those studies, although they made up for one third of all buckthorns. In contrast, the ziziphoids were represented by more than 50% of the accepted species taxa. A re-balancing in taxon sampling might have had an effect on its own, more relevant, however, was the fact that a more complete rhamnoid group allowed for more precise fossil-constraints on nodes within this part of the tree. In total, my reconstructed ages were slightly older, as represented by the mean crown ages of the buckthorn family. While I reconstructed a mean age of 101.6 Ma, Onstein et al. (2015) reconstructed 93 Ma. When the 95% HPD intervals were considered, I reconstructed 102.6 Ma, compared to 101 Ma (Onstein et al., 2015). As both studies relied on data on the one hand, but also used fossil data and unique methods exclusively on the other hand, the high concordance of node ages added further support to the recovered ages. Furthermore, the slight differences in particular node ages were nullified when it comes to biogeographic reconstructions, because the set time-slices were broader, i.e. exceeding the differences (Hauenschild et al., *submitted*). Consequently, I addressed the issue of the age of the buckthorns, presenting a temporal origin around the Albian-Cenomanian boundary, which coincided with the age of †*Ziziphus* sp., the oldest leaf fossil related to Rhamaceae (Spicer et al., 2002). The spatial origin of the buckthorns, however, could not yet be sufficiently solved. Yet, my reconstructions did point to a Northern Hemisphere origin, which is also supported by the locations of the oldest Rhamnaceae-related fossils in Siberia and the United States (Basinger and Dilcher, 1984; Spicer et al., 2002), but the ziziphoid lineage (two thirds of extant Rhamnaceae species) originated from the South, i.e. Africa and South America.

Ancestral area reconstructions and biogeographic scenarios - When it came to the spatial origin of lineages within Rhamnaceae, I could unravel major parts of the biogeographic history of the ziziphoids and the role of the Gondwanan break-up in this group (chapter 3). Furthermore, the biogeographic history of *Alphitonia* s.l., a species poor lineage within the ziziphoids was clarified (chapter 4). As already stated, the ziziphoid lineage originated from the Cretaceous landmasses of South America, Africa, and potentially Madagascar and India, which at this time were partly connected. This finding was backed up by the oldest ziziphoid fossils, such as the South American †*Archaeopaliurus boyacensis* Correa, Manchester, Jaramillo & Gutierrez, as well as the Indian *Ziziphus* sp. and *Paliurus* sp (Chen et al., 2017; Correa et al., 2010; Singh et al., 2010). Also, I highlighted a vicariance event during the Early Phanerozoic: The most recent common ancestors of *Ziziphus* s.s., *Paliurus*, *Hovenia* Thunb. and tribe Gouanieae Reissek ex Endl. inhabited the northwards drifting Gondwanan fragments Africa and India and subsequently colonized the Northern hemisphere, Africa, India and Madagascar. This finding was also backed up by the fossil record, as Mid Cenozoic fossil taxa related to this group were found exclusively on the Northern hemisphere, India and Africa, such as †*Hovenia*, †*Ziziphus* and †*Paliurus* (Burge and Manchester, 2008; Li et al., 2014; Wheeler and Meyer, 2012). Extant species of *Paliurus* and *Ziziphus* s.s. are found throughout the Old World, and tribe Gouanieae is distributed throughout the tropics. Both range expansions were younger LDD events that obscured the older vicariance events. Similar results, i.e. the obscuring of older vicariance events by younger LDD events were highlighted in other groups, such as Boraginales and ferns (Le Péchon et al., 2016; Luebert et al., 2017). Besides those two lineages, the majority of the remaining ziziphoid lineages were reconstructed to have their most recent common ancestors on the remaining southern Gondwanan fragments, such as Australia, South America and Zealandia. In chapter 3, I highlighted that Cenozoic LDD events, not Gondwanan vicariance events, shaped the distinct distributions of *Alphitonia* s.l. and tribes Colletieae Reissek ex Endl., Phyliceae Reissek ex Endl., and Pomaderreae Reissek ex Endl.. This finding was vital, as disjunct distributional patterns in closely related taxa on former Gondwanan fragments, such as Colletieae being present in South America (and one species in North America), Australia, and New Zealand, were often taken as an indication of Gondwanan vicariance. In fact, as tribe Phyliceae is limited to Africa and the Antarctic Islands, and tribe Pomaderreae, as well as *Alphitonia* (s.l.) were overall found in Australia and the Pacific, Gondwanan vicariance would have been easy to justify. The integrative approach, however, incorporated ancestral age estimations, and the divergence times estimates were not at all fitting to events of continental drift. Hence, all those seemingly Gondwanan vicariance patterns had to be the result of Mid to Late Cenozoic LDD events. In chapter four, I illustrated the potential of small studies tackling lineages of limited species numbers, areas, and epochs. The case study revealed that the most recent common ancestor of *Alphitonia* s.l. occurred on Australia and migrated on the Australia plate northwards. Only during the Pliocene, Pleistocene and Holocene, speciation resulting in extant species took place, and the range of the group was continuously extended towards the West into Southeast Asia and the East into the Pacific Islands. Only the dispersal of *A. ponderosa* Hillebr. (and/or its ancestors) remained obscured, as early humans might have played a role in establishing the species on the distant islands of Hawaii and the Marshall Islands.

Both studies also provided a contribution towards understanding the impact of the Gondwanan break-up on plants and the subsequent dispersal of the descendants. I highlighted three major issues: 1) if vicariance is directly excluded by the age of the group, attacking the question if vicariance or long distance dispersal (LDD) was a major driver of a group, is not expedient. Often, vicariance was rejected by recovering ages too young to fit with the timing of break-up events (Beaulieu et al., 2013; Nauheimer et al., 2012). Hence, vicariance cannot be tested by biogeographic analyses. I highlighted that it was vital to use sufficiently old groups as model taxa if the contribution of vicariance was to be investigated. Second, 2) a seemingly fitting extant distribution of a taxon to Gondwanan vicariance, such as disjunct distributions of closely related taxa on Gondwanan fragments, was shown to be a rather bad proxy for an ideal study group. Vicariance events from the Cretaceous and Paleogene were shown to be obscured by younger LDD events, which is hardly surprising, as the taxa had more than 20 million years to disperse, migrate, expand and shrink their distributions in a climatically changing environment throughout the Neogene and Quaternary. Besides my study, and the previously mentioned study within in phylogenetic groups, this obscuring of vicariance pattern can also illustrated on entire plant communities (Carlucci et al., 2017). Third, 3) I highlighted that once an Gondwanan origin of a group was shown (*Alphitonia* s.l., cf. chapter), certain questions became irrelevant, such as the westward or eastward migration through Wallacea, as this is *a priori* defined by the ancestors. If the ancestors were distributed exclusively on the isolated Australian plate in the Paleogene, and are the extant taxa are nowadays found throughout Southeast Asia, a westward expansion is conditional. Hence we could focus on details, such as dispersal scenarios and the impact of early human migrations.

Prospects (Rhamnaceae) – Future projects within the buckthorns include biogeographic reconstructions within the rhamnoid lineage. It comprises about 300 species and three tribes: the species rich Rhamneae, the moderately sized Ventilagineae Hook. f., and the monotypic Maesopsideae Weberb. Within Rhamneae, we observed high taxonomic diversity surrounding the Qinghai-Tibet-Plateau and Eastern Asia (*Rhamnus* sect. *Rhamnus* L.) and the Central American mountain ranges (*Frangula* Mill.), yet only a few species were described within the closely related genera *Endotropis* Raf. in and around the Rocky Mountains and *Atadinus* Raf. from the European Alps to the Caucasus. It should be tested, whether diversification rates vary within the rhamnoids, and especially within tribe Rhamneae, as expected. If this hypothesis was supported, I would investigate why and how some mountain systems and surrounding areas contained more species and the occurring lineages experienced higher diversification rates than others. Potential methods attacking these questions are BAMM and BayesRate analyses, which I already used for *Allium* L. (Hauenschild et al., 2017). Similar questions were answered in other groups, such as rapidly radiations clades within the large genus *Saxifraga* L., and its relation to their geographic distributions and morphological traits (Ebersbach et al., 2017). Concerning the biogeographic history, I proposed a different hypothesis than in the ziziphoid lineages. Most Mesozoic and Early Cenozoic fossil taxa attributed to the rhamnoids were found throughout the Northern Hemisphere or Northern South America, such as Colombia (Correa et al., 2010; MacGinitie, 1953; Manchester, 2001; Peppe et al., 2007; Rueffle and Trostheide, 2000). Furthermore, even in the predominantly African, Australian, Pacific and Southeast Asian tribe Ventilagineae the oldest fossils, i.e. distinct samaras of the *Ventilago*-type, were found in Miocene China (Liu et al., 2015) and Maastrichtian Columbia, in the same site as other rhamnoid taxa (Calvillo-Canadell and Cevallos-Ferriz, 2007). Hence, I hypothesised a Northern Hemisphere origin of the rhamnoid lineage, which has to be tested by using the framework provided in chapter three. Besides this, the addressed remaining taxonomic questions, such as the status of *Ziziphus pubescens*, should be investigated. Last but not least, the case study of *Alphitonia* s.l. highlighted the need to adjust the methods, i.e. implementing methods from population genetics and high-throughput sequencing. Now that I provided a robust framework for the entire ziziphoid lineage, its tribes and genera, the applied biogeographic methods hit their capacity when it came to details on species level. However, it was this level with a single species that caused speciation and will cause speciation in the future. The application of high-throughput sequencing methods in small groups, such as done in *Ziziphus* (Huang et al., 2017), or even within a species provide new opportunities for understanding the evolutionary processes within a taxon that caused its extant distribution and biodiversity.

Prospects beyond the buckthorns – To achieve a better understanding of the evolutionary and biogeographic processes that shaped the extant biodiversity on earth, multiple approaches are possible, thinkable, attacked, and vital. For example, further studies related to the impact of the Gondwanan break-up could tackle multiple issues in order to unravel more general scenarios. Naturally, further case studies in other taxa directly experiencing vicariance are of course advantageous, but this is by far not the only possible way of how to proceed. Vicariance was not limited to the large scale continental drifts of the Mesozoic and beyond, its acting and functionality could also be investigated on a small scale within species or populations. For me, it was advantageous and helpful to go from big to small, i.e.

from the family, to a larger group within that family, to a genus, or species and populations. But, attacking evolutionary processes from small to big is likewise vital. Similarly, the natural incorporation of multiple case studies into a big meta analyses is as vital as integrating findings of meta studies into new or revised case studies. Yet, as big as the impact of vicariance may be on certain taxa, it cannot account for the majority of species diversity on earth. Cretaceous vicariance events could explain divergence in the backbone of higher rank phylogenies (i.e. inter and intra family level), but if a lineage comprises thousands of extant species, or only one, cannot be explained, in most cases, by vicariance (Carlucci et al., 2017; McGlone, 2005). As shown in Appendix 1 for *Allium* L. (Hauenschild et al., 2017) and by a plethora of other studies in multiple taxa, younger events, often related to Quaternary climate oscillations and/or the uplift of mountains, may have acted as major drivers for the differences in biodiversity observed today (Ebersbach et al., 2017; Favre et al., 2015; Hoorn et al., 2013; Mosbrugger et al., 2018). With the onset of advanced diversification rate analyses during the last decade (Silvestro et al., 2011), scientists were eager to test why some species-rich lineages radiated much faster than other closely related lineages, and to identify the one, or multiple triggers for rapidly increased net diversification (Bacon et al., 2013; Ebersbach et al., 2017; Onstein and Linder, 2016; Schwery et al., 2015). Unravelling the early history of those taxa and their close relatives, i.e. the times prior to the increased diversification, might show differences in ancestral distribution. This may shed light on the ancestral distributions of the fast radiating taxon compared to its close relatives that did not show increased net diversification rates. Because in the end, it was the biogeographic history that causes the precondition.

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APPENDIX

Appendix 1: Spatio-temporal evolution of *Allium* L. in the Qinghai-Tibet-Plateau region: Immigration and in situ radiation.

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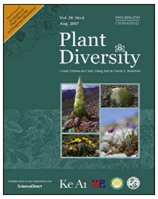

Spatio-temporal evolution of *Allium* L. in the Qinghai–Tibet-Plateau region: Immigration and *in situ* radiation

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ABSTRACT

A plethora of studies investigating the origin and evolution of diverse mountain taxa has assumed a causal link between geological processes (orogenesis) and a biological response (diversification). Yet, a substantial delay (up to 30 Myr) between the start of orogenesis and diversification is often observed. Evolutionary biologists should therefore identify alternative drivers of diversification and maintenance of biodiversity in mountain systems. Using phylogenetic, biogeographic, and diversification rate analyses, we could identify two independent processes that most likely explain the diversity of the widespread genus *Allium* in the Qinghai–Tibet Plateau (QTP) region: (1) While the QTP-related taxa of the subgenus *Melanocrommyum* diversified *in situ*, (2) QTP-related taxa of other subgenera migrated into the QTP from multiple source areas. Furthermore, shifts in diversification rates within *Allium* could not be attributed spatially and temporally to the uplift history of the QTP region. Instead, global cooling and climate oscillations in the Quaternary were major contributors to increased speciation rates in three clades of *Allium*. Our study therefore adds to the growing evidence supporting the "mountain-geo-biodiversity hypothesis", which highlights the role of climate oscillations for the diversification of mountain organisms.

1. Introduction

Why are some regions more species-rich than others? This question has been at the focus of many studies over the past few decades (e.g. Antonelli and Sanmartín, 2011; Barthlott et al., 1996; Condamine et al., 2012; Favre et al., 2015; Graham et al., 2014; Tang et al., 2006). Besides the tropics, mountain systems have been found to be especially diverse, as reflected by the global distribution of species richness of vascular plants (Barthlott et al., 2005). Furthermore, a large proportion of terrestrial centres of diversity is associated with mountain systems (Fjeldså et al., 2012; Hughes and Atchison, 2015). Some have argued that orogenesis could have facilitated the establishment of high levels of biodiversity by providing a diversity of ecological opportunities on a remarkably small geographic scale (Dufour et al., 2006; Hoorn et al., 2013; Linder, 2008; Silvestro and Schnitzler, 2018). In addition, the "mountain geo-biodiversity hypothesis" by Mosbrugger et al. (2018), developed based on research in the QTP and for subtropical mountain systems, highlights the potential importance of the interaction between climatic and geological settings. This hypothesis suggests that, as a pre-requisite for diversification, surface uplift should have created full elevational zonation (from tropical to nival thermal belts), providing both refugia for the persistence of lineages during climate modifications as well as geographic barriers promoting allopatric speciation. Simultaneously to, or following, the uplift, mountains should have acted as "species pumps" (*sensu* Haffer, 1969) during the glacial cycles of the Pliocene–Pleistocene (Ehlers and Gibbard, 2004; Kaufman and Manley, 2004). Thus, a delay between the

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initial uplift and the biological response (diversification) might be observed, particularly in old mountain systems such as the Qinghai–Tibet Plateau (QTP) and others, as reviewed by Hughes and Atchison (2015).

For the QTP, the highest and largest plateau on Earth (Wang et al., 2014) resulting from at least 50 Myr of continuous orogenesis (Favre et al., 2015; Renner, 2016), several studies have uncovered shifts in net diversification rates of plant groups during the last few million years (Myr) (e.g. Ebersbach et al., 2016; Favre et al., 2016; Xing and Ree, 2017). These recent radiations have led to a few plant genera (e.g. *Gentiana*, *Saxifraga*, *Corydalis*, and *Saussurea*) being particularly well-represented in the alpine subnival belt flora of the Hengduanshan (Xu et al., 2014). More generally, the mountains surrounding the QTP (the Tianshan, the Himalayas, and the Hengduanshan) are particularly species-rich. Together with the relatively species-poor QTP interior, they harbour over 12,000 vascular plant species (Zhang et al., 2016 and references therein). Favre et al. (2015) highlighted the importance of studying widespread groups, instead of only endemic taxa, to compare the evolutionary patterns between the region of interest and other mountain systems or ranges. Furthermore, the prevalence of biological interchange should be investigated, in order to better understand whether the QTP acted as a sink or a source area for plants. In the present study, we evaluate the “mountain-geo-biodiversity hypothesis” using the evolutionary and biogeographic history of the genus *Allium* L. (Amaryllidaceae), a taxon occurring in several mountain systems around the world.

Allium L. is one of the largest currently recognised monocotyledon genera, comprising at least 660 temperate and subtropical species (Choi and Oh, 2011; Govaerts et al., 2016; Gregory et al., 1998; Li et al., 2010; Xu and Kamelin, 2000), of which at least 111 occur within or surrounding the QTP. The genus is distributed across the Northern Hemisphere with a few exceptions (e.g. eastern and southern Africa), mainly in regions that are seasonally dry, such as the Irano-Turanian region (Choi and Oh, 2011; Govaerts et al., 2016; Xu and Kamelin, 2000). Its main centre of diversity is located between Southwest and Central Asia and the Mediterranean region, which is also supposed to be the major centre of diversification of *Allium*, besides a second one existing in North America (Choi and Oh, 2011). The genus is the only member of the monotypic tribe Allieae (Borkh.) Dumort., within the subfamily Allioideae Engl. (Amaryllidaceae J. St.-Hil.) (Chase et al., 2009). *Allium* is characterised by bulbs enclosed in membranous (sometimes fibrous) tunics, free or almost free tepals, and often a subgynobasic style (Friesen et al., 2006). Most species produce remarkable amounts of cysteine sulphoxides, causing the specific smell and taste of onion and garlic (Friesen et al., 2006). The taxonomy of *Allium* was revised in 2006 and 2010, based on morphological characters and *rbcL* sequence data (Friesen et al., 2006; Li et al., 2010). Nonetheless, a proliferation of synonyms and disagreement as to the subdivision of the genus still persists (Choi and Oh, 2011; Herden et al., 2016; Li et al., 2016; Sennikov and Seregin, 2015). For example, although there is a phylogenetic clustering of all five groups, the individual delineations of the subgenera *Allium*, *Cepa* (Mill.) Radić, *Polyprason* Radić, *Reticulatobulbosa* (Kamelin) N. Friesen, and *Rhizirideum* (G. Don ex Koch) Wendelbo are lacking phylogenetic support, or are shown to be polyphyletic (Li et al., 2010). On species level, the World Checklist of *Allium* (Govaerts et al., 2016) provides the most recent taxonomic account of the genus. Friesen et al. (2006) detected three distinct phylogenetic lineages in *Allium*, which were named: “First, Second, and Third Evolutionary Line” (EL1, EL2, EL3). It was proposed in 2011 to split the genus into three separate genera based upon these phylogenetic lineages (Banfi et al., 2011). The proposed names are *Nectaroscordum* Lindl. for EL1, *Caloscordum* Herb. for EL2, and *Allium* for EL3, but this new nomenclature has not yet been broadly adopted.

Here we present the most comprehensive molecular data set on *Allium* to date, including species consensus sequences that underwent multiple stages of quality control. Because the application of the correct taxonomy based upon a solid phylogenetic hypothesis is crucial to this study, we first ask (1) Are the current circumscriptions of the evolutionary lineages and subgenera supported by our phylogeny? Subsequently, we use the reconstructed phylogeny, combined with molecular dating, biogeographic analyses, and diversification rates estimation, to answer two further questions: (2) Did *Allium* species in the areas adjacent to the QTP evolve *in situ*, or did they disperse there, and if so, when and from where? and (3) Did shifts of diversification rates occur simultaneously in the QTP and other old mountains, as expected following the “mountain-geo-biodiversity hypothesis”?

2. Materials and methods

2.1. Species coverage and sequence alignments

We downloaded all 4842 *Allium* sequences available from GenBank (accessed in May 2015; Benson et al., 2013), as well as 23 outgroup sequences from the most closely related genera within Alloideae (*Leucocoryne* Lindl., *Nothoscordum* Kunth, *Tristagma* Poepp., and *Tulbaghia* L.) and one more distantly related species, *Dichelostema multiflorum* (Benth.) A. Heller (Chen et al., 2013). Sequences belonging to different molecular regions were aligned individually in Geneious 6.1.6 (Kearse et al., 2012). To take into account possible multiple accessions per species, we proceeded as follows: In a first step, all sequences from each marker with identical organism name (operational taxonomic unit: OTU) were grouped and automatically aligned with MAFFT ver. 7.221 (Katoh and Standley, 2013). In case of markers for which only partial sequences were provided (e.g. ITS, *mat*K), we preferably used complete sequences as a guide to align the smaller fragments. If no complete sequence was available, we concatenated the sequences of the individual parts. The concatenated complete sequences were then aligned using the “auto” option. Fragments were added to these single species alignments with the option “–addfragments”. Some sequences on GenBank were provided as reverse (R), reverse complement (RC) or as complement (C), meaning each marker could potentially be provided as one of four options (incl. the usual forward option F). We always used the “–adjustdirection” option in MAFFT, which tests for each sequence if the reverse complement fits the alignment better, thereby accounting for F and RC options. In addition, for all sequences with a distance larger than 0.1 to the other sequences (within species), we tested a reverse alignment. As this step again uses the “–adjustdirection” option, all four possible options (F, R, RC, C) were covered (F and RC from within MAFFT, and R and C through this additional step). In case this test failed and pairwise distances larger than 0.1 could not be resolved (e.g. reverse complementary fragments or mis-determinations), we excluded the data for this taxon and marker to account for mislabelled or otherwise erroneous data. For all remaining taxa (i.e. single marker alignments with within species distances below 0.1), a consensus sequence was generated for each marker per species, which was then used for the following steps.

If for a given marker at least 40 species were present, all consensus sequences of this marker were aligned in the same fashion as explained above. After a visual check of the individual alignments, they were concatenated and plastid and nuclear partitions were defined. Sequences with lengths shorter than 10 percent of the longest unaligned sequence were removed from the multi-species alignment (MSA) of the markers ITS, *atp*B–*rbc*L, *rbc*L, *mat*K, *psb*A–*trn*H, *rps*16, *trn*L–*trn*F, and *trn*L–*rpl*32, of which the regions ITS, *trn*L–*trn*F, and *trn*L–*rpl*32 were represented by more than 50% of the taxa included. Our final MSA comprised 352 out of 1082 accepted *Allium* species (Govaerts et al., 2016), representing all accepted major lineages and subgenera.

2.2. Phylogenetic analyses and molecular dating

All phylogenetic analyses estimated parameters independently for the nuclear and plastid partitions. To test the monophyly of the ingroup for the subsequent molecular dating analysis and to identify potential problematic long branches, we conducted Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. We produced a best scoring maximum likelihood tree (from 70 ML searches), based on the concatenated data and the partition table using RAxML v. 8.2.8 (Stamatakis, 2014) under the GTRGAMMA substitution model. Node support was calculated by a Bootstrap analysis with 350 iterations determined by the "bootstopping" algorithm (Pattengale et al., 2010) during the analysis. We performed Bayesian phylogenetic inference using a Metropolis-coupled Markov Chain Monte Carlo (MC^3) approach as implemented in MrBayes v. 3.2.5 (Ronquist et al., 2012). Four independent analyses were run for 50 million generations with four incrementally heated chains, sampling from the posterior probability distribution every 5000th generation. In addition, we used the model-jumping approach to sample all possible 203 substitution models according to their posterior probability (Ronquist et al., 2012) in combination with a gamma model of rate heterogeneity. After confirming that the four independent runs converged on the same solution space judged by effective sample size (ESS) values well above 200 and homogeneous "white noise"-like traces of both the individual runs and the combined log, a majority-rule consensus tree was constructed from the combined runs (burn-in: 36%, as indicated by ESS values). Furthermore, we compared the reconstructed phylogeny of a simple concatenated non-partitioned matrix with the one of a partitioned (plastid/nuclear) matrix to visualize potential alternative topologies in *Allium* (Appendix 1). Major topological incongruencies only appeared in the Third Evolutionary Line, and did not affect our analyses. A likelihood-ratio test rejected the null hypothesis of clock-like evolution of the analysed sequences ($p < 0.001$).

Divergence times were therefore estimated under an uncorrelated lognormal relaxed clock, using BEAST v.1.8.2 (Drummond et al., 2012), based on a data set from which we removed the markers with the highest proportions of missing data (*atp*B–*rbc*L, *rbc*L, *mat*K, *psb*A–*trn*H, *rps*16), as these showed to impede MCMC convergence in preliminary analyses. In the absence of known fossils of *Allium* or closely related genera (Smith, 2013), we used two secondary calibration approaches on *Allium*'s root node (i.e. crown node of Allioideae) from a recently published chronogram of Amaryllidaceae (Chen et al., 2013), defining (A) a rather narrow exponential prior distribution around their suggested age of 37 Myr (spanning from 37.0 at the 2.5% quantile to 37.4 at the 97.5% quantile with setting the offset to 37.0 and the mean to 0.1) and (B) a normal prior distribution spanning the age range Chen et al. (2013) found (27.8–44.5 Myr), with a mean of 37 Myr and a standard deviation of 4.5 Myr to tailor the distribution's 2.5% and 97.5% quantiles to 28.2 Myr and 45.8 Myr, respectively. Both analyses were run four times, sampling every 10,000th of the 50 million MCMC generations in (A) and 100 million MCMC generations in (B). ESS values well above 200 for the combined log after removing varying proportions (10–25%) of burn-in from individual runs confirmed that convergence was reached. Therefore, we combined the tree files of the individual runs each in (A) and (B) and afterwards produced a maximum clade credibility (MCC) tree with common ancestor heights as described by Heled and Bouckaert (2013) using LogCombiner and TreeAnnotator (both v. 1.8.3) (Bouckaert et al., 2014). The alignments and consensus/MCC trees are available from the corresponding author upon request.

2.3. Diversification rates

We used BAMM (Rabosky, 2014), including the R package BAMMtools (Rabosky et al., 2014), to assess the diversification rate heterogeneity within *Allium*. We reduced the MCC tree to only one OTU per species (removing all subspecies and varieties), and specified lineage-specific sampling fractions to account for incomplete taxon sampling. We ran four MCMC chains (15 million generations, every 5000th generation sampled, 10% burn-in) to identify distinct configurations of rate shifts. However, some limitations of BAMM have recently been identified (Moore et al., 2016), including strong prior sensitivity and potentially unreliable rate estimates (but see Rabosky et al., 2017). Thus, we additionally used BayesRate (v.1.6.5, Silvestro et al., 2011) to evaluate the different diversification scenarios. Importantly, BayesRate employs a likelihood function (Nee et al., 1994) different from the one implemented in BAMM (Maddison et al., 2007), and thus provides an independent evaluation of the diversification model (number of rate shifts) and the associated rates. We compared the most likely scenario identified by BAMM with both simpler and more complex models of diversification (i.e. fewer or more rate shifts) via thermodynamic integration and estimated model parameters for the best-fit model, again accounting for incomplete taxon sampling.

2.4. Biogeographic analysis

To estimate ancestral geographic distributions we performed biogeographic analyses using the R (R Core Team, 2015) package BioGeoBEARS (Matzke, 2014) under the DEC model (Ree, 2005; Ree and Smith, 2008) and a likelihood version of the DIVA (Ronquist, 1997) model with or without the inclusion of a jump dispersal (i.e. founder event) parameter (Matzke, 2014). The maximum combination of areas parameter was set to 7. We did not set time strata, manual dispersal multipliers, and area restrictions, to avoid sharp temporal borders on a secondarily calibrated reconstruction. The best model for the data set among those four was chosen based on AIC values. Eleven geographic areas were defined with a more fine-scale delineation for the QTP and the regions neighbouring it. These were delineated using their ecological attributes (climate, topography, etc.), while other regions (continental scale) were coded according to their interconnectivity. In detail, the continental regions were coded as follows: A: NA: North America, B: EUR: Europe *s.l.* (eastern borders being the Caucasus mountains and Ural mountains), and C: AFR: Africa. The Asian continent was delimited as follows: D: WIT: Western Irano-Turanian region, E: AA: Arctic Asia, (East of the Ural and North of the southern Siberian mountain chains), F: QTP: QTP *s.l.* (Qinghai–Tibet Plateau, Tian Shan, Hengduanshan, and the Himalayas). G: NEA: Northeast-Asian steppes (surrounded by Area AA in the North, QTP in the West; the border follows the Han River and further the Yangtze River in the South), H: JAP: Japan and Sakhalin. The rest of the Asian land (surrounded by region WIT in the West, QTP in the North, the Pacific Ocean in the East, and the Wallace Line in the South) is separated by both, seasonality and elevation, resulting in I: SUBA: Subtropical Asia and J: TROPA: Tropical Asia (Appendix 2).

Extant species' distributions were based on more than 2.7 million individual occurrence points from the literature and herbarium vouchers. In taxa for which this procedure did not provide data, we added more coarse distribution information from the World Checklist of selected Plant Families (Govaerts et al., 2016). In order to match the taxonomic units in the distribution data to those in the chronogram, we used the synonymy list from Govaerts et al. (2016) to merge entries in both, the occurrence data and in the chronogram. Subsequently, the gathered distributional data was transformed to presence/absence data of the eleven defined geographic areas. To perform the biogeographic analyses we removed the species for which we could not obtain any distribution data, as well as subspecific taxa from the tree. Furthermore, *Allium* comprises a notable number of cultivated species (Mabberley, 2008). As the distribution of those species has been largely influenced by humans, we preferred coding their native areas of distribution.

3. Results

3.1. Phylogenetic analysis, molecular dating

We compiled a data set of 352 OTUs (including 18 subspecies, 18 varieties, one forma, and four undetermined specimens) with 12306 aligned base pairs from eight markers: ITS (1037 bp), *atp*B–*rbc*L (1008 bp), *mat*K (1595 bp), *psb*A–*trn*H (882 bp), *rbc*L (3222 bp), *rps*16 (1327 bp), *trn*L–*trn*F (1214 bp), *trn*L–*rpl*32 (2021 bp). Our phylogenetic reconstructions are overall similar to the reconstructions by Friesen et al. (2006) using ITS, and Li et al.

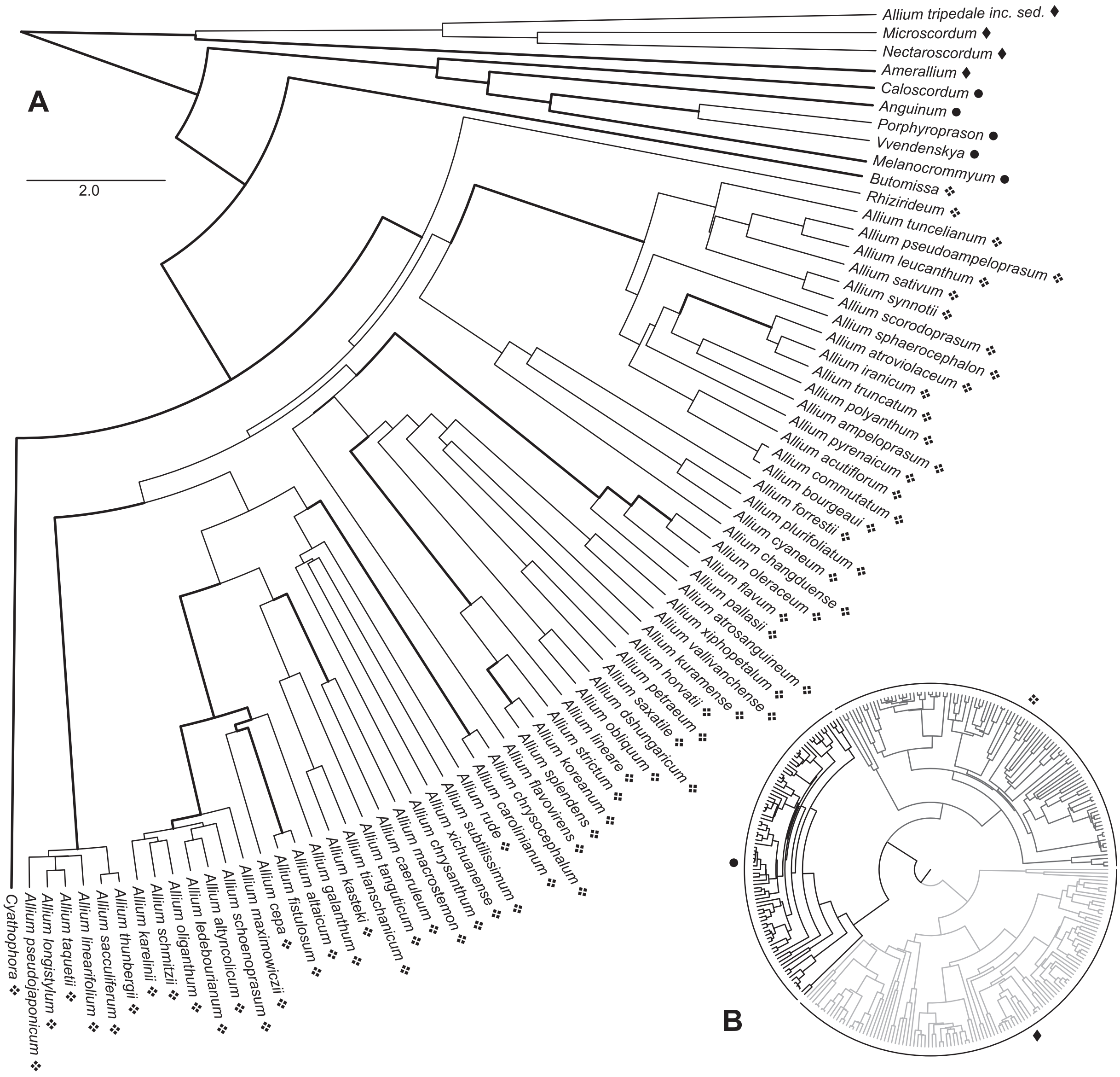

Fig. 1. The maximum clade credibility (MCC) tree of *Allium* as reconstructed by BEAST. A. Monophyletic subgenera are collapsed. The subgenera *Allium*, *Cepa*, *Reticulatobulbosa*, and *Polyprason* do not form monophyletic non-exclusive groups, hence this clade is displayed uncollapsed. Support values above pp = 0.96 are shown by thicker lines. Evolutionary lines are represented by symbols (1st = ◆, 2nd = ●, 3rd = ❖). The classification (Evolutionary Lines) refers to Friesen et al. (2006). B. Full overview of the tree, highlighting the First (light grey), the Second (black) and the Third (dark grey) Evolutionary Lines.

(2010) with ITS and *rps*16 (Fig. 1). Partition-dependent topological variations are present, yet none affects the arrangement of major lineages. All three previously recognized evolutionary lines are present and supported in our phylogeny (Fig. 1; posterior probabilities: 0.97, 1.00, and 1.00 respectively, bootstrap values: 75, 100, and 100), and our reconstruction of EL3 (Friesen et al., 2006) is largely similar to previous topologies. Furthermore, we observed two taxa (*Allium condensatum* Turcz. and *Allium kingdonii* Stearn), which were placed differently in our phylogeny, when compared to Friesen et al. (2006). Yet, consistent with the results of Huang et al. (2014), *A. kingdonii* is nested within subgenus *Amerallium* Traub., as sister to the clade of American *Amerallium*. Our phylogenies support the previously described subgenera *Nectaroscordum* (Lindl.) Asch. & Graebn., *Microscordum* (Maxim.) N. Friesen, *Amerallium*, *Caloscordum* (Herb.) R. M. Fritsch, *Anguinum* (G. Don ex Koch) N. Friesen, *Poryphyproprason* (Ekberg) R. M. Fritsch, *Vvedemskya* (Kamelin) R. M. Fritsch, *Melanocrommyum* (Webb. & Berth.) Rouy., *Butomissa* (Salisb.) N. Friesen, *Cyathophora* (R. M. Fritsch) R. M. Fritsch, and *Rhizirideum* (G. Don ex Koch) Wendelsbo *s.s.* The subgenera *Allium*, *Reticulatobulbosa*, *Polyprason*, and *Cepa*, and the above-mentioned *A. condensatum* (formerly *Rhizirideum*) are nested within each other (Fig. 1).

The crown node of *Allium* was estimated to be at least 12.8 (±1.6) Myr old. The crown of EL1 was reconstructed to be at least 10.2 (±1.4) Myr old. EL2 was estimated to have evolved between 10.0 (±1.4) and 6.6 (±1.4) Ma, and EL3 between 10.0 (±1.4) and 8.7 (±1.2) Ma (Fig. 2).

3.2. Diversification

BAMM identified three shifts of net diversification rates: the North American part of subgenus *Amerallium* (I), a clade comprising the Eurasian subgenera *Allium*, *Cepa*, *Polyprason*, and *Reticulatobulbosa* (II), and the QTP-centred section of the Central Asian subgenus *Melanocrommyum* (III) (Fig. 2). The five most credible scenarios (15.8%, 8.8%, 5.9%, 4.7%, and 4.7% of samples, respectively) all reconstructed rate shifts in the same groups (I, II, III, cf. Fig. 2A), yet in groups II and III, the location of the rate shift is not constant among the scenarios, while the rate shift related to group I occurs at the same branch among all five most credible scenarios. The sixth most credible scenario did not involve any significant rate shift (4.1% of samples), followed by two scenarios with three rate shifts similar in position to the five most credible scenarios (2.3% and 2.0% of samples, respectively). All other scenarios received probabilities below 2.0%. The data can be obtained from the corresponding author upon request.

The analyses with BayesRate confirmed the presence of three diversification rate shifts within *Allium* (Table 1). The highest marginal likelihood was found for a model with three different rates assigned to clades I, II + III, and the remaining lineages, respectively (Fig. 2). The posterior distributions of the diversification rates (Fig. 2B) show an almost two-fold increase in the diversification rate (r) between the QTP-centred section of

Table 1
Diversification scenarios tested in BayesRate. Clades as shown in Fig. 1A. For each model, the marginal likelihood scores and the relative Bayes Factor are presented.

Model	logML	BF
I|(II + III)|Rest	−438.681	0
(I + II + III)|Rest	−440.264	3.166
I|(II + III + Rest)	−443.875	10.388
All linked	−448.748	20.134
All unlinked	−452.78	28.198

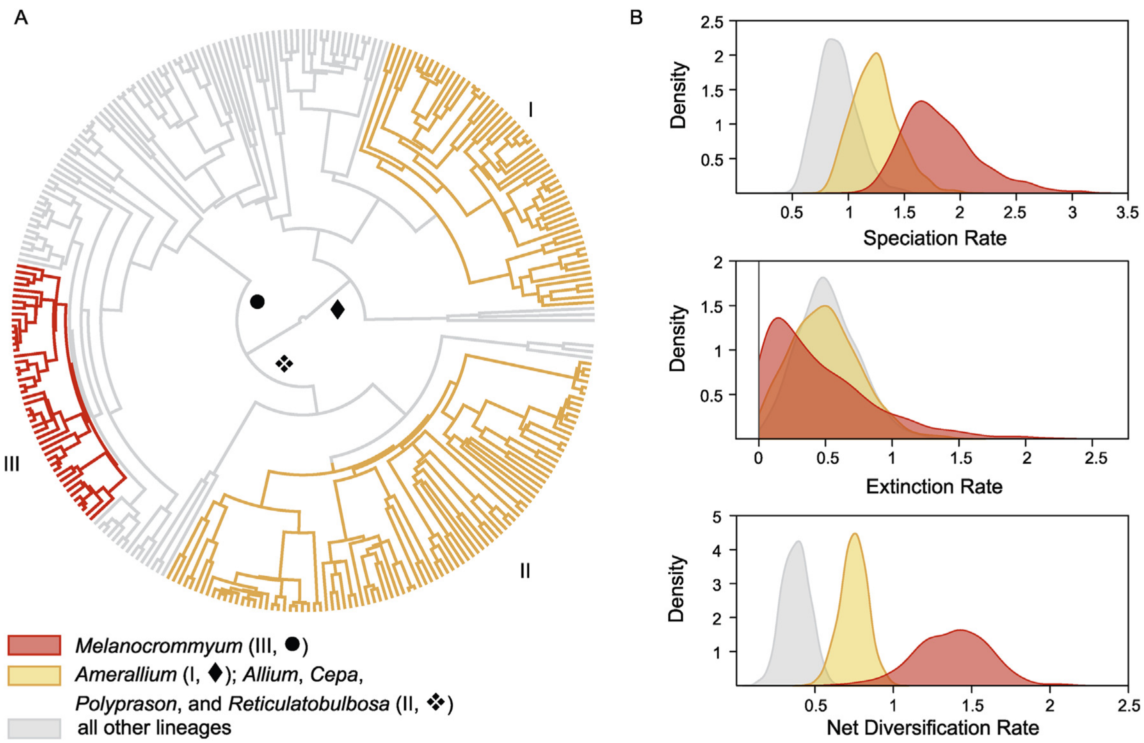

Fig. 2. Diversification dynamics in *Allium*. The tree shows the best-fit model of diversification by BayesRate (A), identical colours indicate that speciation and extinction rates were linked between clades. Marginal posterior densities of the speciation, extinction, and net diversification rates (B) for the clades defined in (A). I: a part of subgenus *Amerallium* in EL1 ◆. II: the subgenera *Allium*, *Cepa*, *Polyprason*, and *Reticulatobulbosa* in EL3❖. III: major parts of subgenus *Melanocrommyum* in EL2●.

subgenus *Melanocrommyum* (III; $r = 1.371$, 95% HPD [0.932; 1.897]) and clades I and II ($r = 0.771$, 95% HPD [0.605; 0.942]), while the posterior rate estimate for the remaining clades is substantially lower ($r = 0.365$, 95% HPD [0.220; 0.528]). These differences are mainly due to variation in speciation rates across *Allium*, while the extinction rate shows a much less pronounced distinction between clades (Fig. 2B).

3.3. Biogeographic analysis

Model evaluation identified the models without founder events as most suitable models (weighted ratio: 2.73), and most likely with the DEC model. However, all models achieved overall similar likelihoods and favoured consistent scenarios. The geographic origin of *Allium* could not be reconstructed with high confidence (eight areas, each with less than 50% probability, Appendix 2). EL1 was reconstructed to be of European (EUR) origin (less than 50% probability). Within subgenus *Amerallium*, the North American taxa were reconstructed to have originated in North America (NA, Appendix 2), while the Asian, African, and European taxa were estimated to be of European (EUR) origin (<50% to >98%, Appendix 2). The ancestral area of EL2 could not be resolved (eight areas, each with less than 50% probability, Appendix 2). Within this lineage, a similar pattern was reconstructed for the subgenera *Caloscordum* (four areas of overall similar probability), and *Anguinum* (eight areas of potential origin). The ancestral area of the subgenera *Melanocrommyum*, *Poryphyroprason*, and *Vvedemskya* were reconstructed to be of Arctic Asian (AA) origin, and within *Melanocrommyum*, major parts of its diversification were reconstructed to have occurred in the Western Irano-Turanian (WIT) and the QTP region (Appendix 2). EL3, and a major part of its internal lineages, was reconstructed to be of Northeast Asian (NEA) origin (Appendix 2). In contrast to EL2, the ancestral area of extant taxa distributed in the QTP was not reconstructed as being the QTP itself.

4. Discussion

We performed phylogenetic, biogeographic, and diversification rate analyses using the widespread genus *Allium* as a model group to test the “mountain geo-biodiversity hypothesis”. By gathering a large data set of available sequence data, combined with two data sets on distributional data, we could reveal new information related to the taxonomy within the genus by providing phylogenetic support to the monophyly of several subgenera (Fig. 1). Furthermore, we could identify a dual biogeographic pattern for *Allium* species occurring within the QTP region: while multiple taxa of one lineage seem to have immigrated from other areas (e.g. the Arctic and Northeast Asia) into the QTP and adjacent regions (e.g. species of EL3), the QTP acted more as a source area in a clade of subgenus *Melanocrommyum* (Appendix 2). Finally, although we recognize three diversification rate shifts occurring either in Asia or North America, the conditions subtending the “mountain-geo-biodiversity hypothesis” were only partially verified.

4.1. Taxonomy and the consensus approach

To arrive at solid biogeographical interpretations, one should ideally rely on a well-supported phylogenetic reconstruction, including a reasonable sampling across taxonomic units (e.g. subgenera, sections) and geographic areas. In our data set, all major lineages of *Allium* were included across the entire distribution range of the genus. Nevertheless, some clades and/or regions were better represented than others. For example, species coverage for areas which had already been targeted by phylogenetic studies, such as Korea and northeastern China (Choi and Oh, 2011), was higher than for regions and subgenera not yet revised in detail (e.g., the Americas). In general, our results are in line with the last revision of *Allium* (including 15 subgenera, organised into three evolutionary lines; Friesen et al., 2006), and with more recent studies targeting some of these subgenera, such as *Anguinum* and *Cyathophora* (Choi and Oh, 2011; Herden et al., 2016; Li et al., 2016). Nevertheless, several subgenera (*Cepa*, *Reticulatobulbosa*, *Polyprason*, and *Allium*) did not receive sufficient phylogenetic support in our analyses, or were placed in a polytomy. In addition, other subgenera, such as *Microscordum*, were only represented by one species, precluding any conclusions regarding their monophyly. Further phylogenetic studies on *Allium* should therefore primarily aim at resolving these phylogenetic uncertainties. Nevertheless, our automated approach was successful in recovering all clades previously described as monophyletic (Fig. 1). This result supports the argument of Banfi et al. (2011) for a nomenclatural revision, encouraging the split of *Allium* into three genera, namely *Nectaroscordum*, *Caloscordum*, and *Allium*. In the absence of major discrepancies between our phylogenies and Friesen et al.'s (2006) recent classification of *Allium*, we consider our data set suitable to reconstruct the evolutionary history of *Allium*.

4.2. Spatio-temporal dynamics of Allium evolution

Allium probably originated between 11.2 and 14.4 Ma (Appendix 2) in a yet undetermined area, potentially in a combination of several areas. Because there is no satisfactory paleobotanical data for *Allium* and closely related taxa, as is often the case for temperate herbaceous plants (mostly) pollinated by insects, we were unable to perform a thorough quality check on the temporal estimates. Yet, our time estimates are in line with other molecular dating approaches targeting larger clades including *Allium* and other genera within Amaryllidaceae (Conrad, 2008; Magallón et al., 2015). However, our estimates do not support the hypotheses of Li et al. (2010), who postulated that the First Evolutionary Line (EL1) originated around the Cretaceous—Paleogene boundary, to fit with possible vicariance events. Moreover, despite the lack of certainty regarding the geographical origin of the genus, we could recover the likely origins of major lineages and subgenera within the three evolutionary lines within *Allium* (Appendix 2).

It is likely that Europe was the centre of origin of the common ancestor of EL1, during the Late Miocene. Subgenus *Amerallium* dispersed from Europe first to North America (where it later diversified), and later on, from Europe to the Western Irano-Turanian region (potentially multiple times). However, our phylogenetic placement of the QTP-related *A. kingdonii* (different when compared to previous phylogenetic work, e.g. Friesen et al., 2006) obscures the precise route for the dispersal towards North America. Hence, our results do not contradict the spatial findings of Li et al. (2010), who proposed dispersal from eastern Asia to western North America. Similarly, subgenus *Microscordum* dispersed from Europe to the QTP, eastern and Tropical Asia. In contrast, ancestral area reconstructions lacked support for the origin and dispersal routes of EL2. Subgenera *Melanocrommyum*, *Poryphyroprason*, and *Vvedemskya* probably originated in an area including Europe and/or northern Asia (AA) around the Miocene/Pliocene boundary. Hence, like in EL1, Europe and/or the Euro-Siberian region seems to constitute the centre of origin, rather than solely eastern Asia, as claimed by Li et al. (2010). During the Early Pliocene, subgenus *Melanocrommyum* dispersed to the Western Irano-Turanian region, and later on back to northern Asia, possibly via the QTP region, which was associated with an increase in the rate of speciation (see below). Clearly, both the Irano-Turanian and the QTP region acted successively as a sink and a source area for dispersal. In fact, the former region likely was a source area for many drought-tolerant taxa (Manafzadeh et al., 2016). These findings are, to a large extent, in agreement with the hypotheses by Li et al. (2010), who also recognized a relatively recent centre of diversification in Central Asia for *Melanocrommyum*. Finally, we could locate the origin of EL3 in northeastern Asia (area NEA, including, among others, Mongolia), by the end of the Miocene. From there, Eurasian subgenus *Butomissa* and its sister clade (comprising *Allium*, *Cepa*, *Cyathophora*, *Reticulatobulbosa*, and *Rhizirideum*) dispersed mostly towards northern Asia (area AA) and Europe, where they diversified and are still abundant today. From northeastern Asia, these subgenera also repeatedly colonised more southern areas as well as the QTP. Overall, we have uncovered a highly complex biogeographic history, with multiple dispersal events between Eurasian areas. In fact, only the colonisation of North America and South or Southeast Asia appears to have been unidirectional.

4.3. Shifts in diversification rates and the "mountain-geo-biodiversity hypothesis"

As postulated by the "mountain-geo-biodiversity hypothesis" (Mosbrugger et al., 2018), diversification in the region of the QTP may have required several conditions to be initiated, including the development of a full elevational zonation and climate oscillations promoting a "species pump" effect. In this region, the start of the uplift largely predated climate oscillations (by several Myr), suggesting that a delay of biological (diversification) processes might have occurred with respect to the uplift's start. Such a delay was indeed observed for some plant genera (*Saxifraga*, Ebersbach et al., 2016, and Gao et al., 2015; *Gentiana*, Favre et al., 2016) that were shown to have been present in the QTP for an extended period of time before the onset of their radiation. In *Allium*, the diversification of subgenus *Melanocrommyum* is generally consistent with the "mountain-geo-biodiversity hypothesis". The QTP-centred clade shows the highest rate of diversification, with the start of the radiation (crown node age estimate 2.34 Ma, 95% HPD [3.04:1.56]) coinciding with the onset of the Pleistocene climatic fluctuations. However, it should be noted that our biogeographic reconstructions suggest that the clade was probably not present in the QTP before that time. Thus, the higher rate of diversification could be either the result of a "species pump" effect following climatic oscillations, or be due to an ecological radiation following colonisation from the Western Irano-Turanian region. In addition, it should be noted that the exact position of this rate shift was not stable among the most credible scenarios. As a result, we cannot ascertain whether the increased speciation rate is associated with only the QTP and Arctic Asia, or extends to the Western Irano-Turanian region (Appendix 2). The other two diversification rate shifts (in the North American clade of subgenus *Amerallium* and the clade encompassing the subgenera *Allium*, *Cepa*, *Cyathophora*, *Reticulatobulbosa*, and *Rhizirideum* in northeastern Asia) predate the Pleistocene climatic oscillations by several Myr, both starting in the Late Miocene. Therefore, *Allium* does not fully support the "mountain-geo-biodiversity hypothesis". Rather, the species richness of *Allium* in the Northern Hemisphere (including its mountain systems) might have been triggered by global cooling facilitating a complex pattern of biological interchange between Eurasian regions, potentially aiding allopatric speciation and ultimately diversification.

The "mountain-geo-biodiversity hypothesis" also postulates that mountains should buffer against extinction during climate modifications by providing refugia and suitable habitats within a short distance, as already suggested by Hoorn et al. (2013). However, we do not find much evidence for this in our analysis. Extinction rates were found to be relatively low across *Allium*, regardless of whether a subclade occurred predominantly in mountain systems (e.g. subgenus *Melanocrommyum* in the QTP and the Irano-Turanian region), in the Euro-Siberian lowlands (subgenera *Allium*, *Cepa*, *Cyathophora*, *Reticulatobulbosa*, and *Rhizirideum*), or across North America (e.g. subgenus *Amerallium*). Consequently, increased speciation rates account for most of the higher net diversification rates. Similar findings were reported in Ericaceae by Schwery et al. (2015). However, in contrast, bellflowers (Lagomarsino et al., 2016), and the Paleo-Patagonian flora (Palazzesi et al., 2014) indicate potentially higher extinction rates in lowland areas. Hence, we need to gather further insights into other widespread taxa to test whether extinction rates in the region of the QTP were lower in comparison to those in the Eurasian lowland, and attest the role of the QTP region as buffer against extinction.

5. Conclusions

Our results show how the interaction among plant taxa, geology, and climate contributes to patterns of plant diversity in the region of the QTP (including the Hengduan Mountains) and beyond. Some of the results presented here indeed provide support for the "mountain-geo-biodiversity hypothesis". However, other processes such as ecological radiations, where species diverge as populations adapt to novel habitats in topographically complex regions, cannot be ruled out. In fact, we suggest that most likely both allopatric speciation (via the "species pump" effect) and ecological divergence act together in driving plant diversification in mountain regions. Both processes might lead to different ecological patterns, with speciation driven by climatic oscillations generally resulting in little ecological differences, whereas ecological radiations should show a high degree of ecological niche divergence between closely related species. Hence, further studies, especially concerning the ecological niche, are needed to evaluate the relative importance of climate-driven and ecological diversification processes.

Acknowledgements

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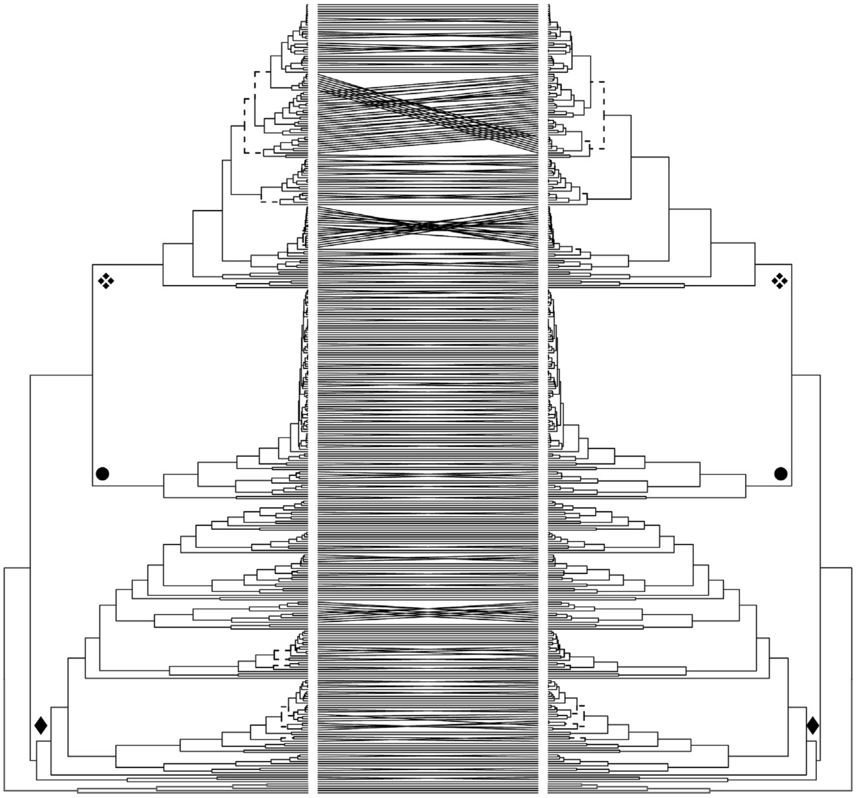
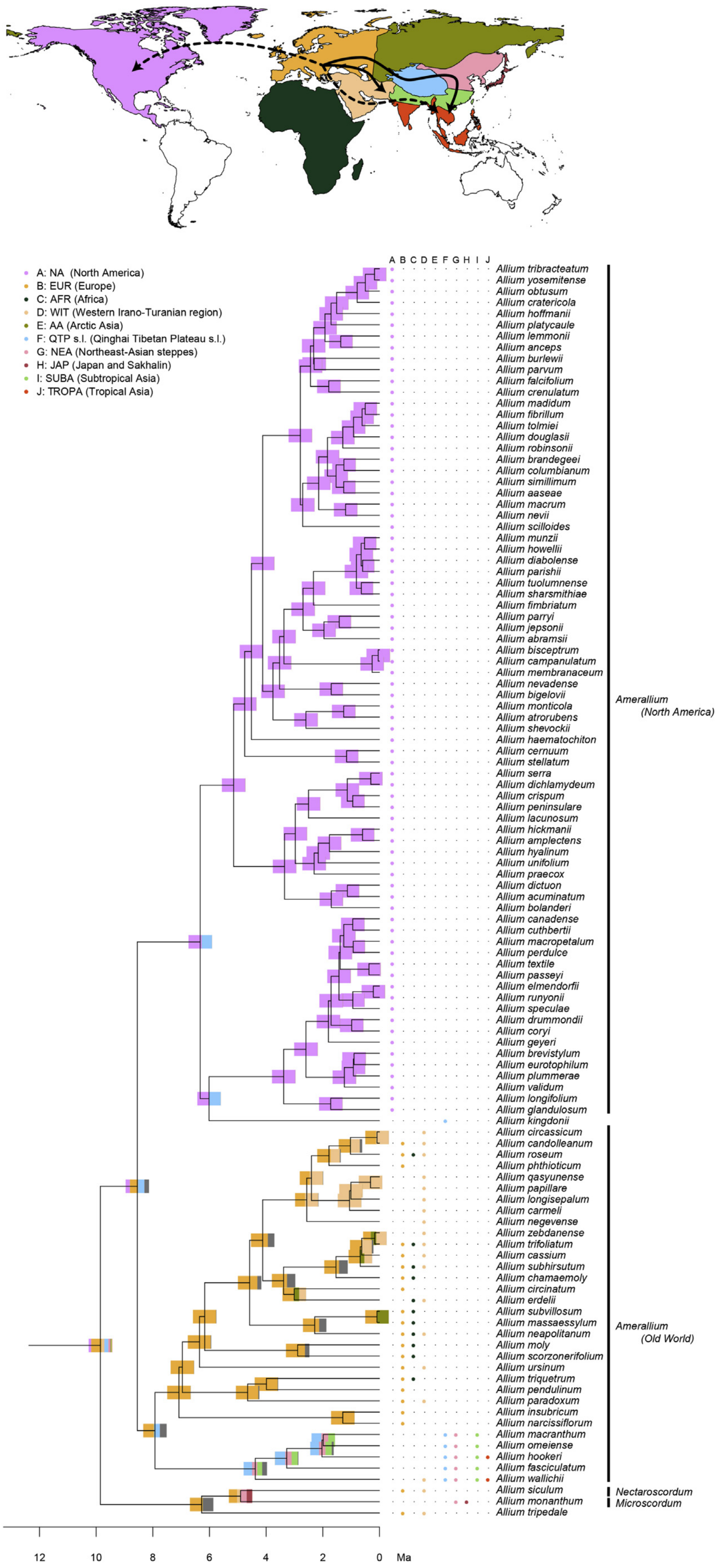
Appendix 1

Comparison of phylogenies (uncalibrated ultrametric trees) of *Allium* (black) and outgroups (grey) on the base of a concatenated non-partitioned data set (left) and a partitioned data set (right, nuclear/plastid). The evolutionary lines one to three (EL 1–3) are labelled by symbols (◆: First, ●: Second, ❖: Third Evolutionary Line).



Appendix 2

Ancestral area reconstructions in *Allium*, divided into three parts (Fig. 1: Evolutionary Line (EL) 1, Fig. 2: EL2, and Fig. 3: EL3). Node bars illustrate the percentage of an area being reconstructed as ancestral area, represented by colours from the map. The column between the tree and the species names displays the extant (non-introduced) range. Arrows on the map show reconstructed dispersal routes (lines) and potential dispersal routes (dotted lines). Subgenera are only given for EL1 and EL2, as the majority of subgenera described in EL3 are not monophyletic.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● B: EUR (Europe)
● C: AFR (Africa)
● D: WIT (Western Irano-Turanian region)
● E: AA (Arctic Asia)
● F: QTP s.l. (Qinghai Tibetan Plateau s.l.)
● G: NEA (Northeast-Asian steppes)
● H: JAP (Japan and Sakhalin)
● I: SUBA (Subtropical Asia)
● J: TROPA (Tropical Asia)
A B C D E F G H I J
Allium tribracteatum
Allium yosemitense
Allium obtusum
Allium cratericola
Allium hoffmanii
Allium platycaule
Allium lemmonii
Allium anceps
Allium burlewii
Allium parvum
Allium falcifolium
Allium crenulatum
Allium madidum
Allium fibrillum
Allium tolmiei
Allium douglasii
Allium robinsonii
Allium brandegeei
Allium columbianum
Allium simillimum
Allium aaseae
Allium macrum
Allium nevii
Allium scilloides
Allium munzii
Allium howellii
Allium diabolense
Allium parishii
Allium tuolumnense
Allium sharsmithiae
Allium fimbriatum
Allium parryi
Allium jepsonii
Allium abramsii
Allium bisceptrum
Allium campanulatum
Allium membranaceum
Allium nevadense
Allium bigelovii
Allium monticola
Allium atrorubens
Allium shevockii
Allium haematochiton
Allium cernuum
Allium stellatum
Allium serra
Allium dichlamydeum
Allium crispum
Allium peninsulare
Allium lacunosum
Allium hickmanii
Allium amplectens
Allium hyalinum
Allium unifolium
Allium praecox
Allium dictuon
Allium acuminatum
Allium bolanderi
Allium canadense
Allium cuthbertii
Allium macropetalum
Allium perdulce
Allium textile
Allium passeyi
Allium elmendorfii
Allium runyonii
Allium speculae
Allium drummondii
Allium coryi
Allium geyeri
Allium brevistylum
Allium eurotophilum
Allium plummerae
Allium validum
Allium longifolium
Allium glandulosum
Allium kingdonii
Allium circassicum
Allium candolleanum
Allium roseum
Allium phthioticum
Allium qasyunense
Allium papillare
Allium longisepalum
Allium carmeli
Allium negevense
Allium zebdanense
Allium trifoliatum
Allium cassium
Allium subhirsutum
Allium chamaemoly
Allium circinatum
Allium erdelii
Allium subvillosum
Allium massaessylum
Allium neapolitanum
Allium moly
Allium scorzonerifolium
Allium ursinum
Allium triquetrum
Allium pendulinum
Allium paradoxum
Allium insubricum
Allium narcissiflorum
Allium macranthum
Allium omeiense
Allium hookeri
Allium fasciculatum
Allium wallichii
Allium siculum
Allium monanthum
Allium tripedale
Amerallium
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Amerallium
(Old World)
Nectaroscordum
Microscordum
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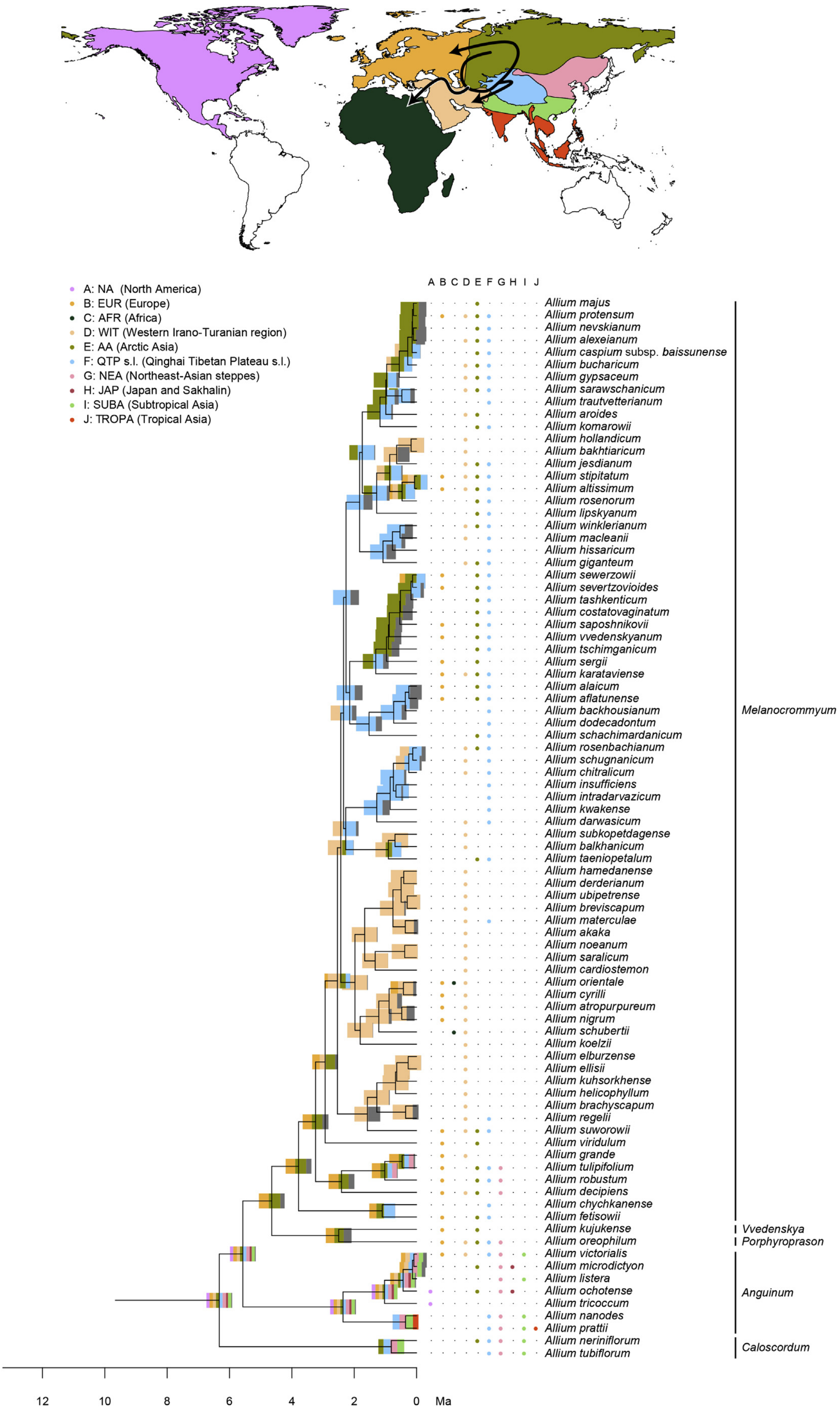
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● H: JAP (Japan and Sakhalin)
● I: SUBA (Subtropical Asia)
● J: TROPA (Tropical Asia)
A B C D E F G H I J
Allium majus
Allium protensum
Allium nevskianum
Allium alexeianum
Allium caspium subsp. baissunense
Allium bucharicum
Allium gypsaceum
Allium sarawschanicum
Allium trautvetterianum
Allium aroides
Allium komarowii
Allium hollandicum
Allium bakhtiaricum
Allium jesdianum
Allium stipitatum
Allium altissimum
Allium rosenorum
Allium lipskyanum
Allium winklerianum
Allium macleanii
Allium hissaricum
Allium giganteum
Allium sewerzowii
Allium severtzovioides
Allium tashkenticum
Allium costatovaginatum
Allium saposhnikovii
Allium vvedenskyanum
Allium tschimganicum
Allium sergii
Allium karataviense
Allium alaicum
Allium aflatunense
Allium backhousianum
Allium dodecadontum
Allium schachimardanicum
Allium rosenbachianum
Allium schugnanicum
Allium chitralicum
Allium insufficiens
Allium intradarvazicum
Allium kwakense
Allium darwasicum
Allium subkopetdagense
Allium balkhanicum
Allium taeniopetalum
Allium hamedanense
Allium derderianum
Allium ubipetrense
Allium breviscapum
Allium materculae
Allium akaka
Allium noeanum
Allium saralicum
Allium cardiostemon
Allium orientale
Allium cyrilli
Allium atropurpureum
Allium nigrum
Allium schubertii
Allium koelzii
Allium elburzense
Allium ellisii
Allium kuhsorkhense
Allium helicophyllum
Allium brachyscapum
Allium regelii
Allium suworowii
Allium viridulum
Allium grande
Allium tulipifolium
Allium robustum
Allium decipiens
Allium chychkanense
Allium fetisowii
Allium kujukense
Allium oreophilum
Allium victorialis
Allium microdictyon
Allium listera
Allium ochotense
Allium tricoccum
Allium nanodes
Allium prattii
Allium neriniflorum
Allium tubiflorum
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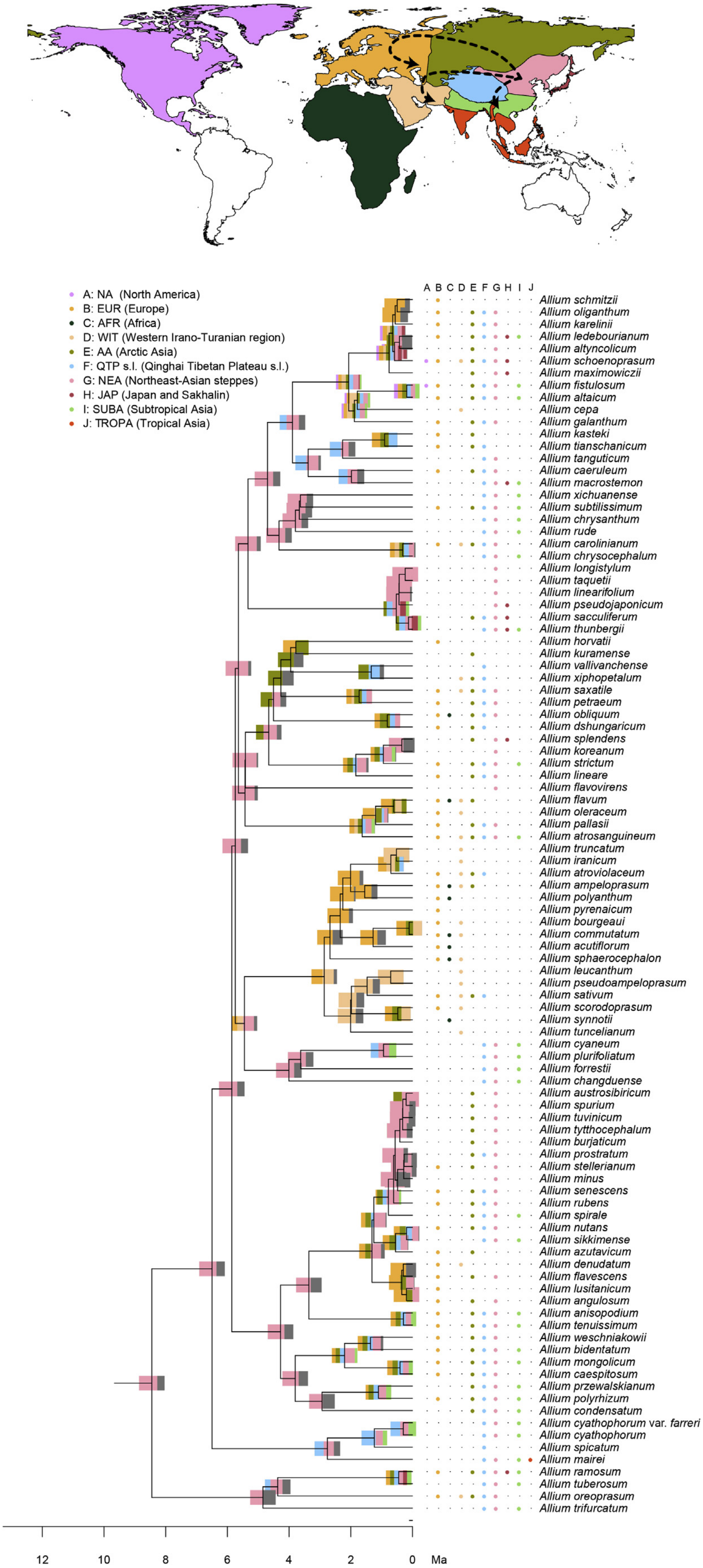
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G: NEA (Northeast-Asian steppes)
H: JAP (Japan and Sakhalin)
I: SUBA (Subtropical Asia)
J: TROPA (Tropical Asia)
A B C D E F G H I J
Allium schmitzii
Allium oliganthum
Allium karelinii
Allium ledebourianum
Allium altyncolicum
Allium schoenoprasum
Allium maximowiczii
Allium fistulosum
Allium altaicum
Allium cepa
Allium galanthum
Allium kasteki
Allium tianschanicum
Allium tanguticum
Allium caeruleum
Allium macrostemon
Allium xichuanense
Allium subtilissimum
Allium chrysanthum
Allium rude
Allium carolinianum
Allium chrysocephalum
Allium longistylum
Allium taquetii
Allium linearifolium
Allium pseudojaponicum
Allium sacculiferum
Allium thunbergii
Allium horvatii
Allium kuramense
Allium vallivanchense
Allium xiphopetalum
Allium saxatile
Allium petraeum
Allium obliquum
Allium dshungaricum
Allium splendens
Allium koreanum
Allium strictum
Allium lineare
Allium flavovirens
Allium flavum
Allium oleraceum
Allium pallasii
Allium atrosanguineum
Allium truncatum
Allium iranicum
Allium atroviolaceum
Allium ampeloprasum
Allium polyanthum
Allium pyrenaicum
Allium bourgeaui
Allium commutatum
Allium acutiflorum
Allium sphaerocephalon
Allium leucanthum
Allium pseudoampeloprasum
Allium sativum
Allium scorodoprasum
Allium synnotii
Allium tuncelianum
Allium cyaneum
Allium plurifoliatum
Allium forrestii
Allium changduense
Allium austrosibiricum
Allium spurium
Allium tuvinicum
Allium tytthocephalum
Allium burjaticum
Allium prostratum
Allium stellerianum
Allium minus
Allium senescens
Allium rubens
Allium spirale
Allium nutans
Allium sikkimense
Allium azutavicum
Allium denudatum
Allium flavescens
Allium lusitanicum
Allium angulosum
Allium anisopodium
Allium tenuissimum
Allium weschniakowii
Allium bidentatum
Allium mongolicum
Allium caespitosum
Allium przewalskianum
Allium polyrhizum
Allium condensatum
Allium cyathophorum var. farreri
Allium cyathophorum
Allium spicatum
Allium mairei
Allium ramosum
Allium tuberosum
Allium oreoprasum
Allium trifurcatum
12
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Selbständigkeitserklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel *„SYSTEMATICS, TAXONOMY AND BIOGEOGRAPHY OF THE COSMOPOLITAN AND FOSSIL-RICH BUCKTHORN FAMILY (RHAMNACEAE JUSS.)*" selbstständig und nur unter Verwendung der angegebenen Hilfsmittel erstellt habe. Alle wörtlichen und sinngemäßen Zitate aus anderen Werken sind als solche gekennzeichnet. Personen, die an der Erstellung von Teilen der Dissertation beteiligt waren, sind zu Beginn des jeweiligen Kapitels benannt. Der jeweilige Beitrag der beteiligten Personen ist am Ende der Dissertation aufgeführt. Weitere als die jeweils genannten Personen waren an der Erstellung der Arbeit nicht beteiligt. Weiterhin versichere ich, dass die vorgelegte Arbeit in gleicher oder in ähnlicher Form keiner anderen wissenschaftlichen Einrichtung zum Zwecke einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde.

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Title: **Phylogenetic relationships within the cosmopolitan buckthorn family (Rhamnaceae) support the resurrection of *Sarcomphalus* and the description of *Pseudoziziphus* gen. nov.**

Journal: *TAXON, 65(1), 47-64 (2016).*

Authors: Hauenschild, F.; Matuszak, S.; Muellner-Riehl, A. N. & Favre, A.

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Adrien Favre:	• Project idea • Conception of project • Contribution of samples • Interpretation • Writing of the manuscript: revision and finalization of the manuscript
Alexandra N. Muellner-Riehl:	• Securing of funds • Contribution of samples • Interpretation of results • Writing of the manuscript: revision and finalization of the manuscript

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Author contributions chapter 2

Title: **Corrigendum to "Analysis of the cosmopolitan buckthorn genera *Frangula* and *Rhamnus* s.l. supports the description of a new genus, *Ventia*"**

Journal: *TAXON, 65(1), 65-78 (2016).*

Authors: Hauenschild, F.; Favre, A.; Salazar, G. & Muellner-Riehl, A. N.

Author	**Contribution**
Frank Hauenschild (first author)	• Taxonomic expertise • Interpretation of results • Writing of the manuscript: drafting the corrigendum and finalization of the corrigendum
Gerardo Salazar:	• Writing of the manuscript: revision and finalization of the manuscript
Adrien Favre:	• Writing of the manuscript: revision and finalization of the manuscript
Alexandra N. Muellner-Riehl:	• Taxonomic expertise • Interpretation of results • Writing of the manuscript: revision and finalization of the manuscript

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Author contributions chapter 3

Title: **The influence of the Gondwanan break-up on the biogeographic history of the ziziphoids (Rhamnaceae)**

Journal: *Journal of Biogeography* (*submitted*)

Authors: Hauenschild, F.; Favre, A.; Michalak, I.; & Muellner-Riehl, A. N.

Author	**Contribution**
Frank Hauenschild (first author)	• Project idea • Conception of project • Sample collection • Laboratory work • Data processing and analysis • Interpretation of results • Writing of the manuscript: drafting first manuscript version and finalization of the manuscript
Adrien Favre:	• Project idea • Conception of project • Interpretation of results • Writing of the manuscript: revision and finalization of the manuscript
Ingo Michalak:	• Conception of project • Interpretation of results • Data processing and analysis • Writing of the manuscript: revision and finalization of the manuscript
Alexandra N. Muellner-Riehl:	• Securing of funds • Contribution to conception of project • Interpretation of results • Writing of the manuscript: revision and finalization of the manuscript

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Author contributions chapter 4

Title: **Biogeographic analyses support an Australian origin for the Indomalesian-Australasian wet forest-adapted tropical tree and shrub genus *Alphitonia* and its close allies (Rhamnaceae)**

Journal: *Botanical Journal of the Linnean Society* (*accepted*)

Authors: Hauenschild, F.; Favre, A., Schulz, M. & Muellner-Riehl, A. N.

Author	**Contribution**
Frank Hauenschild (first author)	• Project idea • Conception of project • Sample collection • Laboratory work • Data processing and analysis • Interpretation of results • Writing of the manuscript: drafting first manuscript version and finalization of the manuscript
Adrien Favre:	• Project idea • Conception of project • Contribution of samples • Interpretation of results • Writing of the manuscript: revision and finalization of the manuscript
Maria Schulz:	• Laboratory work • Data processing and analysis • Interpretation of results • Writing of the manuscript: parts of the manuscript's results and interpretations are based on the master thesis of Maria Schulz
Alexandra N. Muellner-Riehl:	• Securing of funds • Interpretation of results • Writing of the manuscript: revision and finalization of the manuscript

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Author contributions Appendix 1

Title: **Spatio-temporal evolution of *Allium* L. in the Qinghai-Tibet-Plateau region: immigration and *in situ* radiation**

Journal: *Plant Diversity 39(4), 167-179 (2017).*

Authors: Hauenschild, F.; Favre, A., Schnitzler, J., Michalak, I., Freiberg, M. & Muellner-Riehl, A. N.

Author	**Contribution**
Frank Hauenschild (first author)	• Conception of project • Data processing and analysis • Interpretation of results • Writing of the manuscript: drafting first manuscript version and finalization of the manuscript
Adrien Favre:	• Project idea • Conception of project • Interpretation of results • Writing of the manuscript: revision and finalization of the manuscript
Jan Schnitzler:	• Conception of project • Data processing and analysis • Interpretation of results • Writing of the manuscript: drafting the chapters about diversification rates, revision, and finalization of the manuscript
Ingo Michalak:	• Project Idea • Conception of the project • Data processing and analysis • Writing of the manuscript: drafting the materials and methods and parts of the introduction
Martin Freiberg:	• Provision of distribution data • Taxonomic expertise • Data processing and analysis
Alexandra N. Muellner-Riehl:	• Securing of funds • Interpretation of results • Writing of the manuscript: drafting parts of the introduction; revision and finalization of the manuscript

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