Seed germination and genetic structure of two *Salvia* species in response to environmental variables among phytogeographic regions in Jordan (Part I)

and

Phylogeny of the pan-tropical family Marantaceae (Part II).

Dissertation

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“Four years ago I started this project as a PhD project, but it turned out to be a long battle to achieve victory and dreams. This dissertation is the culmination of this long process, where the definition of “Weekend” has been deleted from my dictionary. It cannot express the long days spent in analyzing sequences and data, battling shoulder to shoulder with my ex-computer (RIP), R-studio, BioEdite and Microsoft Words, the joy for the synthesis, the hope for good results and the sadness and tiredness with each attempt to add more taxa and analyses.”

“At the end, no phrase can describe my happiness when I saw the whole dissertation is printed out.”

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SUMMARY

Plant biodiversity is the sum total of all biotic variation from the level of genes to ecosystems. However, the relationship between biodiversity and ecological processes has emerged as a central issue in ecological and environmental sciences during the last decade. Because ecosystems collectively determine the biogeochemical processes that regulate species diversity and distribution, studies investigating the potential ecological consequences on levels of plant diversity have provided valuable information in conservation context. Therefore, phylogenetic and temporal analyses can shed the light on the ecological and evolutionary processes that have shaped current biodiversity.

PART I

Understanding how environmental variation affects medicinal plant population persistence is essential for species conservation and predicting the ecological impact of expected future increases in climate variability. Due to the meeting place of Mediterranean, Irano-Turanian, Saharo-Arabian and Sudanian phytogeographical regions, in Jordan, plant species in this region generally face environmental heterogeneity over short geographical distances. However, the effect of such environmental variability, as well as ecological diversification between regions, may have a biological footprint on genetic diversity and seed germination. Such prints can be detected due to the evolutionary forces that can shape genetic structure, differentiation and promote local adaptation among populations. Therefore, plant species with continuous distribution along sharp environmental gradients are interesting systems to study among-population genotypic and phenotypic divergence at comparably small scales. In this dissertation, the genetic diversity, population structure and seed germination were studied for two Jordanian medicinal *Salvia* species occurring along an environmental gradient spanning from arid habitat (Irano-Turanian and Saharo-Arabian regions) with highly variable and unpredictable rainfall to the moist habitat (Mediterranean region) with less variable and more predictable rainfall. In the first two studies (Part I) of the present dissertation, my main objective is to test if the environmental variability among habitats (phytogeographical regions) of both *Salvia* species plays a role in inducing local adaption in seed germination and in shaping the species' genetic

structure. The collected seeds and leaves from all populations across the three regions were used to test the responses of seed germination to variations in temperature and salinity, in the first study, and detect differentiation in genetic structure from AFLP markers, in the second study. Our results showed that the interaction of the phytogeographic region (seed origin) with different levels of temperature and salinity significantly affected the germination responses in terms of onset, rate and percentage. In terms of population genetic characteristics, Irano-Turanian and Saharo-Arabian populations had a higher genetic diversity than Mediterranean populations, and genetic diversity increased significantly with increasing temperature. In addition, high levels of genetic differentiation were found as well as two well-supported phytogeographical groups of populations, with Mediterranean populations clustering in one group and the Irano-Turanian and Saharo-Arabian populations in another. In fact, the results of this part (I) of my dissertation provide evidence of local adaptation to salinity and temperature in the respective maternal environments of the study populations, particularly in the Irano-Turanian and Saharo-Arabian regions. Also, populations of these two regions were environmentally isolated from the Mediterranean populations. Where the environmental variability affected their flowering phenology, gene flow and thus genetic differentiation grouped the populations phytogeographically. In conclusion, the signature of local adaptation and genetic clustering found in both *Salvia* species, support my assumption concerning the influence of environmental variability among the phytogeographical regions on plant populations. Hence, the results of this dissertation can help to develop improved strategies for conservation and restoration of wild medicinal plants.

PART II

Phylogeny represents the historical pattern of relationships among organisms which has resulted from the actions of many different evolutionary processes. Recently, the ability to achieve detailed phylogenies provides a window to infer speciation and extinction rates, the ecological and biogeographical causes of speciation and extinction, and the timing of these events. Moreover, the topology of a phylogenetic tree is used to establish the best representation of different clades within regions with high priority for conservation. The fusion of such applications with morphological and ecological data can provide baseline data for understanding both the

origin and maintenance of tropical plant diversity. However, the estimates of evolutionary parameters derived from phylogenetic trees are conditionally upon how accurately these trees reflect true biological events. In phylogeny experimental design studies, it has been suggested that increased sampling of taxa, characters (genes) or in both dimensions can improve the estimates of evolutionary parameters, and is thus important for improved applications of phylogenetic analyses.

The geographical history of the pantropical distributed family, Marantaceae, is complex and little known to date. Earlier efforts using molecular phylogenies were hampered by a poor resolution along the backbone of the family tree. Therefore, the central objective in the third study (Part II), was to improve the phylogenetic resolution of the Marantaceae backbone through utilizing more additional molecular data and taxa than in the past. In this study, ~600 sequences from both nuclear and chloroplast genomes covering 187 taxa, representing all genera within the Marantaceae family were included. Results of the combined analyses showed that increasing sampling in both dimensions, taxa and characters improved the Marantaceae backbone support. The resulting tree topology focusing on the resolution of major clades was mostly congruent among applied methods and with preexisting family phylogenies. A few relationships within genera or clades were newly resolved here. A genus, *Monophyllanthe*, added to the phylogeny here for the first time appeared within the *Stachyphrynium* clade as sister to the genus *Marantochloa*. Only the affinity of the genus *Haumania* to one of the other major clades still remained uncertain. Such improved phylogeny based on multiple molecular markers from both genomes and a complete sampling of Marantaceae genera will be a solid base to investigate in the future the timing of speciation and the migration events leading to the currently observed biogeographical patterns in this family.

This study gives an overview of different genetic approaches at several different levels of genetic diversity from intraspecific to genus level, their specific applications to different evolutionary questions and the utilization of their respective results for conservation efforts.

Thereby the overall aim for conservation is to detect and in the following chose the maximum of genetic diversity at all levels from populations, species, genera to families for a maximal stability and survival, respectively.

ZUSAMMENFASSUNG

Der Zusammenhang zwischen ökologischen Prozessen und pflanzliche Biodiversität ist im vergangenen Jahrzehnt zu einer der zentralen Aufgabenstellungen in der Ökologie und den Umweltwissenschaften geworden. Ökosysteme bestimmten die biogeochemische Prozesse, welche die Artdiversität und -verteilung beeinflussen. Daher sind im Naturschutzkotext insbesondere Studien über ihre potentiellen ökologischen Konsequenzen auf die pflanzliche Biodiversität von größtem Interesse. Phylogenetische und populationsgenetische Analysen können in diesem Zusammenhang wichtige ökologische und evolutionäre Prozesse aufzeigen, welche die Biodiversität bestimmen.

Teil 1

Das Verständnis darüber wie Umweltvariation die Fitness von Pflanzenpopulationen beeinflusst ist essentiell um den ökologischen Einfluss der in der nähereren Zukunft zu erwartenden klimatischen Veränderungen auf die lokale Biodiversität vorhersagen zu können. Da in Jordanien die mediterane, die irano-turanische, die saharo-arabische und die sudanesische phytogeographische Region aufeinandertreffen, sind die Pflanzen auf diesem kleinen geografischen Raum enormer Umweltheterogneität ausgesetzt. Die daraus resultierende ökologische Diversifikationzwischen Regionen können einen gewaltigen Einfluss auf die genetische Diversität und Keimfähigkeit von Populationen haben. Diese evolutionären Kräfte können durch Untersuchungen der lokalen Adaptation von Populationen sowie Analysen zur genetischen Struktur und Differenzierung zwischen Populationen veranschaulicht werden. Pflanzenarten mit einer kontinuierlichen Verbreitung entlang starker Umweltgradienten stellen hierbei besonders interesannte Modellsysteme dar, um geno- und phenotypische Divergenz auf vergleichsweise kleinem geografischem Raum zu verstehen.

In dieser Doktorarbeit wurden die genetische Populationsstruktur, die genetische Diversität, und die Keimraten von Populationen zweier medizinisch genutzter *Salvia*-Arten untersucht. Die Populationen beider Arten wurden in Jordanien entlang von

Umweltgradienten in drei der vier vorkommenden phytogeographischen Regionen untersucht: Vollkommen aride Habitate mit stark variablen und unvorherseharen Niederschlägen in der irano-turanischen und der saharo-arabischen Region bis hin zu relativ feuchten Habitaten mit regelmäßigen Niederschlägen in der mediteranen Region. Fokus der ersten beiden Studien dieser Dissertation (Teil 1) war, zu untersuchen, wie Umweltvariabilität zwischen diesen Habitaten die populationsgenetische Struktur und die lokale Adaptation der Keimraten der beiden *Salvia*-Arten beeinflusst. Dazu wurden Samen und Blattmaterial in den drei phytogeographischen Regionen gesammelt. Die Keimreaktion der Samen in Bezug auf Temperatur- und Salinitätsregime wurde in der ersten Studie getestet. In der zweiten Studie wurde mit Hilfe von AFLP-Markern die genetische Struktur und Differenzierung der Populationen dargestellt.

Die Ergebnisse meiner Studien zeigten, dass die Interaktionen der phytogeographischen Region mit dem Temperatur- sowie dem Salinitätsregime die Keimfähigkeit der Samen signifikant beeinflusste. In Bezug auf die populationgenetischen Charakteritika der Populationen zeigte sich, dass die irano-turanischen und saharo-arabischen Populationen eine höhere genetische Diversität aufwiesen als die mediteranen Populationen. Außerdem wurde eine hohe genetische Differenzierung zwischen den phytogeographischen Regionen gefunden, wobei die mediteranen Populationen ein genetisches Cluster darstellten und die irano-turanischen zusammen mit den saharo-arabischen Populationen ein zweites genetisches Cluster umfassten. Auch konnte gezeigt werden, dass sich die Umweltbedingungen dieser beiden Regionen stark von denen der mediteranen Region unterscheiden.

Die Ergebnisse des ersten Teils meiner Dissertation belegen klare Hinweise auf lokale Adaptation zu Salinität und Temperatur, vor allem in der irano-turanischen und der saharo-arabischen Region. Die Umweltvariabilität zwischen den phytogeographischen Regionen beeinflusst die Phänologie der untersuchten jordanischen Populationen sowie den Genfluss und die genetische Differenzierung zwischen den Populationen beider *Salvia*-Arten. Zusammenfassend lässt sich erkennen, dass lokale Adaptation und genetische Differenzierung in beiden *Salvia*-Arten deutliche Effekte aufwiesen. Dies stützt meine

Annahmen bezüglich des Einflusses der Umweltheterogenität zwischen den phytogeographischen Regionen in Jordanien auf die Pflanzenpopulationen. Aus diesem Grund können die Ergebnisse dieser Doktorarbeit dabei helfen, die Strategien für den Schutz und die Renaturierung wilder Medizinalpflanzen in dieser Region zu verbessern.

Teil 2

Phylogenie repräsentiert die historischen Muster der Verwandtschaftsbeziehungen zwischen Organismen, die durch viele verschiedene evolutionäre Prozesse zustande gekommen sind. Neuerdings verschafft uns die Fähigkeit detaillierte Phylogenien zu erstellen die Möglichkeit, Rückschlüsse auf Artbildungs- und Aussterberaten zu ziehen sowie deren ökologische und biogeographische Gründe und zeitliche Einordnung. Des Weiteren kann die Topologie der phylogenetischen Bäume dafür genutzt werden, eine für die genetische Vielfalt eines Gebietes repräsentative Artenauswahl für den Umweltschutz zu erstellen. Die Kombination solcher Anwendungen mit morphologischen und ökologischen Daten kann grundlegende Daten für das Verständnis zu Herkunft und Aufrechterhaltung tropischer Pflanzenvielfalt liefern. Allerdings hängen die auf Grundlage phylogenetischer Bäume geschätzten evolutionären Parameter stark von der Präzision ab mit der biologischen Ereignisse abgebildet werden. Theoretische Modellierungen phylogenetischer Rekonstruktionen besagen, dass sich die Präzision geschätzter evolutionärer Parameter mit zunehmender Anzahl gesammelter Taxa und/oder Merkmale (genetische Marker) erhöht, sodass dies eine wichtige Methode bei der Erzielung verlässlicher Ergebnisse ist.

Die geographische Geschichte der pantropisch verbreiteten Familie der Marantaceae ist komplex und noch nicht vollständig bekannt. Die früheren Verwendungen molekularer Phylogenien der Familie wurden durch eine unzureichende Auflösung der basalen Artaufspaltungsprozesse im Baum verhindert. Daher ist das zentrale Ziel dieser dritten Studie (Teil II), die Auflösung der basalen Aufspaltungsprozesse in der Familie der Marantaceae durch eine erhöhte Anzahl molekularer Marker und Taxa zu verbessern. In dieser Studie wurden ~600 Sequenzen aus nukleärem und chloroplasten Genom von 187 Arten, alle Gattungen der Familie der Marantaceae repräsentierend, verwendet. Die Ergebnisse einer kombinierten Analyse zeigten, dass die erhöhte Anzahl an Taxa und Merkmalen, zu einer verbesserten Auflösung der basalen Aufspaltungsereignisse beitrug.

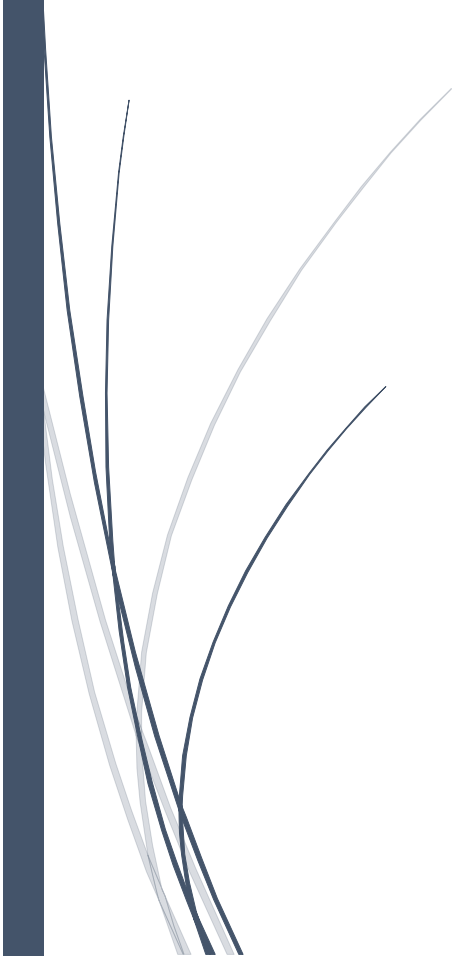
Die erzielte Baumtopologie mit Fokus auf den wichtigsten Kladen stimmte zwischen verwendeten Methoden und mit in der Vergangenheit erzielten Ergebnissen überein. Einige Verwandtschaftsbeziehungen zwischen Gattungen oder Kladen wurden hier erstmals aufgelöst. Die hier erstmals berücksichtigte Gattung *Monophyllanthe* wurde im Stachyphrynium clade als Schwestergattung zu *Marantochloa* positioniert. Nur die nähere Verwandtschaftsbeziehung der Gattung *Haumania* zu einem der anderen Kladen blieb weiterhin offen.

Eine solche verbesserte Phylogenie, basierend auf einer Vielzahl an molekularen Markern beider Genome und einer kompletten Gattungsaufsammlung der Marantaceae, stellt eine solide Basis für die nachfolgenden Untersuchungen zur zeitlichen Einordnung und Abfolge der Artbildungsprozesse und Wanderungsbewegungen dar, die zu den heute beobachteten biogeographischen Mustern der Familie führten.

Diese Studie gibt einen Überblick über verschiedene genetische Herangehensweisen auf unterschiedlichen Ebenen genetischer Diversität, vom innerartlichen bis zum Gattungsniveau, ihrer jeweilige Anwendung auf unterschiedliche evolutionäre Fragestellungen sowie die Nutzung ihrer jeweiligen Ergebnisse für den Umweltschutz. Dabei ist das übergreifende Ziel des Umweltschutzes die Detektion eines Maximums an genetischer Diversität und ihrer darauffolgenden Auswahl auf sämtlichen Ebenen von Populationen, Arten, Gattungen bis hin zu Familien zum Erreichen einer maximalen respektiven Stabilität und Überlebensrate.

CHAPTER 1

General Introduction



Two notable features characterize biodiversity in the twenty-first century: its distribution around the planet's surface is highly uneven and its diversity is declining fast (Pimm *et al.* 1995). In response to these features, numerous approaches have been developed for identifying and exploring biodiversity for different taxonomic groups, different ecosystems, and different countries and regions (Brooks *et al.* 2015). Plant biodiversity is usually explored at three levels: genetic diversity, species diversity and ecosystem diversity (McNeely *et al.* 1990). Genetic diversity is the variety of genes and their alleles within a species, while the variety of species within a habitat or a region is species diversity and the variety of ecosystems in a given place compose the ecosystem diversity. Most plant biodiversity assessments are based on species counts (e.g. total, endemic, threatened), but these may not be the most suitable metrics as they may not adequately represent the processes that gave rise to the observed diversity patterns. It is necessary not only to conserve as much species diversity as possible, but also to conserve sets of species that represent as much 'evolutionary history' as possible to guarantee the maintenance of high levels of biological diversity in the future (Vézquez & Gittleman 1998). The melding of phylogenetics, phylogeography and population genetics into integrative molecular evolution is an important step to improve our understanding of biodiversity at all levels (Cutter 2013). In addition, studies taking into consideration population genetic and phylogenetic information can potentially improve our conservation efforts (Forest *et al.* 2015), as phylogenetic knowledge and historical biogeography hold an important explanatory potential for the understanding of global biodiversity patterns. The later has major implications for our efforts in conservation and thus the future of biodiversity (Dornburg 2008)

In this dissertation, I investigate in Part I how environmental variability among phytogeographic regions induces local adaptation in seed germination (chapter two) and shapes genetic diversity and differentiation in two medicinal *Salvia* species from Jordan (chapter three). While Part II focuses on revealing the phylogenetic relationships among the taxonomic groups and taxa of the pantropically distributed family Marantaceae under complete clade sampling (chapter four). Both parts contribute to our understanding of the ecological and evolutionary processes that have shaped current plant biodiversity.

PART I

SEED GERMINATION AND ENVIRONMENTAL VARIABILITY

Germination is a crucial stage in the life cycle of plants where successful establishment and recruitment are largely dependent on successful germination, a process regulated by several environmental factors (Chauhan *et al.* 2006). Temperature, light, pH, salinity and soil moisture are the major environmental factors that simultaneously affect seed germination (e.g. Martins *et al.* 2000; El-Keblawy & Al-Rawai 2005; Goari *et al*. 2011, 2013) and, thereby affect the distribution of plants in their natural habitats (Guan *et al*. 2009).

Plant populations may face environmental heterogeneity over short geographical distances. Such steep environmental gradients can give rise to predictable variation in life-history traits and physiological tolerances (Vergeer & Kunin 2011) and can induce ecological divergence and lead to local adaptations (Brousseau *et al*. 2013; Cochrane *et al*. 2014), with different individuals and populations potentially expressing different phenotypes and tolerances. However, variation in phenotypes may be determined not only by the genotype and the environment of the individuals but also by maternal effects (Roach & Wulff 1987), and Gutterman (2000) contends that phenotypic variation is caused by the local conditions under which seeds mature. Environmental conditions experienced by a mother plant can have a strong impact on plant fitness (Lacey & Herr 2000), which in turn can affect the quality of the offspring (Mousseau & Fox 1998) and influence seed germination (Rossiter 1996, 1998). Maternal effects are considered to be adaptive when they enhance offspring fitness in an environment similar to that experienced by the parental generation (Rossiter 1996, 1998; Donohue & Schmitt 1998). Therefore, variation in germination under controlled conditions, both within and between species, can be interpreted as an adaptation to specific habitat conditions at local and regional scales (Thompson 1975; Pegtel 1985; Meyer *et al*. 1995, 1997).

Identifying the signature of local adaptation is an increasingly important goal in conservation biology. *Salvia spinosa* L. and *Salvia syriaca* L. (Lamiaceae) are two native medicinal plant species (Oran & Al-Eisawi 1998; Ulubelen 2003) that occur across the Mediterranean, Irano-Turanian and Saharo-Arabian phytogeographic regions of Jordan (Zohary

& Feinbrun 1966; Zohary 1973; Danin 1992). Therefore, the continuous distribution of both *Salvia* species populations along such gradients are an interesting system to study interpopulation divergence and local adaptation at very fine scales.

GENETIC DIVERSITY AND ENVIRONMENTAL VARIABILITY

Genetic diversity is a fundamental feature of species, populations and ecosystems, because it represents the evolutionary potential of species to survive and adapt to environmental changes. High genetic diversity can contribute to the maintenance of population health, by including alleles that may be valuable in resisting upcoming diseases, pests and other stresses. The evolution of genetic diversity is a non-random and structured process and occurs over time at both the molecular and the organism level and across all geographical scales (Nevo 2001; Ahuja & Jain 2015). In general, genetic diversity arises from different biological processes, where mutation and gene flow positively affect the genetic diversity and genetic drift and direct selection affect it negatively (Hamasha *et al*. 2013). Additionally, many other factors such as environmental and geographical variability, dispersal barriers (Wu *et al*. 2016), population demography (e.g. population size, Hensen & Oberprieler 2005) and population fragmentation due to anthropogenic activities (Thompson 2005) can further force and affect these processes.

Genetic diversity is the key pillar of biodiversity: diversity within species, between species, and of ecosystems (CBD, Article 2, Govindaraj *et al*. 2015). On the one hand, maintaining such diversity gives the population a buffer against environmental changes and provides the flexibility to adapt. On the other hand, revealing the role of environmental variability, as well as ecological diversification between habitats, for the maintenance of genetic diversity among populations is crucial to understand the evolutionary forces that shape the genetic structure and differentiation between local populations (Linhart & Grant 1996; Gram & Sork 2001). Therefore, genetic diversity is assumed positively correlated with environmental heterogeneity (Nevo 2001) and can create disruptive selection, and therefore support different genotypes within different populations (Via & Lande 1985). At the same time, however, increasing environmental heterogeneity can lead to decreased population sizes and subsequent genetic drift which in turn decreases genetic diversity / or leads to a loss of alleles (Kahilainen *et*

al. 2014). Therefore, when the genetic diversity within plant populations, is spatially structured due to a heterogeneous environment, indicating habitat selection or restricted gene flow, inbreeding and genetic drift can be expected (Linhart & Grant 1996). Hence, assessing the influence of the environmental and geographic factors on the genetic diversity of populations is one of the fundamental issues of population genetic studies (Wu *et al*. 2016).

The pattern of genetic differentiation of ecologically and environmentally subdivided populations could be explained by the restriction of gene flow either by seeds or pollen (Hamasha *et al*. 2013). Seed dispersal is often strongly spatially restricted. Therefore, gene flow is primarily dependent on pollen dispersal within and into populations (Scheepens *et al*. 2012). Pollen dispersal is highly dependent on the availability of dispersal vectors and the range these vectors cover is usually spatially restricted (Escaravage & Wagner 2004). Thus, pollen dispersal is relatively rare over long-distance (Hardy *et al*. 2004), and isolation by distance is the most common model to explain restricted gene flow (Wu *et al*. 2016) and often serves as an explanation for the effect of geographic isolation on the pattern of genetic differentiation. However, a limited pollen dispersal for short distance may as well result from isolation by environment (IBE) whereby among populations pollen dispersal is governed by environmental variations among different habitats (Wang & Bradburd 2014). Such environmental variations can influence the flowering phenology in terms of onset and duration and lead to a high probability of reproductive isolation (Franks & Weis 2009). Moreover, the presence of physical and landscape barriers between the species populations, such as differences in altitude can limit the gene flow and isolate them (Hensen *et al.* 2012). As such, the hypothesis of chapter three is that environmental variation among the phytogeographic regions in Jordan has played a role in shaping the current genetic diversity and population differentiation of the two investigated *Salvia* species. To test this hypothesis, amplified fragment length polymorphism (AFLP) was used to characterise/detect intra-specific genetic differentiation across the phytogeographic regions (see Herrmann *et al.* 2010).

PHYTOGEOGRAPHIC REGIONS AND STUDY AREA

Biogeographic and bioclimatic regions are the fundamental units of comparison in many broad-scale ecological and evolutionary studies (Crisp *et al*. 2009) and provide an essential tool for conservation planning (Whatteker & Ladle 2011). A phytogeographic region is a natural area characterized by a homogeneous floristic composition (Friis & Balslev 2005). Djamali *et al*. (2012) demonstrated that climate is a primary determinant of phytogeographic regionalization. Therefore, the meeting of different phytogeographical regions results in the presence of different species from different regions, in other words, different species with different response to the prevailing climatic and environmental conditions (Batanouny 2013).

In the Middle East, five main phytogeographical regions meet (Fig. 1): the Saharo-Arabian, Irano-Turanian, Sudanian, Mediterranean and Euro-Siberian regions (Zohary 1973). According to Zohary (1973), this meeting have led to formation of a wide diversity in ecological habitats and flora which renders this region an area of special ecological interest. Jordan, the study area, is a Middle Eastern country and lies between 29° and 11° N latitude and 33° and 22° E longitude. The total area of Jordan is nearly 90,000 km²; and hosts a wide range of diverse habitats that comprise about 1% of the world's flora.

Jordan is part of the biogeographical zones of the Middle East. The four principal bioclimatic or biogeographical regions recognized in Jordan (Fig. 2) are: Mediterranean, Irano-Turanian, Saharo-Arabian and Sudanian (representing tropical penetration) (Kasapligil 1956; Long 1957; Al–Eisawi 1996; GCEP 2000). The Mediterranean region predominates in the highlands of Jordan, from Irbid in the North to Ras al-Naqb in the South. It is generally associated with altitudes above c. 700 m, but in the north, where precipitation is higher, it is found at lower altitudes (Al-Eisawi 1996). This zone has the highest precipitation, between 300 and 600 mm, and the lowest mean maximum temperature at 15-22°C, with minimum annual temperatures of 5-10°C. Most of the region´s vegetation cover consists of forest and maquis (shrubland) communities. The Mediterranean zone is surrounded by the Irano-Turanian region, forming a narrow strip of variable width, except in the north, where it extends eastwards along the Jordanian-Syrian border. Altitude typically ranges between 500-700 m a.s.l. Precipitation is

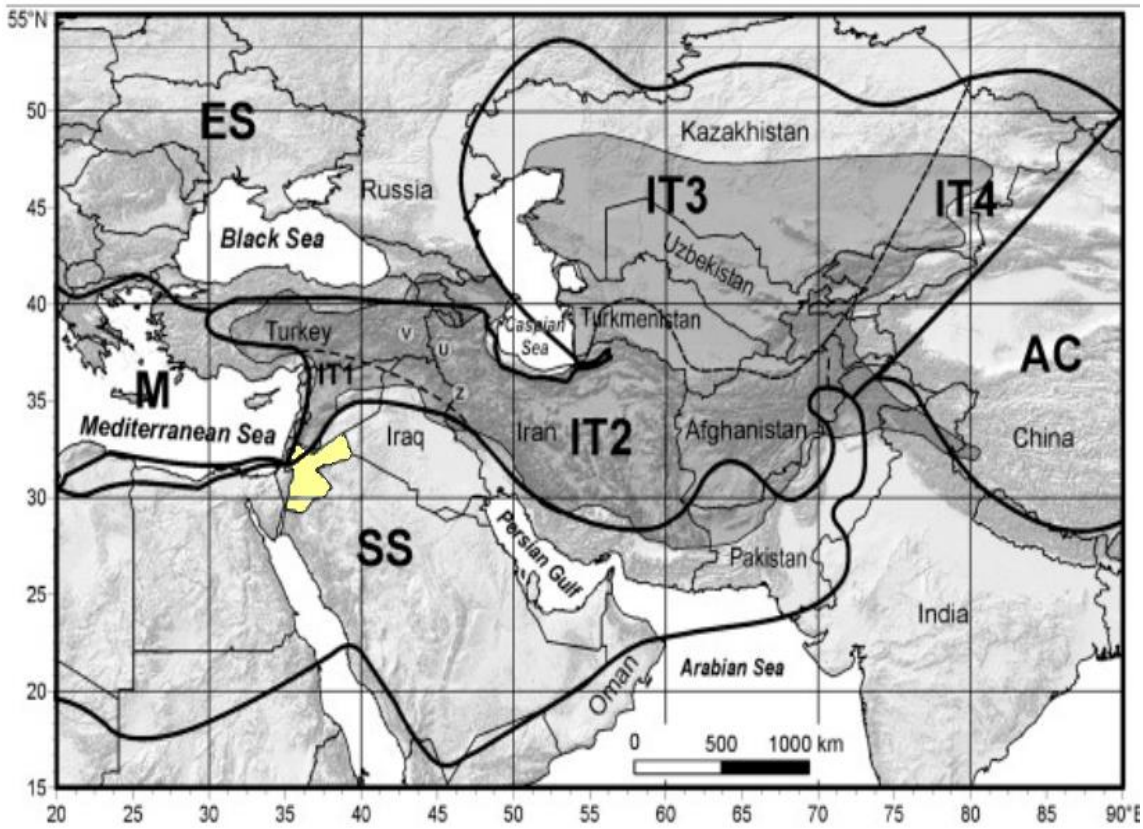


Figure 1. Phytogeographic subdivisions of South-West and Central Asia with adjacent areas (Djamali *et al*. 2012). ES, Euro-Siberian; IT, Irano-Turanian; M, Mediterranean; SS, Saharo-Sindian.

between 150 to 300 mm, and mean minimum annual temperature 2-5°C and mean maximum annual temperature 15-25°C. To the east of the Irano-Turanian region follows the Saharo-Arabian zone. It is characterized by a mostly flat semi-desert and desert, or Badiya, encompassing c. 80 % of the total land area of Jordan. Mean altitude is c. 600-700 m a.s.l., but rises to 1,200 m at the Jordanian-Syrian borders and drops to 520 m in the Azraq basin. Annual precipitation ranges between 100-150 mm on the border with the Irano-Turanian region quickly dropping to 50 mm further east and south. Mean annual minimum temperatures are 2-15°C and mean annual maximum temperature is 15-20°C. This area extends around the southern end of the Dead Sea and the flattened areas in the Rift Valley into the Sudanian region. This region is characterized by low altitude, high temperature and very little amount of rainfall.

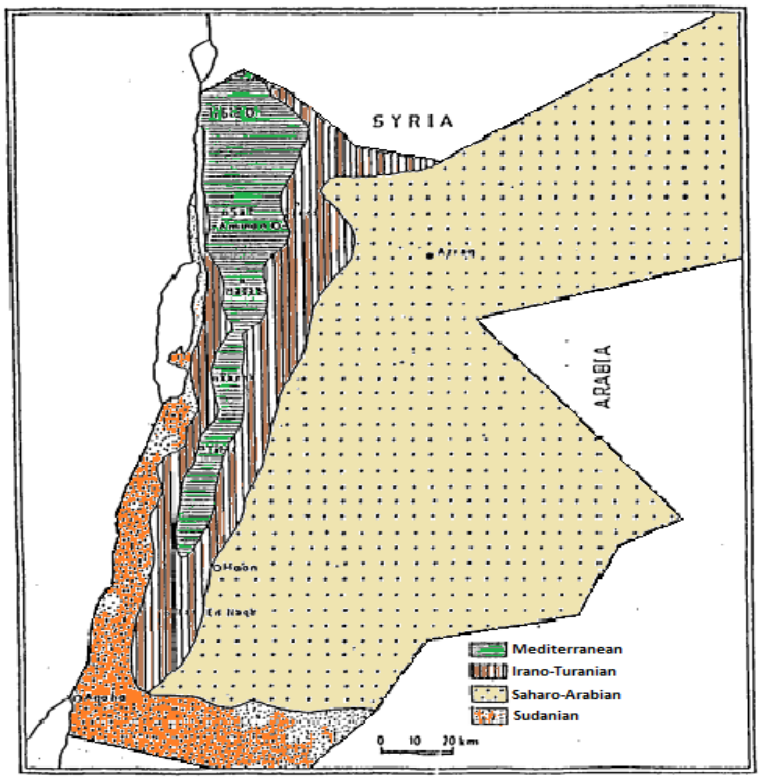


Figure 2. Phytogeographical regions in Jordan taken from Al-Essawi (1996).

MEDICINAL PLANTS AND STUDY SPECIES

Medicinal plants are coming under increasing pressure from habitat destruction and climatic change (Roberson 2008). In addition, overharvesting due to bioprospecting or commercial collection, leading to reduced effective population sizes, soil seed bank depletion and loss of genetic diversity, has placed many wild medicinal species at risk of extinction (Cruse-Sanders *et al*. 2005). Thus, overharvested populations may be driven to extinction more quickly than those exposed to population fragmentation and habitat destruction alone (Vance 2002). In Jordan, medicinal plants have attracted increasing scientific (Hudaib *et al*. 2008; Al-Quran 2011; Nawash *et al*. 2013) and commercial attention (World Bank 2003). For example, recent overharvesting has pushed *Salvia fruticosa* L. to extinction from the wild in Jordan (Al-Eisawi 1996). Therefore, there is increasing pressure on wild plant populations, from which most medicinal plants are harvested, and there is an urgent need to conserve medicinal plants in their habitats for future generations (Al-Quran 2011).

Salvia spinosa L. (2n=2x=20, Al-Turki *et al.* 2000) and *Salvia syriaca* L. (2n=2x=22, Afzal-rafii 1980) are short-lived perennial herbs that can grow from 30 to 60 cm and 30 to 80 cm, respectively (Zohary & Feinbrun 1966). Their flowering season extends from April to June for *S. spinosa* and till July for *S. syriaca*. Flowers of both species receive visits from honey bees and bumble bees in the field (Al-Gharaibeh *et al*. 2016), which is supported by Claßen-Bockhoff *et al*. (2004), who describe bee pollination for the majority of *Salvia* species. Both *Salvia* species produce mucilaginous seeds (Al-Gharaibeh *et al*. 2016). Flowering stems typically break off at a point below the panicle in *S. spinosa* and seeds are subsequently dispersed by windblown tumbling, while gravity is the only dispersal mechanism for *S. syriaca* seeds (personal observation). Populations of *S. spinosa* inhabit the Irano-Turanian region and extend to the Mediterranean and Saharo-Arabian regions. Despite having the same chorotype as *S. spinosa*, *S. syriaca* is more common in the Mediterranean region but much rarer in the Saharo-Arabian region (Zohary & Feinbrun 1966). In the Mediterranean and Irano-Turanian regions, seed-producing populations of both species were found to be highly fragmented, with population sizes (number of individuals) being relatively small due to agricultural practices that treat both species as weeds due to their allelopathic properties (Qasem 2001). In addition, seed production is very low in *S. syriaca* (Al-Gharaibeh *et al*. 2016).

In summary, the main aim of Part I of my dissertation is: to reveal the influences of environmental variability between the Jordanian phytogeographical regions (habitats) on inducing local adaptation in seed germination and shaping genetic diversity of two *Salvia* species. To achieve this aim, in **Chapter 2**, I verify inter-population variability in germination responses (percentage and rate) to temperature and salt stress, and with respect to phytogeographic region; and I link such variation, as well as the levels of temperature and salinity that can trigger or inhibit germination in populations of both species, with their distribution range and local adaptation. In **Chapter 3**, I investigate the distribution of genetic diversity among and within populations of both *Salvia* species with respect to differences in water availability (Drought index) and temperature among their habitats (phytogeographical regions). In addition, I examine how the differences in climatic conditions and altitudinal ranges

among the three phytogeographical regions can influence the *Salvia* flowering phenology and consequently their genetic differentiation.

PART II

PHYLOGENY AND MOLECULAR DATA

In the 18th century, Linnaeus developed the binomial system of nomenclature. He not only gave birth to the field of taxonomy but was the first to draw a phylogenetic tree. Later Charles Darwin added the occurrence of two important processes in phylogeny, mainly, branching and subsequent divergence (Patwardhan *et al*. 2014). A phylogeny represents the historical pattern of relationships among organisms which has resulted from the actions of many different evolutionary processes (Jerrold 2014). Phylogenetic relationships are depicted by branching diagrams called cladograms, or phylogenetic trees (Jerrold 2014). Reconstructing the evolutionary relationships of species is a major goal in biology (Brown 2002). The endeavor of inferring of these relationships is not only of intrinsic interest to biologists, but also has many practical applications throughout biology (Heath *et al*. 2008). The constructed tree-like pattern that describes these relationships between the organisms allows biologists to infer when and where various structures, molecules, or behaviors have evolved in living organisms. Trees also provide information about the expected distribution of these features across taxonomic groups. Moreover, phylogenies facilitate the interpretation of comparative observations by accounting for the historical non-independence of organisms when analyzing across various levels of biological organization (e.g. genes, genomes, individuals, populations, species, or clades) (Heath *et al*. 2008). Briefly, while the classical phylogenetic approach relies on morphological characteristics of an organism, the more recent molecular approach depends on nucleotide sequences of RNA and DNA and sequences of amino acids of a protein. In this approach, the relationships among organisms or genes are studied by comparing homologues of DNA or protein sequences, whereas dissimilarities among the sequences indicate genetic divergence as a result of molecular evolution during the course of time (Dereeper *et al*. 2008). Molecular data, specifically DNA sequences, have received a great deal of attention as a potential source of "phylogenetically informative" characters that are putatively less ambiguous than non-

molecular characters (Chase *et al*. 1993). Since the basic bio-molecular framework of all organisms is similar and morphology of an organism is actually the translation of its genome, therefore, a combination of morphological and molecular analyses can strengthen the exercise of the determination of phylogenetic relationships of organisms to a great extent (Patwardhan *et al*. 2014).

The difficulty in obtaining large mathematical datasets when morphological characters are used was one of the main driving forces behind the gradual shift towards molecular data (Brown 2002). In the 1960s, studies depended largely on indirect assessments of DNA or protein variations (e.g. Protein electrophoresis). Later and during the 1980s, early research using rRNA involved direct reverse transcriptase mediated sequencing of portion of both the small and large subunits of ribosome (Hamby & Zimmer 1992). DNA-based phylogenetics began to be carried out on a large scale after the huge development in the methods utilizing DNA isolation, PCR, automated sequencing and then comparing these DNA or protein sequences. However, DNA is considered to yield more phylogenetic information than protein, because mutations that result in non-synonymous changes alter the DNA sequence but do not affect the amino acid sequence and the ability to examine both the coding and non-coding regions of the genome (Brown 2002). Recently, molecular phylogeny entered yet another rapidly expanding area with great improvements in the techniques and analyses of nucleic acid and protein sequencing (Patwardhan *et al*. 2014). In the next-generation sequencing (NGS), the introduction of instruments capable of producing millions of DNA sequence reads in a single run is rapidly changing the landscape of genetics, providing the ability to answer questions with heretofore unimaginable speed (Mardis 2008).

NUCLEAR AND CHLOROPLAST DNA

Plant cells contain three genomes with different evolutionary origins, ways of transmissions between generations and functions: nuclear, mitochondrial and chloroplastic (Kim *et al*. 2015). These three genomes evolve at different rates, with those from the nuclear genome being the fastest and those from mitochondrial and plastid DNA the slowest (Qiu *et al*. 1999). Since Mitochondrial DNA (mtDNA) shows hyper-variable structure in plant genomes (Park *et al*.

2013), chloroplast (cpDNA) and nuclear ribosomal DNA (nR) units are the primary sequences used to analyze plant genetic diversity as well as evolution (Soltis *et al*. 1999). Nuclear DNA is bi-parentally inherited while DNA in chloroplasts is usually maternally inherited (Wolf *et al*. 1989; Wicke *et al*. 2011). Therefore, the information retrieved separately from both genomes (plastid and nuclear) may appear incongruent (Savolainen & Chase 2003). However, the use of slowly evolving regions may provide only information to recover resolution at the family, order and/or genus level, while the use of faster evolving DNA regions could provide better resolution of relationships within younger taxa or taxa with slow evolutionary rates (Karehed *et al*. 2008). To infer both infrageneric and intrageneric relationships, therefore, the inclusion of different DNA genomes (chloroplast and nuclear) is effective (Mort *et al*. 2007; Karehed *et al*. 2008). In general, the use of molecular markers derived from nuclear and chloroplast genomes helps to obtain the true phylogeny and to improve the resolution. A near fully resolved tree might then provides an unprecedented opportunity to investigate the whole genetic history of a group of taxa, particularly by means of a combined analysis of bi-parentally inherited nuclear and maternally inherited organelle markers (Burban & Petit 2003; Petit *et al*. 2005; Wang & Ge 2006; Zarza *et al*. 2008).

METHODS TO BUILD AND TEST PHYLOGENETIC TREES

The construction of evolutionary trees is now a standard part of exploratory sequence analysis (Holder & Lewis 2003). Several methods to build phylogenetic trees have been developed, but building trees remains a hyper-complex mathematical problem because the number of solutions (possible trees) that ideally should be evaluated increases exponentially with taxon number (Savolanian & Chase 2003). The three main categories of computational techniques which are commonly used to build DNA-based phylogenetic trees are: (i) distance methods, in which pairwise genetic distances among sequences are used to build trees; (ii) maximum parsimony methods, in which overall nucleotide changes are minimized in the tree-building process (usually with equal probabilities for all changes, but which can also incorporate uneven probabilities such as in maximum likelihood methods); and (iii) maximum likelihood methods (including Bayesian analysis), a computational technique in which phylogenetic trees are built according to models of nucleotide evolution (i.e. incorporating different frequencies of change

and nucleotide composition as well as probabilities of change; Savolanian & Chase 2003, Harrison & Langdale 2006). In Bayesian analysis, a further set of assumptions (termed priors) are integrated into the original model and the branch swapping algorithms differ (Doaudy *et al*. 2002). However, the length of the computational time and the required degree of rigor are highly dependent on the analysis type (Harrison & Langdale 2006).

The distance method approach has the advantage of computational simplicity and therefore speed (Harrison & Langdale 2006), with reaching the accurate phylogeny if relatively few evolutionary events have occurred between sequences (Holder & Lewis 2003). Instead, parsimony and likelihood are more complex and time consuming methods as the branches of a starting tree are rearranged many times in the process of tree finding to form the tree that minimizes the number of character state changes (parsimony) or the tree that best fits the data (likelihood). However, parsimony and likelihood perform well most of the time (Hillis *et al*. 1994) and are preferred because they have the potential to rigorously explore the relationship between the tree and the entities included (Harrison & Langdale 2006). In both methods, accuracy in estimating the evolutionary relationships for the same entities varies (Kolaczkowski & Thornton 2004). Maximum parsimony is strongly biased towards recovering an incorrect tree under certain combinations of branch lengths, whereas maximum likelihood is not (Kuhner & Felsenstein 1994; Miyamoto & Fitch 1995; Huelsenbeck 1998). Likelihood-based techniques, however, only recover the true phylogeny when the correct model is used, and nonparametric statistical methods (parsimony) are often applied when the assumptions of parametric techniques are violated. Moreover, parametric methods, including maximum likelihood, are generally more powerful than nonparametric techniques and can be robust to certain violations (Chang 1996; Sullivan & Swofford 2001). On the other hand, computer simulations showed that maximum parsimony performs substantially better than current parametric methods over a wide range of conditions tested, including moderate heterogeneity and phylogenetic problems not normally considered difficult (Kolaczkowski & Thornton 2004) and the probability to recover the true tree topology under a hierarchy of models is higher than likelihood (Yang 1996). However, the development of a statistical methodology for the efficient estimation of the tree topology remains a difficult open problem (Yang 1996).

Since the resulting phylogenetic trees, from just any method, represent a hypothesis of relationship and do not factually depict evolutionary relationships, it is hard to assess how accurately they reflect true biological events (Harrison & Langdale 2006). Nevertheless, a number of methods exist to test the amount of support for a phylogenetic tree by evaluating the support for each sub-tree in the phylogeny (Douady *et al*. 2003). The evaluation of statistical clade support values is an important aspect of phylogenetic analysis as a function of the explanatory power of a given analysis (Grant & Kluge 2008). These support measures are a prerequisite to identify the well supported clades in a tree as a base for any inference of the evolution of the biological system (Huelsenbeck *et a*l. 2000; Lutzoni *et al*. 2001; Pagel & Lutzoni 2002), e.g. to serve as the conceptual framework for the study of trait evolution (Alfaro *et al*. 2003). The quantifiers most commonly used are bootstrap (Felsenstein 1985), jackknife (Farris *et al*. 1996) and posterior probability (Rannala & Yang 1996) support values. Grant & Kluge (2008) proposed the support measures from Parsimony, Maximum likelihood, and Bayesian phylogenetic inference are equivalent. However, bootstrapping provides a robust (though potentially time-consuming) way to assess confidence in phylogenetic estimates (Holder & Lewis 2003), while posterior probability and jackknife differ significantly from an ideal support index (Simmons *et al*. 2003). Posterior probability and jackknife support values have been found to be consistently higher and lower, respectively, than bootstrap values for the same clades in empirical analyses (e.g. Rannala & Yang 1996; Leaché & Reeder 2002; Simmons *et al*. 2003). In general, reasonable support is roughly 70% and high support is more than 95% (Felsenstein 1985; Hillis & Bull 1993).

TAXON SAMPLING, MOLECULAR DATA AND THE ACCURACY OF PHYLOGENETIC ANALYESE

Taxon and character sampling is a major issue in all data collection for molecular phylogenetics (Rosenberg & Kumar 2001). Many recent efforts to improve phylogenetic estimates have focused on taxon sampling rather than increasing sequence length or the number of overall characters in the analysis, and this often does have a beneficial effect on the accuracy of phylogenetic analyses (Heath *et al*. 2008). However, if phylogenetic accuracy increases more by either sampling more characters or more taxa, is still debated among authors (e.g. Kim 1996; Kim 1998; Graybeal 1998; Hillis 1998; Poe 1998; Zwickl & Hillis 2002; Hedtke *et al*. 2006;

Heath *et al*. 2008; Townsend & Lopez-Giraldez 2010). Several authors argued that extensive taxon sampling has a positive and important effect on the accuracy of phylogenetic estimates (e.g. Graybeal 1998; Zwickl & Hillis 2002), as additional taxa can disperse tree homoplasy, reducing the effect of long-branch attraction (Heath *et al*. 2008) and therefore the accuracy for the correct reconstruction increases (Hedtke *et al*. 2006). In contrast, other authors have argued that adding taxa alleviates the inconsistency problem (Kim 1996) and large character data sets alone are sufficient to estimate an accurate phylogeny. For example, results of Rosenberg & Kumar (2001) suggested that longer sequences, rather than extensive sampling, will better improve the accuracy of phylogenetic inference. However, both characters and taxa are shown to provide significantly improved accuracy when sampling includes characters evolving at optimal rates (Townsend & Lopez-Giraldez 2010). Therefore, extensive sampling in both dimensions of taxa and characters (genes) is desirable/necessary (Qiu *et al*. 1999).

PHYLGENIES OF TROPICAL FLORAS

Tropical rainforests are the most biologically diverse of terrestrial biomes (Phillips 1997). The fusion of traditional taxonomy, ecological data and molecular methods will provide baseline data for understanding both the origin and maintenance of tropical plant diversity (Dick & Kress 2009). Evolutionary biologists have focused on the description and classification of tropical diversity, and on factors involved in its origin (Schemske 2002). However, species composition in a biological community results from the interplay of evolutionary and ecological mechanisms (Ricklefs 1987). Phylogenies can help to reveal these mechanisms and provide unique information about the historical changes in the distribution of communities and biomes (Becerra 2005). Hence, phylogenetic trees are fundamental for understanding variation in species diversity (Heath *et al*. 2008). The shape of such trees contains clues about pattern of speciation and extinction (Kirkpatrick & Slatkin 1993). However, the application of fossil calibration points can have a significant impact on the estimates of node ages and therefore the accurate detection of the shifts in diversification rates (Hug & Roger 2007). Moreover, such analyses of the temporal distribution of diversification events use branch lengths obtained from time-adjusted phylogenies can be applied to investigate temporal and biogeographic processes that may have shaped the diversity of the tropical flora (Becerra 2005).

STUDY SPECIES AND AREA

The Marantaceae family, with approximately 29 genera and 550 species (Andersson 1998, Govaerts & Kennedy 2016), is the second largest family in the order Zingiberales (Sass *et al*. 2016). It has long been recognized as a sister of the family Cannaceae based on results of phylogenetic analyses of morphological (Kress 1990) and molecular (Kress *et al*. 2001; Kress & Specht 2006; Barrett *et al*. 2014) data. Marantaceae species are small to moderate-sized perennial, rhizomatous herbs characterized by a pulvinus (Petersen 1889, Kennedy 2000). The family is distributed throughout the tropics (Fig. 3) except in Australia, with 15 genera in tropical America, 9 in tropical Africa, and 6 in tropical Asia (Table 1). *Halopegia* and *Thalia* are the only transcontinental genera, they are represented in both tropical Africa/tropical Asia and tropical Africa/tropical America, respectively. In the Neotropics, the Marantaceae species richness is highest with an estimated 450 species while the remaining species are paleotropical (Africa: ~40 spp. Dhetchuvi 1996, Asia: ~50 spp., Suksathan *et al*. 2009). The morphological diversity found in the Paleotropics is higher than in the Neotropics (Andersson 1981). Only a few genera are found in the temperate regions of South and North America.

Figure 3. Distribution map of the Marantaceae family according to Missouri botanical garden (www.mobot.org).

www.mobot.org

Table 1. List of Marantaceae genera and their continental distribution.

Geographic distribution	Genus name	Genus synonymous
Tropical Africa	***Afrocalathea***	
	Halopegia*	
	Haumania	
	Hypselodelphys	
	Marantochloa	***Ataenidia***
	Megaphrynium	
	Sarcophrynium	
	Thaumatococcus	
	Trachyphrynium	
	Thalia*	
Tropical America	***Calathea***	
	Ctenanthe	
	Goeppertia	***Calathea***
	Hylaeanthe	
	Ischnosiphon	
	Koernickanthe	
	Maranta	
	Monophyllanthea	
	Monotagma	
	Myrosma	
	Pleiostachya	
	Sanblasia	
	Saranthe	
	Stromanthe	
	Thalia*	
Tropical Asia	***Donax***	
	Halopegia*	
	Indianthus	***Schumannianthus***
	Phrynium	***Cominsia, Monophrynium, Phacelophrynium***
	Schumannianthus	
	Stachyphrynium	

,** Genera with transoceanic distribution. ***Halopegia genus occur in mainland Africa, in Madagascar, and in South-East Asia. ***Thalia*** occurs in Africa and America.

Several previous studies estimated the relationships among major clades within the Marantaceae family either based on morphological and anatomical (Petersen 1889; Loesener

1930; Kirchoff 1983; Kress 1990; Andersson 1998; Kress *et al*. 2001) or on molecular data at the family level or for specific taxa (Andersson & Chase 2001; Prince & Kress 2006a; Prince &Kress 2006b; Suksathan *et al*. 2009; Ley & Claßen-Bockhoff 2011; Borchsenius *et al*. 2012). However, no previous phylogenetic study was conducted on the whole Marantaceae family under complete generic sampling. Therefore, in Chapter four, we take the advantage of this optimal sampling (188 taxa representing all genera) and sequences (~600 sequences representing both chloroplast and nuclear markers) to achieve a higher resolution and support for the branching of the Marantaceae backbone.

CHAPTER 2

Local adaptation to different phytogeographic regions: habitat-related variations in seed germination in response to temperature and salinity for two medicinal *Salvia* species from Jordan.

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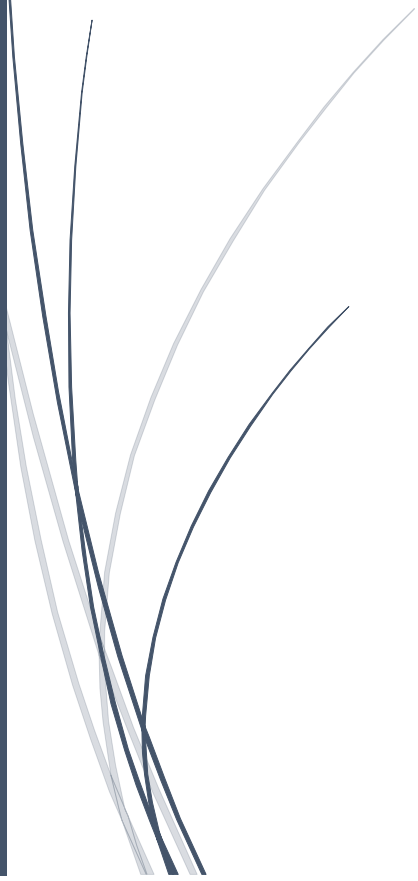

ABSTRACT

Salvia spinosa L. and *Salvia syriaca* L. are perennial medicinal herbs that occur in the Mediterranean, Irano-Turanian and Saharo-Arabian phytogeographic regions of Jordan. With respect to the seed germination requirements, prevailing environmental conditions in each phytogeographic region may promote local adaptation and consequently affect the distribution range of the species. Using seeds of both species collected from populations across the three regions, we tested responses to variations in temperature and salinity under laboratory conditions. Both species showed significant differences in cumulative germination percentages and germination rates (modified Timson Index) with temperature, while origin only significantly affected *S. spinosa* seeds. Both species germinated best under the highest temperature regime (32/20°C). The low temperature regime (8/4°C) completely inhibited germination in *S. syriaca*, whereas it led to 80% to 95% germination in *S. spinosa*, with significant variation being recorded between the phytogeographic regions. For both species, salt solutions of 0, 25 and 50 mM NaCl yielded the highest germination percentages and rates, which sharply and significantly declined at higher concentrations (100 and 200 mM NaCl). Our results provide evidence of local adaptation of the study species to salinity and temperature in the respective maternal environments, particularly in the Irano-Turanian and Saharo-Arabian regions. Such differentiation should be accounted for in future conservation planning.

Keywords: Germination percentage, germination rate, phytogeographic region, *Salvia spinosa*, *Salvia syriaca*.

INTRODUCTION

Medicinal plants are coming under increasing pressure from habitat destruction, bio-prospecting and climatic change (Roberson 2008). In addition, overharvesting has placed many wild medicinal species at risk of extinction as a result of reduced effective population sizes, soil seed bank depletion and loss of genetic diversity (Cruse-Sanders *et al*. 2005). Thus, overharvested populations may be driven to extinction more quickly than those exposed to population fragmentation and habitat destruction alone (Vance 2002).

Detailed information on germination behavior is important to the understanding of species in terms of their establishment, tolerance of abiotic factors, and dynamics across environmental gradients. Furthermore, it can provide clues to a species' survival strategy with respect to seed dormancy, pretreatments, optimal conditions for seed germination and the influence of seed provenance (Baskin & Baskin 1998; Ronnenberg *et al*. 2008; Hamasha & Hensen 2009). The study of seed germination behavior has provided the data required for developing and implementing effective strategies and protocols for the *ex situ* and *in situ* conservation of, for example, *Arabis kennedyae* (Andreou *et al*. 2011) and *Argyranthemum* species (Francisco-Ortega *et al*. 1994).

For habitat restoration projects, the use of native species and local seed provenances is often recommended because they are expected to be better adapted to the local climate and soil conditions (Bischoff *et al*. 2006). Seed provenance has been shown to significantly affect germination, indicating that genetic and environmental factors have a strong influence on seed germination traits (Bischoff *et al*. 2006). Inter-population variability in the response of germination behavior to environmental conditions has been widely reported for many species in accordance with differences among their habitats of origin (e.g. Hamasha & Hensen 2009), and can be attributed to both maternal influences and genetically based local adaptation.

A number of abiotic factors, such as temperature, salinity, light and soil moisture, can have significant individual and cumulative species-specific effects on germination, thereby affecting the distribution of plants in natural habitats (Huang *et al*. 2003; El-Keblawy & Al-Rawai 2005; Gorai *et al*. 2011). In addition, environmental factors can influence the onset,

percentage and rate of germination for different species (Cendán *et al*. 2013). For example, when moisture conditions are favorable, salinity and temperature represent major factors affecting seed germination and initial seedling establishment in arid and saline regions (Huang *et al*. 2003; El-Keblawy & Al-Rawai 2005; Al-Khateeb 2006; Maraghni *et al*. 2010). Knowledge of the effects of temperature and salinity on germination may therefore be useful in evaluating germination characteristics or establishment potential for species in arid regions (Jordan & Haferkamp 1989; Maraghni *et al*. 2010). In addition, such knowledge will complement the limited data available on the influence of soil salinity as a driver in plant ecology and phytogeography (Bui 2013).

Species with wide distribution ranges can show either high phenotypic plasticity (Banta *et al*. 2012) or intraspecific differentiation in germination behavior under varying environmental conditions (Keller & Kollmann 1999; Fenner & Thompson 2005; Ronnenberg *et al*. 2008). However, variation in phenotypes may be determined not only by the genotype and the environment of the individuals but also by maternal effects (Roach & Wulff 1987), and Gutterman (2000) contends that phenotypic variation is caused by the local conditions under which seeds mature. Maternal environmental conditions can have a strong impact on plant fitness (Lacey & Herr 2000), which in turn can affect the quality of the offspring (Mousseau & Fox 1998) and influence seed germination (Rossiter 1996, 1998). Maternal effects are considered to be adaptive when they enhance offspring fitness in an environment similar to that experienced by the parental generation (Rossiter 1996, 1998; Donohue & Schmitt 1998).

In addition, differences in genotype and phenotype can interact with environmental factors such that fitness can vary among populations as a function of both local adaptation and the local environment (Banta *et al*. 2012). In this respect, species exhibiting greater intraspecific variation can have a greater chance of coping with climate change, as it may buffer species against associated adverse effects (Thuiller *et al*. 2004). Genetically determined local adaptation can be evaluated by examining the germination traits of plants with different provenances under a variety of environmental conditions (Luzuriaga *et al*. 2006; Sales *et al*. 2013). However, as plants with broad geographic distributions are generally associated with high colonization

and persistence, plants with limited distribution are expected to have less success in plant recruitment than more widespread species (Astegiano *et al*. 2013).

In Jordan, medicinal plants have attracted increasing scientific (Hudaib *et al*. 2008; Al-Quran2011; Nawash *et al*. 2013) and commercial attention (World Bank 2003). For example, recent overharvesting has pushed *Salvia fruticosa* L. to extinction from the wild in Jordan (Al-Eisawi 1996). Therefore, there is increasing pressure on wild plant populations, from which most medicinal plants are harvested, and there is an urgent need to conserve medicinal plants in their habitats for future generations (Al-Quran 2011).

For the present study, we investigated local adaptation in the germination behavior of two *Salvia* species from Jordan. *Salvia spinosa* L. and *Salvia syriaca* L. (Lamiaceae) are two native medicinal plant species (Oran & Al-Eisawi 1998; Ulubelen 2003) that occur across the Mediterranean, Irano-Turanian and Saharo-Arabian phytogeographic regions of Jordan (Zohary & Feinbrun 1966; Zohary 1973; Danin 1992). We assessed the diversity between seed provenances of the study species in order to test for intraspecific variation in seed germination and local adaptation. For instance, several studies have demonstrated that seeds that originate from populations subjected to high-salinity germinate more readily and earlier than those from less saline environments (Ungar 1991; Van Zandt & Mopper 2004; Yao *et al*. 2010). Therefore, this approach may serve as a model for testing differences in germination between individuals from different provenances, and our results may provide valuable knowledge to support conservation and restoration programs for such species elsewhere. In addition, to test whether germination behavior reflects local adaptation to the prevailing environmental conditions of the three seed-source regions (Mediterranean, Irano-Turanian and Saharo-Arabian), we tested for intraspecific variation in percentages and rates of seed germination between our seed provenances.

The objectives of this study were: (i) to verify inter-population variability in germination responses (percentage and rate) to temperature and salt stress, and with respect to phytogeographic region; and (ii) link any such variation, as well as the levels of temperature and

salinity that can trigger or inhibit germination in populations of both species, with their distribution range and local adaptation.

MATERIALS AND METHODS

Study area and study species

Jordan is situated between longitudes 35°40’ and 39°E and between latitudes 29°30’ and 34°N, and it is unique in its natural diversity. Biogeographically, Jordan consists of four major phytogeographic regions, namely the Mediterranean, Irano-Turanian, Saharo-Arabian and Sudanian regions, and its environmental heterogeneity is very remarkable (Zohary 1973; Al-Eisawi 1996). The climate is characterized by moist cold winters and hot dry summers, resulting in extreme variability in rainfall across the year. Rainfall decreases from west to east and from north to south (Al-Eisawi 1996). Generally, the content of soluble salts in the soils increases with decreasing rainfall. Salinity ranges from 9 to 600 mM within Jordanian soils, with the highest levels occurring in the Jordan Valley (Al Qudah 2001), where environmental conditions of the Sudanian region prevail.

According to geographic information and climatic data from 31 meteorologic stations as presented by Al-Eisawi (1996), the Mediterranean, Irano-Turanian and Saharo-Arabian regions of Jordan are characterized by elevational ranges of 700–1700 m a.s.l., 400–700 m asl. and 600–700 m a.s.l., respectively. Annual rainfall is 300–600 mm, 150–250 mm and 50–100 mm, respectively. Mean temperature in summer is 20°C, 25°C and > 30°C, respectively (Table 1), and soil salinity is equivalent to 9 mM, 24 mM and 150–200 mM NaCl, respectively (Al Qudah 2001).

Salvia spinosa and *S. syriaca* are perennials that can grow to 30–60 cm and 30–80 cm tall (Zohary & Feinbrun 1966), respectively, with the flowering season extending from April to June for *S. spinosa* and until July for *S. syriaca*. The majority of *Salvia* species are pollinated by bees (Claßen Bockhoff *et al*. 2004), whereas flowers of both species receive visits from honeybees and bumblebees (field observation). Seed production per mature plant ranges from 1500 to 10 000 seeds for *S. spinosa*, and from 100 to 2000 for *S. syriaca*. Nutlets of both study species contain a pectinaceous mucilage layer that imbibes a large amount of water when moistened (Oran 1997). Populations of *S. spinosa* inhabit the Irano-Turanian region and extend to the

Mediterranean and Saharo-Arabian regions. Despite having the same Chorotype as *S. spinosa*, *S. syriaca* is more common in the Mediterranean region but much rarer in the Saharo-Arabian region (Zohary & Feinbrun 1966; Danin 1992).

Table 1. Geographic, meteorologic and site descriptions of the seed sources of the *Salvia spinosa and Salvia syriaca* populations

	Phytogeographic	Geography			Climate				
Population	**region**	**Lt**	**Ln**	**Al**	**Rn**	**Tm**	**Ta**	**Tj**	**No. Ind.**
Salvia spinosa									
Gafgafa	Med	32°.37'	35°.92'	782	412	17.2	24.4	9.1	18
Abo Bana	Med	30°.87'	35°.67'	1148	220	14	20.7	6.5	17
Fjaej	Med	30°.57'	35°.63'	1263	300	12.3	19.4	4.4	19
Kings Road	Med	30°.08'	35°.43'	1616	270	14.1	21.6	5.8	67
Sarrot	Ira	32°.17'	35°.95'	534	250	15	27	4	23
Humret Sahen	Ira	32°.10'	35°.66'	650	280	19.6	26.5	11.7	19
JUST	Ira	32°.48'	35°.98'	588	250	15	27	4	173
Al Jezzah	Ira	31°.68'	35°.96'	715	157	16.7	23.6	8.4	53
Safawi	Sah	31°.99'	36°.85'	615	75	18.7	27	9.2	31
Al-Azaraq	Sah	31°.87'	36°.74'	560	91	17.7	25.1	8.7	15
Wadi Rum	Sah	29°.60'	35°.38'	960	59	25.1	39.2	9.3	12
Borqu Castle	Sah	32°.60'	38°.01'	545	82	18	26	7	13
Salvia syriaca									
Ras Yousif	Med	32°.72'	35°.87'	820	473	15.2	22.4	7.1	80
Airport Highway	Med	31°.87'	35°.88'	921	275	12.3	23.6	8.4	35
Madaba	Med	31°.74'	35°.84'	781	320	18.3	24.9	10.5	> 2000
Abo Bana	Med	30°.87'	35°.67'	1148	220	14	20.7	6.5	19
Dieban	Ira	31°.59'	35°.79'	636	301	18.3	24.9	10.5	> 300
Karak	Ira	31°.20'	35°.73'	747	179	17.4	24.1	9.5	250
Rehab	Ira	32°.35'	36°.02'	796	220	15	24	4	277
Sarrot	Ira	32°.17'	35°.95'	534	250	15	27	4	63
Mafraq 2	Sah	32°.37'	36°.21'	526	155	16.9	24.8	7.4	51
Mafraq 1	Sah	32°.33'	36°.19'	722	160	15	24	4	42

Phytogeographic regions based on Al-Eisawi (1996): Med, Mediterranean; Ira, Irano-Turanian; Sah, Saharo-Arabian. The climate data for the sampling sites were obtained from Hamasha and Hensen (2009), and the Jordanian Meteorological Department (unpublished data). Lt, latitude; Ln, longitude; Al, altitude (m); Rn, mean annual rainfall (mm); Tm, temperature mean (°C); Ta, mean hottest month (August) temperature (°C); Tj, mean coldest month (January) temperature (°C). No. ind., number of individuals per sampled population.

Seed collection and germination tests

Sampling of both *Salvia* species aimed to cover most of their natural distribution within Jordanian borders. Seeds were collected from populations of three out of the four phytogeographic regions, covering the whole extent of their occurrence. Sampling from microhabitats that seemed to be extremely saline or that may have been affected by agricultural activities was avoided. In the period between May–June and late June–July 2013, mature seeds were collected from 12 and 10 natural populations of *S. spinosa* and *S. syriaca*, respectively. Four populations per region were sampled, except for *S. syriaca* in the Saharo-Arabian region, where we only found two populations with mature seeds (Table 1). Seeds were collected from three to 10 individuals per population depending on the population size (Table 1). Seeds of both species were placed in paper bags and stored at room temperature (20°C). We germinated a small proportion (20 seeds per population) of the collected seeds directly after collection (August 2013) at the Jordan University of Science and Technology seed laboratory to assess seed dormancy. After 3 months of storage, 50 seeds per population were subjected to triphenyl tetrazolium chloride to assess initial seed viability. In *S. spinosa*, the average mass per seed lot (5 × 25 seeds) was calculated for the four populations from each region. Populations of the Irano-Turanian region produced larger and heavier seeds than those of the Mediterranean and Saharo-Arabian regions (0.164 g, 0.146 g and 0.142 g, respectively). Owing to the large percentage of empty seeds in *S. syriaca*, we were not able to calculate the average mass of seed lots. In order to eliminate the effect of the empty seeds from our results, seeds were soaked in distilled water for 2 minutes before each experiment, with any floating seeds subsequently being discarded.

Experiments were carried out with five replicates of 25 full intact seeds per population. Seeds were germinated on moistened filter paper (Whatman No. 1) in 70-mm-diameter Petri dishes. Germination was carried out at 12 h warm white light /12 h darkness (Light Thermostat, Typ 1301, RUMED, Halle (Saale), Germany) and checked every 2 days, with seedlings being removed. To ensure randomness among treatments and populations, the horizontal and vertical positions for all Petri dishes inside the germination chambers were shifted every 2 days.

Effects of temperature and phytogeographic regions on germination

To examine variation in germination among populations from different phytogeographic regions in response to the various temperature regimes, we simulated the mean temperature regime prevailing in all regions during the main germination period (8/4°C and 20/10°C, November and February–March, respectively) and during summer (32/20°C) (after Hamasha & Hensen 2009). Germination of seeds from all populations was tested with these three simulated temperature regimes on filter paper moistened with 10 mL of distilled water. The tests were terminated after 38 days.

Effects of salinity and phytogeographic region on germination

Due to the limited availability of *S. syriaca* seeds, and based on the results from the former experiment, we examined the influence of salinity and provenance on seed germination in both species by incubating seeds only under the optimum temperature regime (32/20°C). Temperature can interact with salinity to affect seed germination (Luan *et al*. 2014) and the evaluation of tolerance of salinity without temperature interaction can be carried out more effectively at the most suitable germination temperatures than at lower or higher temperatures.

We germinated seeds on one layer of filter paper moistened with 10 mL of either distilled water (control) or different NaCl solutions (25, 50, 100 and 200 mM). Petri dishes with lids were tightly sealed using Parafilm to prevent evaporation. On a weekly basis, seeds were transferred to new Petri dishes with new filter paper, lids and fresh solutions to maintain salt concentrations close to the target levels throughout the germination period. All tests were terminated after 22 days.

Statistical analysis

All statistical analyses were performed in R-3.0.2 (R Core Team 2013). We used the cumulative proportion of germinated seeds per dish by the end of the experiment (i.e. germination probability or percentage) and the modified Timson Index (TI) as response variables. TI describes the germination velocity and is calculated as follows: $TI = \Sigma G/t$, where G is the

percentage of seed germination at 1-day intervals, and *t* is the overall germination period (Pérez-Fernández *et al*. 2006). In this equation, the TI value increases with germination velocity as well as with the number of germinated seeds. We used linear and generalized linear mixed effects models to analyze TI and germination probability, respectively (R-package lme4, Bates *et al*. 2013). While we assumed a normal error distribution for TI based on model analytic plots (Crawley 2013), germination percentage (probability) was analyzed as a binomial variable using the *logit link* function.

In an explorative analysis we tested the effect of seed mass on the probability of *S. spinosa* germination and TI by means of logistic and linear regression, respectively, with both analyses including a random effect of population. As the effects were not significant, we excluded seed mass from further analysis. All models included the main effects (phytogeographic region and temperature or salinity treatment) and two-way interactions of the latter two with phytogeographic region on germination probability and TI, respectively. These models also included a random effect of population. The significance of these main effects and interactions was assessed using Wald χ^2 tests, and pairwise comparisons were performed with Tukey's post-hoc tests ($P < 0.05$).

RESULTS

Neither species showed dormancy at room temperature (25°C in Jordan), with 85% of *Salvia spinosa* seeds and 50% of *Salvia syriaca* seeds germinating. Initial seed viability values for *S. spinosa* and *S. syriaca* were 90% and 60%, respectively. The low percentage of viable seeds for *S. syriaca* was due to the presence of seed predators. Seed mass in *S. spinosa* had no significant effect on germination percentages ($P = 0.156$).

Effects of temperature and phytogeographic regions on germination

The analysis of probability of germination and the TI showed a significant effect of temperature, phytogeographic region and their interactions on both metrics of seed germination of *S. spinosa* (P values ≤ 0.02 in all cases; see Table 2). Phytogeographic region showed no significant effect on the probability of germination ($P = 1$) and TI ($P = 0.191$) of *S. syriaca* seeds, whereas temperature and its interaction with phytogeographic region did (P values ≤ 0.04 in all cases;

see Table 2). In both species, the highest TI was reached under the 32/20°C regime, followed by the 20/10°C regime (Fig. 1). Variation in the day germination commenced and the rate of germination with respect to seed affinity (phytogeographic region) was only significant under the low temperature regime (8/4°C) in *S. spinosa* (Fig. 1). Saharo-Arabian populations of the former species germinated 8 days earlier than Mediterranean populations and achieved the highest germination percentages (95%) under this low temperature regime. In contrast, seeds of all affinities in *S. syriaca* failed to germinate under the low temperature regime (8/4°C). Higher TI values for both species were achieved under the higher temperature regime (32/20°C) than the lower one (20/10°C) (Fig. 1).

Table 2. Results of the mixed model analyses for the effects of temperature regime (8/4°C, 20/10°C and 32/20°C), salinity concentration (0, 25, 50, 100 and 200 mM of NaCl) and phytogeographic region on seed germination for *Salvia spinosa* and *Salvia syriaca* populations

	Salvia spinosa						*Salvia syriaca*					
	Cum-germ (%) †			TI‡			Cum-germ (%)			TI		
Source	DF§	Chisq*	*p*	DF	Chisq	*p*	DF	Chisq	*p*	DF	Chisq	*p*
Treatment (Temperature)	2	68.83	< 0.001	2	346.79	< 0.001	2	572.14	< 0.001	1	105.43	< 0.001
Phytogeographical region	2	12.07	0.002	2	14.76	< 0.001	2	0	1	2	3.31	0.191
Treatment ×Phytogeographical region	4	78.74	< 0.001	4	57.27	< 0.001	4	26.62	< 0.001	2	6.39	0.041
Treatment (salinity)	4	606.87	< 0.001	4	569.4	< 0.001	4	313.52	< 0.001	4	171.88	< 0.001
Phytogeographical region	2	7.78	0.02	2	3.53	0.171	2	14.3	< 0.001	2	1.47	0.479
Treatment ×Phytogeographical region	8	151.05	< 0.001	8	27.45	< 0.001	8	42.35	< 0.001	8	5.49	0.704

† Cum-germ (%), Cumulative germination percentages; ‡ TI, Timson Index; § DF, Degree of freedom; * Chisq, Chi square test (Wald χ^2).

Effects of salinity and phytogeographic region on germination

In both study species, the highest percentages and rates of germination were recorded in 0, 25 and 50 mM NaCl. Seed affinities had slight but insignificant effects on final germination in the saline treatments (Fig. 2). NaCl concentrations > 50 mM increasingly hampered seed germination in both *Salvia* species, but some seeds still germinated at 200 mM. Both probability of germination and TI decreased significantly ($P < 0.001$ in all cases; see Table 2) with increasing

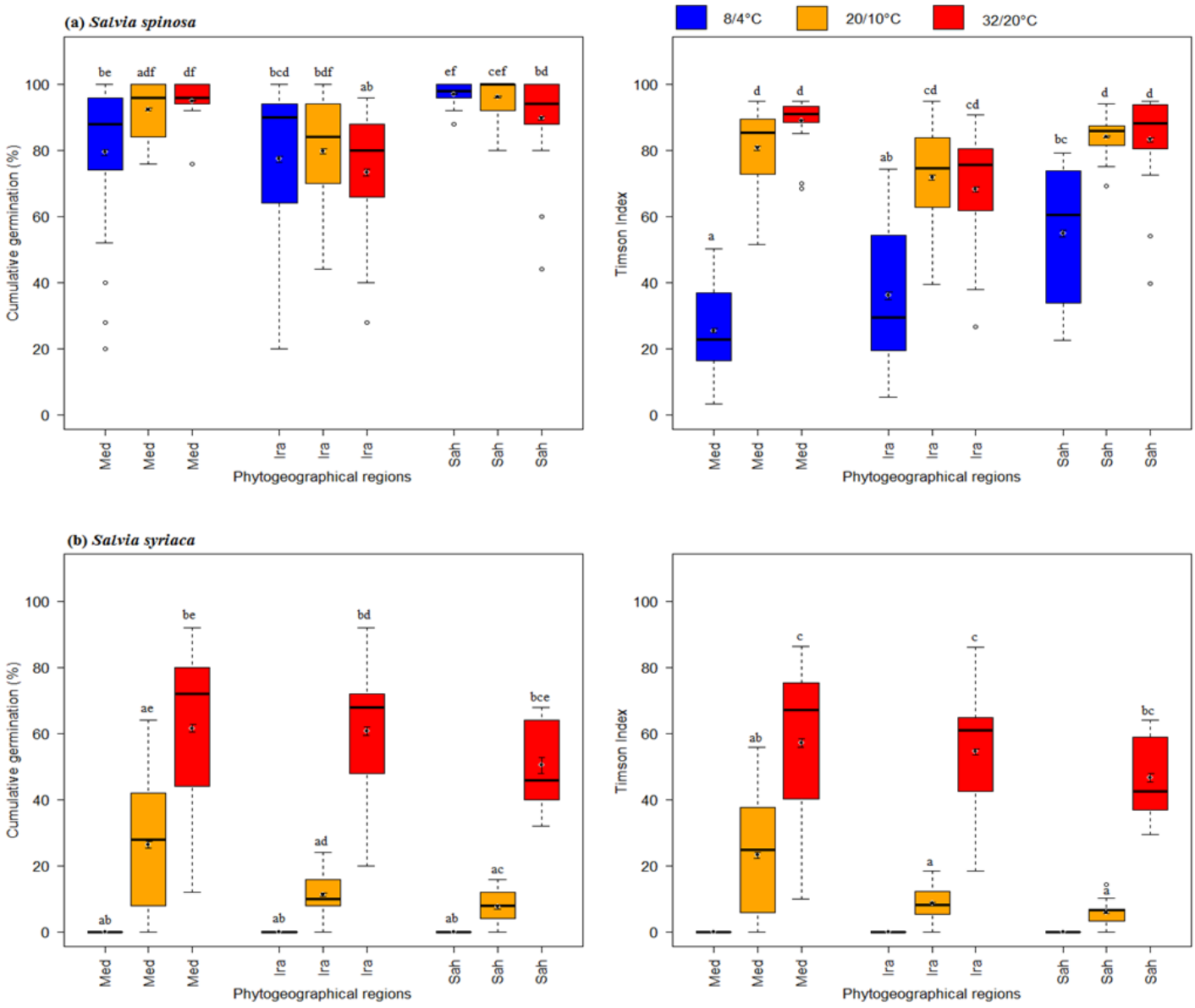

Figure 1. Cumulative germination percentages (analyzed as germination probability up to day 38) and modified Timson Index (TI) for seeds of (a) *Salvia spinosa* and (b)*Salvia syriaca* from different phytogeographic regions and under different day/night temperature regimes. Factor level combinations with the same letter are not significantly different at the 0.05 significance level (post-hoc Tukey's test). Black dots (●) represent arithmetic means while error bars represent standard errors. Boxplots show the following parameters of the original data distributions: boxes, interquartile range; horizontal line, median; whiskers, either maximum/minimum value or 1.5 times the interquartile range of the data (whichever is smaller); points, outliers (with respect to whiskers' range). ■, 8/4°C; ■, 20/10°C; ■, 32/20°C.

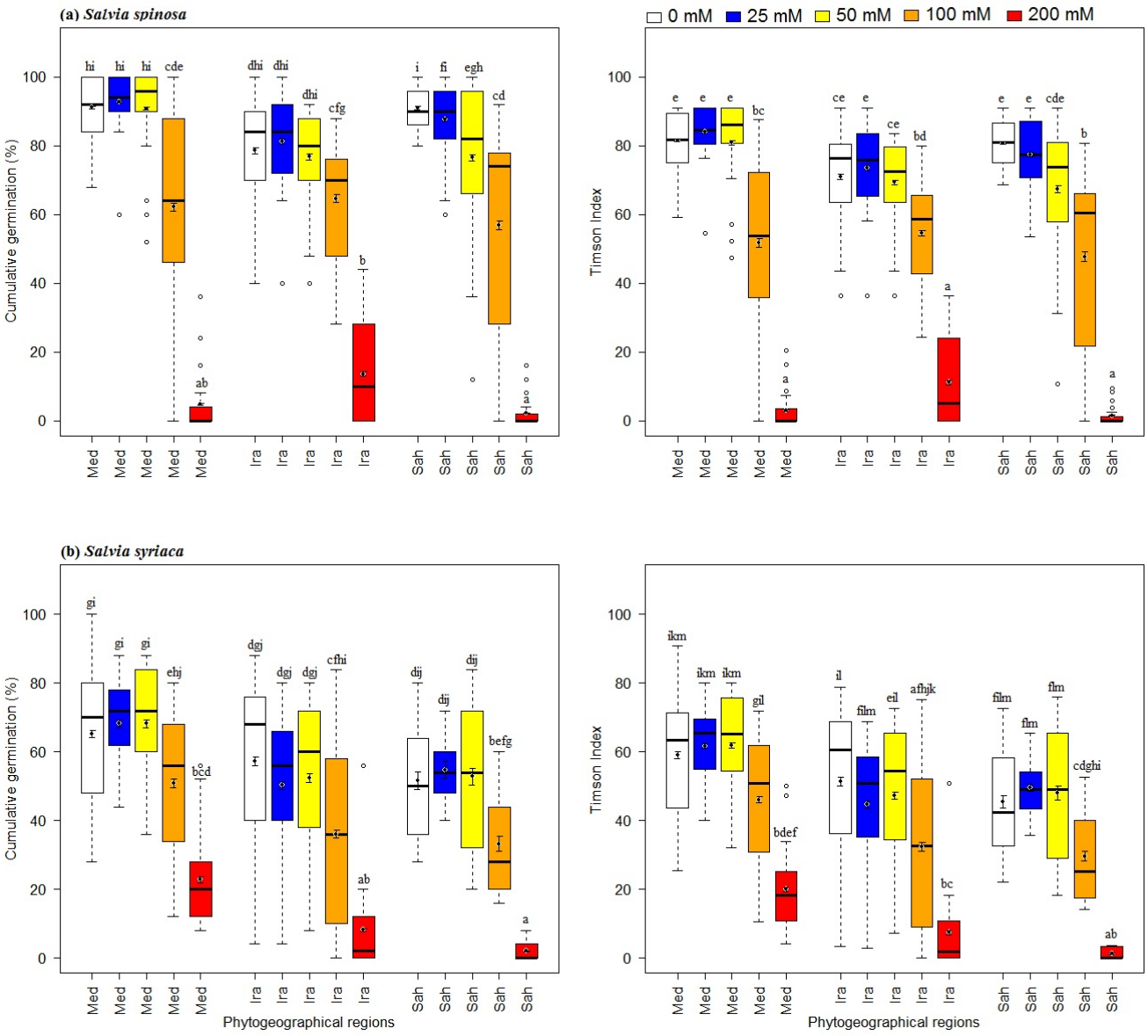

Figure 2. Cumulative germination percentages (analyzed as germination probability up to day 22) and modified Timson Index (TI) for seeds of (a) *Salvia spinosa* and (b)*Salvia syriaca* from different phytogeographic regions (Med, Mediterranean; Ira, Irano-Turanian; Sah, Saharo-Arabian) and at different NaCl concentrations (0, 25, 50, 100 and 200 mM). Factor level combinations with the same letter are not significantly different at the 0.05 significance level (post-hoc Tukey's test). Black dots (●) in boxplots represent arithmetic means while error bars represent standard errors. Boxplots show the following parameters of the original data distributions: boxes, interquartile range; horizontal line, median; whiskers, either maximum/minimum value or 1.5 times the interquartile range of the data (whichever is smaller); points, outliers (with respect to whiskers' range). □, 0 mM; ■, 25 mM; ■, 50 mM; ■, 100 mM; ■, 200 mM.

NaCl concentration, and pairwise comparisons revealed the threshold of 50 mM under which seeds of both species germinated well. Phytogeographic region had a significant effect on probability of germination (*P* values ≤ 0.02 in both species; see Table 2) but a non-significant effect on TI (*P* values ≥ 0·171 in both species; see Table 2). The interaction of salinity with phytogeographic region was highly significant in terms of probability of germination and the TI for *S. spinosa* seeds (*P* < 0.001 in both cases; see Table 2), and for the probability of germination of *S. syriaca* seeds (*P* ≤ 0.001) but not TI (Table 2; *P* = 0.704). Post-hoc tests revealed that *S. spinosa* seeds from the Irano-Turanian region showed a significantly higher probability of germination than seeds from the Saharo-Arabian region under extreme salinity (200 mM; Fig. 2), whereas in *S. syriaca*, seeds from the Mediterranean region showed a significantly higher probability of germination than seeds from the Saharo-Arabian region under extreme salinity (200 mM).

In both species, and for all affinities, seeds started to germinate after 1–3 days of incubation. At each level of salinity (0, 25, 50, 100 and 200 mM), the differences in the germination rates of the two species among the three phytogeographic regions were not significant. In both species, and among populations of the same seed affinity, TI values showed a sharp and mostly significant decline with increasing salinity above 50 mM (Fig. 2). In terms of the Timson Index, and among the phytogeographic regions for *S. spinosa*, the rate of decline with increasing salinity differed significantly (i.e. the rate of decline was higher in both the Mediterranean and Saharo-Arabian populations than in the Irano-Turanian population) (Fig. 2).

DISCUSSION

Our results show that seeds of both species were not dormant after ripening. Germination in *S. spinosa* seeds can be triggered under a wide range of temperature regimes, from 8/4°C to 32/20°C, which indicates that they are able to germinate at any time from spring to winter in habitats where moisture is available. The inability of *S. syriaca* seeds to germinate at 8/4°C indicates a limit around these temperatures for the species. With regard to salinity, both species achieved the highest percentage and rate of germination at 0, 25 and 50 mM NaCl, but they largely failed to germinate at 200 mM NaCl.

Both *Salvia* species achieved optimum germination rates at 32/20°C. In agreement, Côme (1993) found that seeds of *S. officinalis* germinated within a range of 10–25°C, while those of *S. sclarea* showed a slightly broader range of optimal temperatures (10–30°C). Gorai *et al*. (2011) reported that seeds of medicinal Tunisian sage (*S. aegyptiaca*) germinated under a wide range of temperatures and achieved the highest germination percentage (77%) at 30°C. They also concluded that variation in the optimal temperature and germination rates between species might constitute an adaptive strategy to harsh environmental conditions.

Our results support the assumption that adaptive processes generated variation in germination between and among species populations. In a similar study on four Jordanian *Stipa* species, there was significant variation in seed germination with temperature for all four species, and seed provenance had a significant effect for three of the species (Hamasha & Hensen 2009). At the species level, the ability of Saharo-Arabian populations of *S. spinosa* to germinate and achieve a high final germination percentage under an 8/4°C temperature regime more rapidly than Mediterranean and Irano-Turanian populations suggests that the former populations are better adapted to germinating under cold conditions. Among species, the ability to germinate at low temperatures may partially explain the deeper distribution of *S. spinosa* populations in the Saharo-Arabian region. Such adaptation may be interpreted as seeds of populations in arid and semi-arid areas (Irano-Turanian and Saharo-Arabian regions) being able to respond to moisture availability in late autumn and early winter to enable germination when temperatures are relatively low, making them less likely to suffer from desiccation. Our assumption is in line with Hamasha and Hensen (2009), who reported that variability in soil moisture imposes fluctuating selection pressures on different bioclimatic regions, thereby influencing phenologic patterns such as flowering time and timing of seed germination. Moreover, steep climatic gradients might shape the genetic structure of plant populations (Hamasha *et al*. 2013), which is supported by the differences in germination ability between the two *Salvia* species under winter temperatures, as *S. syriaca* populations have limited distribution in the Saharo-Arabian region.

In the present experiment, the seeds of both *Salvia* species showed tolerance of lower salinity concentrations (25 and 50 NaCl mM), with a rapid decline in germination percentages

with increasing salinity above 50 mM. At the lower concentrations, germination percentages were similar to those of the control (Fig. 2). In agreement, Gorai *et al*. (2011) found that seed germination of *Salvia aegyptiaca* at 30°C tolerated moderate salinity levels and was only completely inhibited at 300 mM NaCl. In another study, moderate salinity levels of 50 and 100 mM NaCl had no significant effect on the germination of mucilaginous seeds of *Diplotaxis harra* at the most suitable temperature regime, and germination was completely inhibited at 200 mM (Tlig *et al*. 2008). Therefore, it is possible that for *Salvia* species, mucilage can serve as a kind of ‘‘filter'' and/or ‘‘sorbent'' to help mitigate the effects of low salt concentrations (25 and 50 mM), as proposed by Yang *et al*. (2011). The same experiment demonstrated that intact achenes of *Artemisia sphaerocephala* had higher germination percentages with increasing osmotic potential and NaCl concentration than those with mucilage removed. Elsewhere, mucilage water uptake in *Henophyton deserti* seeds was unaffected by low and moderate salt concentrations but inhibited under high polyethylene glycol (PEG)-6000 concentrations (Gorai *et al*. 2014).

In our study, at salt concentrations of 100 and 200 mM, phytogeographic region started to have a significant effect on germination percentages (Fig. 4). This significant variation may have resulted from adaptive differentiation or maternal effects on seed characteristics and germination behavior or both. Maternal plants can influence seeds by contributing organelles, endosperm, seed coats and other structures (Roach & Wulff 1987; Baskin & Baskin 1998). Van Zandt and Mopper (2004) found that seeds produced by maternal plants growing under high salinity germinated earlier and in greater numbers than seeds from low-salinity plants, and that such plants can produce larger, more salt-tolerant seeds (Yao *et al*. 2010). Therefore, factors that cause maternal effects can potentially affect seed size (Van Zandt & Mopper 2004). Variation in seed mass or size has an adaptive function with respect to germination in many plant species and especially in halophytes (Khan & Weber 2008). Several authors found that tolerance of salinity is proportional to seed mass in species such as *Salicornia europaea* (Ungar 1987), *Atriplex triangularis* (Khan & Ungar 1984) and *Chenopodium album* (Yao *et al*. 2010). Moreover, according to Lu *et al*. (2010), the mucilage layer produced by the larger *Diptychocarpus strictus* seeds increased seed width by 50%, while in smaller seeds it

only increased by 5%. Therefore, we expect that the thicker mucilage layer produced by larger seeds can absorb and filter more water, as may be the case for the largest seeds found in our study, which were of Irano-Turanian origin. We therefore propose that for our study species, populations with larger seed masses may be able to tolerate higher salinity. Moreover, we assume that such tolerance is further enhanced by the higher production of mucilage in larger seeds than in smaller seeds.

In summary, we assume that the ability of Irano-Turanian and Saharo-Arabian populations to produce larger seeds than Mediterranean populations can be interpreted as an advantageous strategy for both *Salvia* species with respect to their responsiveness and adaptation to salinity in different phytogegraphic regions. According to Al Qudah (2001), salinity in Jordanian soils is about 9 mM in the xeric moisture regime (Mediterranean), 24 mM in the transitional xeric-aridic region (Irano-Turanian), 150 mM in xeric-aridic soils (Saharo-Arabian), and it ranges from 200 mM in some locations in the Saharo-Arabian region to reach a maximum of 600 mM in the Sudanian region. In addition, the low germination percentage recorded under 200 mM saline conditions demonstrates that both study species have a low tolerance of salinity at the germination stage. Flowers *et al*. (2010) use a threshold of 200 mM NaCl to separate halophytes from glycophytes, thus both of our study species are glycophytes (Danin 1992). This limit of tolerance partly explains why these species do not exist in the Sudanian region of Jordan, which is dominated by halophytes as salt concentrations can reach up to 600 mM NaCl (Al Qudah 2001). However, our study could be complemented by further investigation of adaptation to small-scale differences in soil salinity at the local level. One way to achieve this would be by involving populations from different microhabitats of varying salinity in each region and to include analyses of soil samples.

Successful seedling establishment is correlated with seed germination traits, which evolve in response to selection regimes characteristic of particular habitats, especially climatic regimes. Species regulate the onset and level of germination in response to environmental conditions. Therefore, we conclude that information on seed germination responses to temperature and salinity for each phytogeographic region should be taken into account before restoration projects commence, as this will facilitate the successful establishment of seeds in

the given region. Seed collection, either for restoration projects or seed banks, should focus on selecting seeds from the respective provenances, as restoration with local seeds is recommended not only for germination but also for seedling survival and plant maturation.

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CHAPTER 4

Phylogeny of the pantropically distributed family Marantaceae

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(Manuscript)

ABSTRACT

Phylogenetic resolution of problematic taxonomic groups can be improved and strengthened by increasing the amount of molecular data and the sampling of ingroup taxa. Here we reassess the phylogeny of the pantropically distributed family Marantaceae compiling a complete genera sampling and using both chloroplast and nuclear markers. Phylogenetic analyses were conducted on a set of four genetic markers (chloroplast markers: *trn*L, *mat*K, *rps*16 and nuclear marker: ITS) for 187 ingroup taxa representing all 29 Marantaceae genera under Maximum Likelihood (ML), Maximum parsimony (MP) criteria and Bayesian Inference (BI). The resulting tree topology focusing on the resolution of major clades was mostly congruent among applied methods and with preexisting family phylogenies. A few relationships within genera or clades were newly resolved here. A genus, *Monophyllanthe*, added to the phylogeny here for the first time appeared within the *Stachyphrynium* clade as sister to the genus *Marantochloa*. Only the affinity of the genus *Haumania* to one of the other major clades still remained uncertain. Four genera, *Calathea*, *Ischnosiphon*, *Maranta* and *Schumannianthus* were identified as being non-monophyletic. Such a robust phylogeny based on multiple molecular markers from both genomes and a complete sampling of Marantaceae genera will be a solid base to investigate in the future the timing of speciation and the migration events leading to the currently observed biogeographical patterns in this family.

Keywords: Backbone, *Haumania*, Marantaceae, *Monophyllanthe*, monophyly.

INTRODUCTION

The Marantaceae family, with approximately 29 genera and 550 species (Andersson 1998, Govaerts & Kennedy 2016), is the second largest family in the order Zingiberales (Sass *et al.* 2016). It has long been recognized as a sister of the family Cannaceae based on results of phylogenetic analyses of morphological (Kress 1990) and molecular (Kress *et al.* 2001; Kress & Specht 2006; Barrett *et al.* 2014) data. Marantaceae species are small to moderate-sized perennial, rhizomatous herbs characterized by a pulvinus (Petersen 1889, Kennedy 2000). The family is distributed throughout the tropics except in Australia. In the Neotropics, the Marantaceae species richness is highest with an estimated 450 species while the remaining species are paleotropical (Africa: ~40 spp., Asia: ~50 spp., Suksathan *et al.* 2009; Dhetchuvi 1996). The morphological diversity found in the Paleotropics was higher than in the Neotropics (Andersson 1981). Only a few genera are found in the temperate regions of South and North America.

Several previous studies estimated the relationships among major clades within the Marantaceae family either based on morphological and anatomical (Petersen 1889; Loesener 1930; Kirchoff 1983; Kress 1990; Andersson 1998; Kress *et al*. 2001) or on molecular data at the family level or for specific taxa (Table 1). Originally, the Marantaceae family has been divided into two tribes based on morphology and the number of fertile locules: Maranteae with one fertile locule, and Phrynieae with three fertile locules per ovary (Petersen1889; Loesener 1930). Later, based on a wider spectrum of morphological characters, Andersson (1998) proposed a world-wide sub division into five informal groups: *Calathea* group, *Donax* group, *Maranta* group, *Myrosma* group and *Phrynium* group. In this wide division, however, four genera were left with unknown affinity (*Haumania*, *Hylaeanthe*, *Thalia* and *Thaumatococus*: Prince & Kress 2006a). Recently, phylogenetic studies provided evidences that the proposed tribes (Petersen1889; Loesener 1930) and the informal groups (Andersson 1998) of the Marantaceae are not monophyletic. Based on a different set of molecular markers and new methods (Table 1), Prince and Kress (2006a and 2006b) proposed a different informal classification of the Marantaceae describing five major clades: *Calathea* clade, *Donax* clade, *Maranta* clade, *Sarcophrynium* clade and *Stachyphrynium* clade.

Table 1. Summary of previous molecular studies on Marantaceae phylogeny.

Target taxa	No. of ingroup genera/species	Molecular marker(s)	Analysis method(s)	Support method(s)	Literature
Marantaceae family	22/59	*rps*16	MP	JK	Andersson &chase 2001
Marantaceae family	27/80	*matK*, *trnL-F*	MP, BI	BS, PP	Prince &Kress 2006a
Marantaceae family	19/25	*matK*, *ndhF*, *rbcL*, *rps*16, *trnL–trnF*, *cox*1 and ITS	MP, BI	BS, PP	Prince &Kress 2006b
Asian Marantaceae taxa	26/79	*rps*16, ITS1 and 5S-NTS	MP, BI	BS, PP	Suksathan *et al*. 2009
***Sarcophrynium* clade, *Marantochloa* clade**	6/43	ITS, 5S, trnL/*trnL-F*	MP, ML, BI	BS, PP	Ley & Claßen-Bockhoff 2011
***Calathea* clade**	6/57	*matK*, *trnK* intron, *trnL* intron, *trnL-F* and ITS	MP	BS	Borchsenius *et al*. 2012

Methods of analysis: BI, Bayesian inference; ML, Maximum likelihood; MP, Maximum parsimony. **Methods of support**: BS, Bootstrap support; JK, Jackknife support; PP, Posterior probability. **Molecular markers**: ITS, internal transcribed spacer.

Resolution and branch support varied among trees produced by both studies, as well as, the placement of the genus *Haumania*. Most recently, Borchsenius *et al*. (2012) proposed a narrowly re-circumscription of the genus *Calathea*. They thereby resurrected the genus *Goeppertia* Nees (1831: 337) to include all members of the former *Calathea* clade I (sensu Prince & Kress 2006a). As a result of this new circumscription and resurrection, *Goeppertia* has become the largest genus in the Marantaceae. Still, all these previous studies are lacking the statistical support for the resolution of the relationships among the five major clades of Marantaceae and the position of the genus *Haumania*.

The evaluation of statistical clade support values is an important aspect of phylogenetic analysis as a function of the explanatory power of a given analysis (Grant & Kluge 2008). These support measures are a prerequisite to identify the well supported clades in a tree as a base for any inference of the evolution of the biological system (Huelsenbeck *et al.* 2000; Lutzoni *et al.* 2001; Pagel & Lutzoni 2002), e.g. to serve as the conceptual framework for the study of trait evolution (Alfaro *et al.* 2003). In both Maximum likelihood (ML) and Maximum parsimony (MP) analyses, tree support can be evaluated by bootstrapping, while the posterior probability support is the evaluation technique in the Bayesian inference (Jill Harrison & Langdale 2006). The bootstrap technique provides an assessment of “confidence” for each clade of an observed tree,

based on the proportion of resampled trees showing that same clade when individual characters in the data set are randomly removed and replaced with data from another character from the same data set (Efron *et al.* 1996). In contrast, the posterior probability is the actual probability of a node being correct (Jill Harrison & Langdale 2006). Bootstrap values > 70% indicate a reasonable support while values of ≥ 95% indicate a high support (Felsenstein 1985; Hillis & Bull 1993). Grant and Kluge (2008) proposed the support measures from parsimony, Maximum likelihood, and Bayesian phylogenetic inference are equivalent. However, when reconstructing the phylogeny of the Marantaceae, Borchsenius *et al*. (2012) found that support values achieved by Maximum likelihood and Bayesian analysis are equivalent, while lower statistical support values achieved in Maximum parsimony analysis. A high support for the accurate resolution in a phylogeny is suggested to be potentially achieved by increasing the total number of characters (Rosenberg and Kumar 2001), taxa (Heath *et al.* 2008; Zwickl & Hillis 2002) or both (Townsend & Lopez-Giraldez 2010).

Currently, the existing studies on the Marantaceae phylogeny could not resolve the relationships of major clades at the back bone of the Marantaceae phylogeny (Prince & Kress 2006a). In addition, the genus *Monophyllanthe* and its affinity to one of the major clades is missing in all previous studies. To overcome these limitations we present here a new phylogenetic analysis using Maximum parsimony (MP) criterion, Maximum likelihood (ML) and Bayesian inference (BI) and three plastid (*matK*, *rps*16 and *trnL-F*) and one nuclear marker (ITS, Internal transcribed spacer). With 187 Marantaceae taxa the full range of morphological variation known in the family is represented and all previously proposed infrageneric entities are covered. By utilizing more additional molecular data and taxa than in the past, the objectives of the current study were: (1) to achieve a higher resolution and support for the branching of the Marantaceae backbone, (2) to ascertain the monophyly of genera and clades, (3) to provide a better statistical support for the placement of the genus *Haumania* and its internal species´ relationships and (4) to locate the genus *Monophyllanthe*.

MATERIALS AND METHODS

Taxon Sequences Assembly

~600 sequences from four genetic markers (chloroplast markers: *trn*L, *mat*K, *rps*16 and nuclear marker: Internal Transcribed Spacer, ITS) covering 188 taxa were included in the analyses, representing all genera within the Marantaceae family and including the outgroup taxon *Canna indica*. All scientific names were updated to the latest synonyms and voucher information for each sample is given in Appendix 1. Datasets were built using on the one hand available published sequences (Andersson & Chase 2001; Prince & Kress 2006a, 2006b; Suksathan *et al.* 2009; Ley & Claßen-Bockhoff 2011; Borchsenius *et al.* 2012; Borchsenius *et al*. in prep.) and on the other hand, 80 sequences of 36 taxa extracted and sequenced newly in the course of this project. However, we could not produce a totally complete dataset. Still, for 16, 14, 17 and 18 taxa out of 188 have no sequence of *matK*, *rps*16, *trnL-F* and ITS, respectively. Furthermore, 18 out of 23 species of the genus *Phrynium* failed to produce the entire matK (mif+867) or ITS (18S+ITS1+5.8S+ITS2+26S) region (Appendix 1).

DNA Extraction, Amplification and Alignment

Total genomic DNA was extracted from leaf tissue using the DNeasy Plant Mini Kit (QIAGEN Inc., California) following the manufacturer's instructions. Amplifications of the target loci *rps16* intron, *matK* gene and *trnL* intron/ *trnL* exon/ *trnLtrnF* intergenic spacer, were conducted in a Mastercycler EP Gradient EPPENDORF via standard PCR. Each 25 µl volume contained 12.5 µl using the PCR mix BioMix (Bioline, Germany) (including the Biotaq DNA polymerase from Ecogen, dNTP Mix, 10x NH4 buffer, MgCl2 solution), 9.5 µl H 2 O and 1 µl genomic DNA extract. Amplification cycles were as follows for *rps16*: one cycle of 2 min at 94°C, 39 cycles of 30 s at 94°C, 60 s at 59°C, 2 min at 72°C with a final extension period of 7 min at 72°C, for *matK*: one cycle of 1.3 min at 94°C, 30 cycles of 1.3 min at 94°C, 2 min at 52°C, 2 min at 72°C with a final extension period of 10 min at 72°C and for *trnL/trnL-F*: one cycle of 2 min at 94°C, 30 cycles of 30 s at 94°C, 60 s at 55°C, 60 s at 72°C with a final extension period of 10 min at 72°C.

Amplification of *rps*16 was performed using the primers *rps*16F and *rps*16R2 (Oxelman *et al.* 1997), for *trnL-F* we used the primers ucp-c and ucp-f (Taberlet *et al.* 1991) and for *matK* the primers *mIF* (Prince & Kress 2006a), *matK*-867F, *matK*-988R and *matK*-1639R (Borchsenius *et al.* 2012). Hereafter, PCR products were purified using ExoSAP-IT™ (USB Corporation) following the

manufactor´s instructions. The products were sent to www.stabvida.com for sequencing in forward and reverse direction.

Sequences from the chloroplast were preliminarily aligned in Muscle 3.6 (Edgar 2004), then manually adjusted in BioEdit 7.2.5 (Hall 1999) and finally exported in Phylip format. Indels were coded with FastGap v. 1.2 (Borchsenius 2007), using the simple indel coding method of Simmons & Ochoterena (2000). Sequences from the highly variable nuclear marker ITS region in the ribosomal RNA gene, including part of the 18S (1-102 bp), through ITS1 (103-390 bp), 5.8S (391-552 bp), ITS2 (553-810 bp), and part of the 26S (811-1048 bp) loci in a single sequence) were aligned by clades identified from our phylogenetic trees reconstructed based solely on the chloroplast sequences. Finally, a partitioned supermatrix dataset of the sequences from *matK*, *rps*16, *trnL-F* and ITS and an indel matrix was prepared manually and exported to the compatible formats for analyses in PAUP, MrBayes and RAxML.

Phylogenetic Analyses and Branch Support

Datasets of the different gene regions were analyzed individually and in combination. The best-fitted model of nucleotide substitution rate for each marker was identified with jModelTest2 2.1.6 (Guindon & Gascuel 2003; Darriba *et al.* 2012) implemented in the CIPRES portal (Miller *et al.* 2010) using default parameters. The Bayesian information criterion (BIC, Schwarz 1978) was used for model choice because of its high accuracy (Darriba *et al.* 2012) and its tendency to favor simpler models than the Akaike information criterion (Posada & Crandall 2001).

Both phylogenetic analyses, Maximum likelihood (ML) and Bayesian inference (BI) were conducted on the CIPRES Science Gateway (Miller *et al*. 2010). Bayesian interferences were calculated including indels in MrBayes 3.2.6 (Ronquist *et al.* 2012). Each analysis consisted of three runs with four sequentially heated chains (temperature set at 0.05) for 5 million generations and sampling a tree every 50 generations with discarding the first 500,000 generations (burnin) prior to the calculation of posterior probability (PP). ML analyses were carried out with default parameters in RAxML-HPC2 BlackBox 8.2.3 (Stamatakis 2006). Maximum Parsimony (MP) analyses were conducted in PAUP* 4.0b10 (Swofford 2002) using a heuristic search with max trees set to 10000 , 100 random addition sequence replicates and branch

swapping algorithm using the tree-bisection-reconnection approach (TBR), holding 2 trees, saving no more than 10 trees per replicate. The consistency (CI), retention (RI) and rescaled consistency (RC) indices were calculated based on the whole data matrix including informative and uninformative characters.

Bootstrap values for Maximum likelihood (with 1,000 fast bootstrap) and Maximum parsimony (BS; Felsenstein 1985) analyses and posterior probabilities (PP) for Bayesian analysis were calculated to estimate branch and clade support. Parsimonious bootstrap percentages were estimated using 1000 replicates (10 random addition replicates, hold 2 trees, saving a maximum of 10 trees per replicate) to maximize the accuracy of the estimation.

RESULTS

DNA sequence summary.

The final data matrix included 688 sequences representing 188 taxa and one outgroup species. The ultimate matrices of aligned regions with indel coding had the following sizes: *matK* - 1375 characters (1362 bp + 13 indels) including 172 taxa*, rps*16 - 1275 characters (1125 bp + 50 indels) including 175 taxa*, trnL-F* - 1131 characters (1039 bp + 92 indels) including 171 taxa for and ITS - 1308 characters (1048 bp + 260 indels) including 170 taxa. Indel events were coded as multistate characters at the end of each data matrix.

Tree topologies.

Tree topologies were almost the same in all combined analyses with only slight variations in branch support, resolution and the placement of the genus *Haumania*. Conflicts in tree topologies among markers were generally for unsupported branches (BS < 50, PP < 0.95) as well as for taxa with a single or two available sequences only. For all Maximum parsimony analyses, tree characteristics and indices are summarized in Table (1). Tree indices indicated lower homoplasy and many more parsimony informative characters in chloroplast markers than in the ITS marker (Table 2).

Table 2. Summary of substitution models and tree scores for each genetic marker in Maximum parsimony analysis.

Maximum parsimony analyses (no. of taxa × no. of characters)	*matK* (173 x1375[a])	*rps*16 (177 x1275[a])	*trnL-F* (172 x1138[a])	ITS (170 x1308 [a])	Combined (188 x 5089 [a])
Substitution Model	TPM1uf+G	TPM1uf+G	TPM1uf+G	TIM1+I+G	
Informative characters	338	325	238	666	1567
Tree length	1032	1103	746	4282	7291
Consistency Index (CI)	0.66	0.65	0.68	0.34	0.46
Retention Index (RI)	0.9	0.88	0.89	0.75	0.81
Rescaled Consistency Index (RCI)	0.59	0.57	0.6	0.25	0.37
Homoplasy Index (HI)	0.34	0.35	0.32	0.66	0.54

[a] Including all characters.

The analyses revealed five well supported clades: *Calathea* clade, *Donax* clade, *Maranta* clade, *Sarcophrynium* clade and *Stachyphrynium* clade. ML-BS and BI-PP revealed a good support for all relationships among these clades except for the *Sarcophrynium* clade and the genus *Haumania*, whereas MP-BS supported only the sister relationship of the *Stachyphrynium* and the *Maranta* clade (BS value: 89, Figure 1).

***Calathea* clade.** The *Calathea* clade was the largest lineage with moderate to high branch support (ML-BS: 71, MP-BS: 85 and BI-PP: 100%). Within the *Calathea* clade there was a highly supported clade in all analyses (ML/MP-BS: 100, BI-PP: 100%) which included the four genera *Calathea* (*Calathea* II sensu Prince & Kress 2006a), *Ischnosiphon*, *Pleiostachya* and *Sanblasia*. The nesting of the two monotypic genera *Sanblasia* and *Pleistachya* within the *Calathea* and *Ischnosiphon* genera, respectively, characterized the latter two genera as non-monophyletic (Fig. 2). The resolution within the *Calathea-Sanblasia* clade was highly supported and formed three groups. The first group included *Sanblasia* and three *Calathea* species (*C. marantina*, *C. plurispicata*, and *C. lutea;* ML/MP-BS: 100 and BI-PP: 100%), where *Sanblasia* was placed at the base. This group was placed as sister (ML-BS: 99, MP-BS: 93 and BI-PP: 100%) to the second *Calathea* group (*C. toroi*, *C. cortalifera*, and *C. utilis;* ML/MP-BS: 100 and BI-PP: 100%). The third *Calathea* group was (*C. guzmanioides*, *C. hagbergi, C. pluriplicata*, and *C. timothei;* ML/MP-BS: 100 and BI-PP: 100%) and placed as sister to the former two groups ML/MP-BS: 100 and BI-PP: 100%). The sister relationship between the *Ischnosiphon-Pleiostachya-Calathea-Sanblasia* clade

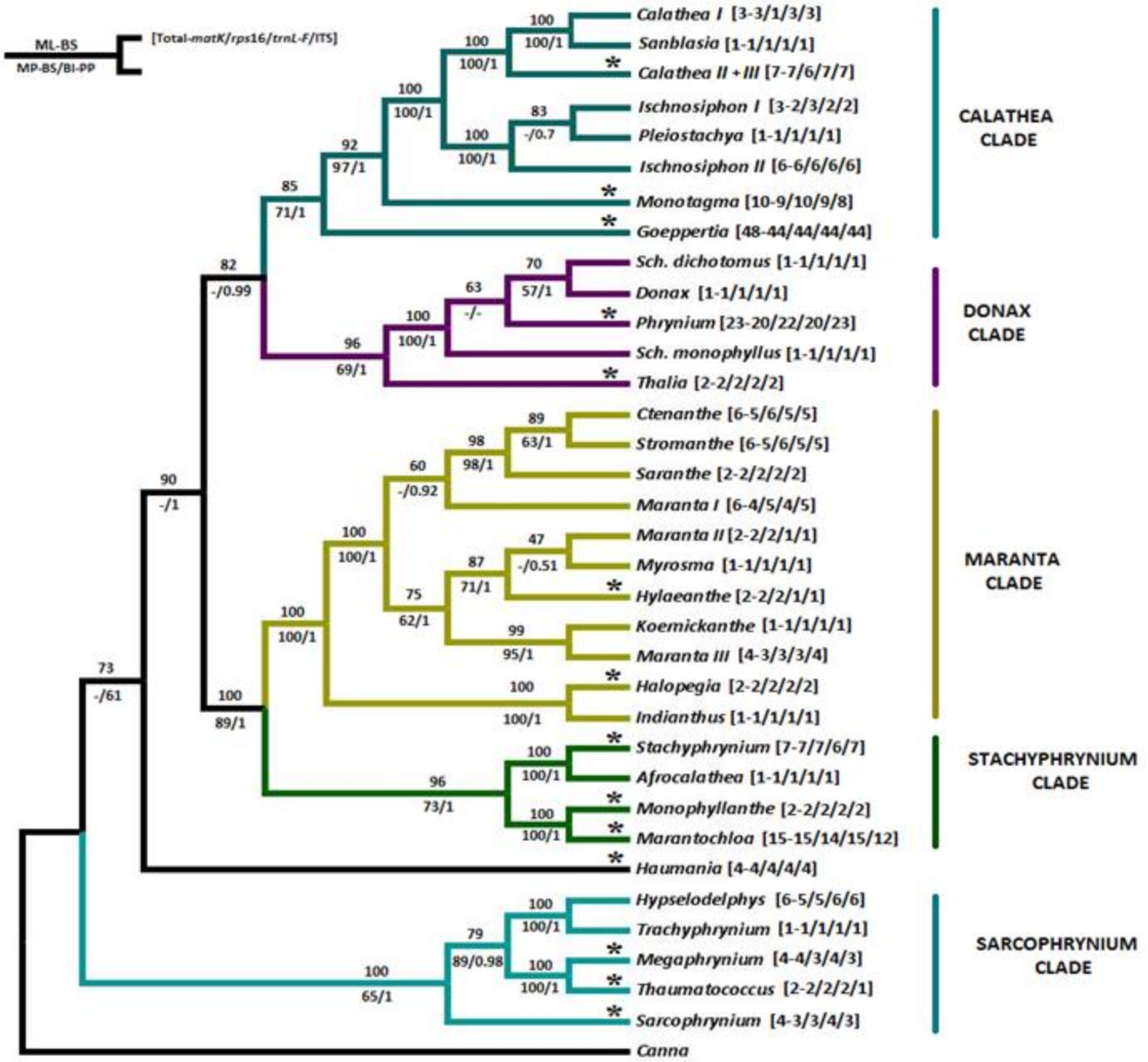

Figure 1. Strict consensus tree (ML) of 188 taxa (here only genera are shown, for the whole tree see Appendix 2.Fig 5a & b) for a combined analysis of the chloroplast markers *matK*, *rps*16 and *trnL-F* and the nuclear marker ITS. Colors indicate major clades (names adopted from Prince and Kress, 2006a). Numbers above and below branches represent support values from ML, MP and BI analyses. -, no branch support in respective analysis. *, high support for the genus or terminal taxa monophyly (ML-BS/BI-PP ≥ 98, MP-BS ≥ 94). Numbers in brackets after the genus name, [total number of known species per genus - number of species with available sequences for *matK*/*rps*16/*trnL-F*/ITS].

and the genus *Monotagma* was highly supported (ML-BS: 92, MP-BS: 97 and BI-PP: 100%), while the sister relationship of the entire clade and the genus *Goeppertia* (*Calathea* I sensu Prince and Kress 1996) was moderately to highly supported (ML-BS: 85, MP-BS: 71 and BI-PP: 100%). The 48 *Goeppertia* species formed a well-supported clade (ML-BS: 96, MP-BS: 98 and BI-PP: 100%). Within this clade, *G. straminea* was placed as sister to all other taxa, which in turn formed five major clades.

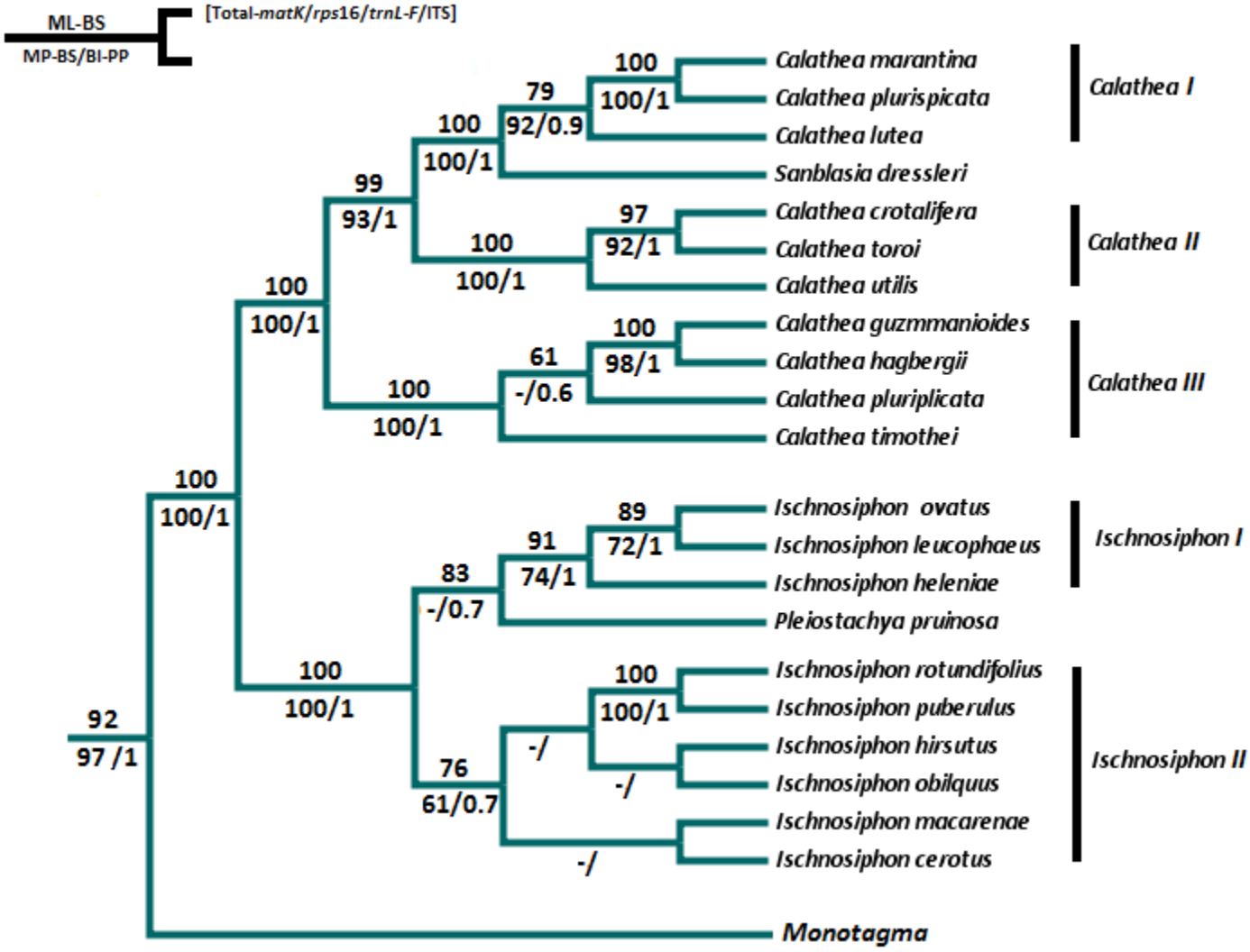

Figure 2. Strict consensus tree (ML, BI) for species relationship within the Ischnosiphon-Pleiostachya-Calathea-Sanblasia clade from a combined analysis. Numbers above and below branches represent support values from ML, MP and BI analyses. -, no branch support in respective analysis. This graph is a selection from the entire tree (Fig. 5a, Appendix 2) and not an independent calculation.

***Donax* clade**. The *Donax* clade included the genera *Donax, Phrynium, Schumannianthus* (two species), and *Thalia.* There was a moderate to high support for the monophyly of the entire clade (ML-BS: 99, MP-BS: 69 and BI-PP: 100%), but low support for the internal branches depicting the relationships among the two *Schumannianthus* species, *Donax* and *Phrynium* expect for the sister relationship of *Thalia* to all other genera of this clade. The monophyly of the genus *Phrynium* including the genera *Cominsia, Monophrynium and Phacelophrynium,* was approved (ML/MP-BS: 99, BI-PP: 100%). The relationships among all species of the genus *Phrynium* were not resolved, however, some subclades and sister relationships were moderately to strongly supported. These relationships were revealed in the subclade including *P. hirtum, P. fissifolium* and *P. villosulum* (ML-BS: 76, MP-BS: 98 and BI-PP: 98 %), the subclade *P. hainanense, P. pedunculiferum* and *P. tokinense* (ML-BS: 100, MP-BS: 97 and BI-PP: 100 %), the subclade *P. imbricatum, P. obscurum, P. pubinerve* and *P. tristachyum* (ML-BS: 93, MP-BS: 77 and BI-PP: 100 %), the sister relationship between *P. kaniense* and *P. macrocephalum* (ML-BS:96, MP-BS: 97 and BI-PP: 100 %), between *P. giganteum* (*Cominsia gigantean* sensu Saksuthan *et al.* 2009) and *P. whitei* (ML-BS:100, MP-BS: 99), between *P. interruptum* and *P. simplex* (*Phacelophrynium interruptum* and *Monophrynium. Simplex,* respectively, sensu Saksuthan *et al.* 2009; ML-BS: 100, MP-BS: 94 and BI-PP: 100 %) and between P*. imbricatum* and *P. pubinerve* (ML/MP-BS: 100 and BI-PP: 100 %; Appendix 2. Fig. 5a). The genus *Schumannianthus* appeared polyphyletic, although, the support of *Schumannianthus dichotomus* as sister to *Donax* was only strong in the BI-PP analysis (100%). Topologies independently from different markers were different with low support regarding the relationships between *Donax*, *S. dichotomous* and *S. monophyllus* (Appendix 2). Only in *rps*16 a moderate support (ML-BS: 79 MP-BS: 75 and BI-PP: 99%) was found for the sister relationship of *Donax* and *S. dichotomous*.

***Maranta* clade**. This clade included the genera *Ctenanthe*, *Halopegia*, *Hylaeanthe*, *Indianthus Koernickanthe*, *Maranta* (in three parts)*, Myrosma, Stromanthe* and *Saranthe*. The monophyly of the *Maranta* clade was strongly support (ML/MP-BS: 100 and BI-PP: 100%). The genus *Maranta* appeared polyphyletic. *Maranta* I (*M. noctiflora, M. rupicola, M. protracta, M. arundinacea, M. sobolifera, M. tuberculate*) was sister to a clade including *Ctenanthe*, *Stromanthe* and *Saranthe* (low support; ML-BS: 60 and BI-PP: 92%). *Maranta* II (*M. ruiziana, M. parvifolia*) formed a

moderate to high supported clade (ML-BS: 87, MP-BS: 71 and BI-PP: 100%) together with the genera *Myrosma* and *Hylaeanthe*. The highest support (ML-BS: 99, MP-BS: 95 and BI-PP: 100%) was found for the clade including *Maranta* III *(M. pohliana, M. friedrichsthaliana, M. humilis, M. leuconeura, M. cristata*) and the genus *Koernickanthe*. A clade including the two genera *Ctenanthe* and *Stromanthe* was moderately supported (ML-BS: 89, MP-BS: 63 and BI-PP: 100%) but with no support for the respective monophyly of the two genera (Appendix 2). *Ctenanthe dasycarpa,* which was represented only by a single *rps*16 sequence, nested with low support within the genus *Stromanthe*. The subclade including *Ctenanthe, Saranthe* and *Stromanthe* was highly supported (Fig. 1). A well supported clade of *Halopegia*/*Indianthus* was placed at the base of the *Maranta* clade.

***Stachyphrynium* clade**. This clade, strongly supported in the combined analysis only by ML and BI analyses (ML-BS: 96 and BI-PP: 100%), included the genera *Afrocalathea, Marantochloa, Monophyllanthe* and *Stachyphrynium*. Analyses conducted independently per markers found only support for the clade monophyly in all chloroplast markers, while this was not confirmed in the analysis of the nuclear marker (ITS). In the three analyses of the ITS region (ML, MP and BI), the two genera *Marantochloa* and *Monophyllanthe* were placed as sister to the *Maranta* clade. The other two genera *Afrocalathea* and *Stachyphrynium* were placed as sister to the *Haumania* clade either at the base of the clade including *Marantochloa*, *Monophyllanthe* and *Maranta* clade (ML), or in a polytomy at the tree base (BI) or apart from *Haumania* in a large polytomy (MP). All genera within this clade were monophyletic with a strong support from all three analyses (Fig. 1). A clear sister relationship was found for *Afrocalathea*/*Stachyphrynium* and for *Monophyllanthe*/*Marantochloa* (Fig. 1 and 3).

***Sarcophrynium* clade.** This strongly supported clade in the ML (BS=100) and BI (PP= 100%) analyses included the genera *Hypselodelphys*, *Megaphrynium*, *Sarcophrynium* and *Thaumatococcus*, *Trachyphrynium*. The sister relationship between *Hypselodelphys*/*Trachyphrynium*, and between *Megaphrynium/Thaumatococcus,* respectively, was highly supported. Topology and support values from the three combined analyses (ML/MP-BS: 100 and BI-PP: 100%) showed the four *Megaphrynium* species clustered in one clade. Within

the clade, *M. trichogynum* was placed at the base while the top branch occupied by both *M. gabonense* and *M. macrostachyum* (Fig. 4).

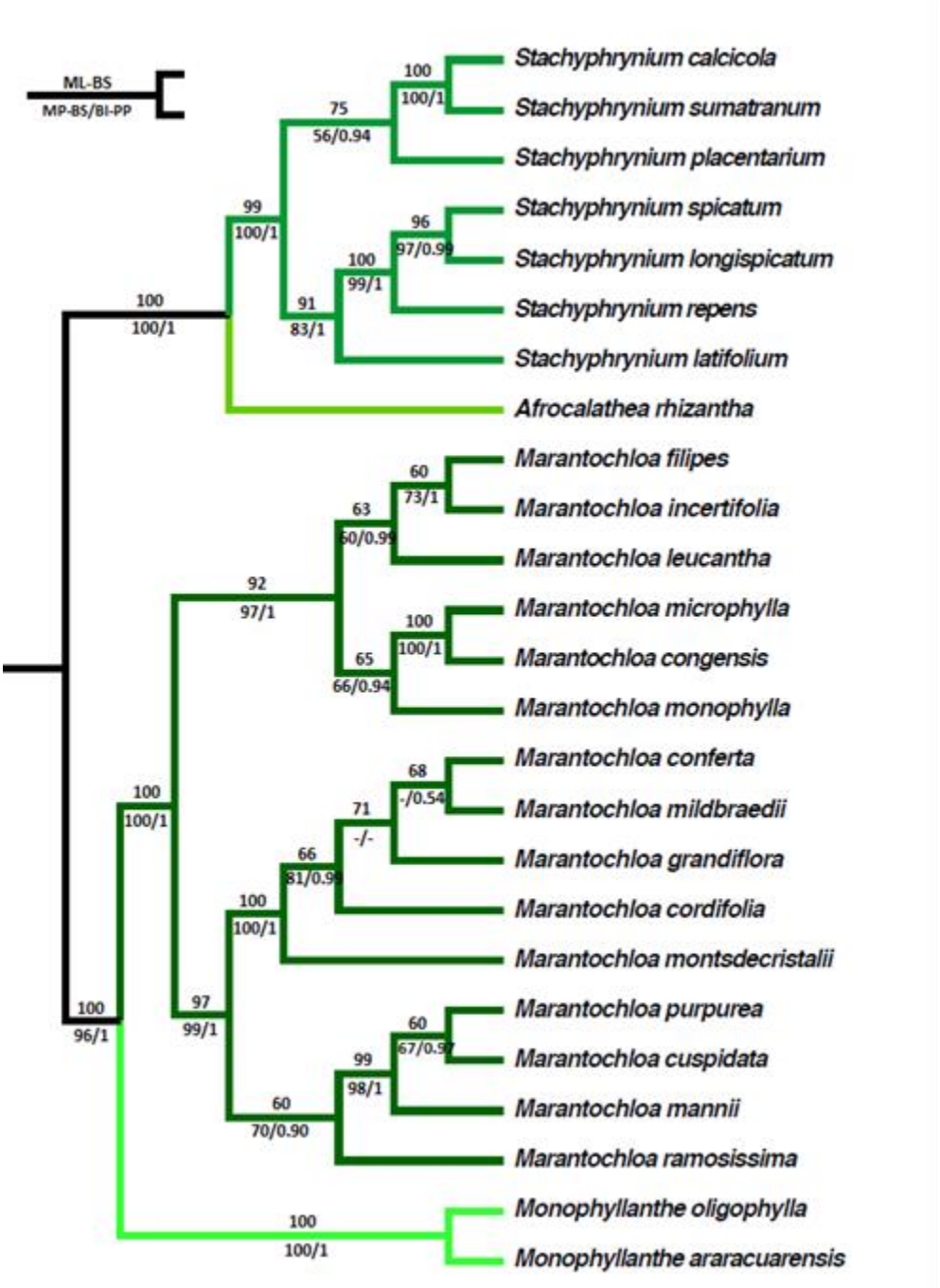

Figure 3. Strict consensus tree (ML) for Species relationship within *Stachyphrynium* clade from combined analyses. Numbers above and below branches represent support values from ML, MP and BI analyses. -, no branch support in respective analysis. This graph is a selection from the entire tree (Fig. 5b, Appendix 2) and not an independent calculation.

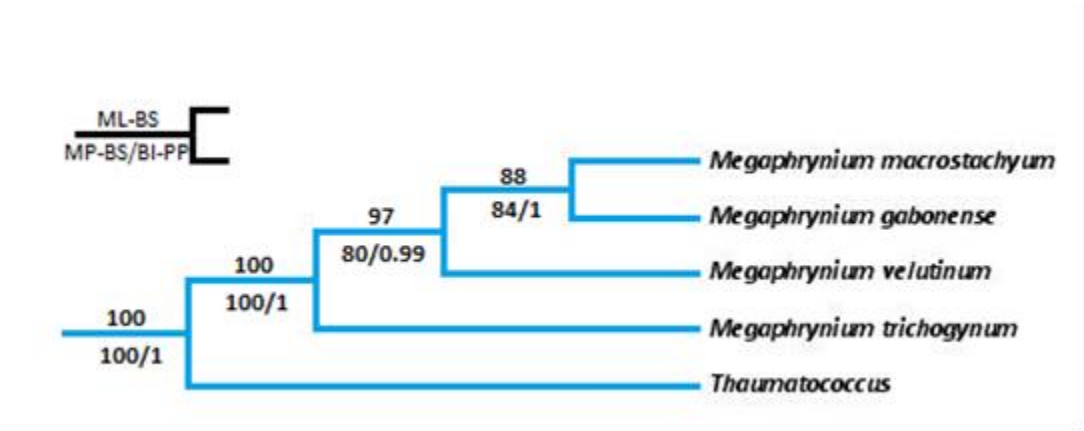

Figure 4. Strict consensus tree (ML, MP and BI) for species relationships within the *Megaphrynium* clade from a combined analyses of all four genetic markers. Numbers above and below branches represent support values from ML, MP and BI analyses. This graph is a selection from the entire tree (Fig. 5b, Appendix 2) and not an independent calculation.

Genus *Haumania*. The genus *Haumania* stood alone in both ML and BI analyses without confirmed affiliation to any of the major clades (Fig. 1), while the MP analysis placed *Haumania* as a member of the *Sarcophrynium* clade (weakly supported, MP-BS: 52). The relationships among the *Haumania* species were fully resolved. In all analyses and for all markers, the unknown *Haumania* species was found closest to *H. dankelmaniana*, where the sister species for both of them was *H. liebrechtsiana* while the species *H. leonardiana* was placed at the base of the *Haumania* clade (Fig. 5).

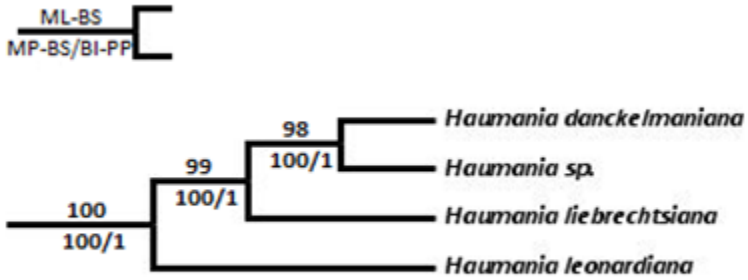

Figure 5. Strict consensus tree (ML, MP and BI) for species relationships within the *Haumania* clade from a combined analyses of all four genetic markers. Numbers above and below branches represent support values from ML, MP and BI analyses. This graph is a selection from the entire tree (Fig. 5b, Appendix 2) and not an independent calculation.

DISCUSSION

The tree topology from this analysis is overall congruent with the topology of the most recent Marantaceae phylogenetic analyses (Andersson & Chase 2001; Prince & Kress 2006a, 2006b) and supports the recognition of the proposed five major lineages. The majority of these lineages were restricted to a single geographical region (continent): tropical America (*Calathea* clade and *Maranta* clade except *Halopegia* from Africa and Asia, *Indianthus* from Asia), tropical Africa (*Sarcophrynium* clade) or tropical Asia (*Donax* clade except *Thalia* from America). While the genera of the *Stachyphrynium* clade can be found in all three tropical regions: Africa (*Afrocalathea* and *Marantochloa*), America (*Monophyllanthe*) and Asia (*Stachyphrynium*). In comparison to the independent marker analyses, the combined analyses improved the Marantaceae backbone support (BS and PP values) and general tree statistics (consistency index, retention index, and rescaled consistency index) against the ITS dataset but not against the chloroplast markers *matK*, *rps*16 and *trnL-F* dataset. Such patterns of higher tree statistics resulting from chloroplast markers rather than from nuclear markers was also found in Ley and Claßen-Bockhoff (2011, *trnL-F* and ITS) and Prince and Kress (2006b, *matK*, *rps*16, *trnL-F* and 18S–26S). Both analyses methods (ML and BI) supported a similar branching of the Marantaceae backbone, but this resolution was not confirmed in the MP analysis. Such results are expected since parsimony and likelihood methods (ML and BI) use different criteria for evaluating topology and choosing the best trees (Kolaczkowski & Thornton 2004). All clade support values (BS and PP) discussed and compared in the following text refer to those of the combined analyses (Fig. 1). If other support values from independent marker analyses are used in the text this is indicated separately in the text.

***Calathea* clade.** The whole clade received a support value of 71 (MP-BS) which is higher than the support values found in previous studies: 65 (Borchsenius *et al.* 2012), 60 (Prince & Kress 2006a) and the MP-JK < 50 (Prince & Kress 2006b). The strong support value (MP-BS: 100) for the *Ischnosiphon-Pleiostachya-Calathea-Sanblasia* clade was also found in (Borchsenius *et al*. 2012). Within this clade, *Calathea* and *Ischnosiphon* are considered potentially paraphyletic with respect to *Sanblasia* and *Pleiostachya,* respectively. The topology of the groups found within the *Calathea-Sanblasia* clade was the same as found by Borchsenius *et al*. (2012) with identical

support values. As more species from *Ischnosiphon* were included in our analyses, more sister species to *Pleiostachya* were found than *I. leucophaeus* (Borchsenius *et al*. 2012). No branch support for the clustering of all *Ischnosiphon* species apart from *Pleiostachya* was found by Prince and Kress (2006a) who used only chloroplast markers. An almost complete sampling of *Monotagma* species (10 species) confirmed the monophyly of the genus found in the previous studies (Andersson and Chase 2001: 2 species, Prince and Kress 2006a: 4 species, Borchsenius *et al*. 2012: 7 species).

The overall clade topology is congruent with the findings of Borchsenius *et al*. (2012), where the *Goeppertia* genus (*Calathea* I sensu Prince and Kress 2006a) was placed as sister to the rest of the clade genera. The support value we found for the *Goeppertia* clade (MP-BS: 98) was higher than in Prince and Kress (2006a, MP-BS: 93) and Borchsenius *et al*. (2012, MP-BS: 90). We could confirm the six major clades within the genus the *Goeppertia: Breviscapus*, *Comosae*, *Microcephalum*, *Ornata, Scapifolia* and *Straminea* found in Borchsenius *et al.* (2012) that supported the infrageneric groupings proposed previously by Kennedy *et al.* (1988). In addition to the similar support value (Appendix 2, Fig. 5a) as in Borchsenius *et al*. (2012) our results added six taxa to the *Comosae* clade (*G. pallidicosta*, *G. picturata*, *G. comosa*, *G. metallica*, *G. neblinensis*, *G. veitchiana*), eight to the *Breviscapus* clade (*G. aemula*, *G. colorata*, *G. concinna*, *G. cylindrical*, *G. laetevirens*, *G. lancifolia*, *G. mirabilis*, *G. zebrine*) and two to the *Ornate* clade (*G. majestica*, *G. splendida*).

***Donax* clade.** Relationships among and within the genera of this clade were not fully resolved. Clade support including 27 taxa was high in both ML and BI analyses (BS: 96 and PP: 100%) but moderate in MP analysis (BS: 69). Our MP support value was higher than in Prince and Kress (2006b, 5 taxa, MP: 53), Borchsenius *et al*. (2012, 4 taxa, MP: 52), slightly lower than in Suksathan *et al*. (2009, 45 taxa, MP: 72) and slightly similar to Prince and Kress (2006a, 9 taxa, MP: 68). The *Donax* clade without the genus *Thalia* was very well supported as in previous studies (Prince & Kress 2006a, 2006b; Suksathan *et al.* 2009; Borchsenius *et al.* 2012). Morphologically, *Thalia* is characterized by one petaloid outer staminode and two equal pendant staminode appendages (Claßen-Bockhoff 1991, see Prince & Kress 2006a) in contrast to all other clade members with two petaloid outer staminodes and one simple appendage at the cucullate staminode. Analyses

at marker level, showed a conflict among markers with respect to the sister relationship of *Donax canniformis* and *Schumannianthus dichotomus* (Appendix 2, Fig. 1a, 2a, 3a, 4b). This resulted in a low support in the ML and MP analyses of the dataset combining all four markers. However, this sister relationship was found strongly supported in the previous studies (Prince & Kress 2006a, 2006b) potentially due to the exclusion of *S. monophyllus* and a reduced taxon sampling within the *Donax* clade. A study with larger sampling from the *Donax* clade but based on *rps*16 only (Suksathan *et al*. 2009) revealed a low supported (MP-BS: 68, BI-PP< 95%) sister relationship between *S. dichotomus* and the genus *Donax* and an unresolved relation to *S. monophyllus*. In the same study but based on a combined analysis of *rps*16 intron + ITS1 + 5SNTS, no sister relationship between *S. dichotomus* and *D. canniformis* was found while *S. monophyllus* was placed at the base of the *Phrynium* clade. Suksathan *et al*. (2006) described *S. monophyllus* by having acaulescent vegetative shoots and short-stemmed, unbranched flowering shoots which is unusual to the stem habit found in *D. canniformis* and *S. dichotomus.* In our data sets probably due to amplification problems in the lab, the whole ITS sequence was of comparable lengths in *S. monophyllus* and the 14 *Phrynium* species (*c*. 240 bp, appendix 1), whereas in *D. canniformis* and *S. dichotomus* it was about 430 and 548 bp longer, respectively. Therefore, we assume that the unique morphology might have placed *S. monophyllus* apart from their closely related species and the low support for this resolution might be due to the variation in sequence length.

The 23 *Phrynium* species formed a well-supported monophyletic group with some resolved internal nodes. This monophyly was also found in Suksathan *et al.* (2009) after the merge of the four genera *Cominsia*, *Monophrynium*, *Phacelophrynium* and *Phrynium* to the genus *Phrynium* (sensu Suksathan *et al.* 2009). Some subclades and sister relationships among the *Phrynium* species were found strongly supported, while the majority was found weakly supported in ML and BI analyses or formed a polytomy in the MP analysis. In general our results support the grouping of the *Phrynium* species based on thier geographical distribution (Suksathan *et al.* 2009) except for the close sister relationships between the two West Malaysian species *P. obscurum* and *P. tristachyum* and the widespread species *P. imbricatum* and *P. pubinerve* (sensu Suksathan *et al.* 2009) rather than to other West Malaysian species *P. hirtum, P. fissifolium* and *P. villosulum*. A new species *P. whitei* was added to the New Guinea clade (see

Suksathan *et al.* 2009) with strong sister relationship to *P. giganteum,* where both species have the same geographical distribution pattern. Variation in the sequences length of ITS and *matK* due to incomplete sequences might have contributed to the poor achieved resolution.

***Maranta* clade.** The Maranta clade received again a high support. This was the first time that all genera of this clade were included in a phylogeny and more than one molecular marker from both chloroplast and nuclear DNA was used (compare to Andersson & Chase 2001; Prince & Kress 2006a, 2006b; Borchsenius *et al.* 2012). For instance, the placement of the genus *Koernickanthe* in the hypothesized tree topology in Prince and Kress (2006a) was based only on the molecular analysis of the *trnL-F* intergenic spacer region and morphological comparisons. Prince and Kress (2006a) further estimated the position of the *Myrosma* genus based on the shared morphological characteristics with other members of *Maranta* clade.

Based on the combined analyses (ML, BI), the 12 *Maranta* species clustered into three groups. This polyphyletic potential was indicated by both chloroplast and nuclear markers independently, as well as in the combined MP analyses but with different topology and support. The monophyly of the genus *Maranta* was already doubted by Andersson and Chase (2001) using only one chloroplast marker (*rps*16) and six *Maranta* species. Based on a limited sampling of *Maranta* (3 species), Prince and Kress (2006a) identified the genus as monophyletic. Polyphyly was neither applicable in Prince and Kress (2006b), as they represented the *Maranta* genus by only one species. Therefore, we assume that *Maranta* in its present circumscription is polyphyletic.

Topologies resulting from ML and BI analyses revealed different sister relationships among the clade taxa than hypothesized by Prince and Kress (2006a). For instance, Prince and Kress (2006a) placed the genus *Koernickanthe* at the base of the *Ctenanth*e-*Hylaeanthe*-*Maranta*-*Myrosma*-*Saranthe*-*Stromanthe* clade, while our analyses strongly supported the sister relationship between *Koernickanthe* and *Maranta III* (ML-BS: 99, MP-BS: 95 and BI-PP: 100%). Moreover, we found that the genus *Saranthe* was more closely related to the two genera *Ctenanthe* and *Stromanthe* than to the genus *Myrosma*. Our results showed a weak (MP-BS: 63) to strong (ML-BS: 89 and BI-PP: 100%) sister relationship for *Ctenanthe* and *Stromanthe*. This

relationship was also found by Andersson & Chase (2001, JK: 60, 8 taxa), Prince and Kress (2006a, BS: 78, 4 taxa), Prince and Kress (2006b, BS: 100, 2 taxa) and Suksathan *et al*. (2009, BS: 87, 3 taxa).

The monophyly of *Ctenanthe* (ML/MP-BS: 100 and BI-PP: 100%) was only supported based on ITS. In the combined analyses, we could only approve the monophyly of the genus when excluding *Ctenanthe dasycarpa* (ML-BS: 93, MP-BS: 99 and BI-PP: 100%) which was only nested with *Stromanthe* genus in the analysis of *rps*16 marker. All study results based only on the *rps*16 marker and including *Ctenanthe dasycarpa* could not show support for the monophyly of the two genera. Here *Ctenanthe dasycarpa* was either placed within the *Stromanthe* clade (Andersson and Chase 2001) or as sister to other *Stromanthe* species rather than *Ctenanthe* species (Suksathan *et al*. 2009). Morphologically, *Ctenanthe* and *Stromanthe* are very similar. While the majority of the species are easily classified at the genus level a few are problematic by sharing characters of both genera (Kennedy 1999). However, Kennedy (1999) found that the placement of the species *Ctenanthe dasycarpa* in *Stromanthe* (sensu Hammel 1986) was incorrect because of the bracts type, as the long and persistent sepals are a characteristic of *Ctenanthe*. However, the corolla tube length in *Ctenanthe dasycarpa* is shorter than in *Ctenanthe* (Kennedy 1978) where this feature is also found in *Stromanthe* (see Prince and Kress 2006a). Therefore, here we suggest to use more genetic markers (chloroplast and nuclear) to reveal the accurate affinity of *Ctenanthe dasycarpa* to the two genera *Ctenanthe* and *Stromanthe.*

The combined analysis very weakly placed the genus *Myrosma* as a sister to the *Maranta* II group and placed the latter taxa moderately supported in one clade with the genus *Hylaeanthe* (ML-BS: 87, MP-BS: 71 and BI-PP: 100%). Analyses based only on *rps*16 data, found no support for the placement of the genus *Myrosma* as sister to the genus *Saranthe* (Andersson & Chase 2001), or placed the five species *Hylaenthe hoffmannii*, *Koernickanthe orbiculata*, *Maranta kerchoviana*, *Maranta massengeana* and *Myrosma cannifolia* in one unsupported polytomy (Suksathan *et al*. 2009). When morphological data was added to the molecular analysis, a sister relationship between *Myrosma* and *Saranthe* was indicated in Prince and Kress (2006a).

***Stachyphrynium* clade**. The monophyly of this clade including the four genera *Afrocalathea, Marantochloa, Monophyllanthe* and *Stachyphrynium* was supported in the chloroplast datasets and the combined analysis. However, a different but only weakly supported clade topology was found in the ITS analysis, where the genera *Marantochloa* and *Monophyllanthe* were always more closely related to the *Maranta* clade than to the genera of the *Stachyphrynium* clade: *Afrocalathea* and *Stachyphrynium.*

The genus *Monophyllanthe* is included here for the first time in the analyses and placed as sister to the genus *Marantochloa*. Thus, the genus *Monophyllanthe* added the American continent to the distribution range of the *Stachyphrynium* clade. Anderson (1998) classified *Monophyllanthe* as a part of the *Maranta* group based on the genus morphology. As this genus was not included in any of the recent molecular studies at the family level (Andersson & Chase 2001; Prince & Kress 2006a, 2006b), no molecular evidence was so far achieved to refute or approve Andersson´s (1998) classification. Despite geographic isolation separating the two genera, *Monophyllanthe* and *Marantochloa* share a number of morphological features, including a solitary and simple cucullate staminode appendage, dehiscent fruits and arillate seeds (see Prince & Kress 2006a). Moreover, stoloniferous rhizomes were found in some *Marantochloa* species (Tomlinson 1961) and in *Monophyllanthe oligophylla* (Andersson 1998).

The here presented combined analyses, confirmed the monophyly of all four included genera. This was not yet the case in Prince and Kress (2006a) where *Ataenidia* was nested within *Marantochloa*, and *Afrocalathea* was embedded within *Stachyphrynium*. However, the genus *Ataenidia* was later included into the genus *Marantochloa* (see Ley & Claßen-Bockhoff 2011): *Marantochloa conferta* (*Ataenidia conferta*). The relationship between *Afrocalathea* and *Stachyphrynium* inferred by Prince and Kress (2006a) was based only on the chloroplast markers (*matK*, *trnL-F*), also a similar result achieved in Suksathan *et al*. (2009) when the analysis was based only on *rps*16. In our study, results from chloroplast markers independently showed also a similar relationship among the two genera. While the nuclear marker (ITS) and later the combined analyses either in our study or in Suksathan *et al.* (2009) confirmed the sister relationship of *Afrocalathea* and *Stachyphrynium*. This result might be achieved because the phylogenetic signal masked by homoplasy in the chloroplast data could be strengthened by a

combined analysis including ITS data which in turn can increases both resolution and support (Karehed *et al.* 2008). However, in our result the Maximum parsimony support value for the monophyly of *Stachyphrynium* genus (BS: 100) was higher than in Suksathan *et al.* (2009, MP-BS: 90). Moreover, the species relationships were almost fully resolved with high support values, while in Suksathan *et al.* (2009) these relationships were not fully resolved with low support values. This example shows, how the addition and concatenation of molecular characters from the chloroplast genome and ribosomal gene regions have a significant impact upon accurate phylogenetic analysis and can improve the resolution of the deep internodes (Townsend & Lopez-Giraldez 2010).

***Sarcophrynium* clade.** The intraclade topology based on all markers from independently evolving genomes (nuclear and chloroplast) are congruent in most parts and confirm the monophyly of all morphologically circumscribed genera within this clade. In addition, the relationships among these genera within the clade are solved with higher support than previously (Ley & Claßen-Bockhoff 2011) potentially due to the addition of further genetic markers. The placement of the genus *Sarcophrynium* at the base of the clade was confirmed by the all analyses (ML, MP and BI) of the combined dataset. This placement was not found in (Prince & Kress 2006b) due to a small sampling but was hypothesized by Prince and Kress (2006a), however, without statistical support.

Concerning the subclade including *Hypselodelphys, Megaphrynium, Thaumatococcus* and *Trachyphrynium,* our results show a moderate support for its monophyly in all three analyses (ML-BS: 79, MP-BS: 89 and BI-PP: 98 %). In a particular study investigating the relationships within the two major African clades (*Sarcophrynium* and *Marantochloa*) based on less molecular data than in our study, Ley and Claßen-Bockhoff (2011) found low support for this subclade (BS > 70). Moreover, the *Megaphrynium* topology was different by placing *M. gabonense* at the base of the genus. This did not yield a morphological support. In our study *Megaphrynium trichogynum* is sister to all other *Megaphrynium* species. In this topology floral (size and arrangement) and pollinator type are congruent between the basal taxon *M. trichogynum* and the sister genus *Thaumatococcus* and shows one shift to bee pollination and its corresponding floral type in the remaining *Megaphrynium* species (see Ley & Claßen-Bockhoff 2011).

Genus *Haumania.* The placement of this African genus *Haumania* as sister to all other major clades (*Calathea*, *Donax*, *Maranta* and *Stachyphrynium* clades) or within the *Sarcophrynium* clade remained unresolved in our analyses, just as in all previous phylogenetic studies. Prince and Kress (2006a) found *Haumania* to be placed at the base of the *Calathea* clade with a poorly supported relationship (less than 50% JK and less than 0.95 PP). However, they found no morphological evidence supporting the inclusion of *Haumania* in the *Calathea* clade. Based on more molecular markers, Prince and Kress (2006b) found *Haumania* in an unresolved polytomy at a more basal position than in the earlier study, or as a weakly supported member of the *Sarcophrynium* clade. To date it is thus still unclear whether the three *Haumania* species belong to the *Sarcophrynium* clade or have to be regarded as the root node for the other four clades. Despite the fact, that our results provide little information concerning the position of *Haumania* the relationships among the *Haumania* species are now fully resolved.

CONCLUSIONS

In conclusion, our results provide a higher resolution and support for the Marantaceae backbone in comparison to what was achieved in all previous studies. The addition of more molecular data and taxa have strengthened the hypothesized topology and suggested generic limits of the relationships within the family Marantaceae (Prince & Kress 2006a; Suksathan *et al.* 2009; Ley & Claßen-Bockhoff 2011; Borchsenius *et al.* 2012). Major new findings include highly supported infrasectional topologies of major clades and many subclades that were not achieved in any of the previous studies. Additionally, our study identified at least four potentially non-monophyletic genera: *Calathea*, *Ischnosiphon*, *Maranta* and *Schumannianthus* and only one genus *Haumania* of uncertain affinity.

Our results strongly supported the monophyly of 12 polytypic genera. The strongly supported sister relationship found between the genera *Monophyllanthe* and *Marantochloa* reveals that the *Stachyphrynium* clade is not restricted to a single geographical region (tropical America, tropical Africa, or tropical Asia). Obtaining a complete and full sequences length for all marker, as well as, more morphological data can enhance the total evidences support Marantaceae phylogeny. Our current results which are based on more molecular data and taxa

can be used in the future to investigate the biogeographical pattern and the timing of divergence in the family Marantaceae.

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APPENDICES

Appendix 1. Source and voucher information for taxa sampled in a phylogenetic study of Marantaceae based on chloroplast and nuclear DNA sequence data.

NO.	Taxa/Synonyms	*matK*	*rps* 16	*trnL-F*	ITS
1	***Afrocalathea rhizantha*** (K.Schum.) K.Schum.	AY140262	EF382847	ley011	EU605908
2	***Calathea crotalifera*** S.Watson	AU1429	AU1429	AU1429	AU1429
3	***Calathea guzmanioides*** L.B.Sm. & Idrobo	AU1423	AU1423	AU1423	AU1423
4	***Calathea hagbergii*** H.Kenn.	AU1424	AU1424	AU1424	AU1424
5	***Calathea lutea*** (Aubl.) E.Mey. ex Schult.	AU1427	AU1427	AU1427	AU1427
6	***Calathea marantina*** (Willd. ex Körn.) K.Koch	JQ341349	NA	JQ341232	JQ341288
7	***Calathea pluriplicata*** H.Kenn.	AY140280	NA	AY140359	JQ341295
8	***Calathea plurispicata*** H.Kenn.	AU1430	AU1430	AU1430	AU1430
9	***Calathea timothei*** H.Kenn.	AU1425	AU1425	AU1425	AU1425
10	***Calathea toroi*** S.Suárez	AU1426	AU1426	AU1426	AU1426
11	***Calathea utilis*** H.A.Kenn.	AY140282	NA	AY140361	JQ341303
12	***Canna indica*** L.	AM114724	AM116859	AM113702	FJ939505
13	***Ctenanthe burle-marxii*** H.Kenn.	AU1809	AU1809	AU1809	AU1809
14	***Ctenanthe dasycarpa*** (Donn.Sm.) K.Schum.	NA	AF141042	NA	NA
15	***Ctenanthe lubbersiana*** (E.Morren) Eichler ex Petersen	AU1811	AU1811	AU1811	AU1811
16	***Ctenanthe marantifolia*** (Vell.) J.M.A.Braga & H.Gomes	AU1813	AU1813	AU1813	AU1813
17	***Ctenanthe oppenheimiana*** (E.Morren) K.Schum.	AU1812	AU1812	AU1812	AU1812
18	***Ctenanthe setosa*** (Roscoe) Eichler	AU1810	AU1810	AU1810	AU1810
19	***Donax canniformis*** (G.Forst.) K.Schum.	AU650	AY914616	AU1931	AU1931
20	***Goeppertia aemula*** (Körn.) Borchs. & S.Suárez/***Calathea aemula***	AY140265	LP327	AY140344	LP327
21	***Goeppertia altissima*** (Poepp. & Endl.) Borchs. & S.Suárez/***Calathea altissima***	AU1444	AU1444	AU1444	AU1444
22	***Goeppertia attenuata*** (H.Kenn.) Borchs. & S.Suárez/***Calathea attenuata***	AU1448	AU1448	AU1448	AU1448
23	***Goeppertia bella*** (W.Bull) Borchs. & S.Suárez/***Calathea bella***	AY140278	LP348	AY140357	JQ341292
24	***Goeppertia capitata*** (Ruiz & Pav.) Borchs. & S.Suárez/***Calathea capitata***	AU1440	AU1440	AU1440	AU1440
25	***Goeppertia colorata*** (Hook.) Borchs. & S.Suárez/***Calathea colorata***	AY140266	LP343	AY140345	LP343
26	***Goeppertia comosa*** (L.f.) Borchs. & S.Suárez/***Calathea comosa***	AY140267	NA	AY140346	Ley025
27	***Goeppertia concinna*** (W.Bull) Borchs. & S.Suárez/***Calathea concinna*** / ***C. leopardina***	AY140272	LP332	AY140351	LP332
28	***Goeppertia curaraya*** (H.Kenn.) Borchs. & S.Suárez/***Calathea curaraya***	AU1438	AU1438	AU1438	AU1438
29	***Goeppertia cyclophora*** (Baker) Borchs. & S.Suárez/***Calathea cyclophora***	AU1431	AU1431	AU1431	AU1431
30	***Goeppertia cylindrica*** (Roscoe) Borchs. & S.Suárez/***Calathea cylindrica***	NA	AF141028	NA	NA
31	***Goeppertia ecuadoriana*** (H.Kenn.) Borchs. & S.Suárez/***Calathea ecuadoriana***	AY140269	NA	AY140348+JN413126	JQ341275
32	***Goeppertia foliosa*** (Rowlee ex Woodson & Schery) Borchs. & S.Suárez/***Calathea foliosa***	AY140270	LP030	AY140349+LP030	JQ341276
33	***Goeppertia fucata*** (H.Kenn.) Borchs. & S.Suárez/***Calathea fucata***	JQ341340	NA	JQ341223	JQ341277
34	***Goeppertia gymnocarpa*** (H.Kenn.) Borchs. & S.Suárez/***Calathea gymnocarpa***	AY140271	LP339	AY140350+LP339	JQ341279
35	***Goeppertia inocephala*** (Kuntze) Borchs. & S.Suárez/***Calathea inocephala***	AU1446	AU1446	AU1446	AU1446
36	***Goeppertia killipii*** (L.B.Sm. & Idrobo) Borchs. & S.Suárez/***Calathea killipii***	AU1451	AU1451	AU1451	AU1451
37	***Goeppertia laetevirens*** (Huber) Borchs. & S.Suárez/***Calathea laetevirens***	Suarez2655+SG132	AU1922	AU1922	Ley026
38	***Goeppertia lanata*** (Petersen) Borchs. & S.Suárez/***Calathea lanata***	AU1433	AU1433	AU1433	AU1433
39	***Goeppertia lancifolia*** (Boom) Borchs. & S.Suárez/***Calathea lancifolia***	ley046+Ley054	ley002	Ley012	NA
40	***Goeppertia latifolia*** (Willd. ex Link) Borchs. & S.Suárez/***Calathea latifolia***	AU1445	AU1445	AU1445	AU1445
41	***Goeppertia leonia*** (Boom bis) Borchs. & S.Suárez/***Calathea leonia***	AU1447	AU1447	AU1447	AU1447
42	***Goeppertia loeseneri*** (J.F.Macbr.) Borchs. & S.Suárez/***Calathea loeseneri***	AY140273	NA	AY140352	JQ341286
43	***Goeppertia majestica*** (Linden) Borchs. & S.Suárez/***Calathea majestica***	AY140274	LP338	AY140353	LP338
44	***Goeppertia metallica*** (Planch. & Linden) Borchs. & S.Suárez/***Calathea metallica***	AY140275	AY656136	AY140354	AY673046
45	***Goeppertia micans*** (L.Mathieu) Borchs. & S.Suárez/***Calathea micans***	AU1435	AU1435	AU1435	AU1435
46	***Goeppertia microcephala*** (Poepp. & Endl.) Borchs. & S.Suárez/***Calathea microcephala***	AU1436	AU1436	AU1436	AU1436
47	***Goeppertia mirabilis*** (Jacob-Makoy ex E.Morren) Borchs. & S.Suárez/***Calathea mirabilis***	AY140277	LP347	AY140356	LP347
48	***Goeppertia mishuyacu*** (J.F.Macbr.) Borchs. & S.Suárez/***Calathea mishuyacu***	AU1443	AU1443	AU1443	AU1443
49	***Goeppertia neblinensis*** (H.Kenn.) Borchs. & S.Suárez/***Calathea neblinensis***	Castro1152	AU1466	ley013	Ley027
50	***Goeppertia pallidicosta*** (H.Kenn.) Borchs. & S.Suárez/***Calathea pallidicosta***	AY140279	ley004	AY140358	Ley028
51	***Goeppertia pavonii*** (Körn.) Borchs. & S.Suárez/***Calathea pavonii***	AU1492	AU1492	AU1492	AU1492
52	***Goeppertia petersenii*** (Eggers) Borchs. & S.Suárez/***Calathea petersenii***	AU1432	AU1432	AU1432	AU1432
53	***Goeppertia picturata*** (K.Koch & Linden) Borchs. & S.Suárez/***Calathea picturata***	NA	AF141033	NA	NA
54	***Goeppertia propinqua*** (Poepp. & Endl.) Borchs. & S.Suárez/***Calathea propinqua***	AU1441	AU1441	AU1441	AU1441
55	***Goeppertia rufibarba*** (Fenzl) Borchs. & S.Suárez/***Calathea rufibarba***	AY140281	AY656138	AY140360	AY673048
56	***Goeppertia silvosa*** (J.F.Macbr.) Borchs. & S.Suárez/***Calathea silvosa***	AU1439	AU1439	AU1439	AU1439
57	***Goeppertia splendida*** (Lem.) Borchs. & S.Suárez/***Calathea splendida***	NA	AF141036	NA	NA
58	***Goeppertia standleyi*** (J.F.Macbr.) Borchs. & S.Suárez/***Calathea standleyi***	AU1442	AU1442	AU1442	AU1442
59	***Goeppertia straminea*** (Petersen) Borchs. & S.Suárez/***Calathea straminea***	AU1450	AU1450	AU1450	AU1450
60	***Goeppertia undulata*** (Linden & André) Borchs. & S.Suárez/***Calathea undulata***	AU1437	AU1437	AU1437	AU1437

61	***Goeppertia varians*** (K.Koch & Mathieu)Borchs. & S.Suárez/***Calathea varians***	**AU1491**	**AU1491**	**AU1491**	**AU1491**
62	***Goeppertia variegata*** (K.Koch) Borchs. & S.Suárez/***Calathea variegata***	**AU1449**	**AU1449**	**AU1449**	**AU1449**
63	***Goeppertia veitchiana*** (Veitch ex Hook.f.) Borchs. & S.Suárez/***Calathea veitchiana***	**NA**	**AY914604**	**NA**	**AY914651**
64	***Goeppertia villosa*** (Lodd. ex G.Don) Borchs. & S.Suárez./***Calathea villosa***	**AU1462**	**AU1462**	**AU1462**	**AU1462**
65	***Goeppertia vinosa*** (H.Kenn.) Borchs. & S.Suárez/***Calathea vinosa***	**AY140284**	**LP359**	**AY140363+LP359**	**JQ341307**
66	***Goeppertia warscewiczii*** (Lem.) Borchs. & S.Suárez/***Calathea warszewiczii***	**AY140285**	**AY656139**	**AY140364+LP024**	**AY673049**
67	***Goeppertia zebrina*** (Sims) Borchs. & S.Suárez/***Calathea zebrina***	**ley047+Ley055**	**AF141038**	**ley014**	**Ley029**
68	***Halopegia azurea*** (K.Schum.) K.Schum.	**AY140291**	**LP364**	**Ley015**	**LP364**
69	***Halopegia blumei*** (Körn.) K.Schum.	**AU446**	**AU446**	**AU446**	**JQ341258**
70	***Haumania danckelmaniana*** (J.Braun & K.Schum.) Milne-Redh	**Al60**	**AL60**	**AL60**	**Ley031**
71	***Haumania leonardiana*** C.M.Evrard & Bamps	**BEB250**	**BEB250**	**BEB250**	**Ley032**
72	***Haumania liebrechtsiana*** (De Wild. & T.Durand) J.Léonard	**Al50**	**Al50_B03**	**ley016**	**Ley030**
73	***Haumania*** sp.	**AY140293**	**AY656143**	**AY140374+JN413109**	**AY673053**
74	***Hylaeanthe hexantha*** (Poepp. & Endl.) A.M.E.Jonker & Jonker	**AU1825**	**AU1825**	**AU1825**	**AU1825**
75	***Hylaeanthe hoffmannii*** (K.Schum.) A.M.E.Jonker & Jonker ex H.Kenn.	**JQ588304**	**AF141051**	**NA**	**NA**
76	***Hypselodelphys hirsuta*** (Loes.) Koechlin	**AU1219**	**AU1219+AL269**	**Al269**	**AU1219**
77	***Hypselodelphys poggeana*** (K.Schum.) Milne-Redh.	**Al168**	**AL168**	**EU647819**	**EU605912**
78	***Hypselodelphys scandens*** Louis & Mullend.	**Al160**	**AL160**	**EU647824**	**EU605917**
79	***Hypselodelphys triangularis*** Jongkind	**NA**	**vMaesen5275**	**EU647822**	**EU605915**
80	***Hypselodelphys violacea*** (Ridl.) Milne-Redh.	**Al28**	**AF141052**	**EU647821**	**EU605914**
81	***Hypselodelphys velutina*** Jongkind	**Ley078**	**NA**	**EU647818**	**EU605911**
82	***Indianthus virgatus*** (Roxb.) Suksathan & Borchs. /***Schumannianthus virgatus***	**AY140328**	**AY914620**	**AU1921**	**AY914666**
83	***Ischnosiphon cerotus*** Loes.	**AY140297**	**LP366**	**AY140378**	**LP366**
84	***Ischnosiphon heleniae*** L.Andersson	**AY140298**	**AY656145**	**AY140379**	**AY673055**
85	***Ischnosiphon hirsutus*** Petersen	**AU1454**	**AU1454**	**AU1454**	**AU1454**
86	***Ischnosiphon leucophaeus*** (Poepp. & Endl.) Körn.	**AY140299**	**LP367**	**AY140380 +LP367**	**JQ341309**
87	***Ischnosiphon macarenae*** L.Andersson	**AU1455**	**AU1455**	**AU1455**	**AU1455**
88	***Ischnosiphon obliquus*** (Rudge) Körn.	**AU1456**	**AU1456**	**AU1456**	**AU1456**
89	***Ischnosiphon ovatus*** Körn., Bull.	**NA**	**AF141054**	**NA**	**NA**
90	***Ischnosiphon puberulus*** Loes.	**AY140300**	**LP368**	**AY140381+LP368**	**JQ341312**
91	***Ischnosiphon rotundifolius*** (Poepp. & Endl.) Körn.	**AY140301**	**LP440**	**AY140382+LP440**	**JQ341313**
92	***Koernickanthe orbiculata*** (Körn.) L.Andersson	**AU1826**	**AU1826**	**AY140383**	**AU1826**
93	***Maranta arundinacea*** L.	**AU1835**	**AU1835**	**AU1835**	**AU1835**
94	***Maranta cristata*** Nees & Mart. /***Maranta bicolor***	**AY140302**	**AY656146**	**AY140385**	**AY673056**
95	***Maranta friedrichsthaliana*** Körn.	**NA**	**NA**	**NA**	**Ley043**
96	***Maranta humilis*** Aubl.	**AU1845**	**AU1845**	**AU1845**	**AU1845**
97	***Maranta leuconeura*** E.Morren	**AU1831**	**AU1831**	**AU1831**	**AU1831**
98	***Maranta noctiflora*** Regel & Körn.	**AU1840**	**AU1840**	**AU1840**	**AU1840**
99	***Maranta parvifolia*** Petersen	**AU1837**	**AU1837**	**AU1837**	**AU1837**
100	***Maranta pohliana*** Körn.	**AU1839**	**AU1839**	**AU1839**	**AU1839**
101	***Maranta protracta*** Miq.	**AU1877**	**AU1877**	**AU1877**	**AU1877**
102	***Maranta ruiziana*** Körn.	**ley075+ley053**	**AF141060**	**NA**	**NA**
103	***Maranta rupicola*** L.Andersson	**Borchsenius lab.**	**NA**	**NA**	**Ley042**
104	***Maranta sobolifera*** L.Andersson	**NA**	**AF141061**	**NA**	**NA**
105	***Maranta tuberculata*** L.Andersson	**AU1842**	**AU1877**	**AU1842**	**AU1877**
106	***Marantochloa conferta*** (Benth.) A.C.Ley/***Ataenidia conferta***	**AY140263+Al115**	**AY656134**	**AY140342**	**AY673044**
107	***Marantochloa congensis*** (K.Schum.) J.Léonard & Mullend.	**Al107**	**AF141062**	**EU647811**	**EU605903**
108	***Marantochloa cordifolia*** (K.Schum.) Koechlin	**Al63**	**Al63**	**EU647802**	**NA**
109	***Marantochloa cuspidata*** (Roscoe) Milne-Redh.	**AU1221**	**AU1221**	**EU647814**	**EU605906**
110	***Marantochloa filipes*** (Benth.) Hutch.	**AU1222**	**AU1222+AL262**	**AU1222**	**AU1222**
111	***Marantochloa grandiflora*** A.C.Ley	**Ley079**	**WPM017T**	**EU647817**	**NA**
112	***Marantochloa incertifolia*** Dhetchuvi	**Al179**	**NA**	**EU647813**	**NA**
113	***Marantochloa leucantha*** (K.Schum.) Milne-Redh.	**AY140305**	**AF141066**	**EU647809**	**EU605901**
114	***Marantochloa mannii*** (Benth.) Milne-Redh.	**Al638**	**ley005**	**EU647806**	**EU605897**
115	***Marantochloa microphylla*** (Koechlin) Dhetchuvi	**ACL2531**	**ACL2531**	**ACl2531**	**AY673057**
116	***Marantochloa mildbraedii*** Koechlin	**Simons14**	**Simons14**	**Simons14**	**EU605893**
117	***Marantochloa monophylla*** (K.Schum.) D'Orey	**Al45**	**ley006**	**EU647810**	**EU605902**
118	***Marantochloa montsdecristalii*** A.C.Ley	**ley048+ley056**	**AL256**	**ley017**	**Ley033**
119	***Marantochloa purpurea*** (Ridl.) Milne-Redh.	**AY140306**	**AY656147**	**AY140389**	**AY673057**
120	***Marantochloa ramosissima*** (Benth.) Hutch	**Al751**	**ley007**	**ley018**	**Ley034**
121	***Megaphrynium gabonense*** Koechlin	**Al155**	**AL155**	**EU647830**	**EU605924**
122	***Megaphrynium macrostachyum*** (K.Schum.) Milne-Redh.	**AU1217**	**AL260+BEB373**	**AU1217**	**AU1217**
123	***Megaphrynium trichogynum*** Koechlin	**Al22**	**AL22**	**EU647828**	**EU605921**
124	***Megaphrynium velutinum*** (K.Schum.) Koechlin	**Ley059+Ley80**	**NA**	**EU652953**	**NA**
125	***Monophyllanthe araracuarensis*** S.Suárez, Galeano & H.Kenn.	**AU1824**	**AU1824**	**AU1824**	**Ley040**
126	***Monophyllanthe oligophylla*** K.Schum.	**AU1823**	**AU1823**	**AU1823**	**Ley041**
127	***Monotagma densiflorum*** (Körn.) K.Schum.	**Ley049+Ley060**	**ley009**	**Ley019**	**NA**
128	***Monotagma dolosum*** J.F.Macbr	**NA**	**AF141069**	**NA**	**NA**
129	***Monotagma juruanum*** Loes.	**AU1458**	**AU1458**	**AU1458**	**AU1458**
130	***Monotagma laxum*** (Poepp. & Endl.) K.Schum.	**AY140309**	**AY656148**	**AY140392+LP 376**	**AY673058**
131	***Monotagma papillosum*** Hagberg & R.Erikss.	**AY140310**	**LP377**	**AY140393+LP243**	**LP377**
132	***Monotagma parvulum*** Loes.	**AY140311**	**LP378**	**AY140394**	**LP378**
133	***Monotagma secundum*** (Petersen) K.Schum.	**AU1459**	**AU1459**	**AU1459**	**AU1459**
134	***Monotagma smaragdinum*** (Linden & André) K.Schum.	**AY140312**	**LP379**	**AY140395+LP379**	**JQ341318**
135	***Monotagma tomentosum*** K.Schum. ex Loes.	**AU1460**	**AU1460**	**AU1460**	**AU1460**
136	***Monotagma tuberosum*** Hagberg & R.Erikss.	**AU1457**	**AU1457**	**AU1457**	**AU1457**
137	***Myrosma cannifolia*** L.f.	**AU1857**	**AU1857**	**AU1857**	**AU1857**
138	***Phrynium aurantium*** (Clausager & Borchs.) Suksathan & Borchs./***Phacelophrynium aurantium***	**Johannsen12_SG120**	**AY914623**	**Johannsen12**	**AY914668*** **
139	***Phrynium fasciculatum*** (C.Presl) Horan./***Monophrynium fasciculatum***	**Suksathan3419_SG91**	**AY914646**	**Suksathan3419**	**AY914691*** **
140	***Phrynium fissifolium*** Ridl.	**NA**	**EF382851**	**NA**	**EF382843*** **

141	***Phrynium giganteum*** Scheff./***Cominsia gigantea***	**AU1862**	**AU1862**	**AU1862**	**AY673050**
142	***Phrynium grandibracteatum*** Clausager & Borchs.	**SJ11_SG139**	**AY914631+SJ11_SG13**	**SJ11_SG139**	**AU532**
143	***Phrynium hainanense*** T.L.Wu & S.J.Chen	**Suksathan296**	**AY914632**	**AU441**	**AU441**
144	***Phrynium hirtum*** Ridl.	**SJ03_SG336**	**AY914633**	**AU1932**	**Ley035*** **
145	***Phrynium imbricatum*** Roxb.	**AY140319**	**LP384 + AU1923**	**AU1923+AY140401**	**AU1923 + LP384**
146	***Phrynium interruptum*** (K.Schum.) Suksathan & Borchs.	**AU618**	**AY914625**	**AU618**	**AU618**
147	***Phrynium kaniense*** Loes. & G.M.Schulze	**NA**	**NA**	**NA**	**EF382844*** **
148	***Phrynium laxum*** (Clausager & Borchs.) Suksathan & Borchs./***Phacelophrynium laxum***	**SJ16_SG142***	**AY914626**	**AU535**	**AU535**
149	***Phrynium macrocephalum*** K.Schum.	**NA**	**EF382852**	**AU697**	**EF 3EF382845****
150	***Phrynium maximum*** Blume/***Phacelophrynium maximum***	**Poulsen1576+Ley050**	**EF382850**	**AU1933**	**AU555**
151	***Phrynium minutiflorum*** Suksathan & Borchs./***Phacelophrynium cylindricum***	**Suksathan3531_SG245**	**AY914624**	**Suksathan3531**	**AY914669*** **
152	***Phrynium obscurum*** Teijsm. & Binn.	**ley066+Ley051**	**AY914636**	**ley020**	**AY91468*** **
153	***Phrynium pedunculiferum*** D.Fang	**ley67 ***	**AY914637**	**ley021**	**AY914683*** **
154	***Phrynium pubinerve*** Blume/***Phrynium philippinense/ P.rheede***	**AU622**	**AY914639**	**AU622**	**AY914684*** **
155	***Phrynium sapiense*** (Clausager, Mood & Borchs.) Suksathan & Borchs/***Phacelophrynium sapien***	**Johannsen2 ****	**AY914630**	**Johannsen2**	**AY914675*** **
156	***Phrynium simplex*** (Elmer) Suksathan & Borchs./***Monophrynium simplex***	**Suksathan_3525_SG115**	**AY914647**	**Suksathan_3525_SG115**	**AY914692*** **
157	***Phrynium tonkinense*** Gagnep.	**Suksathan3543_SG148+Ley**	**AY914641**	**Suksathan3543**	**AY914682*** **
158	***Phrynium tristachyum*** Ridl.	**ley076***	**AY914642**	**NA**	**Ley044**
159	***Phrynium villosulum*** Miq.	**Johannsen13_SG123**	**AY914643**	**Johannsen13**	**AY914688*** **
160	***Phrynium whitei*** (Ridl.) Suksathan & Borchs.	**AU1224**	**AU1224**	**AU1224**	**AU1224**
161	***Pleiostachya pruinosa*** (Regel) K.Schum.	**AU1366**	**AU1366**	**AU1366**	**AU1366**
162	***Sanblasia dressleri*** L.Andersson	**AU1599**	**AU1599**	**AU1599**	**AU1599**
163	***Saranthe klotzschiana*** (Körn.) Eichler	**AU1822**	**AU1822**	**AU1822**	**AU1822**
164	***Saranthe madagascariensis*** (Benth.) K.Schum./***Saranthe unilateralis***	**AU1878**	**AU1878**	**AU1878**	**AU1878**
165	***Sarcophrynium brachystachyum*** (Benth.) K.Schum.	**Al32**	**AL32**	**EU647831**	**EU605926**
166	***Sarcophrynium prionogonium*** (K.Schum.) K.Schum.	**Al55**	**AL55**	**EU647832**	**EU605929**
167	***Sarcophrynium schweinfurthianum*** (Kuntze) Milne-Redh.	**NA**	**NA**	**EU647833**	**EU605928**
168	***Sarcophrynium villosum*** (Benth.) K.Schum.	**Al759**	**AL759**	**ley022**	**NA**
169	***Schumannianthus dichotomus*** (Roxb.) Gagnep.	**AU649**	**AY914619**	**AU649**	**AU649**
170	***Schumannianthus monophyllus*** Suksathan/***Phrynium griffithii***	**AU653**	**AY914621**	**AU653**	**AY914667**
171	***Stachyphrynium calcicola*** A.D.Poulsen & Clausager	**Poulsen_2026_SG154**	**AY914606**	**Poulsen2026**	**AY914652**
172	***Stachyphrynium latifolium*** (Blume) K.Schum.	**AY140329**	**LP386**	**AY140412 + ley023**	**ley036**
173	***Stachyphrynium longispicatum*** Suksathan & Borchs	**Suksathan3321_SG95**	**AY914609**	**Suksathan3321**	**AY914655**
174	***Stachyphrynium placentarium*** (Lour.) Clausager & Borchs.	**Ley073+Ley077**	**AY914610**	**AU447**	**ley037+Ley045**
175	***Stachyphrynium repens*** (Körn.) Suksathan & Borchs.	**AU616**	**AY914611**	**AU616**	**AU616**
176	***Stachyphrynium spicatum*** (Roxb.) K.Schum	**Suksathan3356_SG90**	**AY914612**	**Suksathan335**	**AY914658**
177	***Stachyphrynium sumatranum*** (Miq.) K.Schum.	**AY140318**	**AY914614**	**NA**	**ley038**
178	***Stromanthe jacquinii*** (Roem. & Schult.) H.Kenn. & Nicolson	**NA**	**AF141087**	**NA**	**NA**
179	***Stromanthe papillosa*** Petersen	**AU1879**	**AU1879**	**AU1879**	**AU1879**
180	***Stromanthe schottiana*** (Körn.) Eichler	**AU1817**	**AU1817**	**AU1817**	**AU1817**
181	***Stromanthe stromanthoides*** (J.F.Macbr.) L.Andersson	**AY140334**	**ley010**	**AY140417**	**Ley039**
182	***Stromanthe thalia*** (Vell.) J.M.A.Braga	**AU1814**	**AU1814**	**AU1814**	**AU1814**
183	***Stromanthe tonckat*** (Aubl.) Eichler	**AU1816**	**AU1816**	**AU1816**	**AU1816**
184	***Thalia dealbata*** Fraser	**AU549**	**AY914648.1**	**JQ341215**	**AY914693**
185	***Thalia geniculata*** L.	**AU916**	**EF382853**	**AU916**	**AU916**
186	***Thaumatococcus daniellii*** (Benn.) Benth.	**AU1218**	**AU1218+AL96**	**EU647826**	**EU605919**
187	***Thaumatococcus flavus*** A.C.Ley	**Al56**	**AL56**	**EU647827**	**NA**
188	***Trachyphrynium braunianum*** (K.Schum.) Baker	**AY140339**	**AL171**	**AY140422+ ley024**	**AY673068**

Abbreviations: Sequences name start with (**Ley**) indicate new sequence or part of sequences obtained from DNA extracted in the Institute of Geobotany and Botanical Garden/Halle (Saale), Germany. **NA**, indicate that this sequence is not available. *****, the *mif matK* part is missing from this sequence (≈ 620 bp); ******, the 867 *matK* part is missing from this sequence (≈ 630 bp); *******, part of the 5.8S and the whole ITS2 are missing from this sequence (≈ 620 bp).

Appendix 2. In the following figures a Maximum Likelihood strict consensus tree for each marker analysis: *matK* (Fig 1a and b), *rps*16 (Fig 2a and b), *trnL-F* (Fig 3a and b), ITS (Fig 4a and b) and combined analysis (Fig 5a and b). Numbers above and below branches denote ML and MP bootstrap support of 50 % or higher, respectively. Bold lines indicated branches with posterior probabilities of 0.95 or higher. Dash sign indicates branches not found in the Maximum parsimony strict consensus tree (-/), in the Bayesian analysis(/-) or in both analyses (-/-). Inset upper left corner shows which clades or branches of the Marantaceae family tree is depicted in the large figure.


A
Thalia
B
C
D
Haumania
E
F
Canna
CALATHEA CLADE (A)
DONAX CLADE (B)
CALATHEA CLADE (C)
SARCOPHRYNIUM CLADE (D)
Goeppertia varians
Goeppertia rufibarba
Goeppertia pavonii
Goeppertia concinna
Goeppertia bella
Goeppertia lancifolia
Goeppertia colorata
Goeppertia zebrina
Goeppertia aemula
Goeppertia mishuyacu
Goeppertia standleyi
Goeppertia laetevirens
Goeppertia propinqua
Goeppertia mirabilis
Goeppertia loeseneri
Goeppertia lanata
Goeppertia neblinensis
Goeppertia petersenii
Goeppertia attenuata
Goeppertia metallica
Goeppertia silvosa
Goeppertia pallidicosta
Goeppertia ecuadoriana
Goeppertia villosa
Goeppertia capitata
Goeppertia leonia
Goeppertia comosa
Goeppertia cyclophora
Goeppertia micans
Goeppertia curaraya
Goeppertia fucata
Goeppertia killipii
Goeppertia undulata
Goeppertia microcephala
Goeppertia latifolia
Goeppertia vinosa
Goeppertia warscewiczii
Goeppertia foliosa
Goeppertia altissima
Goeppertia inocephala
Goeppertia variegata
Goeppertia majestica
Goeppertia gymnocarpa
Goeppertia striminea
Thalia geniculata
Thalia dealbata
Phrynium simplex
Phrynium interruptum
Phrynium fascicultatum
Phrynium laxum
Phrynium maximum
phrynium giganteum
Phrynium whitei
Phrynium minutiflorum
Phrynium tonkinense
Phrynium hainanense
Phrynium pedunculiferum
Phrynium villosulum
Phrynium pubinerve
Phrynium imbricatum
Phrynium tristachyum
Phrynium obscurum
Phrynium hirtum
Phrynium aurantium
Phrynium sapiense
Phrynium grandibracteatum
Schumanniathus monophyllus
Donax canniformis
Schumanniathus dichotomus
Calathea timothei
Calathea hagbergii
Calathea guzmanioides
Calathea pluriplicata
Calathea marantina
Calathea crotalifera
Calathea toroi
Calathea utilis
Saniblasia dressleri
Calathea lutea
Calathea plurispicata
Ischnosiphon heleniae
Ischnosiphon leucophaeus
Ischnosiphon obliquus
Pleiostachya pruinosa
Ischnosiphon cerotus
Ischnosiphon macarenae
Ischnosiphon hirsutus
Ischnosiphon puberulus
Ischnosiphon rotundifolius
Monotagma smaragdinum
Monotagma parvulum
Monotagma secundum
Monotagma tomentosum
Monotagma densiflorum
Monotagma papillosum
Monotagma laxum
Monotagma juruanum
Monotagma tuberosum
Hypseladelphys hirsuta
Hypseladelphys scandens
Hypseladelphys poggeana
Hypseladelphys violacea
Trachyphrynium brauniianum
Hypseladelphys velutina
Sarcophrynium villosum
Sarcophrynium prionogonium
Sarcophrynium brachystachyum
Megaphrynium velutinum
Megaphrynium trichogynum
Megaphrynium gabonense
Megaphrynium macrostachyum
Thaumatococcus flavus
Thaumatococcus daniellii
Haumania danckelmaniana
Haumania sp
Haumania liebrechtsiana
Haumania leonardiana

Figure 1a

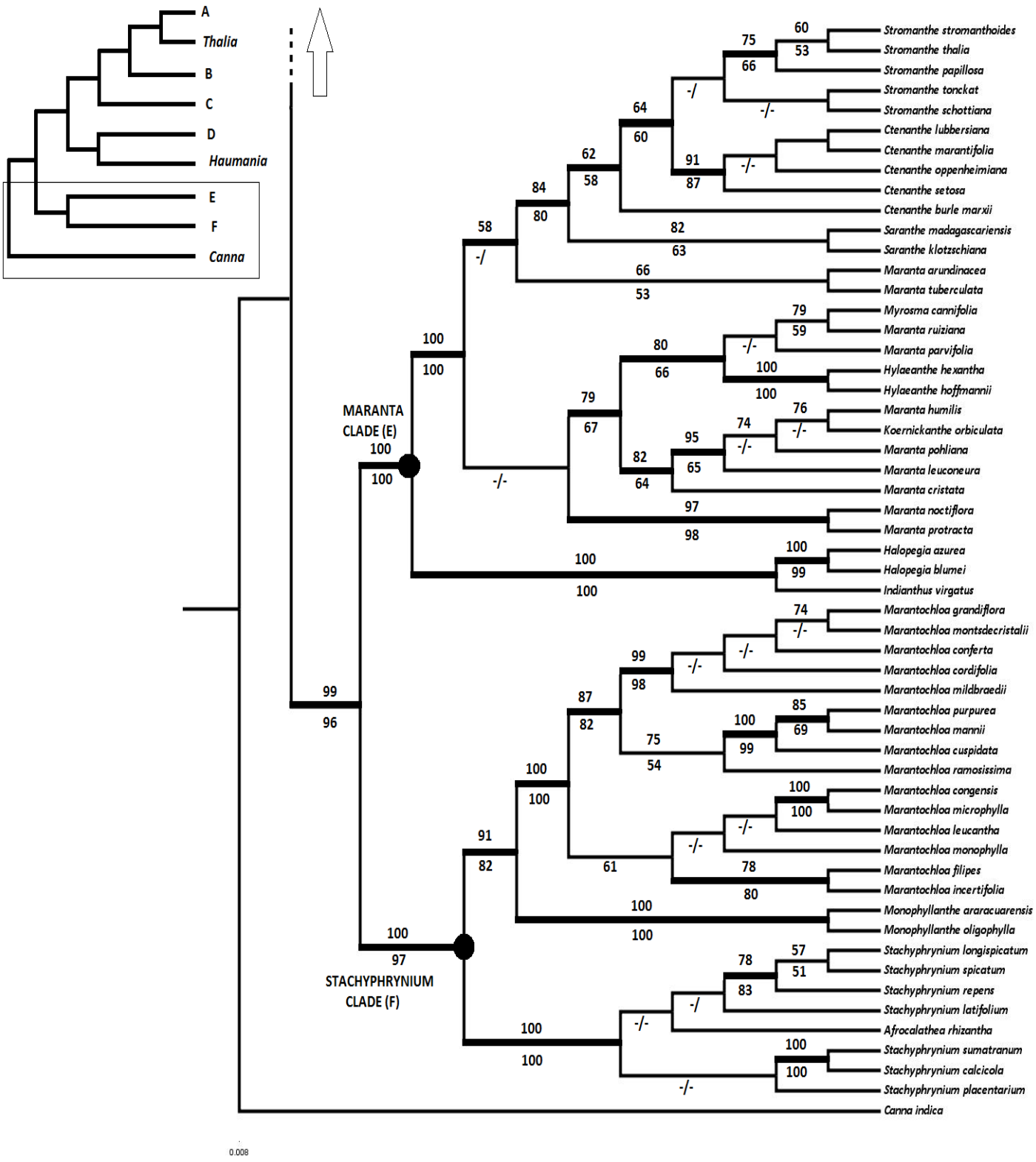
A
Thalia
B
C
D
Haumania
E
F
Canna
MARANTA CLADE (E)
STACHYPHRYNIUM CLADE (F)
Stromanthe stromanthoides
Stromanthe thalia
Stromanthe papillosa
Stromanthe tonckat
Stromanthe schottiana
Ctenanthe lubbersiana
Ctenanthe marantifolia
Ctenanthe oppenheimiana
Ctenanthe setosa
Ctenanthe burle marxii
Saranthe madagascariensis
Saranthe klotzschiana
Maranta arundinacea
Maranta tuberculata
Myrosma cannifolia
Maranta ruiziana
Maranta parvifolia
Hylaeanthe hexantha
Hylaeanthe hoffmannii
Maranta humilis
Koernickanthe orbiculata
Maranta pohliana
Maranta leuconeura
Maranta cristata
Maranta noctiflora
Maranta protracta
Halopegia azurea
Halopegia blumei
Indianthus virgatus
Marantochloa grandiflora
Marantochloa montsdecristalii
Marantochloa conferta
Marantochloa cordifolia
Marantochloa mildbraedii
Marantochloa purpurea
Marantochloa mannii
Marantochloa cuspidata
Marantochloa ramosissima
Marantochloa congensis
Marantochloa microphylla
Marantochloa leucantha
Marantochloa monophylla
Marantochloa filipes
Marantochloa incertifolia
Monophyllanthe araracuarensis
Monophyllanthe oligophylla
Stachyphrynium longispicatum
Stachyphrynium spicatum
Stachyphrynium repens
Stachyphrynium latifolium
Afrocalathea rhizantha
Stachyphrynium sumatranum
Stachyphrynium calcicola
Stachyphrynium placentarium
Canna indica
0.008

Figure 1b

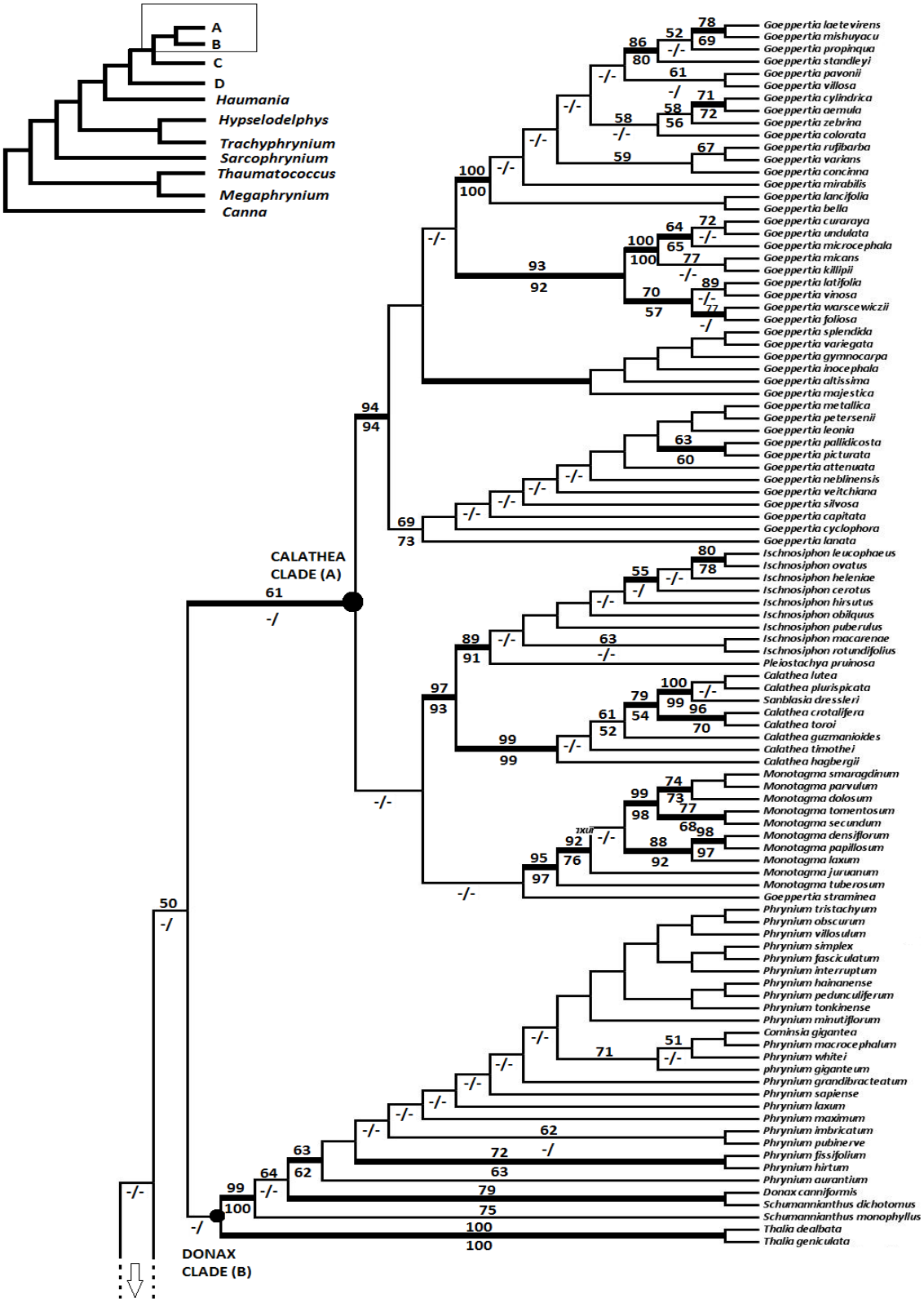
A
B
C
D
Haumania
Hypselodelphys
Trachyphrynium
Sarcophrynium
Thaumatococcus
Megaphrynium
Canna
CALATHEA CLADE (A)
DONAX CLADE (B)
Goeppertia laetevirens
Goeppertia mishuyacu
Goeppertia propinqua
Goeppertia standleyi
Goeppertia pavonii
Goeppertia villosa
Goeppertia cylindrica
Goeppertia aemula
Goeppertia zebrina
Goeppertia colorata
Goeppertia rufibarba
Goeppertia varians
Goeppertia concinna
Goeppertia mirabilis
Goeppertia lancifolia
Goeppertia bella
Goeppertia curaraya
Goeppertia undulata
Goeppertia microcephala
Goeppertia micans
Goeppertia killipii
Goeppertia latifolia
Goeppertia vinosa
Goeppertia warscewiczii
Goeppertia foliosa
Goeppertia splendida
Goeppertia variegata
Goeppertia gymnocarpa
Goeppertia inocephala
Goeppertia altissima
Goeppertia majestica
Goeppertia metallica
Goeppertia petersenii
Goeppertia leonia
Goeppertia pallidicosta
Goeppertia picturata
Goeppertia attenuata
Goeppertia neblinensis
Goeppertia weitchiana
Goeppertia silvosa
Goeppertia capitata
Goeppertia cyclophora
Goeppertia lanata
Ischnosiphon leucophaeus
Ischnosiphon ovatus
Ischnosiphon heleniae
Ischnosiphon cerotus
Ischnosiphon hirsutus
Ischnosiphon obilquus
Ischnosiphon puberulus
Ischnosiphon macarenae
Ischnosiphon rotundifolius
Pleiostachya pruinosa
Calathea lutea
Calathea plurispicata
Sanblasia dressleri
Calathea crotalifera
Calathea toroi
Calathea guzmanioides
Calathea timothei
Calathea hagbergii
Monotagma smaragdinum
Monotagma parvulum
Monotagma dolosum
Monotagma tomentosum
Monotagma secundum
Monotagma densiflorum
Monotagma papillosum
Monotagma laxum
Monotagma juruanum
Monotagma tuberosum
Goeppertia straminea
Phrynium tristachyum
Phrynium obscurum
Phrynium villosulum
Phrynium simplex
Phrynium fasciculatum
Phrynium interruptum
Phrynium hainanense
Phrynium pedunculiferum
Phrynium tonkinense
Phrynium minutiflorum
Cominsia gigantea
Phrynium macrocephalum
Phrynium whitei
phrynium giganteum
Phrynium grandibracteatum
Phrynium sapiense
Phrynium laxum
Phrynium maximum
Phrynium imbricatum
Phrynium pubinerve
Phrynium fissifolium
Phrynium hirtum
Phrynium aurantium
Donax canniformis
Schumannianthus dichotomus
Schumannianthus monophyllus
Thalia dealbata
Thalia geniculata

Figure 2a

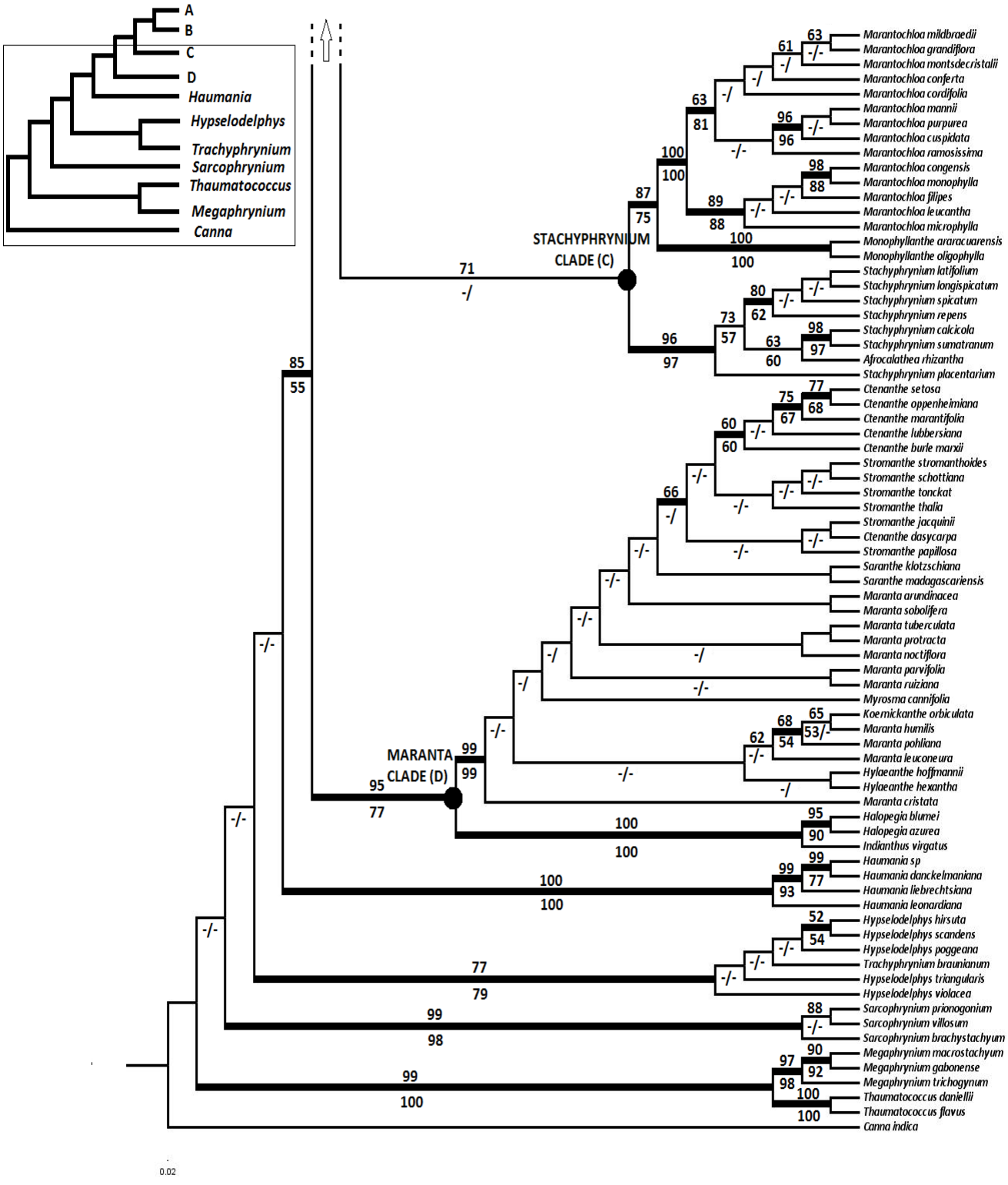
A
B
C
D
Haumania
Hypselodelphys
Trachyphrynium
Sarcophrynium
Thaumatococcus
Megaphrynium
Canna
STACHYPHRYNIUM CLADE (C)
MARANTA CLADE (D)
Marantochloa mildbraedii
Marantochloa grandiflora
Marantochloa montsdecristalii
Marantochloa conferta
Marantochloa cordifolia
Marantochloa mannii
Marantochloa purpurea
Marantochloa cuspidata
Marantochloa ramosissima
Marantochloa congensis
Marantochloa monophylla
Marantochloa filipes
Marantochloa leucantha
Marantochloa microphylla
Monophyllanthe araracuarensis
Monophyllanthe oligophylla
Stachyphrynium latifolium
Stachyphrynium longispicatum
Stachyphrynium spicatum
Stachyphrynium repens
Stachyphrynium calcicola
Stachyphrynium sumatranum
Afrocalathea rhizantha
Stachyphrynium placentarium
Ctenanthe setosa
Ctenanthe oppenheimiana
Ctenanthe marantifolia
Ctenanthe lubbersiana
Ctenanthe burle marxii
Stromanthe stromanthoides
Stromanthe schottiana
Stromanthe tonckat
Stromanthe thalia
Stromanthe jacquinii
Ctenanthe dasycarpa
Stromanthe papillosa
Saranthe klotzschiana
Saranthe madagascariensis
Maranta arundinacea
Maranta sobolifera
Maranta tuberculata
Maranta protracta
Maranta noctiflora
Maranta parvifolia
Maranta ruiziana
Myrosma cannifolia
Koernickanthe orbiculata
Maranta humilis
Maranta pohliana
Maranta leuconeura
Hylaeanthe hoffmannii
Hylaeanthe hexantha
Maranta cristata
Halopegia blumei
Halopegia azurea
Indianthus virgatus
Haumania sp
Haumania danckelmaniana
Haumania liebrechtsiana
Haumania leonardiana
Hypselodelphys hirsuta
Hypselodelphys scandens
Hypselodelphys poggeana
Trachyphrynium braunianum
Hypselodelphys triangularis
Hypselodelphys violacea
Sarcophrynium prionogonium
Sarcophrynium villosum
Sarcophrynium brachystachyum
Megaphrynium macrostachyum
Megaphrynium gabonense
Megaphrynium trichogynum
Thaumatococcus daniellii
Thaumatococcus flavus
Canna indica
0.02

Figure 2b

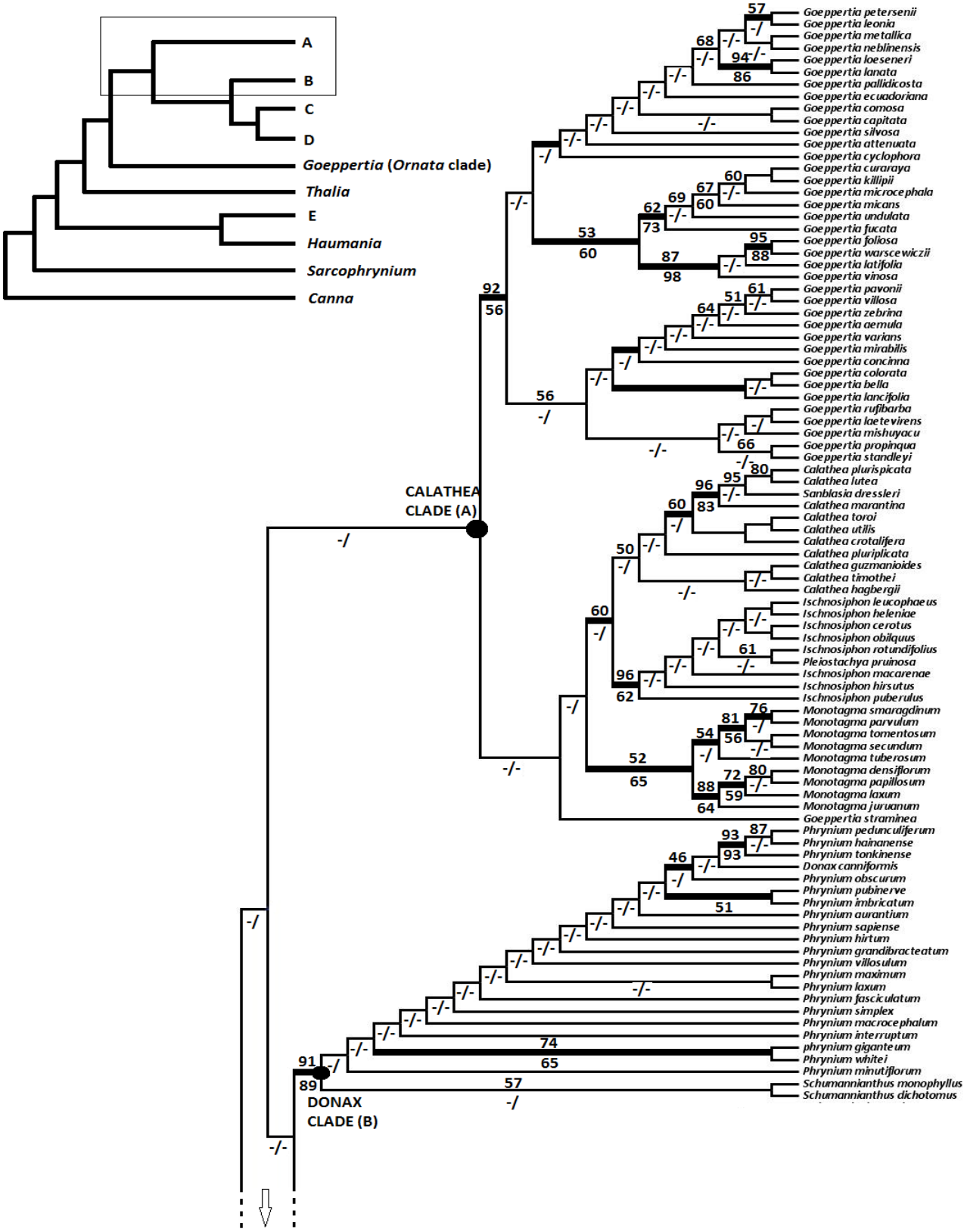
A
B
C
D
Goeppertia (Ornata clade)
Thalia
E
Haumania
Sarcophrynium
Canna
CALATHEA CLADE (A)
DONAX CLADE (B)
Goeppertia petersenii
Goeppertia leonia
Goeppertia metallica
Goeppertia neblinensis
Goeppertia loeseneri
Goeppertia lanata
Goeppertia pallidicosta
Goeppertia ecuadoriana
Goeppertia comosa
Goeppertia capitata
Goeppertia silvosa
Goeppertia attenuata
Goeppertia cyclophora
Goeppertia curaraya
Goeppertia killipii
Goeppertia microcephala
Goeppertia micans
Goeppertia undulata
Goeppertia fucata
Goeppertia foliosa
Goeppertia warscewiczii
Goeppertia latifolia
Goeppertia vinosa
Goeppertia pavonii
Goeppertia villosa
Goeppertia zebrina
Goeppertia aemula
Goeppertia varians
Goeppertia mirabilis
Goeppertia concinna
Goeppertia colorata
Goeppertia bella
Goeppertia lancifolia
Goeppertia rufibarba
Goeppertia laetevirens
Goeppertia mishuyacu
Goeppertia propinqua
Goeppertia standleyi
Calathea plurispicata
Calathea lutea
Sanblasia dressleri
Calathea marantina
Calathea toroi
Calathea utilis
Calathea crotalifera
Calathea pluriplicata
Calathea guzmanioides
Calathea timothei
Calathea hagbergii
Ischnosiphon leucophaeus
Ischnosiphon heleniae
Ischnosiphon cerotus
Ischnosiphon obilquus
Ischnosiphon rotundifolius
Pleiostachya pruinosa
Ischnosiphon macarenae
Ischnosiphon hirsutus
Ischnosiphon puberulus
Monotagma smaragdinum
Monotagma parvulum
Monotagma tomentosum
Monotagma secundum
Monotagma tuberosum
Monotagma densiflorum
Monotagma papillosum
Monotagma laxum
Monotagma juruanum
Goeppertia straminea
Phrynium pedunculiferum
Phrynium hainanense
Phrynium tonkinense
Donax canniformis
Phrynium obscurum
Phrynium pubinerve
Phrynium imbricatum
Phrynium aurantium
Phrynium sapiense
Phrynium hirtum
Phrynium grandibracteatum
Phrynium villosulum
Phrynium maximum
Phrynium laxum
Phrynium fasciculatum
Phrynium simplex
Phrynium macrocephalum
Phrynium interruptum
phrynium giganteum
Phrynium whitei
Phrynium minutiflorum
Schumannianthus monophyllus
Schumannianthus dichotomus

Figure 3a

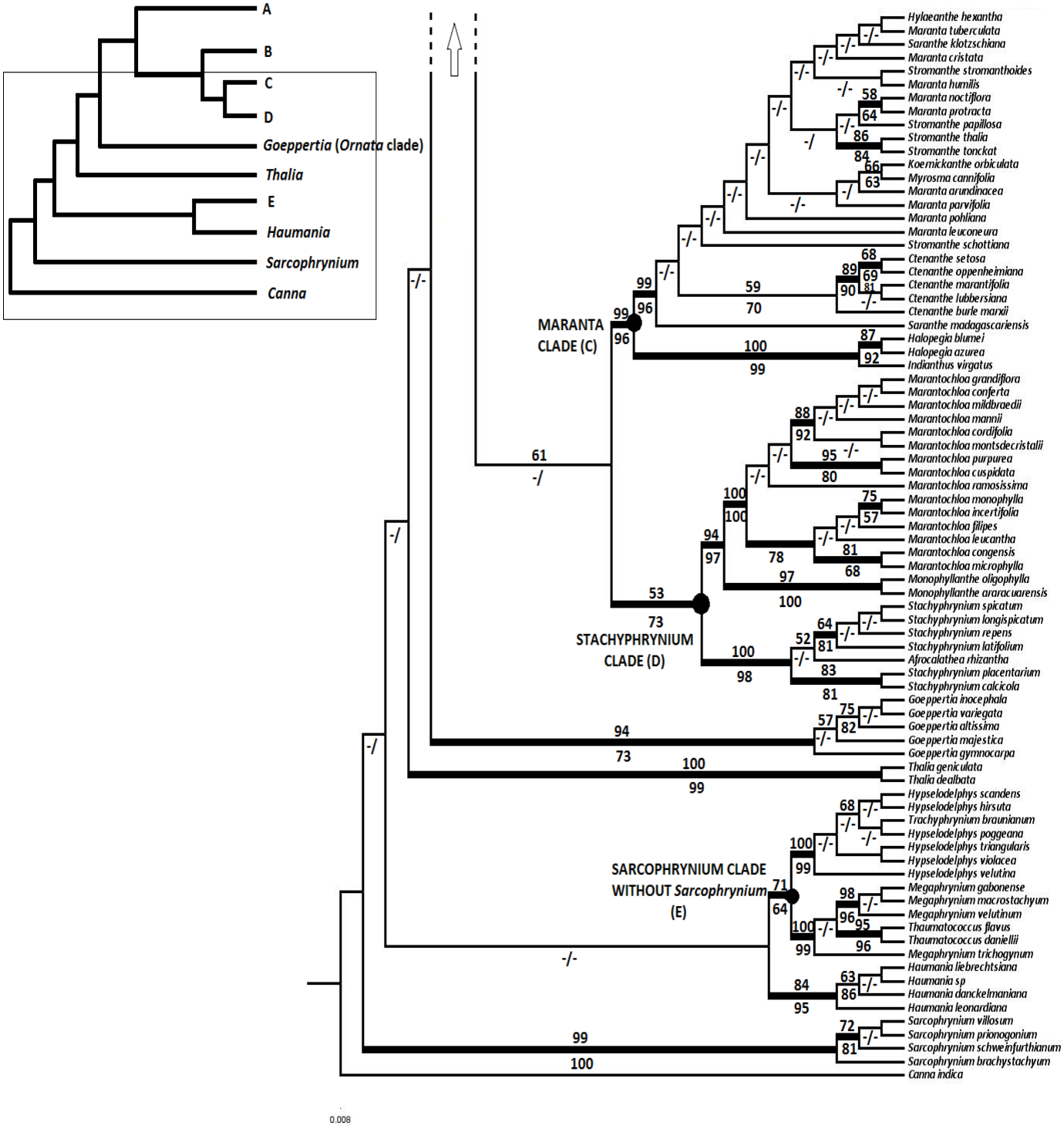
A
B
C
D
Goeppertia (Ornata clade)
Thalia
E
Haumania
Sarcophrynium
Canna
MARANTA CLADE (C)
STACHYPHRYNIUM CLADE (D)
SARCOPHRYNIUM CLADE WITHOUT Sarcophrynium (E)
Hylaeanthe hexantha
Maranta tuberculata
Saranthe klotzschiana
Maranta cristata
Stromanthe stromanthoides
Maranta humilis
Maranta noctiflora
Maranta protracta
Stromanthe papillosa
Stromanthe thalia
Stromanthe tonckat
Koernickanthe orbiculata
Myrosma cannifolia
Maranta arundinacea
Maranta parvifolia
Maranta pohliana
Maranta leuconeura
Stromanthe schottiana
Ctenanthe setosa
Ctenanthe oppenheimiana
Ctenanthe marantifolia
Ctenanthe lubbersiana
Ctenanthe burle marxii
Saranthe madagascariensis
Halopegia blumei
Halopegia azurea
Indianthus virgatus
Marantochloa grandiflora
Marantochloa conferta
Marantochloa mildbraedii
Marantochloa mannii
Marantochloa cordifolia
Marantochloa montsdecristalii
Marantochloa purpurea
Marantochloa cuspidata
Marantochloa ramosissima
Marantochloa monophylla
Marantochloa incertifolia
Marantochloa filipes
Marantochloa leucantha
Marantochloa congensis
Marantochloa microphylla
Monophyllanthe oligophylla
Monophyllanthe araracuarensis
Stachyphrynium spicatum
Stachyphrynium longispicatum
Stachyphrynium repens
Stachyphrynium latifolium
Afrocalathea rhizantha
Stachyphrynium placentarium
Stachyphrynium calcicola
Goeppertia inocephala
Goeppertia variegata
Goeppertia altissima
Goeppertia majestica
Goeppertia gymnocarpa
Thalia geniculata
Thalia dealbata
Hypselodelphys scandens
Hypselodelphys hirsuta
Trachyphrynium braunianum
Hypselodelphys poggeana
Hypselodelphys triangularis
Hypselodelphys violacea
Hypselodelphys velutina
Megaphrynium gabonense
Megaphrynium macrostachyum
Megaphrynium velutinum
Thaumatococcus flavus
Thaumatococcus daniellii
Megaphrynium trichogynum
Haumania liebrechtsiana
Haumania sp
Haumania danckelmaniana
Haumania leonardiana
Sarcophrynium villosum
Sarcophrynium prionogonium
Sarcophrynium schweinfurthianum
Sarcophrynium brachystachyum
Canna indica
0.008

Figure 3b


Goeppertia
Calathea
Sanblasia
Ischnosiphon
Pleiostachya
A
Monotagama
B
Monophyllanthe
Marantochloa
Haumania
Stachyphrynium
Afrocalathea
C
Canna
SARCOPHRYNIUM CLADE (A)
Goeppertia pavonii
Goeppertia villosa
Goeppertia zebrina
Goeppertia aemula
Goeppertia concinna
Goeppertia colorata
Goeppertia varians
Goeppertia rufibarba
Goeppertia laetevirens
Goeppertia mishuyacu
Goeppertia standleyi
Goeppertia propinqua
Goeppertia mirabilis
Goeppertia bella
Goeppertia warscewiczii
Goeppertia foliosa
Goeppertia latifolia
Goeppertia vinosa
Goeppertia lanata
Goeppertia neblinensis
Goeppertia loeseneri
Goeppertia veitchiana
Goeppertia metallica
Goeppertia leonia
Goeppertia petersenii
Goeppertia pallidicosta
Goeppertia ecuadoriana
Goeppertia silvosa
Goeppertia attenuata
Goeppertia capitata
Goeppertia comosa
Goeppertia cyclophora
Goeppertia micans
Goeppertia curaraya
Goeppertia fucata
Goeppertia microcephala
Goeppertia undulata
Goeppertia killipii
Goeppertia altissima
Goeppertia variegata
Goeppertia inocephala
Goeppertia majestica
Goeppertia gymnocarpa
Goeppertia straminea
Calathea plurispicata
Calathea marantina
Calathea lutea
Sanblasia dressleri
Calathea crotalifera
Calathea toroi
Calathea utilis
Calathea hagbergii
Calathea guzmanioides
Calathea pluriplicata
Calathea timothei
Ischnosiphon obilquus
Ischnosiphon hirsutus
Ischnosiphon rotundifolius
Ischnosiphon puberulus
Ischnosiphon macarenae
Ischnosiphon cerotus
Ischnosiphon leucophaeus
Pleiostachya pruinosa
Ischnosiphon heleniae
Megaphrynium trichogynum
Megaphrynium gabonense
Megaphrynium macrostachyum
Thaumatococcus daniellii
Sarcophrynium prionogonium
Sarcophrynium schweinfurthianum
Sarcophrynium brachystachyum
Hypselodelphys violacea
Hypselodelphys scandens
Hypselodelphys triangularis
Hypselodelphys velutina
Hypselodelphys hirsuta
Hypselodelphys poggeana
Trachyphrynium braunianum
Monotagma secundum
Monotagma tomentosum
Monotagma parvulum
Monotagma smaragdinum
Monotagma papillosum
Monotagma laxum
Monotagma juruanum
Monotagma tuberosum

Figure 4a

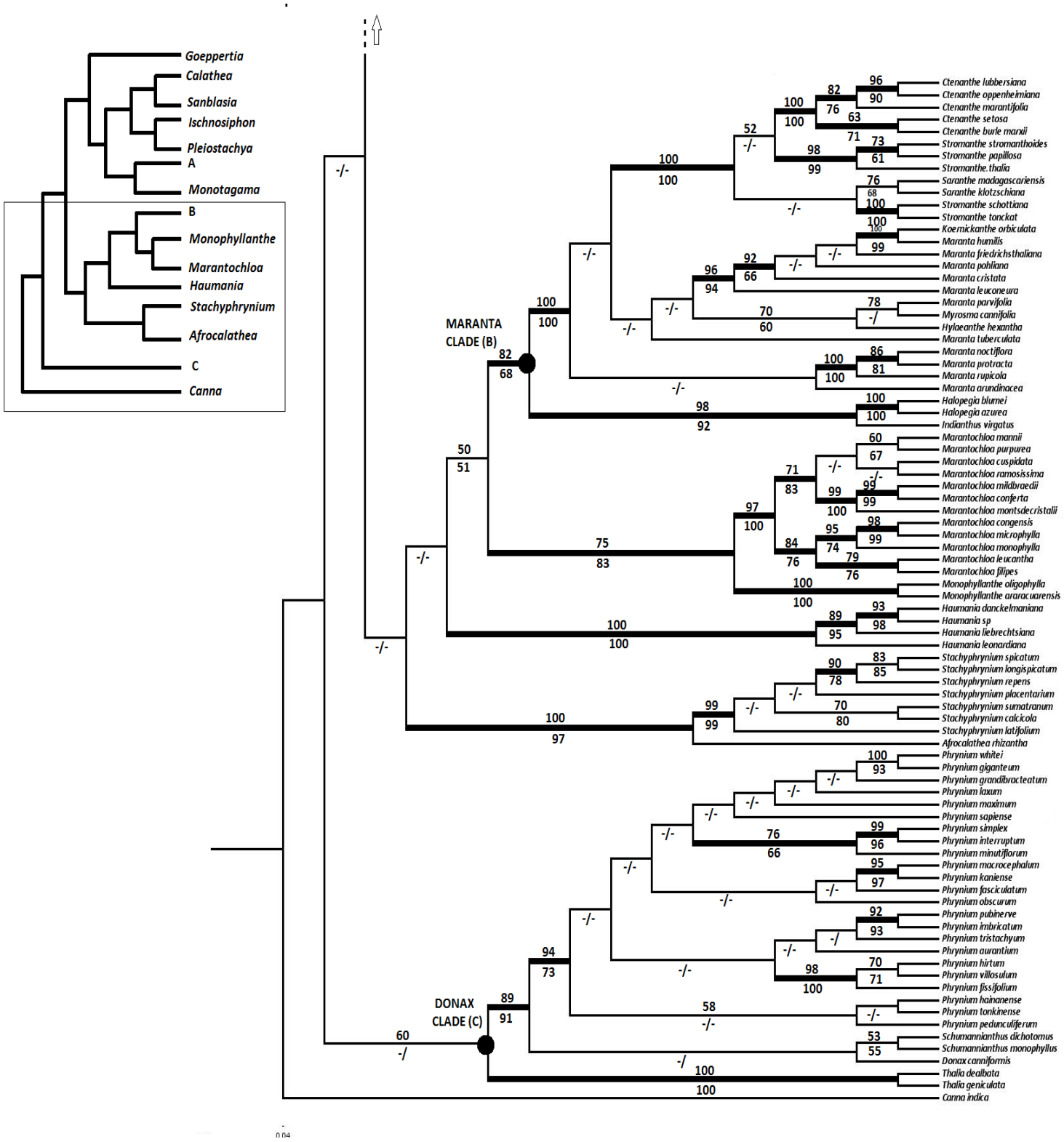
Goeppertia
Calathea
Sanblasia
Ischnosiphon
Pleiostachya
A
Monotagama
B
Monophyllanthe
Marantochloa
Haumania
Stachyphrynium
Afrocalathea
C
Canna
MARANTA CLADE (B)
DONAX CLADE (C)
Ctenanthe lubbersiana
Ctenanthe oppenheimiana
Ctenanthe marantifolia
Ctenanthe setosa
Ctenanthe burle marxii
Stromanthe stromanthoides
Stromanthe papillosa
Stromanthe thalia
Saranthe madagascariensis
Saranthe klotzschiana
Stromanthe schottiana
Stromanthe tonckat
Koernickanthe orbiculata
Maranta humilis
Maranta friedrichsthaliana
Maranta pohliana
Maranta cristata
Maranta leuconeura
Maranta parvifolia
Myrosma cannifolia
Hylaeanthe hexantha
Maranta tuberculata
Maranta noctiflora
Maranta protracta
Maranta rupicola
Maranta arundinacea
Halopegia blumei
Halopegia azurea
Indianthus virgatus
Marantochloa mannii
Marantochloa purpurea
Marantochloa cuspidata
Marantochloa ramosissima
Marantochloa mildbraedii
Marantochloa conferta
Marantochloa montsdecristalii
Marantochloa congensis
Marantochloa microphylla
Marantochloa monophylla
Marantochloa leucantha
Marantochloa filipes
Monophyllanthe oligophylla
Monophyllanthe araracuarensis
Haumania danckelmaniana
Haumania sp
Haumania liebrechtsiana
Haumania leonardiana
Stachyphrynium spicatum
Stachyphrynium longispicatum
Stachyphrynium repens
Stachyphrynium placentarium
Stachyphrynium sumatranum
Stachyphrynium calcicola
Stachyphrynium latifolium
Afrocalathea rhizantha
Phrynium whitei
Phrynium giganteum
Phrynium grandibracteatum
Phrynium laxum
Phrynium maximum
Phrynium sapiense
Phrynium simplex
Phrynium interruptum
Phrynium minutiflorum
Phrynium macrocephalum
Phrynium kaniense
Phrynium fasciculatum
Phrynium obscurum
Phrynium pubinerve
Phrynium imbricatum
Phrynium tristachyum
Phrynium aurantium
Phrynium hirtum
Phrynium villosulum
Phrynium fissifolium
Phrynium hainanense
Phrynium tonkinense
Phrynium pedunculiferum
Schumanniathus dichotomus
Schumanniathus monophyllus
Donax cannifformis
Thalia dealbata
Thalia geniculata
Canna indica

Figure 4b

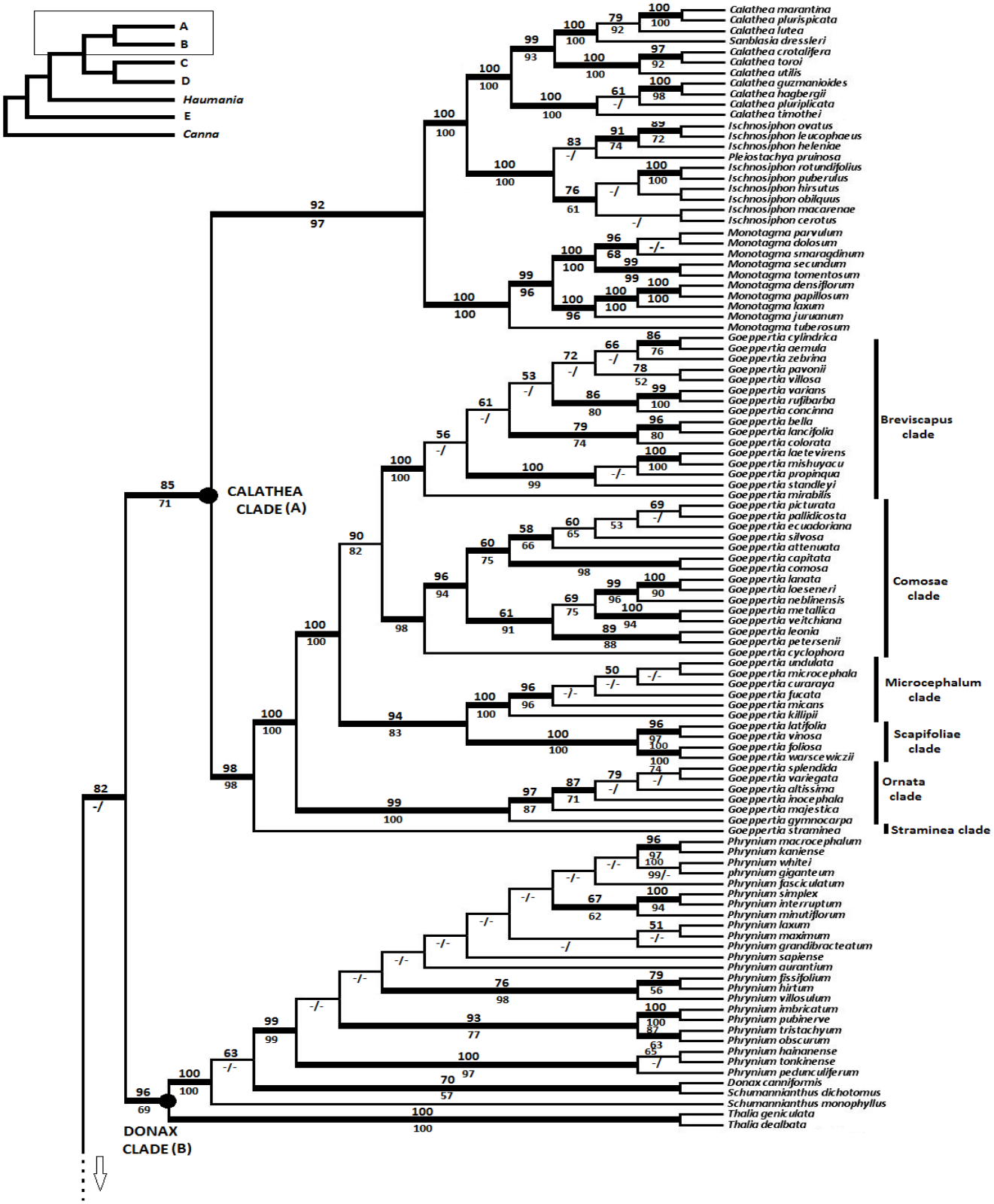
A
B
C
D
Haumania
E
Canna
CALATHEA CLADE (A)
DONAX CLADE (B)
Calathea marantina
Calathea plurispicata
Calathea lutea
Sanblasia dressleri
Calathea crotalifera
Calathea toroi
Calathea utilis
Calathea guzmanioides
Calathea hagbergii
Calathea pluriplicata
Calathea timothei
Ischnosiphon ovatus
Ischnosiphon leucophaeus
Ischnosiphon heleniae
Pleiostachya pruinosa
Ischnosiphon rotundifolius
Ischnosiphon puberulus
Ischnosiphon hirsutus
Ischnosiphon obilquus
Ischnosiphon macarenae
Ischnosiphon cerotus
Monotagma parvulum
Monotagma dolosum
Monotagma smaragdinum
Monotagma secundum
Monotagma tomentosum
Monotagma densiflorum
Monotagma papillosum
Monotagma laxum
Monotagma juruanum
Monotagma tuberosum
Goeppertia cylindrica
Goeppertia aemula
Goeppertia zebrina
Goeppertia pavonii
Goeppertia villosa
Goeppertia varians
Goeppertia rufibarba
Goeppertia concinna
Goeppertia bella
Goeppertia lancifolia
Goeppertia colorata
Goeppertia laetevirens
Goeppertia mishuyacu
Goeppertia propinqua
Goeppertia standleyi
Goeppertia mirabilis
Goeppertia picturata
Goeppertia pallidicosta
Goeppertia ecuadoriana
Goeppertia silvosa
Goeppertia attenuata
Goeppertia capitata
Goeppertia comosa
Goeppertia lanata
Goeppertia loeseneri
Goeppertia neblinensis
Goeppertia metallica
Goeppertia veitchiana
Goeppertia leonia
Goeppertia petersenii
Goeppertia cyclophora
Goeppertia undulata
Goeppertia microcephala
Goeppertia curaraya
Goeppertia fucata
Goeppertia micans
Goeppertia killipii
Goeppertia latifolia
Goeppertia vinosa
Goeppertia foliosa
Goeppertia warscewiczii
Goeppertia splendida
Goeppertia variegata
Goeppertia altissima
Goeppertia inocephala
Goeppertia majestica
Goeppertia gymnocarpa
Goeppertia straminea
Phrynium macrocephalum
Phrynium kaniense
Phrynium whitei
phrynium giganteum
Phrynium fasciculatum
Phrynium simplex
Phrynium interruptum
Phrynium minutiflorum
Phrynium laxum
Phrynium maximum
Phrynium grandibracteatum
Phrynium sapiense
Phrynium aurantium
Phrynium fissifolium
Phrynium hirtum
Phrynium villosulum
Phrynium imbricatum
Phrynium pubinerve
Phrynium tristachyum
Phrynium obscurum
Phrynium hainanense
Phrynium tonkinense
Phrynium pedunculiferum
Donax canniformis
Schumannianthus dichotomus
Schumannianthus monophyllus
Thalia geniculata
Thalia dealbata
Breviscapus clade
Comosae clade
Microcephalum clade
Scapifoliae clade
Ornata clade
Straminea clade

Figure 5a

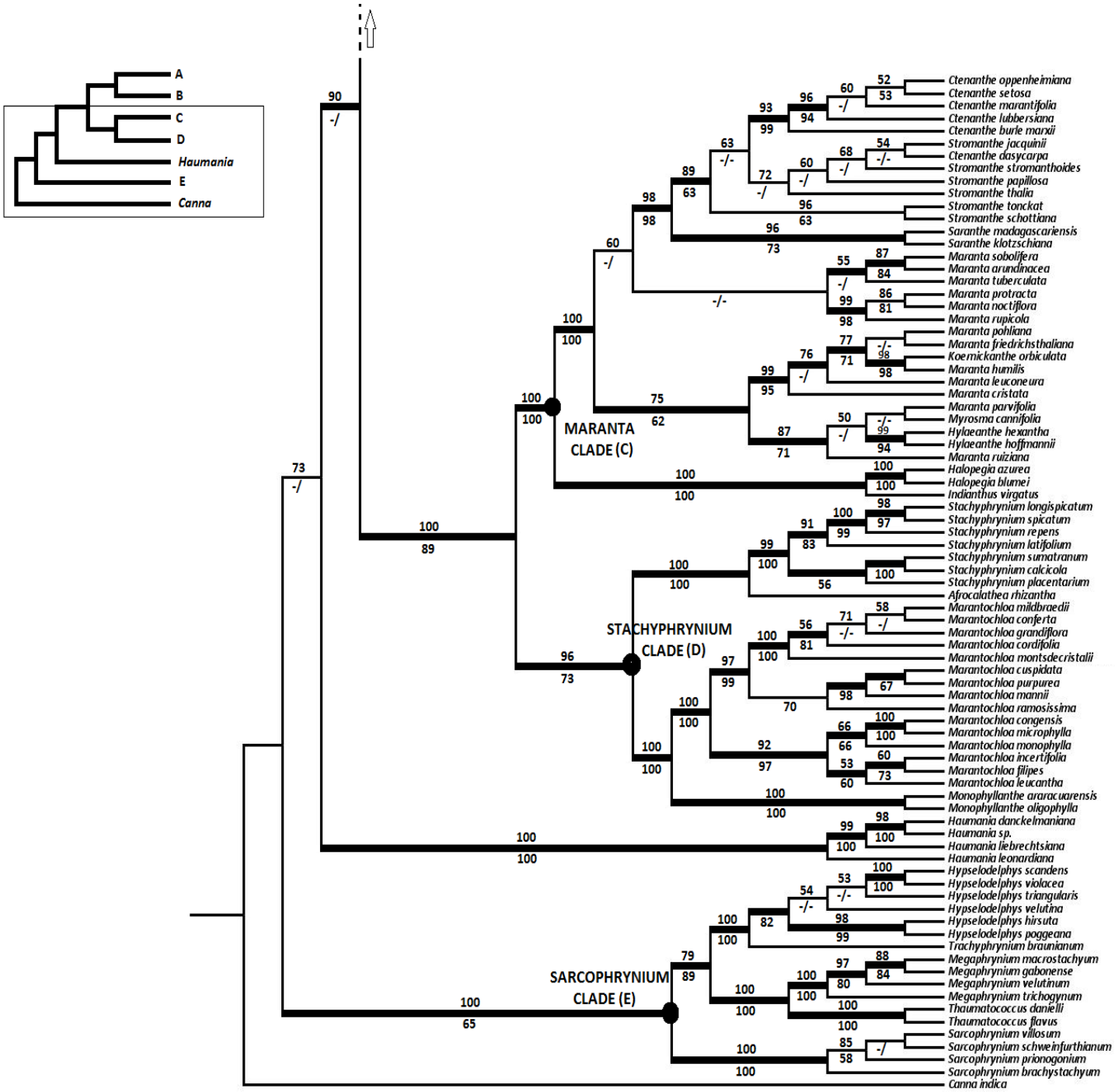
A
B
C
D
Haumania
E
Canna
MARANTA CLADE (C)
STACHYPHRYNIUM CLADE (D)
SARCOPHRYNIUM CLADE (E)
Ctenanthe oppenheimiana
Ctenanthe setosa
Ctenanthe marantifolia
Ctenanthe lubbersiana
Ctenanthe burle marxii
Stromanthe jacquinii
Ctenanthe dasycarpa
Stromanthe stromanthoides
Stromanthe papillosa
Stromanthe thalia
Stromanthe tonckat
Stromanthe schottiana
Saranthe madagascariensis
Saranthe klotzschiana
Maranta sobolifera
Maranta arundinacea
Maranta tuberculata
Maranta protracta
Maranta noctiflora
Maranta rupicola
Maranta pohliana
Maranta friedrichsthaliana
Koernickanthe orbiculata
Maranta humilis
Maranta leuconeura
Maranta cristata
Maranta parvifolia
Myrosma cannifolia
Hylaeanthe hexantha
Hylaeanthe hoffmannii
Maranta ruiziana
Halopegia azurea
Halopegia blumei
Indianthus virgatus
Stachyphrynium longispicatum
Stachyphrynium spicatum
Stachyphrynium repens
Stachyphrynium latifolium
Stachyphrynium sumatranum
Stachyphrynium calcicola
Stachyphrynium placentarium
Afrocalathea rhizantha
Marantochloa mildbraedii
Marantochloa conferta
Marantochloa grandiflora
Marantochloa cordifolia
Marantochloa montsdecristalii
Marantochloa cuspidata
Marantochloa purpurea
Marantochloa mannii
Marantochloa ramosissima
Marantochloa congensis
Marantochloa microphylla
Marantochloa monophylla
Marantochloa incertifolia
Marantochloa filipes
Marantochloa leucantha
Monophyllanthe araracuarensis
Monophyllanthe oligophylla
Haumania danckelmaniana
Haumania sp.
Haumania liebrechtsiana
Haumania leonardiana
Hypselodelphys scandens
Hypselodelphys violacea
Hypselodelphys triangularis
Hypselodelphys velutina
Hypselodelphys hirsuta
Hypselodelphys poggeana
Trachyphrynium braunianum
Megaphrynium macrostachyum
Megaphrynium gabonense
Megaphrynium velutinum
Megaphrynium trichogynum
Thaumatococcus daniellii
Thaumatococcus flavus
Sarcophrynium villosum
Sarcophrynium schweinfurthianum
Sarcophrynium prionogonium
Sarcophrynium brachystachyum
Canna indica

Figure 5b

CHAPTER 5

Synthesis

GENERAL DISCUSSION

PART I

The investigation of germination behavior and genetic diversity in this dissertation (Part I) provided valuable insight into the role of environmental variables in inducing local adaptation as well as isolating plant populations and shaping their genetic structure. Spatial environmental variation is ubiquitous, and populations of a wide range of species are adapted to local abiotic or biotic conditions (Savolainen *et al*. 2013). Phenotypic and genetic differentiation along environmental gradients, or across contrasting habitat types, can also be indicative of local adaptation and environmental isolation (Conover *et al*. 2009, Wang & Bradburd 2014). The study species, *Salvia spinosa* and *Salvia syriaca*, are among the Jordanian plant species which face environmental heterogeneity over short geographical distances due to the existence of different phytogeographical regions (Al-Eissawi 1996). Identifying the consequences of variation in environmental conditions in terms of local adaptation and genetic structure is an increasingly important goal in conservation biology (Harrisson *et al*. 2014). Chapter 2 provided information regarding additive and interactive effects of temperature, salinity and seed origin on germination behavior, which is important for understanding the ecology of species in terms of their establishment, tolerance of abiotic factors, and range dynamics across environmental gradients. Furthermore, evidence of local adaptation of the study species to salinity and temperature regimes in the respective maternal environments has been revealed particularly in the Irano-Turanian and Saharo-Arabian regions. A similar result was also found in Jordanian *Stipa* species (Hamasha & Hensen 2009), where populations from arid and Saharan regions have higher seed germination than populations from Mediterranean region in response to environmental factors (high temperature and low rainfall) experienced by mother plants in arid and Saharan regions. The ability of *Salvia spinosa* seeds from arid and semi-arid populations to germinate faster and higher at cooler temperatures than Mediterranean populations suggests that the former populations are better adapted to germinating under cold conditions. Such adaptation was interpreted as the ability of seeds of populations in arid and semi-arid areas (Irano-Turanian and Saharo-Arabian regions) to respond to moisture availability in late autumn

and early winter to enable germination when temperatures are relatively low, making them less likely to suffer from desiccation. Germination at cooler temperature to escape later desiccation was also suggested as an adaptive mechanism in seed germination of *Malcolmia littorea* (De Vitis *et al*. 2014). In addition, my results suggest that the mucilage layer produced by the seeds contributes to this adaptation. However, mucilage has always been considered ecologically beneficial for plants' adaptation to diverse environments by enhancing seed dispersal, germination and establishment (Yang *et al*. 2012). Although being able to produce mucilage is an advantage for these plants, mucilage might turn into a disadvantage under the pressure of climate change. My field observations indicate the potential of negative interactions between mucilage and late rainfall with respect to seed viability and dispersal, which may open the door for novel investigations on the impact of further climate changes on the persistence of species with mucilaginous seed.

The results of chapter 3 also detected the signature of environmental variation on genetic structure. Genetic diversity was found to increase significantly with increasing temperature and drought, whereby genetic diversity was higher in dry habitats (Saharo-Arabian and Irano-Turanian), suggesting that environmental stress might select for high levels of genetic diversity. Indeed, our results are in line with several studies found that higher genetic diversity resided in populations from habitats with stressful (i.e. dry and hot) environmental conditions (Nevo *et al*. 1998; Turpeinen *et al*. 2001; Sharma *et al*. 2004, Hamasha *et al*. 2013). Moreover, the variation in the environmental conditions alters the flowering phenology among populations, interrupts their connectivity and isolates them environmentally. Such isolation has lead to a clear genetic split between populations belonging to more moist environments (Mediterranean) and those of arid environments (Saharo-Arabian and Irano-Turanian). The interactions between organisms and their environments can shape spatial distributions of genetic variation, resulting in patterns of isolation by environment in which genetic and environmental distances are positively correlated, independent of geographic distance (Wang & Bradburd 2014). Therefore, our results contribute significantly to understanding the role of ecological processes in genetic divergence both within and between species. Indeed, previous studies revealed and describe how ecological processes that can generate patterns of isolation

by environment can answer pressing questions about the ecological basis of genetic diversity (Balkenhol *et al*. 2009; Storfer *et al*. 2010). In the light of current global climate change, it is necessary to study the effects of climatic variation on intraspecific genetic diversity, since this variation provides the basis for any evolutionary adaptation. It is thus the most fundamental level of biodiversity (May & Goodfrey 1994). In summary, I conclude that the results of chapter 3 play an important role in advancing our understanding of how environmental conditions shape the evolution of biological diversity and inducing local adaptation, which has major implications for a wide variety of disciplines, particularly for understanding how plant species will respond to rapid ecological change and how we can conserve them.

PART II

The rise of phylogenetic biology has revolutionized the study of molecular and developmental evolution. As phylogeny has an important implication for gauging the evolutionary potential of species (Vézquez & Gittleman 1998), therefore, it can be a tool to estimate biogeographic ancestral ranges and assess shifts in species diversification rates once a well-resolved phylogenetic tree is achieved (Berger *et al*. 2015). Results of chapter 4 (Part II) provide an improved resolution in the Phylogeny of Marantaceae family backbone. Such results can provide more robust phylogenetic frameworks for further hypothesis testing of the geographic history of Marantaceae since early efforts were hampered by poor resolution due to low taxon and characters sampling. However, the resulting tree topology focusing on the resolution of major clades was mostly congruent among applied methods and with preexisting family phylogenies. Moreover, the addition of more molecular data and taxa have strengthened the hypothesized topology and suggested generic limits of the relationships within the family Marantaceae (Prince & Kress 2006a; Suksathan *et al.* 2009; Ley & Claßen-Bockhoff 2011; Borchsenius *et al.* 2012). The highly supported infrasectional topologies of major clades and many subclades that were not achieved in any of the previous studies indicate that extensive sampling in both dimensions of taxa and characters (genes) is necessary. In conclusion, our current results which are based on more molecular data and taxa compared to all previous studies (Andersson & Chase 2001; Prince & Kress 2006a; Prince & Kress 2006b; Suksathan *et al*. 2009; Ley & Claßen-Bockhoff 2011; Borchsenius *et al*. 2012) can be used in the future to

investigate the biogeographical pattern and the timing of divergence in the family Marantaceae.

IMPLICATION FOR BIODIVERSITY CONSERVATION

Results of both parts (Part I and II) of this dissertation provide valuable information for plant conservation and confirm that genetic diversity is an essential pillar in conservation genetics due to the basis of the evolutionary potential of species to respond to environmental changes (Toro & Caballero 2005). Also, the detailed information on germination behavior in response to the environmental variables helps to understand the species in terms of their establishment, tolerance of abiotic factors, and dynamics across environmental gradients (Al-Gharaibeh *et al*. 2016). Results of part I, therefore, provided the data required for developing and implementing effective strategies and protocols for the *ex situ* and *in situ* conservation. One of the major issues for in situ conservation and restoration is the selection of specific source populations and how geographically close to the in situ site should the source population be, where differences among species as well as among seed provenances should be taken into account. Also, these differences should be considered in collections for the seed bank. Despite its importance for the long-term viability of populations and functioning of ecosystems, the genetic diversity of populations is seldom given explicit consideration in conservation prioritization (Kahilainen *et al*. 2014). The measures of genetic diversity have been largely absent from species conservation assessments (Mace & Purvis, 2008, Taberlet *et al*. 2012; Hoban *et al*. 2013) and are not explicitly considered in the IUCN Red List of Threatened Species. Since the ultimate goal of conservation actions is to ensure the long-term persistence of species, it is therefore advisable to give the conservation of interpopulation genetic diversity a high priority. However, part (I) also indicates a potential conservation implication related to changes in climate, where shifts in rainfall may adversely affect populations connectivity and recruitment via disturbing their flowering phenology and seedlings survival. Therefore, it is necessary to conserve biodiversity not only by maximizing the number of taxa that are saved today, but also to guarantee the maintenance of high levels of biological diversity in the future. To achieve this, Vézquez & Gittleman (1998) recommended that the consideration of phylogeny is essential. Consequently, the results of part (II) have important implications for conservation since the detailed

phylogenies can provide a window into speciation and extinction rates, the ecological and biogeographical causes of speciation and extinction, and the timing of these events. For instance, branch lengths in a phylogenetic tree scaled to the observed genetic divergence between species would provide a quantitative measure of diversity within a clade. Old, monotypic taxa with few or no sister taxa often make relatively large contributions to diversity, and thus should be accorded high priorities in conservation decisions. In addition, the evolutionary distinctiveness provided by phylogeny would be a useful metric for helping rank species for conservation. For example, **Tuatara** package (http://mesquiteproject.org/packages/tuatara/) can be used to analyze conservation priorities in a phylogenetic context.

OUTLOOK

PART-I

In semiarid and arid regions of the world, the low and irregular distribution of rainfall strongly affects the survival of plants. Their effective recruitment and establishment crucially depend on whether their seeds germinate in the right place and at the right time (Gutterman 1994). Moreover, disturbance in rainfall pattern can also interrupt flowering synchrony among populations of these plants and limit their pollen connectivity (Domínguez & Dirzo 1995). *Salvia* is one of the plant genera producing mucilaginous seeds. The finding of several authors suggested that mucilage layer plays an ecologically important role in successful seed dispersal, germination and later seedling establishment (e.g. Garwood 1985; Yang *et al*. 2012; Gorai *et al*. 2014). In addition, mucilage facilitates adaptation of plants to diverse environments, especially extreme desert conditions (Yang *et al*. 2012). Despite our results suggested that mucilage in *Salvia* seeds can mitigate the stress of drought and salinity conditions on germination by absorbing and filtering more water, it may adversely affect their seed dispersal, limit population's connectivity and enhance their isolation under further climate change. In the field, I observed that late spring rainfall events after seed maturation release mucilage and glue the seeds together as well as to the calyx wall. However, in part (I), the field observations and results raise a series of questions regarding the ecological role of mucilage in response to

climatic changes, which I plan to address in future experiments. Among these questions are: to which degree do changes in climate, particularly shifts in rainfall time, hamper the dispersal of mucilaginous seeds? Do rain events after maturation of mucilaginous seeds (late spring-early summer) trigger germination in relation to the duration and intensity of rainfall? According to previous research, mucilage can initiate or enhance seed germination by retaining moisture needed for imbibition (Garwood 1985; Hedge 1970; Swarbrick 1971). However, I conducted a small-scale preliminary experiment at spring temperatures (20/10°C), which showed that rainfall events after seed maturation in both *Salvia* species can initiate germination due to the water uptake from mucilage. The experiment also showed that seeds germinated (seed coat ruptured and radicle emerged) in first trial lose their viability after five days desiccation and were not able to germinate in the second trial. According to these initial results, the very interesting question arises, what the expected future effects of such rainfall events on the depletion of soil seed banks and recruitment on species with mucilaginous seed will be. Therefore, such investigations to determine the threshold of rainfall events in term of intensity and time that can trigger germination of mucilaginous seed will be necessary. Such studies enable a deeper understanding and tracing of the expected effects of climate change on vegetation from another perspective in the context of conservation.

PART-II

The geographic history of Marantaceae is complex and uncertain. Early efforts using molecular phylogenies were hampered by poor resolution along the backbone of the family tree (Prince & Kress 2006). The lack of a well-resolved phylogenetic framework makes the estimation of the biogeographic ancestral ranges and the shifts in species diversification rates more difficult (Berger *et al*. 2016). However, increased amounts of phylogenetic data and improved analytical tools to evaluate these data are providing more robust phylogenetic frameworks for hypothesis testing (Soltis *et al*. 2011; Davis *et al*. 2014; Ruhfel *et al*. 2014; Zeng *et al*. 2014; Magallón *et al*. 2015). As our current data provides improved resolution and better estimates based on more molecular data and complete generic sampling, therefore, we plan in the future to investigate the biogeographical pattern and the timing of divergence in the family Marantaceae. The complete generic sampling with large number of taxa will facilitate the identification center of

diversity and origin for Marantaceae, as well as, the role of dispersal or vicariance events on shaping their current pantropical distribution. Moreover, a detailed molecular dating will be conducted as we found a new record of a fossil from Asia, which will allow us to calibrate divergence time and contrast that with previous studies.

REFERENCES

REFERENCES

Ababsa M. (2014) *Atlas of Jordan : History, Territories and Society*. Presses de l'Ifpo, Beyrouth.

Afzal-rafii Z. (1980) In Chromosome number reports LXVII. *Taxon* **29**: 365–366.

Ahmad P., Prasad M. (2012) *Abiotic stress responses in plants: metabolism, productivity and sustainability*. Springer Science & Business Media.

Ahuja M.R., Jain S.M. (2015) *Genetic Diversity and Erosion in Plants: Indicators and Prevention*. Springer.

Akaike H. (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**: 716–723.

Al Qudah B. (2001) Soils of Jordan. In: Zdruli P, Steduto P, Lacirignola C, Montanarella L (eds) Soil resources of Southern and Eastern Mediterranean countries. Options Méditerranéennes, Bari : CIHEAM, pp 127–141.

Al-Eisawi D. (1996) *Vegetation of Jordan*. UNESCO-Regional Office for Science and Technology for the Arab States, Cairo.

Alfaro M.E., Zoller S., Lutzoni F. (2003) Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molecular Biology and Evolution* **20**: 255–266.

Al-Gharaibeh M.M., Hamasha H.R., Lachmuth S., Hensen I. (2016) Local adaptation to different phytogeographic regions: habitat-related variations in seed germination in response to temperature and salinity for two medicinal *Salvia* species from Jordan. *Plant Species Biology*. doi:10.1111/1442-1984.12123

Alhamad M. (2006) Ecological and species diversity of arid Mediterranean grazing land vegetation. *Journal of Arid Environments* **66**: 698–715.

Al-Khateeb S.A. (2006) Effect of salinity and temperature on germination, growth and ion relations of *Panicum turgidum* Forssk. *Bioresource Technology* **97**: 292–298.

Al-Quran S. (2011) Conservation of medicinal plants in Ajlun woodland / Jordan. *Journal of Medicinal Plants Research* **5**: 5857–5862.

Al-Quran S. (2012) Species level conservation of four rare endangered medicinal plants in Wadi Rum, southern Jordan. *Acta Botanica Hungarica* **54**: 17–26.

Al-Turki T.A., Filfilan S.A., Mehmood S.F. (2000) A cytological study of flowering plants from Saudi Arabia. *Willdenowia* **30**: 339–358.

Andersson L. (1981) The neotropical genera of Marantaceae. Circumscription and relationships. *Nordic Journal of Botany* **1**: 218–245.

Andersson L. (1998) *Marantaceae*. In: Kubitzki K (ed) Families and Genera of Vascular Plants: 4. Flowering Plants: Monocotyledons Alismatanae and Commelinanae (Except Gramineae). Springer-Verlag, Berlin, pp 278–293.

Andersson L., Chase M. (2001) Phylogeny and classification of Marantaceae. *Botanical Journal of the Linnean Society* **135**: 275–287.

Andreou M., Delipetrou P., Kadis C., Tsiamis G., Bourtzis K., Georghiou K. (2011) An integrated approach for the conservation of threatened plants: The case of *Arabis kennedyae* (Brassicaceae). *Acta Oecologica* **37**: 239–248.

Astegiano J., Funes G., Galetto L. (2013) Plant recruitment and range size: Transition probabilities from ovule to adult in two *Ipomoea*. *Acta Oecologica* **48**: 76–82.

Bacles C.F.E., Ennos R.A. (2008) Paternity analysis of pollen-mediated gene flow for *Fraxinus excelsior* L. in a chronically fragmented landscape. *Heredity* **101**: 368–380.

Badri M., Zitoun A., Ilahi H., Huguet T., Aouani M.E. (2008) Morphological and microsatellite diversity associated with ecological factors in natural populations of *Medicago laciniata* Mill. (Fabaceae). *Journal of Genetics* **87**: 241–255.

Balkenhol N., Gugerli F., Cushman S.A., Waits L.P., Coulon A., Arntzen J.W., Holderegger R., Wagner H.H. (2009) Identifying future research needs in landscape genetics: where to from here? *Landscape Ecology* **24**: 455.

Banta J.A., Ehrenreich I.M., Gerard S., Chou L., Wilczek A., Schmitt J., Kover P.X., Purugganan M.D. (2012) Climate envelope modelling reveals intraspecific relationships among flowering phenology, niche breadth and potential range size in *Arabidopsis thaliana*. *Ecology Letters* **15**: 769–777.

Barrett C.F., Specht C.D., Leebens-Mack J., Stevenson D.W., Zomlefer W.B., Davis J.I. (2014) Resolving ancient radiations: can complete plastid gene sets elucidate deep relationships among the tropical gingers (Zingiberales)? *Annals of Botany* **113**: 119–133.

Baskin C.C., Baskin J.M. (1998) *Seeds: Ecology, Biogeography, and, Evolution of Dormancy and Germination*. Academic Press, San Diego.

Batanouny K.H. (2013) *Plants in the Deserts of the Middle East*. Springer Science & Business Media.

Bates D., Maechler M., Bolker B., Walker S. (2013) lme4: Linear mixed-effects models using Eigen and S4. R Package Version

Bauert M.R., Kälin M., Baltisberger M., Edwards P.J. (1998) No genetic variation detected within isolated relict populations of *Saxifraga cernua* in the Alps using RAPD markers. *Molecular Ecology* **7**: 1519–1527.

Becerra J.X. (2005) Timing the origin and expansion of the Mexican tropical dry forest. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 10919–10923.

Berger B.A., Kriebel R., Spalink D., Sytsma K.J. (2016) Divergence times, historical biogeography, and shifts in speciation rates of Myrtales. *Molecular Phylogenetics and Evolution* **95**: 116–136.

Bischoff A., Vonlanthen B., Steinger T., Müller-Schärer H. (2006) Seed provenance matters — Effects on germination of four plant species used for ecological restoration. *Basic and Applied Ecology* **7**: 347–359.

Bonin A., Ehrich D., Manel S. (2007) Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology* **16**: 3737–3758.

Borchsenius F. (2009) FastGap 1.2. Department of Biosciences, Aarhus University, Denemark.

Borchsenius F., Suárez L.S.S., Prince L.M. (2012) Molecular Phylogeny and Redefined Generic Limits of *Calathea* (Marantaceae). *Systematic Botany* **37**: 620–635.

Braglia L., Casabianca V., Benedetti L.D., Pecchioni N., Mercuri A., Cervelli C., Ruffoni B. (2011) Amplified fragment length polymorphism markers for DNA fingerprinting in the genus *Salvia*. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology* **145**: 274–277.

Brooks T.M., Cuttelod A., Faith D.P., Garcia-Moreno J., Langhammer P., Pérez-Espona S. (2015) Why and how might genetic and phylogenetic diversity be reflected in the identification of key biodiversity areas? Philosophical Transactions of the Royal Society B **370**: 20140019.

Brousseau L., Bonal D., Cigna J., Scotti I. (2013) Highly local environmental variability promotes intrapopulation divergence of quantitative traits: an example from tropical rain forest trees. *Annals of Botany* **112**: 1169–1179.

Brown T.A. (2002) *Molecular Phylogenetics*. Wiley-Liss.

Bui E.N. (2013) Soil salinity: A neglected factor in plant ecology and biogeography. *Journal of Arid Environments* **92**: 14–25.

Burban C., Petit R.J. (2003) Phylogeography of maritime pine inferred with organelle markers having contrasted inheritance. *Molecular Ecology* **12**: 1487–1495.

Cendán C., Sampedro L., Zas R. (2013) The maternal environment determines the timing of germination in *Pinus pinaster*. *Environmental and Experimental Botany* **94**: 66–72.

Chang J.T. (1996) Inconsistency of evolutionary tree topology reconstruction methods when substitution rates vary across characters. *Mathematical Biosciences* **134**:189–215.

Chase M.W., Soltis D.E., Olmstead R.G., Morgan D., Les D.H., Mishler B.D., Duvall M.R., Price R.A., Hills H.G., Qiu Y.L., Kron K.A., Rettig J.H., Conti E., Palmer J.D., Manhart J.R., Sytsma K.J., Michaels H.J., Kress W.J., Karol K.G., Clark W.D., Hedren M., Gaut B.S., Jansen R.K., Kim K.J., Wimpee C.F., Smith J.F., Furnier G.R., Strauss S.H., Xiang Q.Y., Plunkett G.M., Soltis P.S., Swensen S.M., Williams S.E., Gadek P.A., Quinn C.J., Eguiarte L.E., Golenberg E., Learn G.H., Graham S.W., Barrett S.C.H., Dayanandan S., Albert V.A. (1993) Phylogenetics of Seed Plants: An Analysis of Nucleotide Sequences from the Plastid Gene rbcL. *Annals of the Missouri Botanical Garden* **80**: 528–580.

Chauhan B.S., Gill G., Preston C. (2006) Influence of Environmental Factors on Seed Germination and Seedling Emergence of Oriental Mustard (*Sisymbrium orientale*). *Weed Science* **54**: 1025–1031.

Claßen-Bockhoff R. (1991) Investigations on the Construction of the Pollination Apparatus of *Thalia geniculata* (Marantaceae). *Botanica Acta* **104**: 183–193.

Claßen-Bockhoff R., Speck T., Tweraser E., Wester P., Thimm S., Reith M. (2004) The staminal lever mechanism in *Salvia* L. (Lamiaceae): a key innovation for adaptive radiation? *Organisms Diversity & Evolution* **4**: 189–205.

Cochrane A., Yates C.J., Hoyle G.L., Nicotra A.B. (2015) Will among-population variation in seed traits improve the chance of species persistence under climate change? *Global Ecology and Biogeography* **24**: 12–24.

Côme D. (1993) Identification, Preservation, Adaptation, and Cultivation of Selected Aromatic and Medicinal Plants Suitable for Marginal Lands of the Mediterranean Region. Mediterranean Agronomic Institute of Chania.

Conord C., Gurevitch J., Fady B. (2012) Large-scale longitudinal gradients of genetic diversity: a meta-analysis across six phyla in the Mediterranean basin. *Ecology and Evolution* **2**: 2600–2614.

Conover D.O., Duffy T.A., Hice L.A. (2009) The covariance between genetic and environmental influences across ecological gradients: reassessing the evolutionary significance of countergradient and cogradient variation. *Annals of the New York Academy of Sciences* **1168**: 100–129.

Cortés A.J., Monserrate F.A., Ramírez-Villegas J., Madriñán S., Blair M.W. (2013) Drought Tolerance in Wild Plant Populations: The Case of Common Beans (*Phaseolus vulgaris* L.). *PLOS ONE* **8**: e62898.

Crawley M. (2013) *The R Book*. John Wiley & Sons, Ltd, Chichester, United Kingdom.

Crisp M.D., Arroyo M.T.K., Cook L.G., Gandolfo M.A., Jordan G.J., McGlone M.S., Weston P.H., Westoby M., Wilf P., Linder H.P. (2009) Phylogenetic biome conservatism on a global scale. *Nature* **458**: 754–756.

Cruse-Sanders J.M., Hamrick J.L., Ahumada J.A. (2005) Consequences of harvesting for genetic diversity in American ginseng (*Panax quinquefolius* L.): a simulation study. *Biodiversity & Conservation* **14**: 493–504.

Cutter A.D. (2013) Integrating phylogenetics, phylogeography and population genetics through genomes and evolutionary theory. *Molecular Phylogenetics and Evolution* **69**: 1172–1185.

Danin A. (1992) Flora and vegetation of Israel and adjacent areas. *Bocconea* **3**: 18–42.

Danin A. (1999a) Desert Rocks as Plant Refugia in the near East. *Botanical Review* **65**: 93–170.

Danin A. (1999b) Sandstone Outcrops—a Major Refugium of Mediterranean Flora in the Xeric Part of Jordan. *Israel Journal of Plant Sciences* **47**: 179–187.

Danin A. (2008) Desert Rocks – A Habitat Which Supports Many Species That Were New to Science in the Last 40 Years. *Turkish Journal of Botany* **32**: 459–464.

Darriba D., Taboada G.L., Doallo R., Posada D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.

Davis C.C., Xi Z., Mathews S. (2014) Plastid phylogenomics and green plant phylogeny: almost full circle but not quite there. *BMC Biology* **12**: 11.

De Vitis M., Seal C.E., Ulian T., Pritchard H.W., Magrini S., Fabrini G., Mattana E. (2014) Rapid adaptation of seed germination requirements of the threatened Mediterranean species *Malcolmia littorea* (Brassicaceae) and implications for its reintroduction. *South African Journal of Botany* **94**: 46–50.

Khan M.A., Weber D.J. (2008) *Ecophysiology of High Salinity Tolerant Plants*. Springer Netherlands, pp 255–278.

Dereeper A., Guignon V., Blanc G., Audic S., Buffet S., Chevenet F., Dufayard J.F., Guindon S., Lefort V., Lescot M., Claverie J.M., Gascuel O. (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* **36**: W465–W469.

Dhetchuvi J. (1996) *Taxonomie et phytogéographie des Marantaceae et des Zingiberaceae de l'Afrique Centrale (Gabon, Congo, Zaire, Rwanda et Brundi)*. Ph. D. dissertation, Bruxelles, Belgium

Dick C.W., Kress W.J. (2009) Dissecting Tropical Plant Diversity with Forest Plots and a Molecular Toolkit. *BioScience* **59**: 745–755.

Djamali M., Brewer S., Breckle S.W., Jackson S.T. (2012) Climatic determinism in phytogeographic regionalization: A test from the Irano-Turanian region, SW and Central Asia. *Flora - Morphology, Distribution, Functional Ecology of Plants* **207**: 237–249.

Domínguez C.A., Dirzo, R. (1995) Rainfall and flowering synchrony in a tropical shrub: Variable selection on the flowering time of *Erythroxylum havanense*. *Evolutionary Ecology* **9**: 204–216.

Donoghue M.J. (2008) A phylogenetic perspective on the distribution of plant diversity. *Proceedings of the National Academy of Sciences* **105**: 11549–11555.

Donohue K., Schmitt J. (1998) Maternal environmental effects in plants: adaptive plasticity? In: Mousseau TA, Fox CW (eds) Maternal Effects as Adaptations. Oxford University Press, New York, NY, pp 137–158.

Douady C.J., Delsuc F., Boucher Y., Doolittle W.F., Douzery E.J.P. (2003) Comparison of Bayesian and Maximum Likelihood Bootstrap Measures of Phylogenetic Reliability. *Molecular Biology and Evolution* **20**: 248–254.

Duarte J.F., Carvalho D. de, Vieira F. de A. (2015) Genetic conservation of *Ficus bonijesulapensis* R.M. Castro in a dry forest on limestone outcrops. *Biochemical Systematics and Ecology* **59** :54–62.

Eckert C.G., Samis K.E., Lougheed S.C. (2008) Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology* **17**: 1170–1188.

Edgar R.C. (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC bioinformatics* **5**: 113.

Efron B., Halloran E., Holmes S. (1996) Bootstrap confidence levels for phylogenetic trees. *Proceedings of the National Academy of Sciences* **93**: 13429–13429.

El-Keblawy A., Al-Rawai A. (2005) Effects of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw.) D.C. *Journal of Arid Environments* **61**: 555–565.

Escaravage N., Wagner J. (2004) Pollination effectiveness and pollen dispersal in a *Rhododendron ferrugineum* (Ericaceae) population. *Plant Biology* **6**: 606–615.

Evanno G., Regnaut S., Goudet J. (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology* **14**: 2611–2620.

Excoffier L., Lischer H.E.L. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**: 564–567.

Excoffier L., Smouse P.E., Quattro J.M. (1992) Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data. *Genetics* **131**: 479–491.

Farris J.S., Albert V.A., Källersjö M., Lipscomb D., Kluge A.G. (1996) Parsimony Jackknifing Outperforms Neighbor-Joining. *Cladistics* **12**: 99–124.

Felsenstein J. (1985) Confidence Limits on Phylogenies: An Approach Using the Bootstrap. *Evolution* **39**: 783–791.

Fenner M., Thompson K. (2005) *The Ecology of Seeds*. Cambridge University Press, Cambridge.

Fitzgerald T.L., Shapter F.M., McDonald S., Waters D.L.E., Chivers I.H., Drenth A., Nevo E., Henry R.J. (2011) Genome diversity in wild grasses under environmental stress. *Proceedings of the National Academy of Sciences* **108**: 21140–21145.

Flowers T.J., Galal H.K., Bromham L. (2010) Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology* **37**: 604–612.

Forest F., Crandall K.A., Chase M.W., Faith D.P. (2015) Phylogeny, extinction and conservation: embracing uncertainties in a time of urgency. *Philosophical Transactions of the Royal Society B: Biological Sciences* **370**: 20140002.

Fox G.A. (2003) Assortative mating and plant phenology: evolutionary and practical consequences. *Evolutionary Ecology Research* **5**: 1–18.

Francisco-Ortega J., Ellis R.H., González-Feria E., Santos-Guerra A. (1994) Overcoming seed dormancy in ex situ plant germplasm conservation programmes; an example in the endemic *Argyranthemum* (Asteraceae: Anthemideae) species from the Canary Islands. *Biodiversity & Conservation* **3**: 341–353.

Frankham R., Ballou J., Briscoe D. (2010) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, U.K.

Franks S.J., Sim S., Weis A.E. (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences* **104**: 1278–1282.

Franks S.J., Weis A.E. (2009) Climate change alters reproductive isolation and potential gene flow in an annual plant. *Evolutionary Applications* **2**: 481–488.

Friis I., Balslev H. (2005) *Plant Diversity and Complexity Patterns: Local, Regional and Global Dimensions :* Proceedings of an International Symposium Held at the Royal Danish Academy of Sciences and Letters in Copenhagen, Denmark, 25-28 May, 2003. Kgl. Danske Videnskabernes Selskab.

Garwood N.C. (1985) The Role of Mucilage in the Germination of Cuipo, *Cavanillesia platanifolia* (H. & B.) H. B. K. (Bombacaceae), a Tropical Tree. *American Journal of Botany* **72**: 1095–1105.

GCEP (General Corporation for Environmental Protection) (2000) Jordan country study on biological Diversity, Plant Biodiversity and Taxonomy. With technical support from UNEP and funding from GEF, Amman, Jordan.

Gilpin M., Soulé M. (1986) Minimum viable populations, processes of species extinction. In: Soulé M. (ed) Conservation Biology: the Science of Scarcity and Diversity. Soulé Sinauer Associates, Sunderland, Massachusetts, pp 19–34.

Gomaa N.H., Montesinos-Navarro A., Alonso-Blanco C., Picó F.X. (2011) Temporal variation in genetic diversity and effective population size of Mediterranean and subalpine *Arabidopsis thaliana* populations. *Molecular Ecology* **20**: 3540–3554.

Gorai M., Aloui W.E., Yang X., Neffati M. (2013) Toward understanding the ecological role of mucilage in seed germination of a desert shrub *Henophyton deserti*: interactive effects of temperature, salinity and osmotic stress. *Plant and Soil* **374**: 727–738.

Gorai M., Gasmi H., Neffati M. (2011) Factors influencing seed germination of medicinal plant *Salvia aegyptiaca* L. (Lamiaceae). *Saudi Journal of Biological Sciences* **18**: 255–260.

Govaerts R., Kennedy H. (2016) World Checklist of Marantaceae. Facilitated by the Royal Botanic Gardens, Kew. URL: Published on the Internet; http://apps.kew.org/wcsp/ Retrieved 2016.01.23.

Govindaraj M., Vetriventhan M., Srinivasan M. (2015) Importance of Genetic Diversity Assessment in Crop Plants and Its Recent Advances: An Overview of Its Analytical Perspectives. *Genetics Research International* **2015**: e431487.

Gram W.K., Sork V.L. (2001) Association between Environmental and Genetic Heterogeneity in Forest Tree Populations. *Ecology* **82**: 2012–2021.

Grant T., Kluge A.G. (2008) Clade support measures and their adequacy. *Cladistics* **24**: 1051–1064.

Graybeal A. (1998) Is it better to add taxa or characters to a difficult phylogenetic problem? *Systematic Biology* **47**: 9–17.

Grechka D.A., Berezin S.B., Emmott S., Lyutsarev V., Smith M.J., Purves D.W. (2016) Universal, easy access to geotemporal information: FetchClimate. *Ecography*. doi:10.1111/ecog.02321

Guan B., Zhou D., Zhang H., Tian Y., Japhet W., Wang P. (2009) Germination responses of *Medicago ruthenica* seeds to salinity, alkalinity, and temperature. *Journal of Arid Environments* **73**: 135–138.

Guindon S., Gascuel O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**: 696–704.

Gutterman Y. (1994) *Seed Germination in Desert Plants*. Springer Berlin Heidelberg, Berlin, Heidelberg.

Gutterman Y. (2000) Maternal effects on seeds during development. In: Fenner M (ed) Seeds: the ecology of regeneration in plant communities, 2nd edn. CABI, Wallingford, pp 59–84.

Hamasha H.R., Hensen I. (2009) Seed germination of four Jordanian *Stipa spp*: differences in temperature regimes and seed provenances. *Plant Species Biology* **24**: 127–132.

Hamasha H.R., Schmidt-Lebuhn A.N., Durka W., Schleuning M., Hensen I. (2013) Bioclimatic regions influence genetic structure of four Jordanian *Stipa* species. *Plant Biology* **15**: 882–891.

Hamby R.K., Zimmer E.A. (1992) Ribosomal RNA as a Phylogenetic Tool in Plant Systematics. In: Soltis PS, Soltis DE, Doyle JJ (eds) Molecular Systematics of Plants. Springer US, pp 50–91.

Hammel B.E. (1986) The vascular flora of La Selva biological station, Costa Rica, MARANTACEAE. *Selbyana* **9**: 234–242.

Hardy O.J., González-Martínez S.C., Fréville H., Boquien G., Mignot A., Colas B., Olivieri I. (2004) Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae) I. Pattern of pollen dispersal. *Journal of Evolutionary Biology* **17**: 795–806.

Hardy O.J., Vekemans X. (2002) SPAGeDi: a versatile computer program to analyze spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**: 618–620.

Harrison J., Langdale J.A. (2006) A step by step guide to phylogeny reconstruction. *The Plant Journal* **45**: 561–572.

Harrisson K.A., Pavlova A., Telonis-Scott M., Sunnucks P. (2014) Using genomics to characterize evolutionary potential for conservation of wild populations. *Evolutionary Applications* **7**: 1008–1025.

Heath T.A., Hedtke S.M., Hillis D.M. (2008) Taxon sampling and the accuracy of phylogenetic analyses. *Journal of Systematics and Evolution* **46**: 239–257.

Hedge I. (1970) Observation on the mucilage of *Salvia* fruits. Notes from the Royal Botanic Garden, Edinburgh **30**: 79–95.

Hedtke S.M., Townsend T.M., Hillis D.M. (2006) Resolution of Phylogenetic Conflict in Large Data Sets by Increased Taxon Sampling. *Systematic Biology* **55**: 522–529.

Hensen I., Cierjacks A., Hirsch H., Kessler M., Romoleroux K., Renison D., Wesche K. (2012) Historic and recent fragmentation coupled with altitude affect the genetic population structure of one of the world's highest tropical tree line species. *Global Ecology and Biogeography* **21**: 455–464.

Hensen I., Oberprieler C. Effects of population size on genetic diversity and seed production in the rare *Dictamnus albus* (Rutaceae) in central Germany. *Conservation Genetics* **6**: 63–73.

Herrmann D., Poncet B.N., Manel S., Rioux D., Gielly L., Taberlet P., Gugerli F. (2010) Selection criteria for scoring amplified fragment length polymorphisms (AFLPs) positively affect the reliability of population genetic parameter estimates. *Genome* **53**: 302–310.

Hillis D.M. (1998) Taxonomic Sampling, Phylogenetic Accuracy, and Investigator Bias. *Systematic Biology* **47**: 3–8.

Hillis D.M., Bull J.J. (1993) An Empirical Test of Bootstrapping as a Method for Assessing Confidence in Phylogenetic Analysis. *Systematic Biology* **42**: 182–192.

Hillis D.M., Huelsenbeck J.P., Cunningham C.W. (1994) Application and accuracy of molecular phylogenies. *Science* **264**: 671–677.

Hoban S.M., Hauffe H.C., Pérez-Espona S., Arntzen J.W., Bertorelle G., Bryja J., Frith K., Gaggiotti O.E., Galbusera P., Godoy J.A., Hoelzel A.R., Nichols R.A., Primmer C.R., Russo I.R., Segelbacher G., Siegismund H.R., Sihvonen M., Vernesi C., Vilà C., Bruford M.W. (2013) Bringing genetic diversity to the forefront of conservation policy and management. *Conservation Genetics Resources* **5**: 593–598.

Holder M., Lewis P.O. (2003) Phylogeny estimation: traditional and Bayesian approaches. *Nature Reviews Genetics* **4**: 275–284.

Holman J.E., Hughes J.M., Fensham R.J. (2003) A morphological cline in *Eucalyptus*: a genetic perspective. *Molecular Ecology* **12**: 3013–3025.

Huang Z., Gutterman Y. (1999) Water Absorption by Mucilaginous Achenes of *Artemisia monosperma*: Floating and Germination as Affected by Salt Concentrations. *Israel Journal of Plant Sciences* **47**: 27–34.

Huang Z., Zhang X., Zheng G., Gutterman Y. (2003) Influence of light, temperature, salinity and storage on seed germination of *Haloxylon ammodendron*. *Journal of Arid Environments* **55**: 453–464.

Hudaib M., Mohammad M., Bustanji Y., Tayyem R., Yousef M., Abuirjeie M., Aburjai T. (2008) Ethnopharmacological survey of medicinal plants in Jordan, Mujib Nature Reserve and surrounding area. *Journal of Ethnopharmacology* **120**: 63–71.

Huelsenbeck J.P. (1998) Systematic bias in phylogenetic analysis: is the Strepsiptera problem solved? *Systematic Biology* **47**: 519–537.

Huelsenbeck J.P., Rannala B., Masly J.P. (2000) Accommodating Phylogenetic Uncertainty in Evolutionary Studies. *Science* **288**: 2349–2350.

Hug L.A., Roger A.J. (2007) The impact of fossils and taxon sampling on ancient molecular dating analyses. *Molecular Biology and Evolution* **24**: 1889–1897.

Izawa T., Kawahara T., Takahashi H. (2007) Genetic diversity of an endangered plant, *Cypripedium macranthos* var. *rebunense* (Orchidaceae): background genetic research for future conservation. *Conservation Genetics* **8**: 1369–1376.

Jennersten O. (1988) Pollination in *Dianthus deltoides* (Caryophyllaceae): Effects of Habitat Fragmentation on Visitation and Seed Set. *Conservation Biology* **2**: 359–366.

Jerrold D. (2014) Plant phylogeny. Access Science McGraw-Hill Education

Jordan G.L., Haferkamp M.R. (1989) Temperature Responses and Calculated Heat Units for Germination of Several Range Grasses and Shrubs. *Journal of Range Management* **42**: 41–45.

Jugran A.K., Bhatt I.D., Rawal R.S., Nandi S.K., Pande V. (2013) Patterns of morphological and genetic diversity of *Valeriana jatamansi* Jones in different habitats and altitudinal range of West Himalaya, India. *Flora - Morphology, Distribution, Functional Ecology of Plants* **208**: 13–21.

Kahilainen A., Puurtinen M., Kotiaho J.S. (2014) Conservation implications of species–genetic diversity correlations. *Global Ecology and Conservation* **2**: 315–323.

Kårehed J., Groeninckx I., Dessein S., Motley T.J., Bremer B. (2008) The phylogenetic utility of chloroplast and nuclear DNA markers and the phylogeny of the Rubiaceae tribe Spermacoceae. *Molecular Phylogenetics and Evolution* **49**: 843–866.

Kasapligil B. (1956) An ecological survey of the vegetation in relation to forestry and grazing. FAO Report to the Government of the Hashemite Kingdome of Jordan.

Keller M., Kollmann J. (1999) Effects of seed provenance on germination of herbs for agricultural compensation sites. *Agriculture, Ecosystems & Environment* **72**: 87–99.

Kennedy H. (1978) *Systematics and Pollination of the "closed-flowered" Species of Calathea (Marantaceae)*. University of California Press, California.

Kennedy H. (1999) *Calathea singularis* and *Stromanthe palustris*, Two New Species of Neotropical Marantaceae. *Novon* **9**: 61–65.

Kennedy H. (2000) Diversification in pollination mechanisms in the Marantaceae. In: Wilson KL, Morrison DA (eds) Monocots: systematics and evolution. Csiro Publishing, Melbourne, pp 335 – 343.

Kennedy H., Andersson L., Hagberg M. (1988) Marantaceae. In: Harling G, Andersson L (eds) Flora of Ecuador. pp 14–191.

Khan M.K., Pandey A., Thomas G., Akkaya M.S., Kayis S.A., Ozsensoy Y., Hamurcu M., Gezgin S., Topal A., Hakki E.E. (2015) Genetic diversity and population structure of wheat in India and Turkey. *AoB Plants*: plv083.

Khan M.A., Ungar I.A. (1984) Seed Polymorphism and Germination Responses to Salinity Stress in *Atriplex triangularis* Willd. *Botanical Gazette* **145**: 487–494.

Khan M.A., Weber D.J. (2008) *Ecophysiology of High Salinity Tolerant Plants (Tasks for Vegetation Science)*, 1st edn. Springer, Amsterdam, Netherland.

Kim J. (1996) General Inconsistency Conditions for Maximum Parsimony: Effects of Branch Lengths and Increasing Numbers of Taxa. *Systematic Biology* **45**: 363–374.

Kim J. (1998) Large-Scale Phylogenies and Measuring the Performance of Phylogenetic Estimators. Systematic Biology **47**:43–60.

Kim K., Lee S.C., Lee J., Yu Y., Yang K., Choi B.S., Koh H.J., Waminal N.E., Choi H.I., Kim N.H., Jang W., Park H.S., Lee J., Lee H.O., Joh H.J., Lee H.J., Park J.Y., Perumal S., Jayakodi M., Lee Y.S., Kim B., Copetti D., Kim S., Kim S., Lim K.B., Kim Y.D., Lee J., Cho K.S., Park B.S., Wing R.A., Yang T.J. (2015) Complete chloroplast and ribosomal sequences for 30 accessions elucidate evolution of *Oryza* AA genome species. *Scientific Reports* **5**: 15655.

Kirchoff B.K. (1983) Floral Organogenesis in Five Genera of the Marantaceae and in *Canna* (Cannaceae). *American Journal of Botany* **70**: 508–523.

Kirkpatrick M., Slatkin M. (1993) Searching for Evolutionary Patterns in the Shape of a Phylogenetic Tree. *Evolution* **47**: 1171–1181.

Kolaczkowski B., Thornton J.W. (2004) Performance of maximum parsimony and likelihood phylogenetics when evolution is heterogeneous. *Nature* **431**: 980–984.

Kress W.J. (1990) The Phylogeny and Classification of the Zingiberales. *Annals of the Missouri Botanical Garden* **77**: 698–721.

Kress W.J., Prince L.M., Hahn W.J., Zimmer E.A. (2001) Unraveling the Evolutionary Radiation of the Families of the Zingiberales Using Morphological and Molecular Evidence. *Systematic Biology* **50**: 926–944.

Kress W.J., Specht C.D. (2006) The evolutionary and biogeographic origin and diversification of the tropical monocot order Zingiberales. *Aliso* **22**: 619–630.

Kuhner M.K., Felsenstein J. (1994) A simulation comparison of phylogeny algorithms under equal and unequal evolutionary rates. *Molecular Biology and Evolution* **11**: 459–468.

Kutiel P., Lavee H. (1999) Effect of Slope Aspect on Soil and Vegetation Properties Along an Aridity Transect. *Israel Journal of Plant Sciences* **47**: 169–178.

Lacey E.P., Herr D. (2000) Parental Effects in *Plantago Lanceolata* L. Iii. Measuring Parental Temperature Effects in the Field. *Evolution* **54**: 1207–1217.

Leaché A.D., Reeder T.W. (2002) Molecular systematics of the Eastern Fence Lizard (*Sceloporus undulatus*): a comparison of Parsimony, Likelihood, and Bayesian approaches. *Systematic Biology* **51**: 44–68.

Leimu R., Mutikainen P., Koricheva J., Fischer M. (2006) How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* **94**: 942–952.

Ley A.C., Claßen-Bockhoff R. (2011) Evolution in African Marantaceae - Evidence from Phylogenetic, Ecological and Morphological Studies. *Systematic Botany* **36**: 277–290.

Ley A.C., Hardy O.J. (2013) Improving AFLP analysis of large-scale patterns of genetic variation – a case study with the Central African lianas *Haumania spp* (Marantaceae) showing interspecific gene flow. *Molecular Ecology* **22**: 1984–1997.

Liber Z., Židovec V., Bogdanović S., Radosavljević I., Pruša M., Filipović M., Han Dovedan I., Jug-Dujaković M., Šatović Z. (2014) Genetic Diversity of Dalmatian Sage (*Salvia officinalis* L.) as Assessed by RAPD Markers. *Agriculturae Conspectus Scientificus* (ACS) **79**: 77–84.

Linhart Y.B., Grant M.C. (1996) Evolutionary Significance of Local Genetic Differentiation in Plants. *Annual Review of Ecology and Systematics* **27**: 237–277.

Little C.J., Wheeler J.A., Sedlacek J., Cortés A.J., Rixen C. (2015) Small-scale drivers: the importance of nutrient availability and snowmelt timing on performance of the alpine shrub *Salix herbacea*. *Oecologia* **180**: 1015–1024.

Loesener T. (1930) Marantaceae. In: Engler A, Parntl K (eds) Die natürlichen Pflanzenfamilien, 2nd edn. Engelmann, Leipzig, pp 654–693.

Long G. (1957) *The bioclimatology and vegetation of east Jordan*. UNESCO/FAO, Rome.

Löwenstern A., Al-Eisawi D., Garbari F. (2000) Studies on the Flora of Jordan 14. The species of the Hisma Basin (Wadi Rum desert). *Webbia* **55**: 195–277.

Lu J., Tan D., Baskin J.M., Baskin C.C. (2010) Fruit and seed heteromorphism in the cold desert annual ephemeral *Diptychocarpus strictus* (Brassicaceae) and possible adaptive significance. *Annals of Botany* **105**: 999–1014.

Luan Z., Xiao M., Zhou D., Zhang H., Tian Y., Wu Y., Guan B., Song Y., Luan Z., Xiao M., Zhou D., Zhang H., Tian Y., Wu Y., Guan B., Song Y. (2014) Effects of Salinity, Temperature, and Polyethylene Glycol on the Seed Germination of Sunflower (*Helianthus annuus* L.). *The Scientific World Journal* **Volume 2014**, Article ID 170418, 9 pages

Lutzoni F., Pagel M., Reeb V. (2001) Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* **411**: 937–940.

Luzuriaga A.L., Escudero A., Pérez-García F. (2006) Environmental maternal effects on seed morphology and germination in *Sinapis arvensis* (Cruciferae). *Weed Research* **46**: 163–174.

Lynch M., Milligan B.G. (1994) Analysis of population genetic structure with RAPD markers. *Molecular Ecology* **3**: 91–99.

Mace G.M., Purvis A. (2008) Evolutionary biology and practical conservation: bridging a widening gap. *Molecular Ecology* **17**: 9–19.

Magallón S., Gómez-Acevedo S., Sánchez-Reyes L.L., Hernández-Hernández T. (2015) A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytologist* **207**: 437–453.

Manel S., Schwartz M.K., Luikart G., Taberlet P. (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution* **18**: 189–197.

Mantel N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**: 209–220.

Maraghni M., Gorai M., Neffati M. (2010) Seed germination at different temperatures and water stress levels, and seedling emergence from different depths of *Ziziphus lotus*. *South African Journal of Botany* **76**: 453–459.

Mardis E.R. (2008) The impact of next-generation sequencing technology on genetics. *Trends in Genetics* **24**: 133–141.

Måren I.E., Karki S., Prajapati C., Yadav R.K., Shrestha B.B. (2015) Facing north or south: Does slope aspect impact forest stand characteristics and soil properties in a semiarid trans-Himalayan valley? *Journal of Arid Environments* **121**: 112–123.

Martins C.C., Martins D., Negrisoli E., Stanguerlim H. (2000) Seed germination of *Peschiera fuchsiaefolia*: effects of temperature and light. *Planta Daninha* **18**: 85–91.

May R.M., Godfrey J. (1994) Biological Diversity: Differences between Land and Sea. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **343**: 105–111.

McNeely J.A., Miller K.R., Reid W.V., Mittermeier R.A., Werner T.B. (1990) *Conserving the world's biological diversity*. International Union for the Conservation of Nature and Natural Resources, World Resources Institute, Conservation International, WWF-US and World Banks. pp. 174

Meyer S.E., Allen P.S., Beckstead J. (1997) Seed Germination Regulation in *Bromus tectorum* (Poaceae) and Its Ecological Significance. *Oikos* **78**: 475–485.

Meyer S.E., Kitchen S.G., Carlson S.L. (1995) Seed Germination Timing Patterns in Intermountain *Penstemon* (Scrophulariaceae). *American Journal of Botany* **82**: 377–389.

Migliore J., Baumel A., Juin M., Fady B., Roig A., Duong N., Médail F. (2013) Surviving in mountain climate refugia: new insights from the genetic diversity and structure of the relict shrub *Myrtus nivellei* (Myrtaceae) in the Sahara Desert. *PloS One* **8**: e73795.

Miller M., Holder M., Vos R., Midford P., Liebowitz T., Chan L., Hoover P., Warno T. (2010) The CIPRES Portals. URL: http://www.phylo.org/sub_sections/portal.

Miyamoto M.M., Fitch W.M. (1995) Testing the covarion hypothesis of molecular evolution. *Molecular Biology and Evolution* **12**: 503–513.

Mousseau T.A., Fox C.W. (1998) *Maternal Effects As Adaptations*. Oxford University Press.

Mráz P., Gaudeul M., Rioux D., Gielly L., Choler P., Taberlet P., IntraBioDiv Consortium (2007) Genetic structure of *Hypochaeris uniflora* (Asteraceae) suggests vicariance in the Carpathians and rapid post-glacial colonization of the Alps from an eastern Alpine refugium. *Journal of Biogeography* **34**: 2100–2114.

Muscarella R., Uriarte M., Erickson D.L., Swenson N.G., Zimmerman J.K., Kress W.J. (2014) A Well-Resolved Phylogeny of the Trees of Puerto Rico Based on DNA Barcode Sequence Data. *Plos One* **9**: e112843.

Nawash O., Shudiefat M., Al-Tabini R., Al-Khalidi K. (2013) Ethnobotanical study of medicinal plants commonly used by local bedouins in the badia region of Jordan. *Journal of Ethnopharmacology* **148**: 921–925.

Nevo E. (2001) Evolution of Genome-Phenome Diversity under Environmental Stress. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 6233–6240.

Nevo E., Baum B., Beiles A., Johnson D.A. (1998) Ecological correlates of RAPD DNA diversity of wild barley, *Hordeum spontaneum*, in the Fertile Crescent. *Genetic Resources and Crop Evolution* **45**: 151–159.

Nosrati H., Feizi M.A.H., Mazinani M., Haghighi A.R. (2012) Effect of population size on genetic variation levels in *Capparis spinosa* (Capparaceae) detected by RAPDs. *EurAsian Journal of BioSciences* **6**: 70–75.

Nybom H. (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* **13**: 1143–1155.

Oksanen J., Blanchet F.G., Kindt R., Legendre P., Minchin P.R., O'Hara R.B., Simpson G.L., Solymos P., Stevens M.H.H., Wagner H. (2013) Vegan: Community Ecology Package. R Package Version. 2.0-10.

Oran S. (1997) Nutlet anatomy of the genus *Salvia* L. in Jordan. *Flora Mediterranea* **7**: 27–40.

Oran S., Al-Eisawi D. (1998) Check-list of medicinal plants in Jordan. *Dirasat, Medicinal and Biological Sciences* **25**: 84–112.

Oxelman B., Lidén M., Berglund D. (1997) Chloroplastrps16 intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution* **206**: 393–410.

Pagel M., Lutzoni F. (2002) Accounting for phylogenetic uncertainty in comparative studies of evolution and adaptation. In: Lässig M, Valleriani A (eds) Biological Evolution and Statistical Physics. Springer Berlin Heidelberg, pp 148–161.

Park J.Y., Lee Y.P., Lee J., Choi B.S., Kim S., Yang T.J. (2013) Complete mitochondrial genome sequence and identification of a candidate gene responsible for cytoplasmic male sterility in radish (*Raphanus sativus* L.) containing DCGMS cytoplasm. *Theoretical and applied genetics* **126**: 1763–1774.

Patwardhan A., Ray S., Roy A. (2014) Molecular Markers in Phylogenetic Studies-A Review. *Journal of Phylogenetics & Evolutionary Biology* **2**: 131.

Pegtel D.M. (1985) Germination in Populations of *Solanum dulcamara* L. from Contrasting Habitats. *New Phytologist* **100**: 671–679.

Peleg Z., Saranga Y., Krugman T., Abbo S., Nevo E., Fahima T. (2008) Allelic diversity associated with aridity gradient in wild emmer wheat populations. *Plant, Cell & Environment* **31**: 39–49.

Pérez-Fernández M.A., Calvo-Magro E., Montanero-Fernández J., Oyola-Velasco J.A. (2006) Seed germination in response to chemicals: effect of nitrogen and pH in the media. *Journal of Environmental Biology* **27**: 13–20.

Petersen O. (1889) Marantaceae. In: Engler A, Parntl K (eds) Die natürlichen Pflanzenfamilien. Engelmann, Leipzig, pp 31–43.

Petit R.J., Duminil J., Fineschi S., Hampe A., Salvini D., Vendramin G.G. (2005) Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Molecular Ecology* **14**: 689–701.

Phillips O. (1997) Biodiversity and Conservation of Neotropical Montane Forests. *Biodiversity & Conservation* **6**: 1179–1180.

Pimm S.L., Russell G.J., Gittleman J.L., Brooks T.M. (1995) The Future of Biodiversity. *Science* **269**: 347–350.

Poe S. (1998) Sensitivity of Phylogeny Estimation to Taxonomic Sampling. *Systematic Biology* **47**: 18–31.

Posada D., Crandall K.A. (2001) Selecting the Best-Fit Model of Nucleotide Substitution. *Systematic Biology* **50**: 580–601.

Pramuk L.A., Runkle E.S. (2005) Photosynthetic Daily Light Integral during the Seedling Stage Influences Subsequent Growth and Flowering of *Celosia*, *Impatiens*, *Salvia*, *Tagetes*, and *Viola*. *HortScience* **40**: 1099–1099.

Prince L.M., Kress W.J. (2006a) Phylogenetic Relationships and Classification in Marantaceae: Insights from Plastid DNA Sequence Data. *Taxon* **55**: 281–296.

Prince L.M., Kress W.J. (2006b) Phylogeny and biogeography of the prayer plant family: getting to the root problem in Marantaceae. *Aliso* **22**: 643–657.

Pritchard J.K., Stephens M., Donnelly P. (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Qasem J.R. (2001) Allelopathic potential of white top and Syrian sage on vegetable crops. *Agronomy Journal* **93**: 64–71.

Qian X., Wang C., Tian M. (2013) Genetic Diversity and Population Differentiation of *Calanthe tsoongiana*, a Rare and Endemic Orchid in China. *International Journal of Molecular Sciences* **14**: 20399–20413.

Qiu Y.L., Lee J., Bernasconi-Quadroni F., Soltis D.E., Soltis P.S., Zanis M., Zimmer E.A., Chen Z., Savolainen V., Chase M.W. (1999) The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. *Nature* **402**: 404–407.

R Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rannala B., Yang Z. (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* **43**: 304–311.

Ricklefs R.E. (1987) Community Diversity: Relative Roles of Local and Regional Processes. *Science* **235**: 167–171.

Roach, Wulff R.D. (1987) Maternal Effects in Plants. *Annual Review of Ecology and Systematics* **18**: 209–235.

Roberson E. (2008) Medicinal plants at risk. Center for biological diversity, Tucson, AZ, USA.

Ronnenberg K., Wesche K., Hensen I. (2007) Germination ecology of Central Asian *Stipa spp*: differences among species, seed provenances, and the importance of field studies. *Plant Ecology* **196**: 269–280.

Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A., Huelsenbeck J.P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Systematic Biology* **61**: 539–542.

Rosenberg M.S., Kumar S. (2001) Incomplete taxon sampling is not a problem for phylogenetic inference. *Proceedings of the National Academy of Sciences* **98**: 10751–10756.

Rossiter M. (1996) Incidence and Consequences of Inherited Environmental Effects. *Annual Review of Ecology and Systematics* **27**: 451–476.

Rossiter M. (1998) The role of environmental variation in parental effects expression. In: Mousseau TA, Fox CW (eds) Maternal Effects as Adaptations. Oxford University Press, New York, pp 112–134.

Ruhfel B.R., Gitzendanner M.A., Soltis P.S., Soltis D.E., Burleigh J.G. (2014) From algae to angiosperms–inferring the phylogeny of green plants (Viridiplantae) from 360 plastid genomes. *BMC Evolutionary Biology* **14**: 23.

Sales N.M., Pérez-García F., Silveira F.A.O. (2013) Consistent variation in seed germination across an environmental gradient in a Neotropical savanna. *South African Journal of Botany* **87**: 129–133.

Sass C., Iles W.J.D., Barrett C.F., Smith S.Y., Specht C.D. (2016) Revisiting the Zingiberales: using multiplexed exon capture to resolve ancient and recent phylogenetic splits in a charismatic plant lineage. *PeerJ* **4**: e1584.

Savolainen V., Chase M.W. (2003) A decade of progress in plant molecular phylogenetics. *Trends in Genetics* **19**: 717–724.

Savolainen O., Lascoux M., Merilä J. (2013) Ecological genomics of local adaptation. *Nature Reviews Genetics* **14**: 807–820.

Scheepens J.F., Frei E.S., Armbruster G.F.J., Stöcklin J. (2012) Pollen dispersal and gene flow within and into a population of the alpine monocarpic plant *Campanula thyrsoides*. *Annals of Botany* **110**: 1479–1488.

Schemske D. (2002) Ecological and evolutionary perspectives on the origins of tropical diversity. In: Chazdon RL, Whitmore TC (eds) Foundations of Tropical Forest Biology. University of Chicago Press, pp 163–173.

Schwarz G. (1978) Estimating the Dimension of a Model. *The Annals of Statistics* **6**: 461–464.

Simmons M.P., Ochoterena H. (2000) Gaps as Characters in Sequence-Based Phylogenetic Analyses. *Systematic Biology* **49**: 369–381.

Simmons M.P., Pickett K.M., Miya M. (2004) How Meaningful Are Bayesian Support Values? *Molecular Biology and Evolution* **21**: 188–199.

Soltis D.E., Smith S.A., Cellinese N., Wurdack K.J., Tank D.C., Brockington S.F., Refulio-Rodriguez N.F., Walker J.B., Moore M.J., Carlsward B.S., Bell C.D., Latvis M., Crawley S., Black C., Diouf D., Xi Z., Rushworth C.A., Gitzendanner M.A., Sytsma K.J., Qiu Y.L., Hilu K.W., Davis C.C., Sanderson M.J., Beaman R.S., Olmstead R.G., Judd W.S., Donoghue M.J., Soltis P.S. (2011) Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany* **98**: 704–730.

Soltis P.S., Soltis D.E., Chase M.W. (1999) Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature* **402**: 402–404.

Sommer S., McDevitt A.D., Balkenhol N. (2013) Landscape genetic approaches in conservation biology and management. *Conservation Genetics* **14**: 249–251.

Song Y., Gao Z., Luan W. (2012) Interaction between temperature and photoperiod in regulation of flowering time in rice. *Science China Life Sciences* **55**: 241–249.

Sørensen T.J. (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. Kongelige Danske Videnskabernes Selskab, København.

Stein K., Rosche C., Hirsch H., Kindermann A., Köhler J., Hensen I. (2014) The influence of forest fragmentation on clonal diversity and genetic structure in *Heliconia angusta*, an endemic understory herb of the Brazilian Atlantic rain forest. *Journal of Tropical Ecology* **30**: 199–208.

Still D.W., Kim D.H., Aoyama N. (2005) Genetic Variation in *Echinacea angustifolia* Along a Climatic Gradient. *Annals of Botany* **96**: 467–477.

Storfer A., Murphy M.A., Spear S.F., Holderegger R., Waits L.P. (2010) Landscape genetics: where are we now? *Molecular Ecology* **19**: 3496–3514.

Suksathan P., Borchsenius F., Poulsen A.D. (2006) *Schumannianthus monophyllus* (Marantaceae)-A New and Unusual Species from Borneo. *Novon: A Journal for Botanical Nomenclature* **16**: 139–141.

Suksathan P., Gustafsson M.H., Borchsenius F. (2009) Phylogeny and generic delimitation of Asian Marantaceae. *Botanical Journal of the Linnean Society* **159**: 381–395.

Sullivan J., Swofford D.L. (2001) Should we use model-based methods for phylogenetic inference when we know that assumptions about among-site rate variation and nucleotide substitution pattern are violated? *Systematic Biology* **50**: 723–729.

Swarbrick J.T. (1971) External mucilage production by the seeds of British plants. *Botanical Journal of the Linnean Society* **64**: 157–162.

Swofford D.L. (2002) PAUP- Phylogenetic Analysis Using Parsimony. Sinauer Associates, Sunderland, Massachusetts.

Taberlet P., Gielly L., Pautou G., Bouvet J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105–1109.

Taberlet P., Zimmermann N.E., Englisch T., Tribsch A., Holderegger R., Alvarez N., Niklfeld H., Coldea G., Mirek Z., Moilanen A., Ahlmer W., Marsan P.A., Bona E., Bovio M., Choler P., Cieślak E., Colli L., Cristea V., Dalmas J.P., Frajman B., Garraud L., Gaudeul M., Gielly L., Gutermann W., Jogan N., Kagalo A.A., Korbecka G., Küpfer P., Lequette B., Letz D.R., Manel S., Mansion G., Marhold K., Martini F., Negrini R., Niño F., Paun O., Pellecchia M., Perico G., Piękoś-Mirkowa H., Prosser F., Puşcaş M., Ronikier M., Scheuerer M., Schneeweiss G.M., Schönswetter P., Schratt-Ehrendorfer L., Schüpfer F., Selvaggi A., Steinmann K., Thiel-Egenter C., van Loo M., Winkler M., Wohlgemuth T., Wraber T., Gugerli F., IntraBioDiv Consortium (2012) Genetic diversity in widespread species is not congruent with species richness in alpine plant communities. *Ecology Letters* **15**: 1439–1448.

Thompson P.A. (1975) Characterization of the germination responses of *Silene dioica* (L.) Claiv., populations from Europe. *Annals of Botany* **39**: 1–19.

Thompson J.D. (2005) *Plant Evolution in the Mediterranean*. Oxford University Press.

Thuiller W., Brotons L., Araujo M.B., Lavorel S. (2004) Effects of restricting environmental range of data to project current and future species distributions. *Ecography* **27**: 165–172.

Till-Bottraud I., Gaudeul M. (2002) Intraspecific genetic diversity in alpine plant. In: Körner C, Spehn M (eds) Mountain biodiversity: a global assessment. Springer, Parthenon, London, pp 23–34.

Tlig T., Gorai M., Neffati M. (2008) Germination responses of *Diplotaxis harra* to temperature and salinity. *Flora - Morphology, Distribution, Functional Ecology of Plants* **203**: 421–428.

Tomlinson P.B. (1961) Morphological and anatomical characteristics of the Marantaceae. *Journal of the Linnean Society of London, Botany* **58**: 55–78.

Toro M., Caballero A. (2005) Characterization and conservation of genetic diversity in subdivided populations. *Philosophical Transactions of the Royal Society B: Biological Sciences* **360**: 1367–1378.

Townsend J.P., Lopez-Giraldez F. (2010) Optimal selection of gene and ingroup taxon sampling for resolving phylogenetic relationships. *Systematic Biology* **59**: 446–457.

van Treuren R., Bijlsma R., van Delden W., Ouborg N. (1991) The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. *Heredity* **66**: 181–189.

Turpeinen T., Tenhola T., Manninen O., Nevo E., Nissilä E. (2001) Microsatellite diversity associated with ecological factors in *Hordeum spontaneum* populations in Israel. *Molecular Ecology* **10**: 1577–1591.

Ulubelen A. (2003) Cardioactive and antibacterial terpenoids from some *Salvia* species. *Phytochemistry* **64**: 395–399.

Ungar I.A. (1979) Seed Dimorphism in *Salicornia europaea* L. *Botanical Gazette* **140**: 102–108.

Ungar I.A. (1991) *Ecophysiology of Vascular Halophytes*. CRC Press, Boca Raton, FL.

Van Zandt P.A., Mopper S. (2004) The effects of maternal salinity and seed environment on germination and growth in *Iris hexagona*. *Evolutionary Ecology Research* **6**: 813–832.

Vance N. (2002) *Ecological Considerations in Sustainable Use of Wild Plants*. University Press, Kansas: pp 151–162.

Vekemans X., Beauwens T., Lemaire M., Roldán-Ruiz I. (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology* **11**: 139–151.

Ventura Y., Myrzabayeva M., Alikulov Z., Omarov R., Khozin-Goldberg I., Sagi M. (2014) Effects of salinity on flowering, morphology, biomass accumulation and leaf metabolites in an edible halophyte. *AoB Plants*: plu053.

Vergeer P., Kunin W.E. (2011) Life history variation in *Arabidopsis lyrata* across its range: effects of climate, population size and herbivory. *Oikos* **120**: 979–990.

Vézquez D.P., Gittleman J.L. (1998) Biodiversity conservation: Does phylogeny matter? *Current Biology* **8**: R379–R381.

Via S., Lande R. (1985) Genotype-Environment Interaction and the Evolution of Phenotypic Plasticity. *Evolution* **39**: 505–522.

Volis S. (2007) Correlated patterns of variation in phenology and seed production in populations of two annual grasses along an aridity gradient. *Evolutionary Ecology* **21**: 381–393.

Wang I.J., Bradburd G.S. (2014) Isolation by environment. *Molecular Ecology* **23**: 5649–5662.

Wang H.W., Ge S. (2006) Phylogeography of the endangered *Cathaya argyrophylla* (Pinaceae) inferred from sequence variation of mitochondrial and nuclear DNA. *Molecular Ecology* **15**: 4109–4122.

Wheeler J.A., Schnider F., Sedlacek J., Cortés A.J., Wipf S., Hoch G., Rixen C. (2015) With a little help from my friends: Community facilitation increases performance in the dwarf shrub *Salix herbacea*. *Basic and Applied Ecology* **16**: 202–209.

Whittaker R.J., Ladle R.J. (2011) The Roots of Conservation Biogeography. In: Ladle RJ, Whittaker RJ (eds) Conservation Biogeography. John Wiley & Sons, Ltd, pp 1–12. 2016).

Wicke S., Costa A., Muñoz J., Quandt D. (2011) Restless 5S: The re-arrangement(s) and evolution of the nuclear ribosomal DNA in land plants. *Molecular Phylogenetics and Evolution* **61**: 321–332.

Wilcock C., Neiland R. (2002) Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science* **7**: 270–277.

Wolfe K.H., Gouy M., Yang Y.W., Sharp P.M., Li W.H. (1989) Date of the monocot-dicot divergence estimated from chloroplast DNA sequence data. *Proceedings of the National Academy of Sciences of the United States of America* **86**: 6201–6205.

World Bank (2003) Jordan - Conservation of Medicinal and Herbal Plants Project. The World Bank, Washington, DC. http://documents.worldbank.org/curated/en/864781468752124171/Jordan-Conservation-of-Medicinal-and-Herbal-Plants-Project.

Wu Z., Yu D., Li X., Xu X. (2016) Influence of geography and environment on patterns of genetic differentiation in a widespread submerged macrophyte, Eurasian watermilfoil (*Myriophyllum spicatum* L., Haloragaceae). *Ecology and Evolution* **6**: 460–468.

Yang Z. (1996) Phylogenetic analysis using parsimony and likelihood methods. *Journal of Molecular Evolution* **42**: 294–307.

Yang X., Baskin C.C., Baskin J.M., Liu G., Huang Z. (2012) Seed Mucilage Improves Seedling Emergence of a Sand Desert Shrub. *Plos One* **7**: e34597.

Yang X., Zhang W., Dong M., Boubriak I., Huang Z. (2011) The Achene Mucilage Hydrated in Desert Dew Assists Seed Cells in Maintaining DNA Integrity: Adaptive Strategy of Desert Plant *Artemisia sphaerocephala*. *Plos One* **6**: e24346.

Yao S., Lan H., Zhang F. (2010) Variation of seed heteromorphism in *Chenopodium album* and the effect of salinity stress on the descendants. *Annals of Botany* **105**: 1015–1025.

Zarza E., Reynoso V.H., Emerson B.C. (2008) Diversification in the northern neotropics: mitochondrial and nuclear DNA phylogeography of the iguana *Ctenosaura pectinata* and related species. *Molecular Ecology* **17**: 3259–3275.

Zohary M. (1973) *Geobotanical Foundations of the Middle East*. CRC Press, Stuttgart.

Zohary M., Feinburn D. (1966) *FLORA PALAESTINA, Part Three: TEXT*. Israel Academy of Sciences and Humanities, Jerusalem.

Zwickl D.J., Hillis D.M. (2002) Increased taxon sampling greatly reduces phylogenetic error. *Systematic Biology* **51**: 588–598.

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APPENDIX 1

PUBLICATIONS OF THE DISSERTATION

Al-Gharaibeh M, Hamasha H, Lachmuth S, & Hensen I (2016) Local adaptation to different phytogeographic regions: habitat-related variations in seed germination in response to temperature and salinity for two medicinal *Salvia* species from Jordan. ***Plant Species Biology***. doi: 10.1111/1442-1984.12123

Al-Gharaibeh M, Hamasha H, Rosche C, Lachmuth S, Wesche K & Hensen I (submitted 23.03.2016) Environmental gradients shape the genetic structure of two medicinal *Salvia* species in Jordan. ***Plant Biology****.*

Al-Gharaibeh M, Borchsenius F, McKechnie L, Sanmartin I, & Ley A (manuscript) Phylogeny of the pantropically distributed family Marantaceae.

OTHER PUBLICATIONS BY THE AUTHOR

2015:

Albach, D. C., and **Al-Gharaibeh, M.** (2015). Systematics and biogeography of *Veronica* subg. Pentasepalae from the Levant. ***Willdenowia***. 45: 5–14.

Alali, F., El-Elimat, T., Albataineh, H., Al-Balas, Q., **Al-Gharaibeh, M.,** Falkinham III, J. O., Swanson, S. M., and Oberlies, N. H. (2015). Cytotoxic Homoisoflavones from the Bulbs of *Bellevalia eigii*. ***Journal of Natural Products***. 78: 1708-1715.

2014:

Alali, F. Q., Amrine, C.S., El-Elimat, T., Alkofahi, A. S., Tawaha, K., **Al-Gharaibeh, M.,** Swanson, S. M., Falkinham III, J. O., Cabeza, M., Sánchez, A., Figueroa, M., and Oberlies, N. H. (2014). Bioactive withanolides from *Withania obtusifolia*. ***Phytochemistry letters***. 9: 96-101.

2013:

Alali, F. Q., Njadat, R. M., Mhaidat, N. M., Alkofahi, A. S., **Al-Gharaibeh, M.,** and El-Elimat,T. (2013). Alkaloids from *Glaucium aleppicum* Papaveraceae. ***Jordan Journal of Pharmaceutical Sciences***. 6:308-313.

2012:

El-Alali, A., Al Zoubi, A., **Al-Gharaibeh, M.,** Tawaha, K. and Alali, F. Q. (2012) Phytochemical and Biological Investigation of *Nitraria retusa* Asch. ***Jordan Journal of Pharmaceutical Sciences***. 5:155-163.

2011:

Al-Gharaibeh, M. In: W. Greuter & T. Raus (eds.) (2011). *Rosa pulverulenta* M. Bieb, Med-checklist Notulae 30. ***Willdenowia*** 41: 323.

2010:

Alhamad, M.N., Oswald, B.P., Bataineh, M.M., Alrababah, M.A. and **Al-Gharaibeh, M.** (2010) Relationships between herbaceous diversity and biomass in two habitats in arid Mediterranean rangeland. ***Journal of Arid Environment***. 74:277-283.

Tawaha, K., **Al-Gharaibeh, M.,** El-Elimat, T. and Alali, F. Q. (2010) Determination of Hypericin and Hyperforin Content in Selected Jordanian *Hypericum* Species. ***Industrial Crops and Products***. 32: 241-245.

2009:

Alali, F. Q., Tawaha, K. and **Al-Gharaibeh, M.** (2009) LC-MS and LC-PDA Analysis of *Hypericum empetrifolium* and *Hypericum sinaicum*. Zeitschrift für Naturforschung C, ***Journal of biosciences***. 64:476-82.

Lahham, J. and **Al-Gharaibeh, M.** (2009) An Ecosystematic study of a semi-arid ecotone in the northern part of Jordan. ***The Arab Journal for Arid Environment***. 2:1-13.

2007:

Alrababah, M., Alhamad, M., Suwaileh, A and **Al-Gharaibeh, M.** (2007) Biodiversity of semi-arid Mediterranean grassland: Impact of grazing and afforestation. ***Applied Vegetation Science***. 10:257-264.

Tawaha, K., Alali, F. Q., **Al-Gharaibeh, M.,** Mohammad, M. and El-Elimat, T. (2007) Antioxidant activity and total phenolic content of selected Jordanian plant species. ***Food Chemistry*** 104:1372-1378.

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Languages

- Arabic Language (Native speaker).
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- Field manager of scientific J.U.S.T forestry arboretum, Jordan University of Science and Technology. Dep. of Natural Resources and Environment. 2002-2012.
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Training Courses

- West Asia Red List Training of Trainers: Jan, 17th -21st January 2011, IUCN, International Union for the Conservation of Nature-Regional Office for West Asia (ROWA), Dead Sea, Jordan.
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Fellowships and Awards

- Research fellowship for project Bridging the Rift (BTR)/ Ecology and Biodiversity of Wadi Araba, Stanford University, California, USA, 2008.
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Conferences

- First International Congress, Documenting, Analyzing and Managing Biodiversity in the Middle East, Intercontinental Hotel, Aqaba, Jordan, 20 th -23 th, October, 2008.
- Phylogenetic symposium “The time for phylogenetics: inferring and applying time trees in evolutionary biology", 22nd to 24th November 2013, Carl von Ossietzky Universität, Oldenburg, Germany.

APPENDIX 2

Declaration of own contributions to the original article /Erklärung über den persönlichen Anteil an den Publikationen

Study (Chapter 2):

Al-Gharaibeh M, Hamasha H, Lachmuth S, & Hensen I (2016) Local adaptation to different phytogeographic regions: habitat-related variations in seed germination in response to temperature and salinity for two medicinal *Salvia* species from Jordan. ***Plant Species Biology***. doi: 10.1111/1442-1984.12123

Field work:	**Mohammad Al-Gharaibeh:** 100 %
Laboratory work:	**Mohammad Al-Gharaibeh:** 90 %
Analysis:	**Mohammad Al-Gharaibeh:** 50 % (Susanna Lachmuth 50 %)
Writing:	**Mohammad Al-Gharaibeh:** 80 % (Corrections by all co-authors)

Study (Chapter 3):

Al-Gharaibeh M, Hamasha H, Rosche C, Lachmuth S, Wesche K & Hensen I (in review) Environmental gradients shape the genetic structure of two medicinal *Salvia* species in Jordan. ***Plant Biology****.*

Field work:	**Mohammad Al-Gharaibeh:** 100 %
Laboratory work:	**Mohammad Al-Gharaibeh:** 90 %
Analysis:	**Mohammad Al-Gharaibeh:** 70 % (Susanna Lachmuth, Karsten Wasche and Christoph Rosche 30 %)
Writing:	**Mohammad Al-Gharaibeh:** 80 % (Corrections by all co-authors)

Study (Chapter 4):

Al-Gharaibeh M, Borchsenius F, McKechnie L, Sanmartin I, & Ley A (manuscript) Phylogeny of the pantropically distributed family Marantaceae.

Genebank sequences assembly:	**Mohammad Al-Gharaibeh:** 100 %
Laboratory work:	Alexandra Ley: 20 %, Co-authors: 80 %
Phylogenetic Analysis:	**Mohammad Al-Gharaibeh:** 100 %
Writing:	**Mohammad Al-Gharaibeh:** 80 % (Alexandra Ley 20%)

Declaration of self-contained work / Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit nicht bereits zu einem früheren Zeitpunkt der Naturwissenschaftlichen Fakultät I – Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg oder einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde. Darüber hinaus erkläre ich, dass ich die vorliegende Arbeit eigenständig und ohne fremde Hilfe verfasst sowie keine anderen als die im Text angegebenen Quellen und Hilfsmittel verwendet habe. Textstellen, welche aus verwendeten Werken wörtlich oder inhaltlich übernommen wurden, wurden von mir als solche kenntlich gemacht. Im Übrigen erkläre ich, dass ich mich bisher noch nie um einen Doktorgrad beworben habe.

Halle (Saale), den

Unterschrift: .....

(Mohammad Al-Gharaibeh)